Application of metabolomics to the analysis of ancient organic residues

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Abstract

The grape is arguably one of the oldest cultivated products in human history and the analysis of its main product, wine, reveals clues to trade and associations of previous civilizations. In ancient times, wine was stored in clay amphorae, which, if not properly sealed with resin or pitch allowed the wine to wick into clay matrices, dry, and polymerize producing insoluble, intractable materials that may remain within the matrix for several thousand years. Presently, identification of wine residue is based upon the extraction of these polymeric materials from the ceramic matrix and analyzing/identifying the chemical fingerprints.

Two main biomarkers have historically been employed for the identification of wine residue: tartaric and syringic acids. In some cases, the presence of one of these biomarkers has been designated as the confirmatory signature of wine often leading to false positives as amphorae were re-used in antiquity. Herein, a novel approach utilizing metabolomics has been applied to archaeological objects in order to further mine possible biomarkers for a more accurate assessment of the original foodstuff. An untargeted metabolic profiling method was combined with a targeted analytical method resulting in the successful validation of eight representative biomarkers in two separate archaeological sites.

Acknowledgements

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List of Abbreviations

ANOVA	Analysis of Variance
B.C.E.	Before Christian era
B.P.	Before present
CV	Coefficient of variation
DAG	Diacylglycerol
DHB	Dihydrobenzoic
DIMS	Direct Infusion (Mass Spectrometry)
EIC	Extracted ion chromatogram
FT-ICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography-mass spectrometry
g-log	Generalized – log
H	hour
HCl	Hydrochloric acid
HESI	Heated electrospray ionization source
HILIC	Hydrophilic Interaction liquid chromatography
HPLC	High Performance Liquid Chromatography
IT	Ion trap
KCl	Potassium chloride
KOH	Potassium hydroxide
LC-MS	Liquid chromatography-mass spectrometry
LTQ-FT-ICR-MS	Linear ion trap Fourier transform ion cyclotron resonance mass spectrometry
ml	Millilitre

mm	Millimetre
mM	Millimolar
MS	Mass spectrometry
MS/MS	Tandem Mass Spectrometry
m/z	mass/charge
N	Normal
NCE	Normalized collision energy
PPM	Parts per million
PSI	Pound-force per square inch
QC	quality control
RPM	revolutions per minute
RSD	Relative standard deviation
RT	Retention time
S	seconds
SDS	Sodium dodecyl sulfate
SIM	Selected Ion Monitoring
SNR	Signal to Noise Ratio
SOP	Standard operating procedure
SPE	Solid phase extraction
TAG	triacylglycerol
TIC	Total ion chromatogram
µl	Microlitre
v/v	Volume to volume

1. Introduction

In this thesis, a metabolomics approach to analytical testing was applied to the identification of organic residue in archaeological artifacts. The purpose was to develop a suite of biomarkers, in particular, for the identification of aged wine residue. The approach included a global untargeted analysis to model sherds in order to discern indicative biomarkers for materials aged under laboratory conditions and then apply those newly found biomarkers using a highly selective, targeted analytical method to archaeological artifacts. The extraction steps applied in this analysis produced three separate aliquots: polar, non-polar, and an alkaline fusion aliquot. However, an attempt was made to limit sample manipulation in order to increase the sensitivity for trace analyses of ancient organic residues.

The analysis of archaeological residues by chemical testing unofficially began in 1970 with the gas chromatography analysis of bog butter by Thornton, et al. (Thornton, et al., 1970). Bog butter was an ancient form of lard found in the British Isles; the waxy material that remained was analysed for its fatty acid constituents in order to identify the lipid origins. This was the first successful application of analytical chemistry to the study of archaeological materials. The hydrophobic lipid constituents proved a very useful material for analysis, as the fatty substituents were impervious to groundwater leaching and therefore lasted for several millennia, although in many circumstances the chemical fingerprint represented the degraded or altered version of the original. Modern day gas-chromatography in combination with mass spectrometry, as well as gas chromatography/combustion/isotope ratio monitoring mass spectrometry are currently utilised for the determination of the archeological fats in an attempt to identify the original plant or animal sources. The results of these types of analyses answer long standing archaeological questions which include the start of dairying in Britain, the effect of Roman rule on British diets during the Iron Age,

and the prevalence of pork production in the Neolithic era (Copley et al., 2005; Redfern et al., 2012; Mukherjee et al., 2008).

The analysis of fermented beverages in archaeological materials has not been as successful and suffers from the lack of a consistently applied analytical method. In 1993, an article in *Analytical Chemistry* detailed the analysis of amphorae from an early Mesopotamian society, dating from 3500 B.C. and 2900 B.C. and located in the western part of Iran (Michel et al., 1993). Sherds taken from representative amphorae were extracted in boiling solvent; the residue was analysed by Fourier transform infrared spectroscopy (FTIR) and identified as a calcium tartrate salt, purportedly the altered form of tartaric acid, a common acid found in grape products. The results are questionable since FTIR is a gross analytical technique often used for the initial determination of chemical classes such as resins, waxes, proteins, oils, and fats (Regert, 2011). Also, there is a great risk of confusing the infrared pattern of calcium tartrate with calcium oxalate, a common mineral deposition found on ancient pottery and stone. Therefore, the use of FTIR should be relegated to an initial survey of materials rather than as the definitive descriptor. However, FTIR was again used to determine a tartrate salt and ultimately the presence of wine in pottery dating c.6200-5800 B.C from the Hunan province in China (McGovern et al., 2004).

A substantial improvement in the analytical methodology applied to the analysis of archaeological wine within the past decade was the application of highly sensitive instruments including liquid and gas chromatography combined with mass spectrometry. In 2004, a group of researchers applied reversed phase liquid chromatography in combination with triple quadrupole mass spectrometry to a dried remnant of (presumably) wine from the 18th Dynasty of the Egyptian Kingdom (Guasch-Jane et al., 2004). Tartaric acid and syringic acid were both reported, strongly indicating the presence of wine. However, a separate research group at the University of California was unable to repeat the 2004 published methods and in 2010 published their own methodology for the analysis of

archaeological wine (Barnard et al., 2010). The researchers developed a new LC-MS/MS method using reversed phase liquid chromatography to analyse the samples from artifacts dating to 4100 B.C.E. and ultimately identified syringic acid. Recently, a new analytical method was developed that utilises gas chromatography-mass spectrometry. Using GC-MS, researchers have identified several characteristic acids including tartaric acid indicative of the presence of wine in archaeological plasters (Pecci et al., 2013). Although the method offers an improvement in the number of markers identified, the authors ascribe the origin of tartaric acid to a salt or to the free acid that has been preserved over time. This origin seems unlikely and the preservation of a small organic acid over an archaeological lifetime is more likely due to esterification within a polymeric network. A separate method employing GC-MS utilised a dual solvent extraction with heat on intact sherds to remove tartaric and syringic acids (Koh and Betancourt, 2010). The method was applied to a more recent group of artifacts by the same researcher and the results were nearly 100% positive for tartaric acid, and only slightly less for syringic acid (Koh et al., 2014). These unusually successful results certainly require further investigation.

Due to the chemical complexity of wine and its multiple constituents, section 1.3 of this introduction discusses the building blocks of wine (Cooper and Marshall, 2001). Based upon the enological studies on the polymerisation of wine over time, the discussion also includes how a polymeric network is formed during aging. It is assumed that a similar polymerisation has occurred in antiquity thereby allowing a polymeric network to reside within a clay matrix for an extended period of time. Sections 1.1 and 1.2 briefly describe the production of wine in ancient time and introduce the two archeological sites explored in this work. Section 1.4 introduces the triacylglycerol analysis of the VIndolanda samples. Section 1.5 briefly discusses the metabolite extraction procedures, with a focus on the alkaline attack utilised to break apart the polymeric network; sections 1.6 and 1.7 discuss the analytical and statistical procedures applied in this research. The final objectives are then described in section 1.8.

1.1 Introduction to Ancient Wine

The grape vine (*Vitis vinifera*) is one the oldest cultivated crops in human history having been domesticated from the original wild vine nearly 8000 years before present (This et al., 2006). Archaeological evidence and genetic determination suggest the domesticated western grape vine originated from the wild type (*Vitis vinifera*, subspecies *sylvestris*) in Transcaucasia, spreading to the Mediterranean through the Iberian peninsula and into Europe often transported by the major empires of the day, the Phonecians, the Carthaginians, and the Romans (McGovern, 2003: 39; Myles et al., 2011). Archaeobotanical evidence of a domesticated grape vine was excavated from a Neolithic site in Georgia, south of the modern day capital, Tblisi (McGovern, 2003: 23). Dated to 6000 B.C. by radiocarbon dating, this represents the earliest archaeological evidence of the domesticated grape vine. Later excavations unearthed the oldest (currently) large scale production of wine in neighboring Armenia dated to approximately 4100 B.C.E. (Barnard et al., 2010). At this site were the archaeobotanical remains of grapes including grape vines and grape seeds surrounding a press. This find pre-dates the next oldest site of wine production and consumption, 3150 B.C., from the tomb of Scorpion 1 one of the very earliest Egyptian kings. Grape seeds, skins, and dried pulp were identified in the tomb. The function of wine in these contexts is at present purely speculative and thought to support ceremonial or religious functions.

Due to the time required to nurture the young vines, ancient vineyards were developed in stable societal structures in order to provide the several years necessary for cultivation. The production of wine from those grapes was often organised around substantial infrastructure including immovable plaster bases or limestone monolith wine presses, as well as transport amphora (van Dommelen and Bellard, 2008). When the grapes were harvested, the vineyard workers would crush the grapes underfoot (tread). In the case of a two tier wine press (Figure 1.1), the grapes were crushed on the upper portion of the press allowing the juice to flow through an opening or gutter into the lower

portion in preparation for storage and transport in clay amphorae. Grapes are fruits that are high in sugar, with glucose and fructose accounting for approximately 20-25% of the berry's weight (Cheynier et al., 2010). Once crushed the uptake of glucose by the yeast *Saccharomyces cerevisiae* (naturally occurring on the surface of the grapes) initiates a cascade of reactions resulting in the production of ethanol (Ribereau-Gayon et al, 2006: 56).

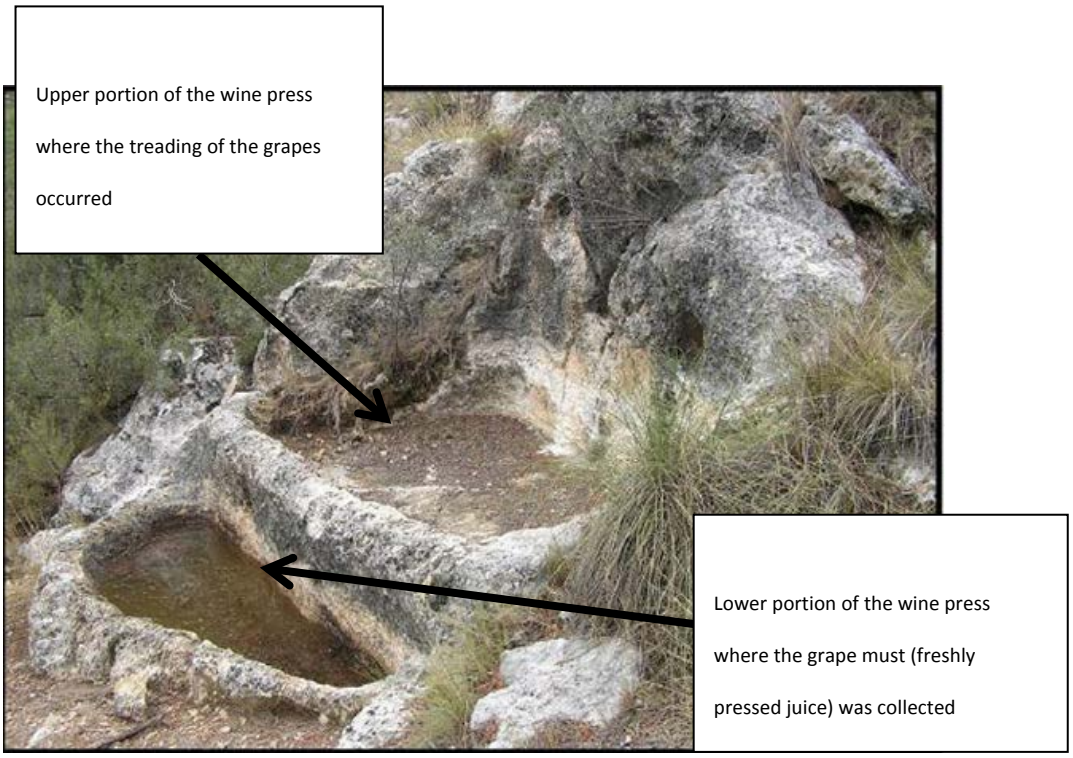


Figure 1.1. An intact double wine press in Valencia, Spain, active between 5th to 3rd centuries B.C.E. (Pérez Jordà et al., 2011).

Besides the ethanol content of the wine, its organoleptic properties depend upon very reactive molecules originating from the flesh, skin, and seeds of the grape. The monomeric species, once extruded from the grape during crushing, interact chemically with one another to form polymeric compounds. This translates to a change in color, for example, from orange-red to reddish-brown, accompanied by a change in taste (Waterhouse and Kennedy, 2004:144). The resulting polymers develop into an intractable material, often precipitating out of the liquid. It is, therefore, not so

preposterous to theorise that ancient wine may still exist as a long lasting polymeric network stored within the interstices of the amphora's clay matrix. In order to determine the presence or absence of wine in an ancient artifact, the presumed polymer can be broken down chemically thereby releasing the original monomeric species.

1.2 Archaeological Samples

Two archaeological sites were chosen for the targeted biomarker analysis. The first site in Sardinia, Italy, experiences a dry climate with sandy soil in an area known for a history of wine production. The actual archaeological site is undisturbed and there were limited vegetal remains beyond carbonised grape pips. This site suggests limited interaction with other foodstuffs such as olives or cereals. The second site at Vindolanda, Northumberland, was a military/civilian encampment with a wet environment and heavy, peat soil. The materials gathered from this excavation included vegetal and animal remains suggesting a site that was inherently multi-use. Sardinia offered the optimum site for the application of this newly developed suite of biomarkers, whereas Vindolanda offered a further test of the robustness in the application of the same set of biomarkers due to the intrinsic nature of a multi-use environment.

1.2.1 The History of Grape Cultivation and Production of Wine in Sardinia

Sardinia is an island in the Mediterranean well known for its Nuragic civilization, c. 3700 B.C.E. and famous for the prehistoric settlements containing nuraghi, large square towers that dot the island's landscape which remain to this day. The island was overrun several times in the ancient world, by the Phoenicians in the 8th century B.C.E., the Carthaginians in 510 B.C.E., and then the Romans in 238 B.C.E. Sardinia was the 'breadbasket' for these ruling nations, its agricultural economy fueled by the Mediterranean climate with hot and dry summers and an average rainfall of 500 mm. Sandy soil in the southern part of the island is useful for growing grapes, many wild types of which are found on

the island. However, the exponential growth of vineyards determined from archaeobotanical research indicates the greatest evidence of *Vitis* domestication occurred from the middle Bronze Age to the start of Punic times, 3600-2300 B.C.E., a timeline that overlaps with the influx from Phoenicia (Di Rita and Melis, 2013).

1.2.1.1 The Punic Rural Settlement, Terralba, Sardinia

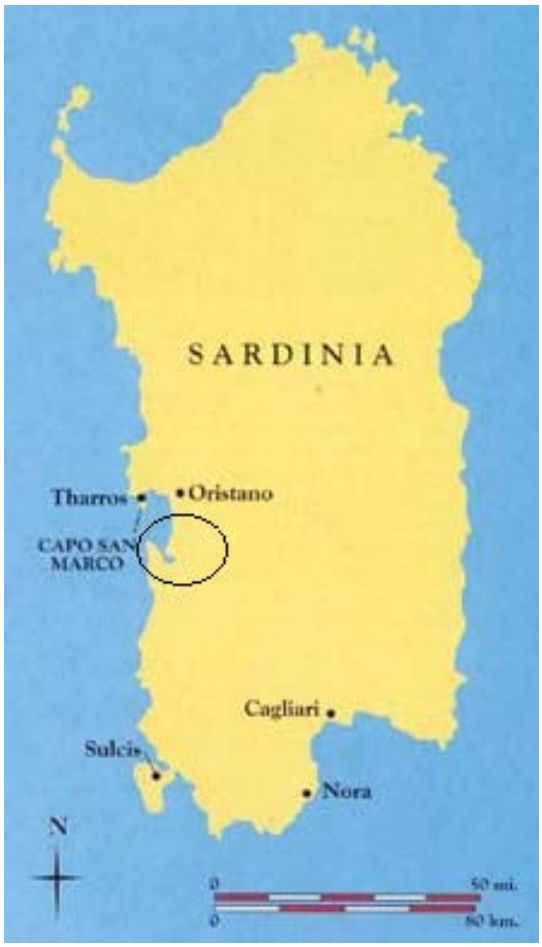


Figure 1.2. The location of the Terralba rural district circled on the map in the southern portion of Oristano Bay, Sardinia, Italy (reproduced from http://members.bib-arch.org/publication.asp?PubID=BSBA&Volume=16&Issue=1&ArticleID=5).

In 2002, the Terralba rural research project began excavating in rural southwestern Sardinia, Figure 1.2, in order to understand the agricultural underpinnings of Punic Sardinia, particularly in relation to the larger Carthaginian economy. Whereas earlier archaeological teams focused on the urban centres created under Phoenician and Roman rule, these excavations were focused on the rural

villages. By the mid 6th century B.C.E., there was an increase in Carthaginian settlements and the number of rural settlements in southwestern Sardinia increased measurably towards the end of the 5th century B.C.E. (van Dommelen and Bellard, 2008). Over several years, the team studied ten rural sites in the western coast of Sardinia, south of the Bay of Oristano.

In the summer of 2007, an area known as Trunce e' Molas, a Punic farmstead active from the 5th to the 2nd century B.C.E. was excavated. The materials collected from the site numbered 14,500 and included iron tools (including sickles), coins, and pottery. Vegetal remains consisted mainly of carbonised grape pips, with some beet root; however, cereals were notably absent from the site. The archaeologists determined three phases of habitation within Trunc e' Molas. Phase I, from 2nd century B.C.E. to the present day, was seriously disrupted by modern ploughing that overturned the soil and damaged stone walls from earlier inhabitation. Phase I also included trenches dug for modern vineyards that had since been pulled. No samples were taken from Phase I because of the disruption of the site and because of its intermingling with modern viticulture. Phase II represented the human inhabitation between the 4thand 2nd century B.C.E. Although there was some intermingling between phase I and II, the lower portions of phase II were considered secure, separated from modern materials. Phase III consisted of layers of sterile sand where no human habitation was found or preserved.

Excavated from Phase II were two large basins thought to be wine presses due to their sheer size as well as the numerous carbonised grape pips surrounding the area, Figure 1.3. The basins were well preserved and untouched by the modern ploughing. The two large (0.9 mx1.4 m) basins were described as coeval, but with differing methods of construction. One basin was carved from a limestone monolith whereas the second was built with stones and finished with thick layers of plaster and a thinner upper coating of earthenware, referred to as the 'preparatory layer.' A lip around the edge suggests the basins were used to hold liquid. Based upon other archaeological

excavations, grape presses were rare in Punic settlements, particularly when compared to the proliferation of grape presses under Roman rule (van Dommelen and Bellard, 2008). Of the presses that were excavated in Punic establishments, there were three basic types: an exterior platform with a lime and sand mortar coating, lime coating over an interior platform, and a platform carved directly in the rock (Jordà et al., 2011).

The wine press excavated at Trunce e' Molas might represent the lower level of a two-tiered wine press; the upper level is believed not to have survived (personal communication: Prof. P. van Dommelen). Again, the fruit was placed on the upper level of the double wine press where it was crushed by treading. The extracted juiced flowed down into the lower basin, from which it would await transfer into clay amphorae for fermentation or immediate trade. Well-preserved double platforms for a wine press have been found in the Iberian coast at Alt de Benimaquia on Valencia also dating from between the 4thand 2nd century B.C.E. (Jordà et al., 2011).

Figure 1.3. The double collection basins excavated in 2007 by the Terralba rural project (van Dommelen, et al., 2007).

The excavated pottery found in Phase II included domestic ware, pieces of amphorae, and three nearly intact amphorae found in a well. Based upon macroscopic and microscopic examination including petrographic thin sections, two types of pottery fabric were excavated from this site (personal communication, Dr. Helen Loney). The sherds examined in this analysis were defined as Fabric B, a granular calcareous matrix with mica inclusions. The color of the fabric varied from pale brown to yellow to pink. The calcareous material was not local to Terralba, but thought to originate from western central Sardinia near the coast (van Dommelen and Trapichler, 2011).

1.2.2 The Vindolanda Settlement

The Vindolanda settlement located in modern day Northumberland, England, was settled in 85 A.D. by Roman soldiers stationed there to guard the Stanegate Road, the *de facto* frontier line prior to the establishment of Hadrian's Wall 37 years later (Figure 1.4). The settlement grew and receded in size over its 500 year history, with the greatest expansion to 8 acres during the years of 105-120 A.D. (Birley, 2010). Roughly nine Roman military forts have been excavated within the settlement, each fort representing a specific period. Archaeological excavations have also unearthed the remnants of a vibrant civilian encampment along the perimeter of the fort walls that included pottery, glass, metal workings, leather footwear and horse reins, jewelry, coins, and sundry objects representative of daily life.

Figure 1.4. A map of the England and Scotland border, with the location of Vindolanda settlement and Hadrian's wall.

The most famous archaeological finds are the Vindolanda Tablets, the earliest examples of handwritten documentation in Britain. The tablets are pieces of thin wood shavings similar to veneer, written in Latin with carbon based ink (Figure 1.5). Many of the tablets were excavated in 1973-1975 under the direction of Dr. Robin Birley, The Vindolanda Trust, and originate from the 1st and the 2nd centuries A.D. Translation of the tablets reveal soldiers' letters home, invitations to social gatherings, dinner menus for high ranking officials, as well as general grocery lists.

Figure 1.5. Vindolanda Tablet 203 representing a menu, or grocery list: pork cutlet, bread, wine, olive oil. Reproduced from Vindolanda Tablets online: http://vindolanda.csad.ox.ac.uk/TVII-203.

The landscape around the fort (and then Hadrian's Wall) was open pasture land, suggested by pollen analysis from cored soil samples; the silver birch woodlands were already cleared by the 2500-1500 B.C.E. (early Bronze Age) and land use under the Roman occupation resulted in an increase in the cultivation of rye, *Secale cereale* (Dark, 2005). A multi-use product, the grain was often used for animal feed and stock supplies for the soldiers, whereas the plant was used for thatch work (Alcock, 2001:18). The main cereal for the Roman soldier was, however, wheat used for making bread (*Triticum aestivum*). Other foodstuffs included locally sourced meat and dairy from cattle, sheep, goat, and pig. Supplementing the soldiers' diet were provisions brought from Gaul, Italy and Spain, including walnuts, beer, fish sauce, olive oil, and wine (Alcock, 2001: 27-89). The civilians living and working around the fort were the probable providers of the local foodstuffs, through herding and farming on land leased to them by the military (Davies, 1971).

For this thesis, samples were gathered during an active excavation in late spring/early summer 2012 and represent amphorae, mortaria, and cooking vessels. All samples gathered represent Periods VII (212-280A.D.), VIII (300-367A.D.) and represent a mixture of military and civilian environs (Birley, 2010). Area A represents a military fort originally constructed in 213 A.D., rebuilt in the 4th century,

and includes the centurion's apartment as well as general soldiers' barracks. Area B represents the civilian encampment active from 213-300 A.D. All contexts contained within each area that pertain to the samples analysed were described by Kate Sheehan-Finn, the Archaeological and Research Assistant, and are listed in Chapter 6.

1.3 Building Blocks of Wine and Polymer Formation

As mentioned earlier, wine is a chemically complex material and is composed of acids, flavonols, anthocyanidins, and tannins. The combinations of these materials maintain the polymeric network described in the following section.

1.3.1 Acids

Organic acids found in the must or the body of the grape gives wine its acidity and tartness; malic acid and tartaric acid account for the majority, contributing approximately 90% of the acidic content (Dinsmore-Webb, 1974:17). Citric, lactic, and succinic acids are also important acidic constituents of wine, with citric originating in the grape must and succinic and lactic acids originating as major byproducts from the fermentation process, Figure 1.6 (Thoukis et al.,1965; Kliewer, 1966).

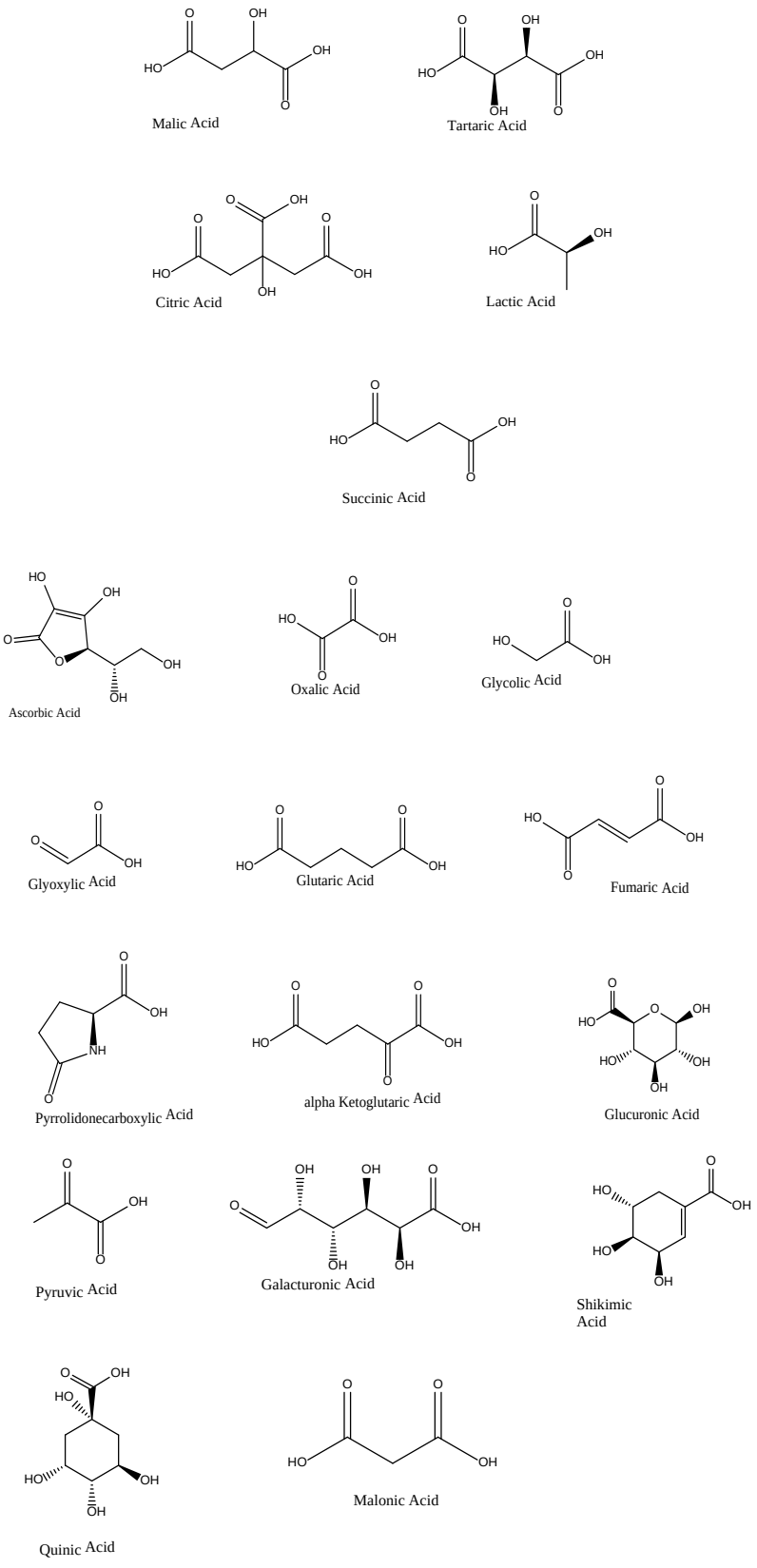
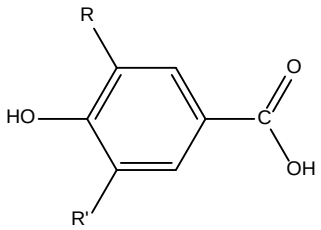


Figure 1.6. Representative organic acids found in wine; malic and tartaric acid constitute 90% of the wine's acidity.

Phenolic acids also originate from the flesh of the grape and are classified as either benzoic acids or cinnamic acids (Figure 1.7 and 1.8); there is a subset of cinnamic acids preferentially esterified with tartaric acid known as hydroxycinnamoyl tartrates (Ribereau-Gayon, 1972:87; Flamini, 2003; Ong and Nagel, 1978). In wine, these tartrates (Figure 1.9) are the most abundant phenolic group and are found in both red and white grapes (Ferrandino et al., 2012).

Benzoic Acids



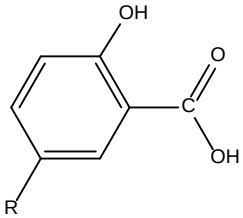


Figure 1.7. Carbon skeletons of benzoic acids.

R=R'=H: p-hydroxybenzoic acid

R=OH, R'=H: protocatechuic acid

R=OCH_3, R'=H: vanillic acid

R=R'=OH: gallic acid

R=H: salicylic acid

R=OH: gentisic acid

R=R'=OCH_3: syringic acid

Cinnamic Acids

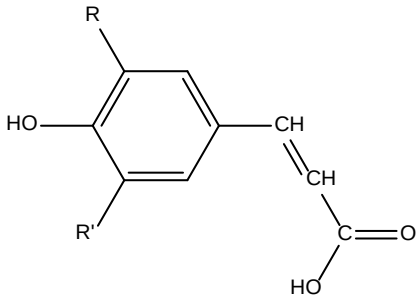


Figure 1.8. Carbon skeleton of the cinnamic acids.

R=R'=H: p-coumaric acid

R=OH, R'=H: caffeic acid

R=OCH_3, R'=H: ferulic acid

R=R'=OCH_3: sinapic acid

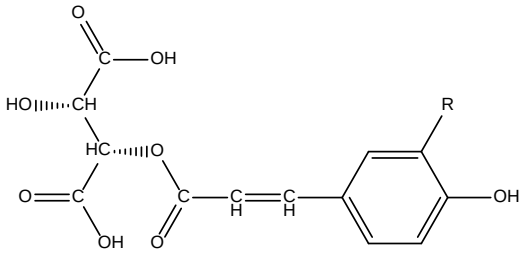


Figure 1.9. Example of an esterified cinnamic acid.

R=H: mono-p-coumaroyltartaric acid

R=OH: monocaffeoyltartaric acid

R=OCH_3: monoferuloyltartaric acid

1.3.2 The Flavonoids

The flavonoids comprise the classes of flavonols, anthocyanidins, and tannins, built around the flavonoid skeleton seen in Figure 1.10. These compounds are found in the grape skin, stems, and seeds and are highly reactive molecules that account for the majority of the organoleptic properties in wine (Fulcrand et al., 1999). During polymerisation, the most reactive sites in the flavonoid backbone are the nucleophilic centres at carbon 6 (C-6) and carbon 8 (C-8), as well as reactive electrophilic centres at carbon 4 (C-4) (Figure 1.10).

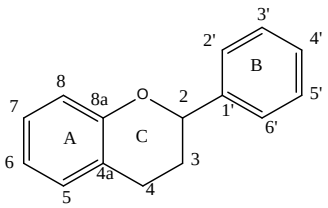
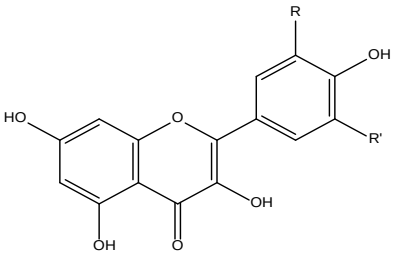


Figure 1.10. The flavonoid skeleton, the backbone for the classes of compounds including flavonols, flavanols, anthocyanidins, and tannins. The carbon number is designated on the skeleton.

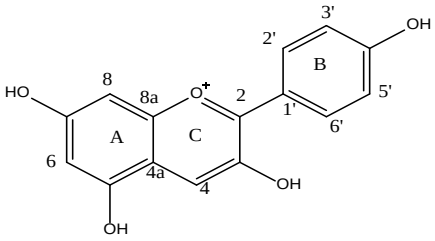
Flavonols, located in the grape skin, are often glycosylated on carbon 3 and include: kaempferol, quercitin, isohamnetin, myricetin, laricitrin, and syringetin (Figure 1.11). All six compounds are identified in the red grape, whereas only the first three compounds are found in the white grape (Ferrandino et al., 2012).



R=R'=H: kaempferol
R=OH, R'=H: quercitin
R=OCH_3, R'=H: isohamnetin
R=R'=OH: myricetin
R= H, R'= OCH_3: laricitrin
R=R'= OCH_3: syringetin

Figure 1.11. Backbone of a flavonol molecule.

Anthocyanidins are the main colorant for red grapes and are concentrated in grape skin. The five recognised compounds are: delphinidin, malvidin, petunidin, cyanidin, and paeonidin (Figure 1.12). They are often glycosylated at C-3 and sometimes acylated with organic or cinnamic acids as seen in Figure 1.13 (Saucier, 2010; Fong et al., 1971). Diglucoside (carbons 3 and 5) are found in modern *Vitis* hybrids (Dinsmore-Webb, 1974:69).



delphinidin, 3'=5'=OH
malvidin, 3'=5'=OCH3
petunidin, 3'=OCH3, 5'=OH
cyanidin, 3'=OH, 5'=H
paeonidin, 3'=OCH3, 5'=H

Figure 1.12. The carbon skeleton of the anthocyanidins the main colorants in the skin of red grapes, often glycosylated at carbon 3. The molecule has reactive nucleophilic centres at carbon 6 and at carbon 8.

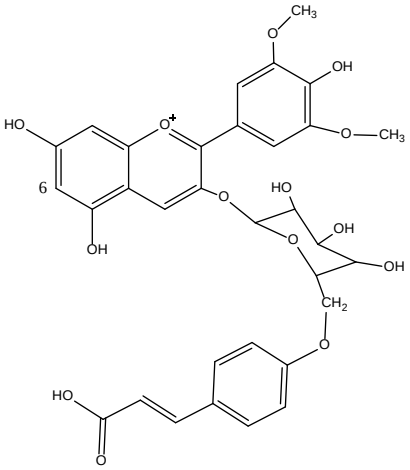
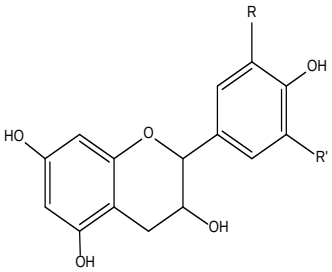


Figure 1.13. Malvidin-3-monoglucoside-p-coumarate.

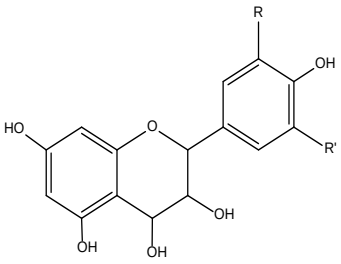
Tannins are highly reactive species originating from the grape skin, stems, and the seeds and are categorised as flavan-3-ols (catechins) and flavan-3,4-diols (leucoanthocyanidins), Figure 1.14 (Dinsmore-Webb, 1974:63).

Flavan-3-ol



R=OH, R'=H: catechin
R=R'=OH: gallocatechin

Flavan-3,4-diol



R=R'=H: leucopelargonidin
R=OH, R'=H: leucocyanidin
R=R'=OH: leucodelphindin

Figure 1.14. Two representative tannin structures.

1.3.3 Formation of Polymers in Wine

As shown, the building blocks of wine are highly reactive species that polymerise over time resulting in a change of flavor and color. One research group suggests this highly reactive nature is due to the lack of substitution found on the tannins and anthocyanidins, describing those molecules found in grapes to be the 'simplest encountered in higher plants' (Brouillard et al., 2003). For example, anthocyanins found in morning glory, *Pharbitis nil,* contain multiple glycosylated and cinnomylated residue side-chains resulting in fairly stable molecules. In contrast, the anthocyanins of the *Vitis* species are limited mainly to glycosylation, with rare exceptions of extending side chains, leaving open reactive nucleophilic and electrophilic centres to interact with similar centres in flavanol and tannin molecules.

The mechanisms of how wines age due to molecular polymerisation is an active branch of study in the field of enology. One polymerising mechanism was suggested by Timberlake and Bridle (1976) in which a model solution was prepared from a pure anthocyanin, a pure flavan-3-ol, and acetaldehyde (the acetaldehyde arising from at least two origins: fermentation and oxidation of ethanol). It was theorised that reactions formed a polymeric species from all three original components (Timberlake and Bridle, 1976). Since then, with the availability of more sensitive analytical techniques, researchers confirmed this mechanism, identifying dimers between catechins and anthocyanin, linked via an ethyl bridge (Es-Safi et al. 1999). Figure 1.15 is based upon the 1976 proposed mechanism. Here, the protonated acetaldehyde, which is prevalent in the acidic environment (A), attacks the C-8 nucleophilic centre of a flavan-3-ol (B). There is a loss of water, which provokes another attack on the C-8 of the anthocyanin (C), thereby joining the flavan-3-ol and the anthocyanin via an ethyl bridge.

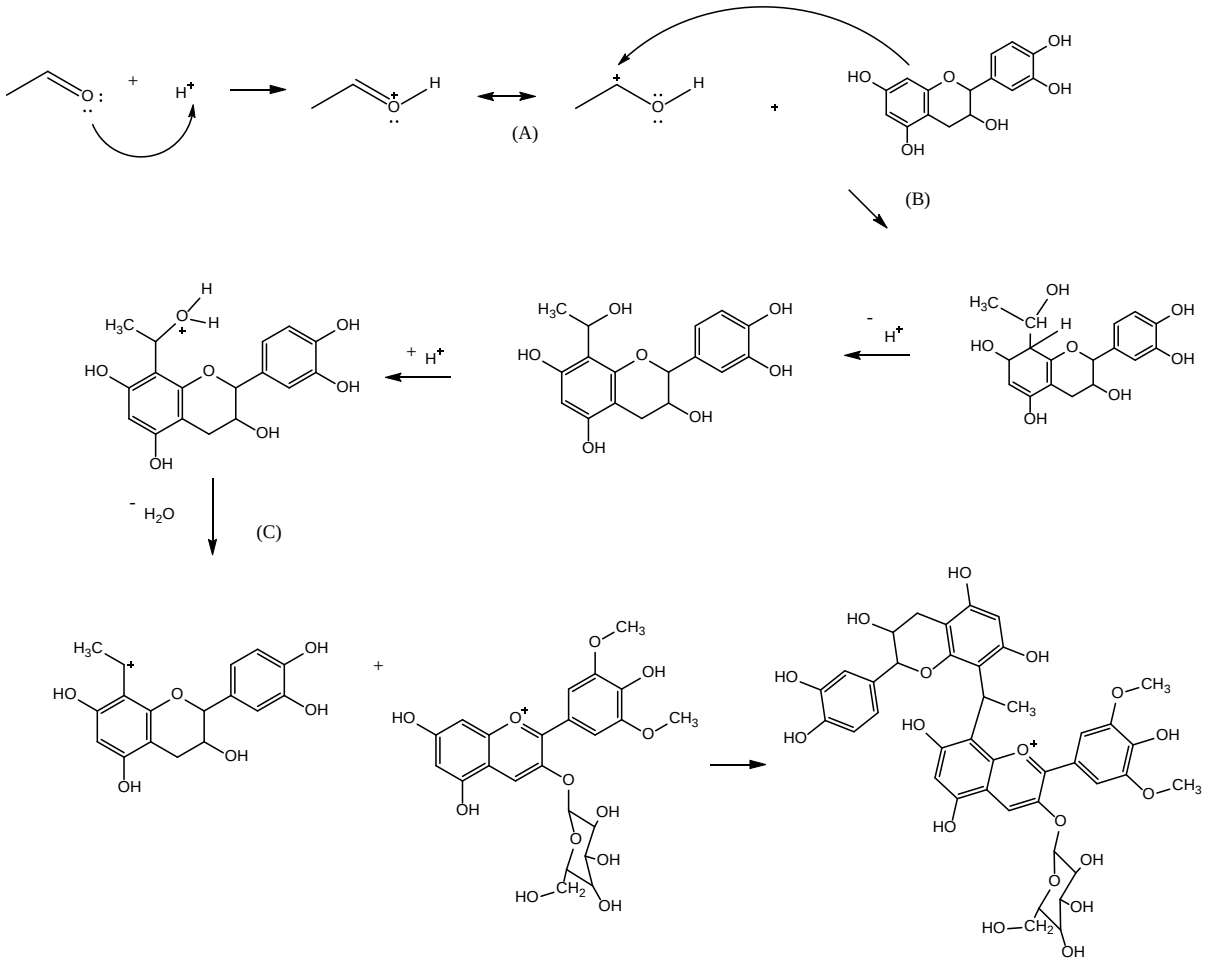


Figure 1.15. The acetaldehyde, prevalent in the acidic environment, attacks the C-8 nucleophilic centre of a flavan-3-ol. A loss of water provokes another attack on the C-8 of the anthocyanin.

A second proposed mechanism involves the direct condensation between tannins and tannins, and tannins and anthocyanins (Jurd, 1969; Haslam, 1980; Singleton and Trousdale, 1992; Somers, 1971). The suggested initiator of this polymerisation is the electrophilic C-4; the result of a positive charge formed in acidic solution seen here in Figure 1.16.

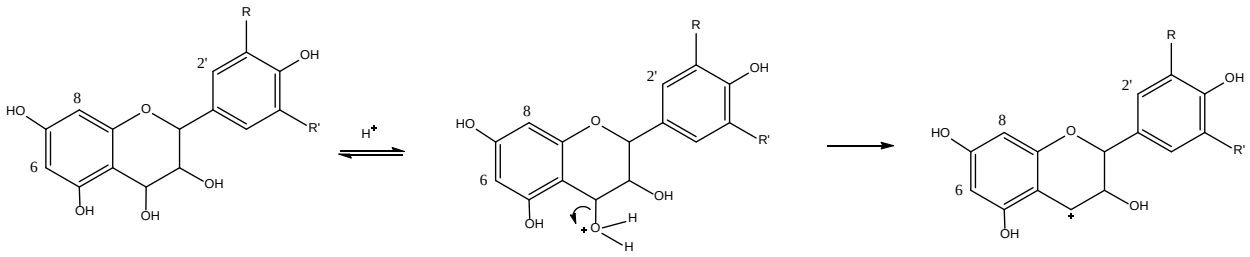


Figure 1.16. Formation of carbonium ion on C-4 from flavan-3,4-ol in acidic solution.

Two types of polymeric formation are suggested: an A-T (anthocyanin-tannin) polymer formed from anthocyanin electrophilic C-4 linked to C-6 or C-8 nucleophilic centres of the condensed tannins, or a T-A polymer (tannin-anthocyanin) whereby the electrophilic C-4 from the tannin dimer links to the nucleophilic centre (C-6 or C-8) of the anthocyanin. The first analytical proof of this polymeric condensation resulting from direct anthocyanin–tannin reactions was reported in 2000 (Remy, et al., 2000). Three years later an octamer formed by a proanthocyanidin heptamer and an anthocyanin was confirmed (Hayasaka and Kennedy, 2003). Figure 1.17 illustrates the formation of an A-T polymer. Initially two tannin molecules condense to form a dimer, followed by a linkage between the electrophilic C-4 on the malvidin molecule and the C-8 nucleophilic centre of the tannin dimer.

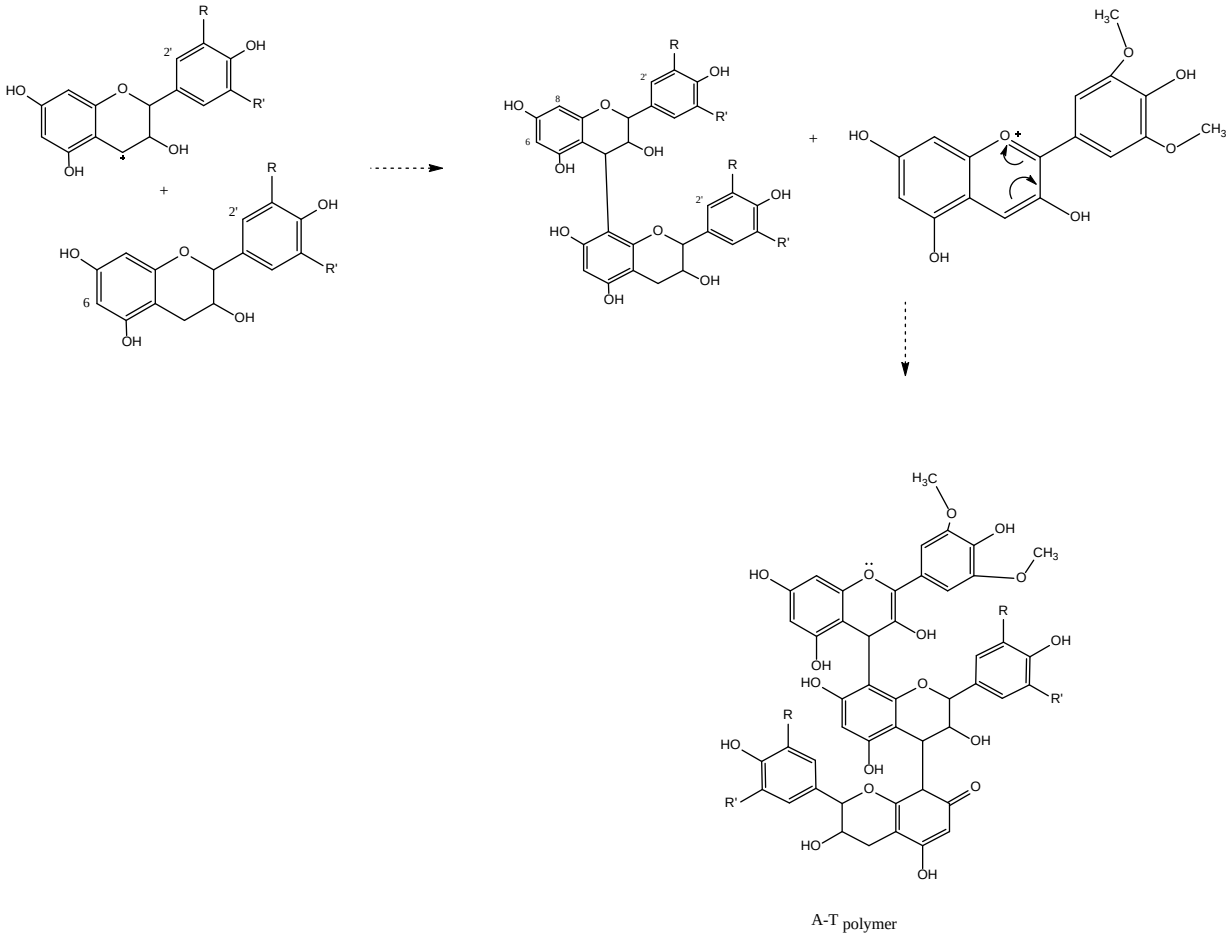


Figure 1.17. Formation of the A-T polymer.

Figure 1.18 was reproduced from the literature and illustrates a hypothetical wine polymer formed during aging; the illustration was based upon compounds identified after depolymerisation of red wine pomace (Wollmann and Hofmann, 2013). One of the compounds identified in the depolymerisation was tartaric acid. As one of the two main biomarkers in the analysis of archaeological wine, the origin of tartaric acid has been consistently attributed to either a salt, calcium tartrate, or to the free tartaric acid having remained due to an arid environment (Michel, et al., 1993; McGovern et al., 2004; 2009; 2013; Romanus et al., 2009; Guasch-Jane et al., 2004; Pecci et al., 2013). From the results presented in this thesis in Chapters 3, 5, and 6, it is far more likely that the tartaric acid was locked within a wine polymeric network over the course of an archaeological timeframe, and released only upon chemical attack of the sample.

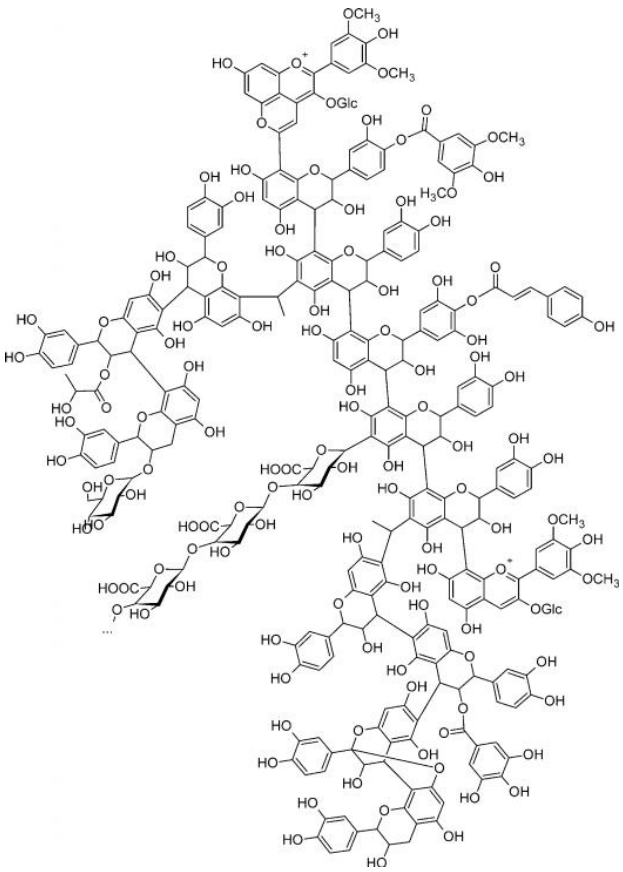


Figure 1.18. Hypothesised wine polymer reproduced from Wollmann and Hofmann (2013).

1.4 Archaeological Triacylglycerols

There has been a voluminous amount of research devoted to the analysis of triacylglycerols (TAGs). The importance of these compounds lay in the fact that proper characterisation of the identified TAGs offers insight into the original animal or plant lipid used in the archaeological context. There have been multiple historical records written about cooking with animal fat and wine for Roman British soldiers and a portion of this research examined the presence of TAGs in the Vindolanda samples (Renfrew, 1985: 38-40; Alcock, 2010: 83). TAGs are abundant lipids consisting of three fatty acids of varying saturation esterified to a glycerol backbone, Figure 1.19. Prevalent in fats and oils, archaeological TAGs have been classified based upon their carbon distribution by high temperature

gas chromatography mass spectrometry resulting in specific profiles that distinguish between plant and animal fats, ruminant and non-ruminant fats, as well as the difference between adipose fat and dairy fats (Charters et al., 1995; Evershed et al., 1997; Dudd and Evershed, 1998). As an example, non-ruminant fats are classified based upon a narrow carbon distribution (C_{48}-C_{54}) whereas ruminant fats and/or dairy have been classified with a broad range from $C_{40}/_{42}$-C_{54} (Regert, 2011). Recently, increased sensitivity and greater structural information in the analysis of archaeological TAGs has been accomplished with nanoelectrospray combined with high resolution mass spectrometry (Garnier et al., 2009; Mirabaud et al., 2007). TAGs are labile compounds, however, and several studies have identified the modes of degradation within a laboratory context, ultimately assisting in the identification of the degraded archaeological TAGs (Dudd et al., 1998). Under oxic conditions, TAGs hydrolyse to their mono-, diacylglycerol and free acid components; however, under anoxic conditions, that hydrolysis slows considerably (Evershed, 2008a). It was presumed that TAG residue representative of foodstuffs within cook pots would still be present in the artifacts excavated from the anoxic waterlogged conditions of Vindolanda.

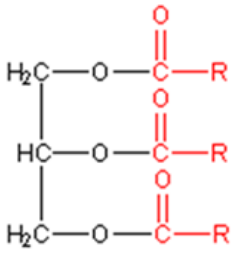


Figure 1.19. A general description for triacylglycerol; the black nomenclature denotes the glycerol backbone and the red nomenclature denotes the attached fatty acids.

In this thesis, lithiated TAGs were examined and fragmented for analysis by high resolution mass spectrometry. For the fatty acid descriptions of TAGs, the neutral losses were compared with published proposed fragmentation mechanisms (Hsu and Turk, 2010). Comparisons were also made

between experimental results and published results of lithiated archaeological TAGs (Garnier et al., 2009; Mirabaud et al., 2007).

1.5 Metabolite Extraction

Any good analytical scheme requires a uniform and reproducible extraction process so that accurate comparisons can be made between analytical runs as well as between experiments separated by time and different laboratories (Lin et al., 2007). For this research, samples were homogenised using the Bligh-Dyer method, a commonly used liquid-liquid extraction approach which utilises a methanol/water/chloroform mixture to separate polar and non-polar metabolites into separate phases (Bligh and Dyer, 1959). Those aliquots were removed and a strong alkaline solution was added to the remaining solid which contained the intractable polymer. Alkaline fusion followed by an acidic wash hydrolysed the ester bonds within the polymer releasing monomeric species which were then extracted for later analysis as seen in Figure 1.20. Alkaline fusion is a common method for the analysis of non-extractable phenolics in foodstuffs, and has also been applied to the analysis of ancient wine residue (Perez-Jimenez and Torres, 2011; Wollmann and Hofmann, 2013; Guasch-Jane et al., 2004; Pecci et al., 2013).

To concentrate low-level metabolites, a pre-concentration step was sometimes necessary prior to analysis. This was accomplished by solid phase extraction (SPE), using a silica or polymer based cartridge often functionalised with hydrophobic, anionic or cationic groups. The extracted sample was poured over the cartridge and the analytes of interest were bonded to the functionalised groups by a variety of interactions, or the analyte of interest washed through the cartridge whilst the interfering matrices remained bonded to the cartridge (Dettmer et al., 2007). SPE was used in several instances during this research. A strong cation exchange cartridge was used to clean up the alkaline fusion protocol in order to remove aluminate ions, a result of matrix interference; this protocol is described in more detail in Chapter 5. Also, an aminopropyl cartridge was used to focus

and concentrate low level TAGs found in the Vindolanda samples. This approach is described in more detail in Chapter 6.

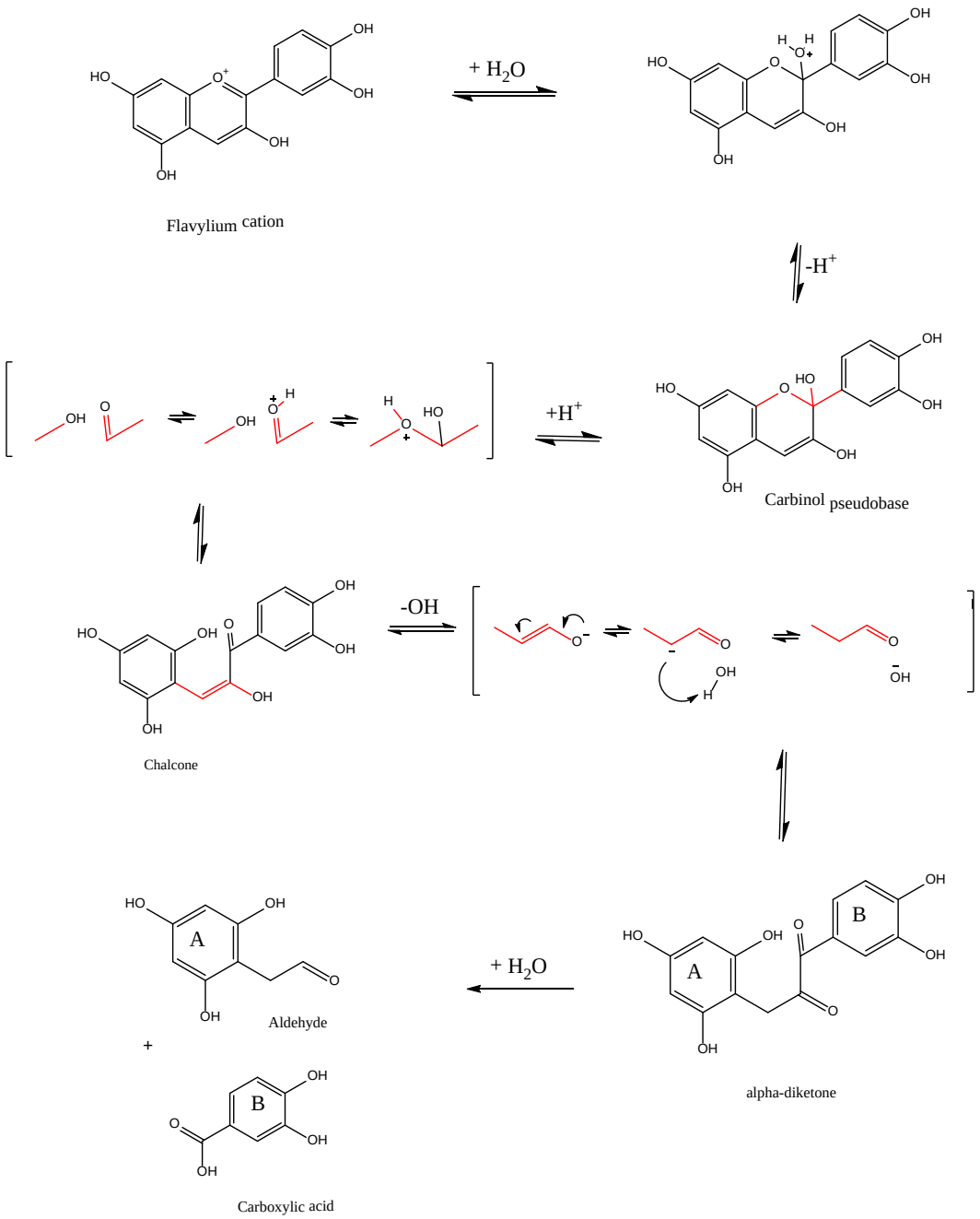


Figure 1.20. Variable species of anthocyanidins based upon pH. The flavylium cation predominates at acidic pH<3. Increasing the pH to 4-5 and the carbinol pseudobase predominates, with an increasing level of chalcone formation. At pH above 8, chalcone species convert to the unstable alpha-diketone via a base catalysed keto-enol tautomerism, which breaks down to form the subsequent aldehyde and carboxylic acid (reproduced from Brouillard et al., 1989).

1.6 Analytical Techniques

There are a number of analytical techniques utilised for metabolomics experiments (Dunn and Ellis, 2005). Rather than provide an overview of available instrumentation, only the techniques that were integral to this thesis are included and concern sample introduction, sample ionisation, and analyte detection: direct infusion and liquid chromatography, electrospray ionisation, and mass spectrometry, specifically the Fourier transform mass spectrometer and the triple quadrupole mass spectrometer. Initially, for an untargeted analysis, high throughput is required; this is accomplished with direct infusion nanoelectrospray. Combining direct infusion with high resolution mass spectrometry (MS), in this case Fourier transform MS, offers the most accurate masses in combination with an untargeted metabolite description. In order to increase sensitivity and focus on the most characteristic metabolites, a targeted sensitive approach is then utilised, typically by combining liquid chromatography with a triple quadrupole mass spectrometer.

1.6.1 Sample Introduction

1.6.1.1 Direct Infusion Nanoelectrospray

Samples introduced into the mass spectrometer via direct infusion electrospray (DIMS) are infused continuously without chromatographic separation. Coupling DIMS with high resolution instruments allows accurate mass to be determined for hundreds to thousands of analytes. However, the lack of chromatographic separation, combined with ion suppression due to salts and matrix interferences sometimes masks compounds of interest.

The first nanoelectrospray was described in the mid 1990's whereby analytes in solution were delivered via an electrically conductive capillary (1-2 µm diameter) under a very low flow rate of

20 nl/min (Wilm and Mann, 1996). Each sample was loaded directly into a unique capillary tip which prevented (or certainly limited) cross contamination between samples. In 2000, a microchip electrospray device was developed whereby 400 nozzles at 20 micrometres in diameter were etched into a silicon wafer to produce a nanoelectrospray chip (Schultz et al., 2000). In this instance, an electrically conductive carbon coated tip picks up several microlitres of sample, connecting with one of the nozzles creating a current and thereby an ionised electrospray. The advent of this device allowed high-throughput analysis of hundreds of samples in only a few hours of analysis time and was utilised in this research.

1.6.1.2 Liquid Chromatography

Liquid chromatography (LC) is an analytical technique often utilised in biological applications whereby a complex mixture of compounds are separated based upon each compound's partitioning between a column stationary phase and the solvents (mobile phase) polarity, Figure 1.21. The mobile phase composition is either maintained (isocratic) or changed throughout the run (gradient elution). Because temperatures in the column compartment are often kept between 25-40^{o} C, LC is useful for compounds that easily degrade at high temperature or are difficult to volatilise without chemical modifications, thereby making them incompatible with gas chromatography.

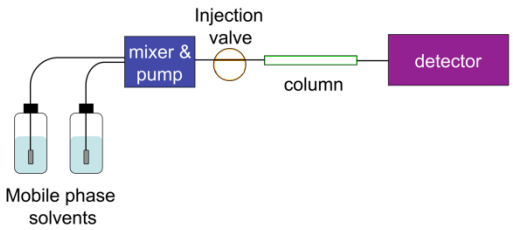


Figure 1.21. A schematic of a liquid chromatograph: the sample is introduced via the injection valve and carried through to the column with a prescribed solvent mixture. The compounds in the sample separate and elute from the column to be detected.

The ability to resolve two adjacent compounds is critical for a successful LC experiment and from Equation 1.1, relies upon the interplay of three factors: capacity factor k', selectivity α, and efficiency N. The capacity factor is a compound's retention time on the column in reference to the void volume, that volume of completely unretained compounds. A higher value represents a compound that is well retained on the column and a lower value represents a compound that elutes earlier. The greatest effect on the capacity factor is the strength of the mobile phase composition. The separation selectivity is described as the ratio of two capacity factors and is often maneuvered by changing the composition or polarity of the mobile phase.

$$R = \left(\frac{1}{4}\right)(\alpha - 1)\sqrt{N}\,[\frac{k'}{1 + k'}]$$

Equation 1.1. The parameters that are necesary for proper resolution in an LC experiment include: α, the selectivity factor, the ability to separate two eluting analytes; N, the efficienicy of a column to successfully separate a chosen group of analytes; k' the capacity factor, or an analytes' retention time in relation to an unretained material.

1.6.1.2.1 Hydrophilic Interaction Liquid Chromatography (HILIC)

Earlier analyses of archaeological wine residue relied upon reversed phase liquid chromatography for the separation of low molecular weight polar compounds (Guasch-Jane et al., 2004; Barnard et al. 2010; McGovern et al., 2009). Since reversed phase chromatography is used primarily for the separation of hydrophobic materials, polar compounds elute quickly off the column. The result is an analyte of interest eluting near the unretained void volume which may lead to irreproducible or questionable results. Chapter 4 of this thesis describes a HILIC elution protocol developed to increase the capacity factors and retention times of polar compounds on the column.

The term 'HILIC' (Hydrophilic Interaction Liquid Chromatography) was coined in 1990 to describe a type of chromatography primarily based on two factors: the elution of hydrophilic analytes from a

stationary polar phase into an increasingly polar mobile phase and the electrostatic effects between analytes and the stationary phase (Alpert, 1990). The initial mobile phase composition consists of a high percentage (>80%) of organic solvent, which is preferred for electrospray ionisation (Nguyen and Schug, 2008). The chromatographic mechanism of HILIC is still not completely elucidated (Buszewsky and Noga, 2012; Dinh et al., 2011; Gama et al., 2012). However, the following parameters are considered integral to the elution procedure: partition of the analyte between the mainly organic mobile phase and an aqueous enriched stationary phase, adsorption between a charged analyte and the charged surface of the stationary phase, ion exchange between the charged analytes, the charged surface of the stationary phase, and the buffer salts, and size exclusion between analytes and the stationary phase (Jandera, 2011; Buszewsky and Noga, 2012).

1.6.2 Sample Ionisation

1.6.2.1 Electrospray Ionisation

In terms of sample ionisation, electrospray ionisation (ESI) describes a 'soft ionisation' technique whereby analytes are ionised with minimal fragmentation allowing detection of the deprotonated (in negative ion mode analyses) molecular ion. This is an advantage over the harsher methods such as electron impact ionisation where multiple mass fragments are produced from one ion. Originally designed by Dole in 1968 and later perfected by Fenn in the 1980's, ESI revolutionised biomolecular analysis and earned John Fenn a Nobel Prize in Chemistry in 2002 (Dole et al., 1968; Fenn et al.,1989; Fenn, 2003).

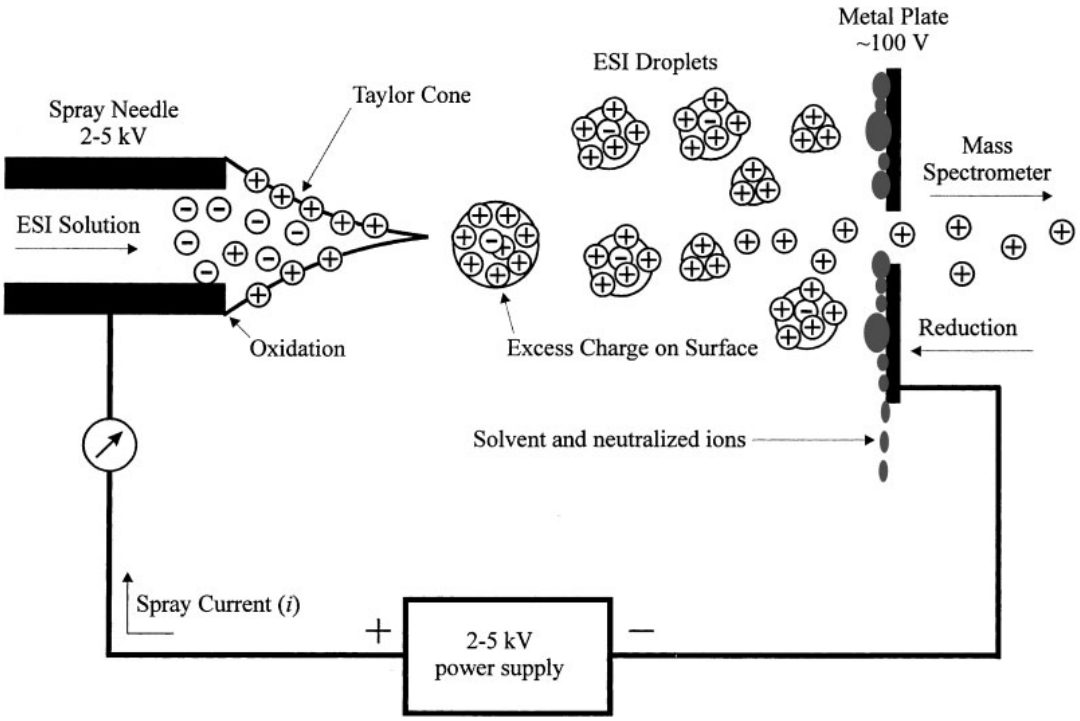


Figure 1.22. Reproduced from (Cech and Enke, 2001), image of a Taylor cone forming at the tip of a positively charged capillary needle. The smaller and highly charged 'offspring' droplets of smaller radius are attracted to the counter electrode and enter the mass spectrometer.

Figure 1.22 offers a schematic description of the ESI methodology. The analyte in solution travels down a metal capillary tube to which a voltage has been applied. The formation of a potential gradient at the capillary tip results in charge separation between the positive and the negative ions forming a Taylor cone; this misshapen liquid is a result of the opposing forces between the highly charged ions' attraction for the counter-electrode at the mass spectrometer entrance and the surface tension of the liquid.

$$E_c = 2\left(\frac{V_C}{r_C}\right)\ln\left(\frac{4d}{r_C}\right)$$

Equation 1.2 The charge formed at the tip of a capillary is described by: V_C , the voltage applied to the capillary; r_C , the radius of the capillary; d , the distance from the tip of the capillary to the counter-electrode.

The electric field formed at the tip of the capillary (E_c) is described by Equation 1.2 where V_C is the voltage applied to the capillary, r_C is the radius of the capillary, and d is the distance to the counter-electrode. Smaller and more highly charged droplets break from the larger cone when the electrostatic repulsion exceeds the surface tension (the Comloumbic repulsion). This process continues while the droplets become smaller and smaller until the highly charged, smallest droplet enters the gas phase.

Two models describe this desolvation phenomenon. The charged residue model states that Coloumbic repulsion occurs continuously until each droplet represents one analyte ion (Dole et al., 1968). The second model, the Ion evaporation model, states that the fully desolvated ions evaporate from the highly charged surface, overcoming the surface tension of the liquid (Iribarne and Thomson, 1976). However, both references agree that the transfer from the charged droplet to a gas phase ion is more efficient with a small droplet radius as shown by equation 1.3:

$$R \infty (\rho V_f 2\gamma)1/2$$

Equation 1.3 A charged droplets radius is proportional to three conditions of the encasing solvent: the flow rate, the solvent's density and its surface tension.

Where V_f is the flow rate, ρ is the density of the solvent, and γ is the solvent surface tension. The droplet formation and desolvation are aided by the addition of an inert gas such as nitrogen, known as sheath gas, as well as the use of an organic solvent with low surface tension and high volatility.

1.6.3 Analyte Detection

1.6.3.1 High Mass Accuracy Mass Spectrometry

Two of the most important parameters in mass spectrometry are mass resolution, the ability to resolve each peak in a spectrum and mass accuracy. Equation 1.4 is the definition of resolution based upon m the mass of the peak, and Δm the difference in *m/z* at 50% of the peak's height, also known as full width at half maximum (FWHM).

$$R = \frac{m}{\Delta m}$$

Equation 1.1 One way to explain resolution of a peak in a mass spectrum is to take the mass of the interested peak and divide by the width of that peak at 50% height, where m is actually the mass to charge value

Mass resolution is critically linked to accurate mass measurement as is seen in Figure 1.23. In this illustration, the resolution is increased from 15,000 up to 80,000 thereby resolving the peaks such that the experimental mass to charge of the peak is within a 0.3ppm mass accuracy. The mass accuracy is the difference between the actual mass and the experimental value; an accurate mass is critical in order to limit the number of possible empirical formulae which can be attributed to that compound.

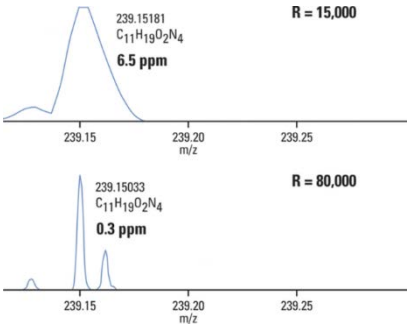


Figure 1.23. High mass resolution is critical for the mass accuracy. The top spectrum taken at a resolution of 15,000 yielded a *m/z* peak at mass 239.15181. The bottom spectrum collected at a higher resolution at 80,000 yielded a more accurate mass of 239.15033 at 0.3ppm mass error (reproduced from Scigelova et al., 2011).

1.6.3.1.1 Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS)

Developed in 1974 by Alan Marshall and Melvin Comisarow, the FT-ICR-MS is currently the mass spectrometer of choice for high mass accuracy of less than 1ppm as well as extremely high resolution of $1e^6$ thus allowing accurate description of extremely complex samples (Brown et al., 2005). The detection component of the FT-ICR-MS is the ion cyclotron resonance cell, seen in Figure 1.24. Kept at high vacuum (10^{-9}mbar), the cell is shielded and surrounded by a magnet of varying strength, 1-12 Tesla, based upon the instrument. The ions introduced into the cell are traveling at a specific velocity in a homogeneous magnetic field and subjected to the Lorentz force, a force acting perpendicular to the travel of the ion path. This force pushes the ions in a trajectory which begins a circular motion maintaining a constant distance from the centre of the cell whilst being electrostatically captured within the cell by opposing trapping plates (Comisarow, 1985; Marshall et al., 1988).

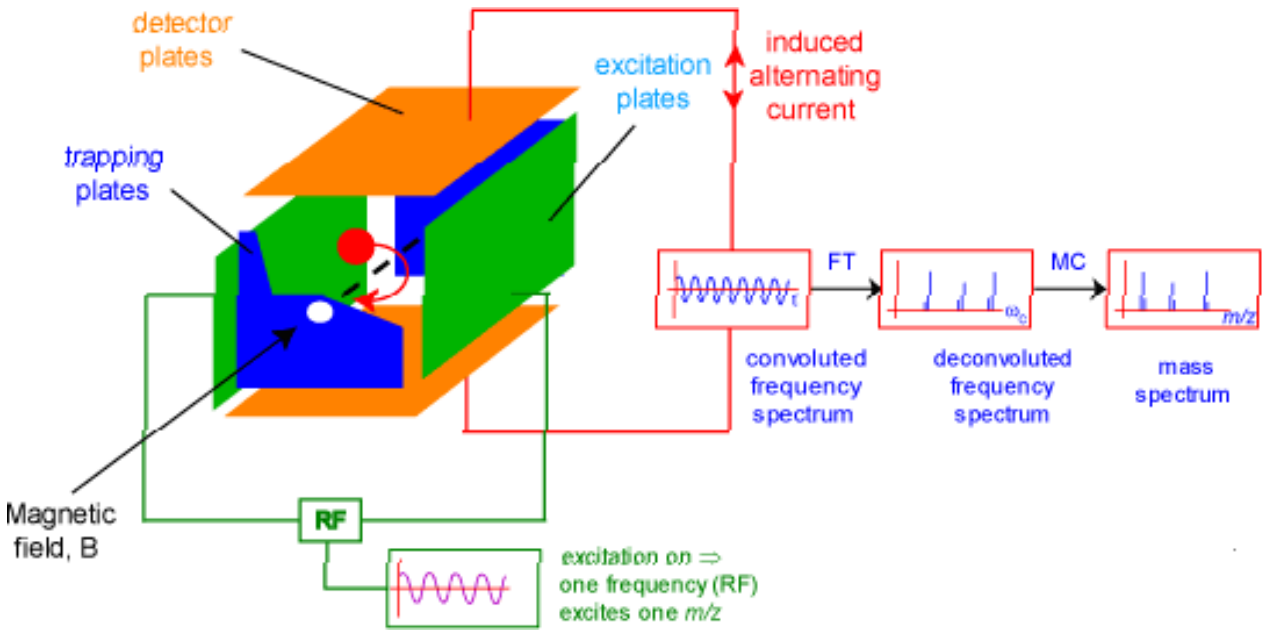


Figure 1.24. Schematic of an ICR cell. In this diagram, the ions traveling in a homogeneous magnetic field are subjected to the Lorentz force, a force acting perpendicular to the travel of the ion path. This force causes the ions to begin a circular trajectory around the centre of the cell, trapped within ICR cell by potentials from the trapping plates. Ions are detected once their radii have increased sufficiently to cause an 'imprint" on the trapping plates. Reproduced from http://www.chm.bris.ac.uk/ms/theory/fticr-massspec.html.

Equation 1.5 describes the cyclotron frequency of the ions:

$$w_c = \frac{qB}{m}$$

Equation 1.2 An ions cylcotron frequency (w_c) is dependent upon: q, the charge of the ion; B, the strenfth of the magnetic field in Tesla; m, the mass of the ion.

Where w_c is the ion cyclotron frequency, q is the charge of the ion, B is the strength of the magnetic field (Tesla), and m is the mass of the ion. From this equation, the frequency of a moving ion must be calculated in order to determine its mass. For this, a pulse of energy is applied to the cell increasing the ions' trajectory radii until the ions are close enough to imprint an 'image current' or transients upon the detector plates. Since every one of the ions' cyclotron frequencies are detected at once, a Fourier transform is needed in order to deconvolute the transient signal from the time domain to the frequency domain, which is then used to produce a mass spectrum.

The high mass accuracy of the FT-ICR-MS limits the number of possible empirical formulae that can be attributed to a mass feature thus assisting in compound identification. One drawback with detection by FT-ICR-MS that may affect resolution and therefore mass accuracy is space charging effects caused by Coulombic interactions between the ion packets within the ICR cell (Dienes et al., 1996; Uechi and Dunbar, 1992). One way to prevent space charging is to limit the packet of ions delivered to the ICR cell. This can be achieved by combining the FT-ICR-MS with a separate mass spectrometer which gauges the amount of ions, such as the linear ion trap mass spectrometer.

1.6.3.1.2 Hybrid Instruments: linear ion trap Fourier transform ion cyclotron resonance mass spectrometry

The high resolution instrument used for this research was the hybrid linear ion trap Fourier transform ion cyclotron resonance mass spectrometer (LTQ-FT-ICR-MS), Figure 1.25. The LTQ is a linear ion trap which can act as a stand-alone instrument and is comprised of four rods with the two opposite rods having the same polarity, Figure 1.26. The linear ion trap operates by trapping ions radially within the quadrupole radiofrequency field and axially by the application of an electrostatic field in the lenses found at the front and back end of the trap. The trapped ions are focused in the centre of the trap by the addition of an inert gas such as helium. This effectively 'dampens' the kinetic energy of the ions and focuses the packet of ions within the z, or axial, direction.

Within this trap ions are isolated, fragmented (by collisional induced dissociation with the helium gas), and ejected for immediate detection or for transfer into the ICR cell. Ions are ejected either axially out the back end of the trap, or radially along the slits in the centre section allowing for greater ejection efficiency. One of the most important features is the automatic gain control (AGC) value (Finnigan™LTQ FT™Hardware Manual, 2005). This user defined value determines the amount of ions ejected into the ICR cell, thereby preventing space charging and decreased mass resolution.

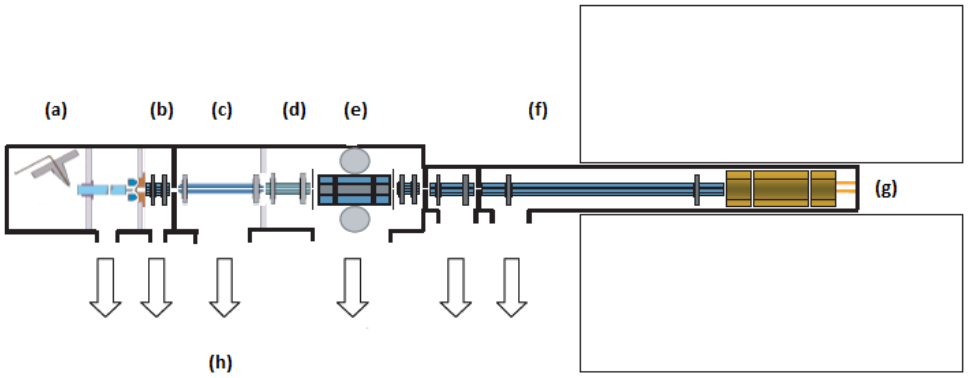


Figure 1.25. A schematic diagram of a hybrid LTQ-FT-ICR-MS. Ions are directed from the source (a) through the tube lens and skimmer (b), continuing through (c) Q0 (quadrupole) and (d) Q1 (octopole) into the (e) linear ion trap. A pre-determined packet of ions are ejected from the linear ion trap through the octopoles (f) for detection in the ICR cell (g). The ICR cell is shielded on either side by a cryogenically cooled magnet. The ions travel through an atmosphere of increasing vacuum due to the process of differential pumping (h). Diagram reproduced from Finnegan LTQ FT hardware manual.

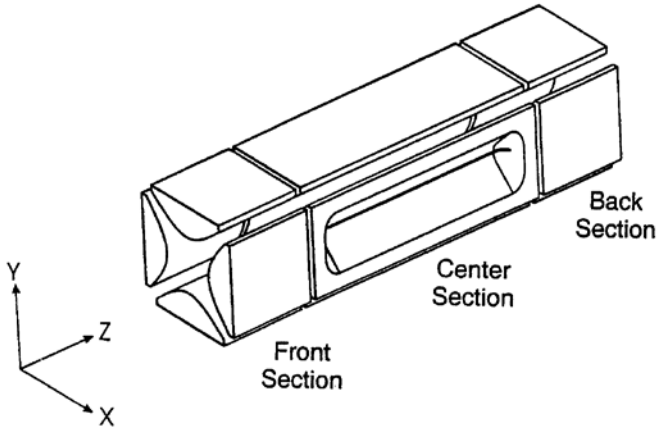


Figure 1.26. A representation of the linear ion trap's quadrupole assembly. Reproduced from the Finnigan™ LTQ FT™ Hardware Manual.

1.6.3.2 Triple Quadrupole Mass Spectrometry

For targeted analysis of trace materials in this thesis, the mass detector of choice was the triple quadrupole mass spectrometer. The underlying principle of operation is based upon the four hyperbolic rods found within each quadrupole. The two rods opposite one another are held at the same potential. Alternating direct current and radio frequency (dc/rf) voltages applied to the quadrupoles allow chosen *m/z* to follow a stable trajectory to the exit, whereas all other *m/z* will follow an unstable trajectory, careen into the sides of the poles and be neutralized, Figure 1.27. The instrument was initially described by Wolfgang Paul in 1953 for which he received the Nobel Prize in 1989 (Paul, 1990).

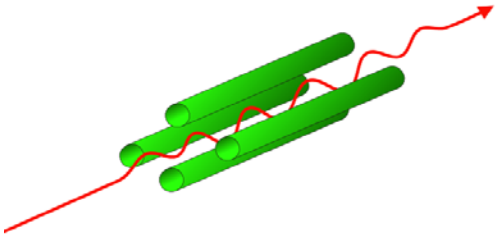


Figure 1.27. An image of four rods within a quadrupole; the red directional arrow represents a successful transmission of ions.

The triple quadrupole mass spectrometer, considered a low/medium resolution instrument, was introduced in 1978 for the isolation and fragmentation of chosen ions using tandem quadrupoles in sequence, Figure 1.28 (Yost and Enke, 1978). Quadrupoles 1 and 3 are mass filters, directing particular masses by varying dc/rf. Quadrupole 2 has two functions: a collisional cell used to fragment the precursor ion by bombardment with a stable gas such as argon, and as an ion guide that focuses and transmits the product ions to quadrupole 3. (Douglas, 2009).

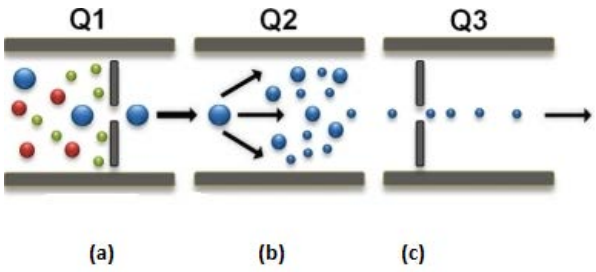


Figure 1.28. A schematic of a triple quadrupole mass spectrometer where (a) ions enter the first quadrupole and mass selectively filtered for transfer into the collision cell (b). The fragmented ions are then filtered through the third quadrupole.

The triple quadrupole instrument can be run in several modes including:

- SIM/scan mode where a precursor ion is selected in Q1, fragmented in Q2 and Q3 scans for all product ions.
- Scan/SIM mode where Q1 is scanned across the whole mass range, all ions are fragmented in Q2 and only the chosen product ions are monitored and detected in Q3.
- neutral scan mode where Q1 is scanned across the chosen mass ranges, the precursor ions are fragmented in Q2, and the product ions are scanned across the chosen mass ranges in Q3 offset by the mass of a neutral loss.
- multiple reaction monitoring (MRM) where Q1 is set to a specific precursor ion that is fragmented in Q2 and Q3 is set to the specific product ions.

MRM mode was chosen for this research in order to achieve greater specificity and sensitivity for trace analysis of compounds in a complex sample matrix. This targeted method isolates and analyses only user defined specific ions, thus increasing sensitivity by isolating a chosen set of masses, as opposed to scanning the whole mass range. The MRM method was developed with pure standards dissolved in a solvent mixture most accurately representing the samples' elution profile off a column. In this way, the proper and most significant fragmentations were recorded and applied to the final method.

1.7 Data Analysis

1.7.1 SIMStitch Method

Since the examination of small molecules in a sample requires the identity of high as well as low abundance ions, the dynamic range of the analytical methodology is crucial. For LTQ-FT-ICR-MS, the manufacturers list a dynamic range of 5000; that is, at a maximum signal of 100%, there is a minimum signal intensity of .02% (LTQ FT hardware manual, 2004; Payne, 2011). In order to maximise this dynamic range, a methodological approach was utilised which allows identification of low abundance ions without increasing the amount of sample introduced into the ICR cell thus maintaining high resolution and mass accuracy, SIMstitch method (Payne et al., 2009; Southam et al., 2007). This approach to spectral processing was utilised for all untargeted MS analyses collected by DIMS-LTQ-FT-ICR-MS in this thesis.

For the SIMStitch method, a chosen mass range is analysed by collecting several small scan windows in a series of specified mass ranges. Only those masses of that particular window are analysed in the ICR cell at one time, thus preventing the deleterious effects of space charging. Transient files are then collected and averaged for each window; for example, for a window of 100 *m/z* mass range, 24 transients were collected and averaged (Southam et al., 2007). A Fourier transformation is then applied to the averaged transients and the overlapped regions of all windows are "stitched" together with a unique algorithm written in the Matlab (The Mathworks, Natick, Massachusetts) environment resulting in a complete mass spectrum.

For the research undertaken in this thesis, each sample was run in triplicate or quadruplicate and in order to eliminate noise artifacts and false peaks, a three stage filtering step was applied. After a SNR was chosen, a 2/3 or 2/4 replicate filter was chosen whereby the peak must be present in two out of the three (or four) replicate samples in order to be determined as 'real'. Finally, a sample

filtering step was applied whereby the percentage chosen was applied to a sample group such that a particular peak, (e.g., over a certain SNR and found in 2/3 replicates) was also found in x% of samples analysed.

1.7.2 Matrix Processing

Data gathered in a metabolomics experiment are often treated as a matrix, with a row for each sample and a column for each of the features or variables such as *m/z* values. The intensities of each variable are listed in the corresponding locations. The data stored within this matrix are then 'preprocessed' in preparation for statistical analysis including: normalisation, imputing missing values, and g-log transformation (a variable scaling function). Analysed along with individual samples are data from quality control samples, or QCs. These samples are a representative mixture of all individual samples to be analysed and are evenly distributed throughout the analyses in order to identify any technical drift during an analytical run (Dunn et al., 2012).

For this thesis, the data matrix was normalised in order to correct for the technical variation or sample dilution that may occur during analysis (Dieterie et al., 2006). For this, a reference spectrum was prepared from the QCs by calculating a median value of all descriptive features. Each sample spectrum was then normalised against the reference spectrum by dividing the features in the sample spectrum by the same features in the reference spectrum, resulting in a normalised data matrix. Due to the thousands of features that were often found in a DIMS experiment, there were invariably missing values in the original data matrix, that is, features that were identified in one sample set were not evident in a separate sample set. The missing values were imputed by several methods including K-Nearest Neighbors (KNN), or in some cases, by returning to the original mass spectrum to determine the noise peak (Hrydziuszko and Viant, 2011).

In order to limit systemic variability and to raise the relative importance of sample variability, the normalised data matrix was g-log transformed resulting in the attenuation of the most intense peaks while amplifying many of the less intense peaks. The result was a matrix with peaks that were of more similar mean intensity and variation. This transformation removed the biases that occur in multivariate statistics, including principal components analyses (which focus on the most varying peaks).

1.7.3 Statistical Analyses

Broadly speaking, the statistics applied to metabolomics data are defined as either univariate, determining the behavior of one variable throughout an experiment, or multivariate, following the changes of many variables using a single test. Univariate tests utilised for this thesis included the student's t-test and 1-way ANOVA. In order to determine significance amongst the tested variables when multiple variables were tested, the Benjamini-Hochberg correction was applied such that the user defined false discovery rate (FDR) was multiplied by the (p-value/total number of features); the resultant adjusted p-value was a more robust descriptor for significance (Benjamini and Hochberg, 1995). Those compounds determined as significant were then examined for their fold change, that is, the difference in intensity amongst timed events.

Multivariate testing in this thesis consisted of principal components analysis (PCA), an unsupervised method of describing multivariate data in reduced dimensions by combining thousands of features (e.g., *m/z*) for each sample and reducing them into a few principal components (PC). The PCs are vectors in a '*n*' dimensional space that collectively identify the greatest variation amongst samples. PC1 is plotted between the greatest variability; PC2 is orthogonal to PC1 and contains the next greatest amount of variation, with each subsequent PC orthogonal to its previous one until the total variability was described. A 'scores plot' was plotted between top ranking PCs, e.g., PC 1 and PC2, to visualise natural clustering in the data. These scores represent how well each sample spectrum was

represented or described by each PC. When patterns and clusters were observed in these reduced dimensionality plots, it was meaningful to look at the 'loadings' for each PC.

Other terms in statistics used in this thesis include the basic description of how well data points agree with one another in a particular measurement or experiment:

1. Mean: sum of measurements divided by the number of measurements.

2. Median: all measurements are collated in descending order and the middle value is chosen as the median value. In the case of an even number of values, the mean value of the two middle values is chosen as the median.

3. Standard Deviation: The standard deviation measures the spread of data from the measurements' mean,

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1}}$$

Equation 1.3 Equation of standard deviation where: σ represents the standard deviation, *i* =total number of measurements, x represents specific data measurements, and $\overline{x}$ is the mean value for all data measurements in experiment.

4. Coefficient of Variation: CV describes the variation of the data with regards to the mean of the total measurements,

$$CV = \frac{\sigma}{\overline{x}}$$

Equation 1.4 The coefficient of variation as described by the standard deviation, σ, divided by the mean of all data measurements, $\overline{x}$.

5. Relative Standard Deviation: RSD describes the coefficient of variation in terms of a percentage,

$$RSD = 100 * \frac{\sigma}{\overline{x}}$$

Equation 1.8 The relative standard of deviation as described by the standard deviation, σ, divided by the mean of all data measurements, $\overline{x}$, multiplied by 100.

1.7.4 Metabolite Identification

Oftentimes, the identification of a significant *m/z* is the 'bottleneck' in a metabolomics study. In order to prepare a targeted method from the differentiated metabolites, the identity of those original metabolites (*m/z*) must be confirmed. There are four levels of confidence (or criteria) in the identification of a metabolite starting at an unknown, to a putative identification of a compound class, to a putative identification of a compound, leading to a definitive identification. The definitive identification is based upon the comparison between the metabolite in question and an actual standard with at least two orthogonal properties such as retention time, fragmentation pattern, and *m/z* (Dunn, et al. 2013).

1.8 Objectives

The aims of this research are to apply a metabolomics strategy to the analysis of organic residue in an archaeological object. Specific aims were addressed in the following chapters:

1. In Chapter 3, to utilise an untargeted metabolomics approach to laboratory aged wine and determine a suite of biomarkers that are most discerning for aged wine.

2. In Chapter 4, to develop a targeted LC-MS/MS method based upon those biomarkers in order to increase the sensitivity for trace level analysis.

3. In Chapter 5, to apply the targeted method to archaeological samples from Sardinia, Italy, in order to determine the presence/absence of ancient polymerised wine.

4. In Chapter 6, to apply that targeted method for wine residue to a multi-use site, in addition to utilise bioinformatics in the analysis and fragmentations of TAGs.

2. Materials and Methods

The methods presented here describe sample collection, sample extraction, and instrumental analysis. Specific description of the wine aging study is discussed in Chapter 3. Descriptions of the ageing of foodstuffs are in Appendix, Chapter 6. Data processing is introduced in this chapter and more fully explained for each specific experiment in the following chapters.

2.1 Materials

2.1.1 Chemicals

Purchased from Fluka:
Vanillic acid 94770-10g
Ammonium acetate 17636-50g
Ammonium hydroxide >=25% in water, 09857-100ml

Purchased from Sigma-Aldrich:
Malic acid 240176-50g
2-hydroxycinnamic acid, predominantly trans H22809-5g
4-hydroxyphenylpyruvic acid 114286-1g
2,3-dihydroxybenzoic acid 126209-5g
Trans-ferulic acid 126708-5g
Citramalic acid 5415375-250mg
2-ketobutyric acid K401-5g
Malonic acid 01236-5g
3-hydroxybenzoic acid 120000-5g
L tartaric acid 281530-5g
l-ascorbic acid A5960-25g
Syringic acid S6681-5g
Succinic acid S-7501-100g
Potassium hydroxide 221473-500g
p-coumaric acid C9008-1g
2-isopropyl malic acid 333115-10mg
Oxaloacetic acid sigma O4126-1g
Ethyl acetate Sigma Aldrich 650528-1L
Hydrochloric acid, 37% 320331-500ML

Purchased from Acros organics:
m-hydroxybenzoic acid 14755-02-03(CAS)
Lithium chloride 199880050

Purchased from Fischer Scientific:
Citric acid C/6200/53-500g
Chloroform C/4966/17-2.5L. 1149533.
Acetonitrile HPLC grade A/0627/17-2.5L

Purchased from Scientific Laboratory Supplies:
Gallic acid 91215-100mg

Purchased from J.T. Baker:
Water HPLC grade 4218-1L
Methanol LC-MS reagent 9822-1L

2.1.2 Miscellaneous

SPE cartridges:
Phenomenex strata-x-c-33u polymeric strong cation. 30mg/1ml part number: 8B-S029-TAK 100/box. Sorbent lot: S229-109
Supelco discovery DSC-NH_2, 1ml tubes, part number: 52636-U, lot number: 2297701
Thermo Scientific C18 100/ml, part number: 60108-302

Champagne vials:
Cronus, part#: VZM-1508CC-100. Vial Champagne clear 8mm narrow mouth, screw top 1.5ml. lot number 1314932Ca08.
Cronus, part#: VCA-0804TB-100. Assembled black cap w/sil/PTFE septa B-425. lot number 130490-1-1. 100/pk

Poulten and Graf, disposable glass Pasteur pipettes 150mm, 250/box.
1.8ml glass vials with aluminum lined caps
Sarstedt 1000ul pipette tips lot 0220/3190011. 250/bag
Fisher brand 200 µl pipette tips. 1000/bag.
Sarstedt 1.5ml eppendrof tubes lot 0000/2069001
Whatman pH indicator strips catalog number 2613991. 100 strips
Glass wool Merck, K93007686
Precellys 7ml hard tissue homogenizing tubes. Product #: CK28-7ml. Catalog number: 0904-01.
Precellys 2 ml hard grinding tubes. Product number MK28 R. Catalog number 03961-1-008.
Pyrex 15 ml glass centrifuge tubes, lot number: 07413036
96-well sample plate, Abgene, Epsom, UK
Fisher Scientific 500ml glass jar with cover, BTF-625-110K

2.1.3 Model Wine Samples

For the laboratory ageing experiments, model wines were taken from Three Choir Vineyards in Gloucestershire, UK. The wines were selected to meet the criteria that they were from a known source, were fermented, but had not been aged. The white wine was pressed from the Seyval Blanc grape. Seyval Blanc is a modern hybrid grape whereby the *Vitis vinifera*, European grape vine, is

crossed with a North American species in order to increase tolerance to cold weather and disease. Any of the North American *Vitis* species could be involved with its genetic description, including: *riparia, labrusca, aestivalis, rupestris*. This wine was fermented in a cold tank for three weeks. The fermentation was complete, based upon residual sugar analysis performed by an employee at Three Choirs Vineyards. Seven aliquots of this white wine were collected into clean glass bottles, each of 400 ml. The red grape varietal was Triomphe d'Alsace, another modern hybrid consisting of three *Vitis* species: *vinifera, riparia, and rupestris*. The grapes were pressed, left in a warm room at 25° C for 5 days before being transferred to a storage tank for a 4 week fermentation period. Three aliquots of this red wine were collected into clean glass bottles, each of 400 ml. All bottles were stored at -80° C until further analysis.

2.1.4 Model Sherds

Model sherds were prepared by Graham Taylor, Experimental Archaeologist and Pottery Specialist in Northumberland, UK. The sherds were meant to mimic ancient amphorae and consisted of Devon ball clay (70%), red Etruria marl (10%), coquet river sand (15%), and Ande site grit (5%). The clay was fired electrically to a temperature of 1050° C, estimating the temperature at which a Roman kiln was fired. The sherds were then hand sawn to a dimension of 3cm in length by 1 cm in width.

2.1.5 Archaeological Samples from Sardinia, Italy

Two sections of the wine press, TM.003.3 and TM.027.3, as well as a separate portion of the preparatory layer from TM.003.3, were received from Professor Peter van Dommelen, University of Glasgow in 2012. Fifteen sherds representing transport amphorae were collected from the University of Leicester in January, 2013 and include representative pieces from the neck, the side wall, and the base. Sample identifications are given in Chapter 5. There were no soil samples accompanying the sherds. The wine press was chosen as a 'positive' control for archaeological wine.

Concerning the amphorae, the neck samples were originally chosen as the 'negative' control, whereas the base portions were considered separate 'positive' controls.

2.1.6 Archaeological Samples from Vindolanda, Northumberland, England

Seventy samples were chosen from those collected by volunteer excavators in June, 2012, during an active excavation under the direction of staff archaeologists. The samples represent mortaria, transport amphorae, cooking pots, soil, and storage jars, from various contexts within the site. All context descriptions were described by Kate Sheehan-Finn, Archaeological and Research Assistant of the Vindolanda Trust and are compiled in chapter 6.

2.1.7 Modern Foodstuffs

Representative food samples common to Roman British cooking were weighed out and aged for three months (lipids) or six months (bulk foodstuffs) at 40°C before extraction and analysis. The foodstuffs were purchased at local markets and include: clear honey, spelt flour, barley malt (barley originating from Australia), and three dried herbs: thyme, oregano, and basil. Foods purchased for the lipid aging experiment included: bacon back, goat cheese, sheep cheese, cows milk 4% fat, olive oil, and sardines packed in water. High temperature cooking experiments were not performed with these samples.

2.2 Methods

Attention was given to cleanliness in order to limit contamination. All glassware including Pyrex glass bottles holding the liquid chromatography mobile phase were autoclaved, solvent rinsed, and covered with methanol cleaned aluminum foil prior to use.

2.2.1 Laboratory Aging Study: Model Sherds

For the wine/water permeated model sherds, approximately 350 ml of HPLC grade water were added to a 500 ml glass jar containing 12 randomly selected model pottery sherds. A low flow of nitrogen gas was added for thirty seconds in order to produce an inert atmosphere. The jar was covered tightly with a threaded cap, secured with parafilm, and allowed to sit at room temperature for seven days. The procedure was repeated for white wine and for red wine.

After the seven day period, the model sherds were removed from the liquid and allowed to air dry on methanol-cleaned glass surfaces. There was heavy residue visible on the white wine sherds and the red wine sherds were heavily stained. After drying overnight, tweezers were used to transfer 4 randomly chosen sherds to clean glass jars that were either empty, or contained 2 cm of dry sand or 2 cm of wet peat. Within the 'dry sand' jars, a further 2 cm of sand were added to cover the sherds. Within the 'wet peat' jars, a further 2 cm of wet peat were added to cover the sherds. The final labeled jars were: water/empty, water/sand, water/peat, red/empty, red/sand, red/peat, white/empty, white/sand, and white/peat. The different environments imitated a dry sandy soil and a heavy, wet peat soil; the plain glass jars were utilised as baseline environments. All jars were placed in the ageing oven at 40° C. After six months, the samples were removed from the oven and any heavy debris from sand or peat was removed. The samples were wrapped in aluminum foil (methanol rinsed) and stored in the -80° C freezer until analysis.

2.2.2 Extraction Method: Model Sherds

A tile cutter was used to remove 100 mg of laboratory aged sherd; the tile cutter was rinsed with methanol between samplings. The sample was weighed on an analytical balance and the weight recorded; the sample was then transferred to a 2 ml Precellys hard grinding tissue tube. The amount of solvent to be added to the sample for a biphasic solution is based on the following ratio in full:

methanol:chloroform:water, 2:2:1.8 (v/v) (Bligh and Dyer, 1959). Four hundred microliters of methanol and 160 µl of water were added to the sample Precellys tube. The sample tube was homogenised in the Precellys 24 homogeniser at 2x6800 rpm/30 s. A glass pipette was used to transfer the slurry into a 1.8 ml glass vial; the Precellys tubes were then discarded. Four hundred microlitres of chloroform were added to the glass vial with a cleaned Hamilton syringe. Two hundred microlitres of water were then added with a 1 ml pipette. The sample was vortexed for 20 seconds and allowed to sit at room temperature for five min. The glass vials were then centrifuged for 10 min at 4000 rpm at 20° C and then transferred to a fume hood. Four hundred microlitres of the top polar layer were transferred to a 1.5 ml Eppendorf tube with a Hamilton syringe. The syringe was rinsed three times with methanol between samples. The polar aliquots were dried down in a Speedvac with no heat and stored at -80° C until analysis. The non-polar fraction was transferred to a 1.8 ml glass vial and blown down to dryness under a stream of nitrogen, capped and stored at -80° C until analysis.

The original sample containing the pottery residue was air dried overnight in the fume hood. One hundred fifty microlitres of 4 M potassium hydroxide (KOH) were then added to the glass vial which was vortexed for 30 s and then centrifuged at 6000 rpm for 2 min (Zsuga and Kiss, 1987; Guasch-Jane et al., 2004). The vial was then heated at 50° C for 10 min on a heating block and then cooled to room temperature. The sample was slowly acidified with 300 µl of 2 N HCl, the pH was gauged with pH paper. Four hundred fifty microlitres of ethyl acetate were then added to the glass vial and the vial was vortexed for 30 s and then centrifuged at 4000rpm for 10 min at 18^{o} C. Approximately 400 µl of the organic extract was transferred into a 1.5 ml champagne vial and then blown down to dryness with nitrogen. The vials were capped and stored at -80° C until analysis.

2.2.3 Extraction Method: Archaeological Sherds

With the side of the drill bit, the top 2 mm surface from the sherd was removed and discarded. A tile cutter was used to remove 1 g of sample sherd. The sample was weighed on an analytical balance, the weight recorded, and the sample transferred to a 7 ml Precellys hard tissue homogenising tube. The biphasic extraction was very similar to that which was described in Section 2.2.2, differing in the increased volume of solvents. Four millilitres of methanol and 1.6 ml of water in full were added to the sample Precellys tube. The sample tube was homogenised in the Precellys 24 homogeniser at 2x6800 rpm/30 s. A glass pipette was used to transfer the slurry and small particles into a 15 ml glass centrifuge tube. Two millilitres of water were added to the Precellys tube to dislodge any remaining particles and added to the centrifuge tube. The Precellys tube was then discarded. Four millilitres of chloroform were added with a graduated cylinder. The centrifuge tube was vortexed for 10 s and then centrifuged for one minute at 500 rpm, room temperature. The polar layer was removed and prepared for storage/analysis as above. The non-polar layer was removed and prepared for solid phase extraction. The remaining solid residue was allowed to air dry in the fume hood overnight.

For solid phase extraction, the non-polar layer was dried down to approximately 400 µl under a stream of nitrogen. Otherwise, the non-polar fraction was dried down under a stream of nitrogen, capped and stored at -80° C. In preparation for SPE, the sample was removed from the freezer and reconstituted in 400 µl of chloroform. The aminopropyl cartridge was added to an SPE manifold and conditioned with 2 ml of hexane. The non-polar aliquot was added to the cartridge. One point four millilitres of ethyl acetate:hexane, 15:85 (v/v), were added to the cartridge to elute off the neutral TAGs which were collected, dried down and stored at -80° C until analysis (Bodennec et al., 2000; Kaluzny et al., 1985).

For alkaline fusion, 600 µl of 1 M KOH were added to the dried residue. The tube was vortexed for 10 s and heated at 50° C for 1 h in a water bath. The sample was allowed to cool to room temperature and the sample acidified by slowly adding 2.5 ml of 2 N HCl. The pH was gauged with pH paper. The sample was then allowed to sit for 24 h at room temperature. In preparation for solid phase extraction, the strong cation exchange cartridge was added to an SPE manifold and conditioned with 1 ml methanol followed by 1 ml HPLC water, per manufacturer's recommendation. A new 15 ml centrifuge tube was added to the SPE tank in order to catch the column runoff. The acidified sample was loaded onto the column and a vacuum drawn. The tube containing the run off was then extracted with 3 ml of ethyl acetate. The tube was vortexed for 10 s and centrifuged for 1 min at 500 rpm. The top organic layer was continuously transferred to a 1.5 ml champagne vial for nitrogen blow down. The vial was then capped and stored at -80° C until analysis.

2.2.4 Sample Analysis: HILIC/LC-Triple quadrupole analysis

The final elution parameters for the liquid chromatography are shown in Table 2.1. The identity of each transition between parent and product ion with its collision energy was transferred to the targeted method, as shown in Table 2.2. These transitions between parent and product ions were determined by choosing the most intense fragment ions. The transitions were also limited to the elution time from the HILIC column, such that the final targeted method contained four segments: segment 1 from 0-17 min collected in full scan mode, segment 2 collected from 17-23 min SRM mode for 8 transitions of the early eluting acids, segment 3 from 23-33 min for the transitions of the later eluting acids, and segment 4 analysed at full scan from 33-43 min.

New mobile phases were prepared each time in clean, solvent rinsed 500 ml Pyrex glass bottles. Mobile phase B was prepared as 100 mM ammonium acetate in HPLC water. In a fume hood, several drops of ammonium hydroxide were added to the solution in order to raise the pH to above 8. The pH was gauged with pH paper and confirmed with a pH meter.

Table 2.1 Final elution parameters of the zic-pHILIC LC method used for the targeted analysis of the archaeological samples.

Column	**SeQuant zic-pHILIC column, 150x2.1, 5 µm**
Mobile Phase A	**Acetonitrile**
Mobile Phase B	**100 mM ammonium acetate, pH = 8.2**
Mobile Phase C	**Water**
Temperature	**30°C**
Sample Volume	**1 µl**
Step 1	**Time 0-7 minute 10%B, 0%C**
Step 2	**Time: 7-21 minutes 10%B, 50%C**
Step 3	**Time: 21-29 minutes 10%B, 50%C**
Step 4	**Time 29-33 minutes 10%B, 0%C**
Step 5	**Time: 33-35 minutes 10%B, 0%C**
Flow Rate	**70 µl/minute**
Detector	**TSQ mass spectrometer**
Electrospray voltage	**-3600**
Vaporisation temperature	**0**
Sheath gas	**10**
Auxillary gas	**0**
Capillary temperature	**275**

Table 2.2. The final parent-product ion transitions used for the analysis of the archaeological samples.

Segment 1:	0-17 minutes; full scan mode				**Segment 3:**	23-33 minutes; SRM mode			**Segment 4:**	33-43 minutes; full scan mode
Segment 2:	17-23 minutes; SRM mode				**Parent Ion**	**Product Ion**	**Parent Ion**	**Product Ion**		
Parent Ion	**Product Ion**	**Parent Ion**	**Product Ion**		***isopropylmalic acid***		***tartaric acid***			
ferulic acid		***meta-hydroxy acid***			175	85	149	73.1		
193.1	134.1	162.9	91		175	113	149	87.1		
193.1	149.1	162.9	93		175	115	149	103.1		
193.1	178.1	162.9	119		***ascorbic acid***		***citric acid***			
syringic acid		***para-coumaric acid***			175	70.8	191.1	85.1		
197.1	95.1	162.9	93		175	86.9	191.1	87.1		
197.1	123.1	162.9	117		175	114.9	191.1	111.1		
197.1	167.1	162.9	119				191.1	173.1		
197.1	182.1				***succinic acid***		***gallic acid***			
2,3-DHB acid		***vanillic acid***			117.1	73.1	168.9	78.9		
152.8	80.9	167.1	108.1		117.1	99.1	168.9	125		
152.8	90.5	167.1	123.1		***malonic acid***		***citramalic acid***			
152.8	108	167.1	152.1		102.7	40.6	146.9	57		
152.8	109				102.7	58.6	146.9	85		
gentisic acid		***keto-butyric acid***					146.9	87		
152.9	52.7	100.7	56.7		***malic acid***		146.9	129		
152.9	80.9	100.7	73.1		133	71.1				
152.9	108				133	73.1				
					133	115.1				

2.2.5 Sample Analysis: DIMS-LTQ-FT-ICR-MS

Initially, an aliquot of 10 mM ammonium acetate in methanol/water (80/20) (v/v), was used to achieve a stable negative ion mode electrospray using a nanoelectrospray chip. Once a stable spray was achieved, the linear ion trap was tuned using a mixture of Thermo Scientific Pierce ESI Negative Ion Calibration Solution, Figure 2.1, epigallocatechin (*m/z* 305), and epigallocatechin gallate (m/z 457).

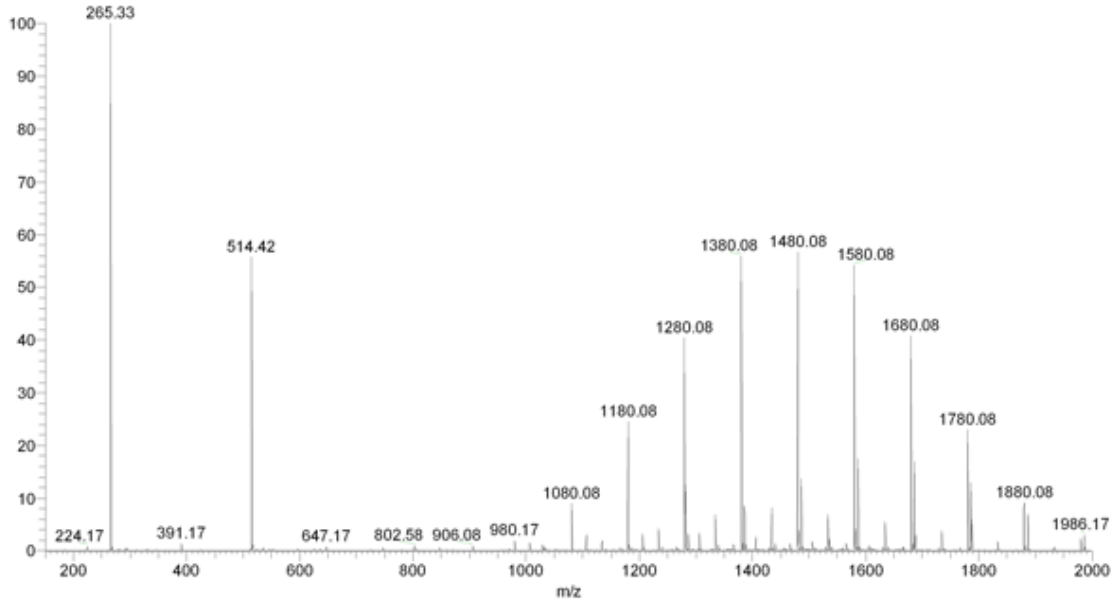


Figure 2.1. A mass spectrum of Thermo Scientific Pierce ESI Negative Ion Calibration Solution, a mixture of SDS (*m/z* 265), sodium taurocholate (*m/z* 514) and Ultramark 1621 (*m/z* 980-1986), reproduced from http://www.piercenet.com/product/esi-negative-ion-calibration-solution.

Five separate tune files were collected over the following m/z ranges: 135-450, 420-730, 700-1010, 980-1030, and 1400-2000, tuning on the largest mass within the middle of the window. Each tune file was then added to the instrument method, with one tune often utilised for several SIM windows.

2.2.5.1 Liquid Wine Aliquot Analysis

The liquid wine aliquots were prepared for analysis as described in Chapter 3; the *m/z* range analysed was 135-2000. The DIMS conditions for the sample delivery included a flow rate of approximately 200 nl/minute with a 0.5 psi gas pressure. Samples were run in the negative ion mode at 1.7 kV; sample volume was 5 µl. For the mass spectrometer, the resolution was maintained at 100,000 (measured from *m/z* 400) with an AGC target value of $1x10^6$. Each SIM window acquisition was 0 .3 s and the total run time was 6.5 min.

2.2.5.2 Sherd and Foodstuff Analysis; polar and alkaline fusion aliquot

Sherd samples were prepared for analysis as described in Chapter 3. For the polar sherds, the *m/z* range analysed was 70-2000. The DIMS conditions for these samples were 5 µl sample volume, run with a gas pressure of 0.5 psi at a negative voltage of 1.75 kV. Resolution was maintained in the mass spectrometer at 100,000 at m/z 400 with an AGC target value of $1x10^6$. Each SIM window acquisition was 0 .3 s for a total run time of 6.5 min.

The alkaline fusion aliquots were analysed over a *m/z* of 70-800. The DIMS conditions for these samples were 5 µl sample volume, run with a gas pressure of 0.5 psi at a negative voltage of 1.75 kV. Again, resolution was maintained in the mass spectrometer at 100,000 at *m/z* 400 with an AGC target value of $1x10^6$. Ten SIM windows were collected. Each SIM window acquisition was 0 .25 s for a total run time of 3.0 min.

2.2.5.3 Lithiated TAG Analysis

The non-polar aliquots were prepared for analysis as described in Section 2.2.3 and in Chapter 6. Briefly, the extracted samples were reconstituted in 2 mM lithium chloride (LiCl) in chloroform:methanol 1:4 (v/v). Ten microlitres of sample were added to the sample well; 8 µl of sample were collected followed by 2 µl of air aspirated into the nozzle. The sample was delivered for 15 min and 10 s at a gas pressure of 0.9 psi and a positive

voltage of 1.5 kV. The mass spectrometer was run in a data dependent mode for MS^n fragmentation. After collecting in full scan mode for 1 min, an initial scan was analysed in MS^1. The most intense ion was then chosen for isolation, fragmentation (MS^2) and identification. The three most intense ions identified in the MS^2 scan were then isolated, fragmented (MS^3) and identified in the linear ion trap. The ion initially fragmented in the full scan mode was put on an exclusion list for the remainder of the analysis thus preventing the ion from being re-fragmented during the analysis. This process cycled through the entire spectrum until the completion of the run. The scan event details are given in Table 2.3.

Table 2.3 The scan event details for the data dependent analysis of TAGs using the LTQ-FT-ICR-MS.

1: FTMS. res=100000 *m/z* (600.0-1000.0)	
2: FTMS. res=100000 Dep MS/MS Most intense ion from scan even 1	**4: ITMS. MS^3 2nd most intense ion from scan event 2**
Activation Type: CID	**Activation Type: CID**
Min. Signal Required: 500.0	**Min. Signal Required: 500.0**
Isolation Width: 1.00 Da	**Isolation Width: 1.00 Da**
Normalised Coll. Energy: 35.0 eV	**Normalized Coll. Energy: 35.0 eV**
Default Charge State: 1	**Default Charge State: 1**
Activation Q: 0.250	**Activation Q: 0.250**
Activation Time: 30.000 ms	**Activation Time: 30.000 ms**
3: ITMS. Dep MS^3 Most intense ion from scan event 2	**5: ITMS. MS^3 3rd most intense ion from 2**
Activation Type: CID	**Activation Type: CID**
Min. Signal Required: 500.0	**Min. Signal Required: 500.0**
Isolation Width: 1.00 Da	**Isolation Width: 1.00 Da**
Normalised Coll. Energy: 35.0 eV	**Normalised Coll. Energy: 35.0 eV**
Default Charge State: 1	**Default Charge State: 1**
Activation Q: 0.250	**Activation Q: 0.250**
Activation Time: 30.000 ms	**Activation Time: 30.000 ms**

2.3 Instrument Settings

2.3.1 LTQ-FT-ICR-MS

Prior to analysis, a tune was performed on the front end of the instrument as mentioned above. If there was a noticeable drop in intensity, the transfer tube was removed and solvent cleaned.

2.3.1.1 Weekly External Calibration

A weekly calibration was performed by laboratory personnel. The automated procedure calibrates and corrects the voltages for ion transmission between the LTQ and the FT-ICR, as well as performs the mass calibration within the ICR cell determining the mass accuracy.

2.3.2 Triple Quadrupole Mass Spectrometer

Prior to analysis, a resolution check was performed on the instrument (described below). If the resolution failed due to mass shift, a calibration of the instrument was performed. Failure due to loss of intensity required cleaning of the S-lens, the main ion transmission device from the ion transfer tube into the first quadrupole, Figure 2.2.

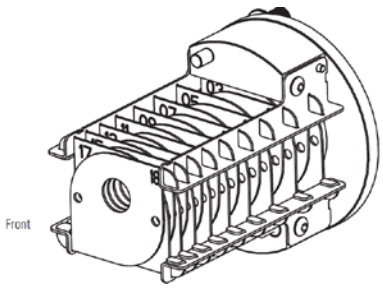


Figure 2.1 A schematic drawing of the S-lens showing its spaced electrodes. Changing the S-lens voltages during tuning procedures allows for the maximum amount of ions to be transmitted to the first quadrupole (reproduced from TSQ Series Hardware Manual, Thermo Scientific, March 2009).

2.3.2.1 Resolution Check of the Mass Spectrometer

A portion of the Pierce Triple Quadrupole Calibration Solution (Thermo Scientific product number 88325) was directly infused into the instrument. Conditions including capillary temperature and sheath gas were varied in order to maintain an optimal spray stability, that is, the point where the RSD of the total ion current does not vary more than 2%. For negative ion analysis, the peak at m/z 506 ak for polytyrosine was monitored. In profile mode, the peak at half maximum must lie between a certain defined criteria for quadrupole 1 and quadrupole 3. Mass accuracy was determined in centroid mode and should lie within .05 m/z. A successful resolution check for all parameters in the negative ion mode was essential before attempting an analytical run.

2.4 Data-Pre-processing

All univariate statistical scripts, normalisation and g-log transformations protocols were written by Dr. Rob Davidson in the Matlab environment. The missing values script was written by Dr. Olga Hrydziuszko also written in the Matlab environment. Compound identification was searched using the MI-Pack program written by Dr. Ralf Weber in the Python environment. The TAG fragmentation algorithm was also written by Dr. Ralf Weber in the python environment.

2.4.1 Matrix Processing

Spectral requirements for DIMS data processing are described in Chapter 1 and specifically for each experiment in Chapter 3. In general, the collected mass spectra were 'binned' into SIM windows of 100 *m/z*. The edges of each window were 'stitched' together by the SIM-stitch program written in the MATlab environment. The SNR was set at 100, using 2/3 or 2/4 replicates and the intragroup sample filter was based on the number of samples in each class/ total number of samples. The blank was subtracted from the samples in order to remove solvent peaks as well as contamination from the extraction procedures.

For DIMS analysis, any missing values were filled in by a separate code in Matlab, MVImpute version 05 which imputes the missing value with a feature from the original spectrum below the calculated SNR threshold. For LC analysis, missing values were removed by replacing a zero/not detected with half the value of the least intense feature for each particular peak (Dr. Warwick Dunn, personal communication). The final data matrix was then normalised by taking the ratio of the median of the intensity of the QCs and the corresponding peak intensity for each feature in a sample. The normalised matrix was then g-log transformed in order to limit intrasample variability (Parsons et al., 2007). Univariate statistics including ANOVA and the student's t-test were performed on the normalised matrix whereas the multivariate statistics including principal components analysis were performed on the normalised and g-log matrix.

2.4.2 MI-Pack and KEGG Database

As explained in Chapter 3, putative identifications were based upon a search of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using MI-Pack, a script written in the Python environment (Weber and Viant, 2010). The putative identifications utilised for this thesis included the 'single peak search' approach which compared the mass of the experimental compound with the mass of an identified compound found in the KEGG database within a user defined mass error. Calculated elemental composition includes adducts most commonly found in negative ion analyses, e.g., $[M-H]^-$. This approach results in a high number of false positives and suggested identifications should be confirmed with mass spectral fragmentation or orthogonal analytical approaches. In order to compare these results with the current published results, only the negative ions were analysed. The positive ion results were not examined for this preliminary research.

2.4.3 Triacylglycerol (TAG) Code

Lithiated TAGs were analysed by DIMS-FT-ICR-MS^3 fragmentation and annotated by a code written in the python environment which compared the neutral losses from each level (MS^2 and MS^3) to a user generated library. The

user generated library was based upon published fragmentation mechanisms (Hsu and Turk, 2010). Robust statistical additions with user defined criteria were added to guard against spurious results. This included coverage of the times the identified peak is found in each scan. For example, 60% coverage was achieved when the chosen peak was identified three times in five scans. A second criterion determined the relative standard deviation of the peak's intensity. A variable intensity throughout the examined scans suggested noise and was not confirmed as a peak. The code is fully described in Chapter 6. The TAG fragmentation MS protocol is described in Section 2.2.5.3.

3. Laboratory Ageing of Wine and the Identification of Aged Wine Biomarkers

The objectives of the research described in this chapter were to monitor the ageing of wine in a controlled laboratory setting over several months using liquid wine samples as well as wine permeated sherds and to identify the extractable metabolites in order to develop a list of aged wine biomarkers. Initially, liquid aliquots of wine were aged over a four point time frame: zero, one, three, and six months in order to determine whether an ageing 'plateau' existed. The aging plateau as described in this research is the point at which the change in metabolic signature had largely ceased and the difference in two consecutive time points is insignificant based upon the PCA scores plot. The results suggested a six-month time frame was needed to reach this plateau. At that point it is hypothesised that a polymeric network had formed and that a chemical attack of the sample was necessary to release the monomeric species useful for a biomarker list. Using this six-month time frame, the laboratory aged sherds were extracted with a modified Bligh and Dyer biphasic extraction protocol resulting in a polar and a nonpolar aliquot (Bligh and Dyer, 1956). To the remaining pottery, a strong alkaline solution was added hydrolysing ester bonds and releasing monomeric species which were then extracted with ethyl acetate (Zsuga and Kiss, 1987). The extraction procedure resulted in three separate aliquots: polar, non-polar, and an alkaline fusion portion. The alkaline fusion portion, considered most useful for archaeological samples, was chosen to determine the relevant biomarkers.

3.1 Ageing of the Model Wine

3.1.1 Sample Preparation for Ageing Experiments

For the liquid wine aliquots, twenty-two individual 1 ml aliquots (22x1 ml) for each of the red wine and the white wine were pipetted into 1.8 ml glass vials for a total of 44 vials. The vials were then plugged with glass wool (Figure 3.1). For blanks, 8x1 ml aliquots of HPLC water were pipetted into 1.8 ml glass vials and plugged with glass wool (Figure 3.2). All vials were placed in a 40° C oven (Zimman and Waterhouse, 2004). Samples removed from the ageing oven after one, three, and six months of incubation included four vials of red wine and four vials of white wine along with associated water blanks. Collected samples were capped and stored in a -80° freezer prior to extraction and analysis. For time point zero, t_0, 100 µl aliquots of red wine, white wine, and water were pipetted into 1.8 ml glass vials and allowed to dry in the ageing oven within two days, capped and stored at -80°C until analysis.

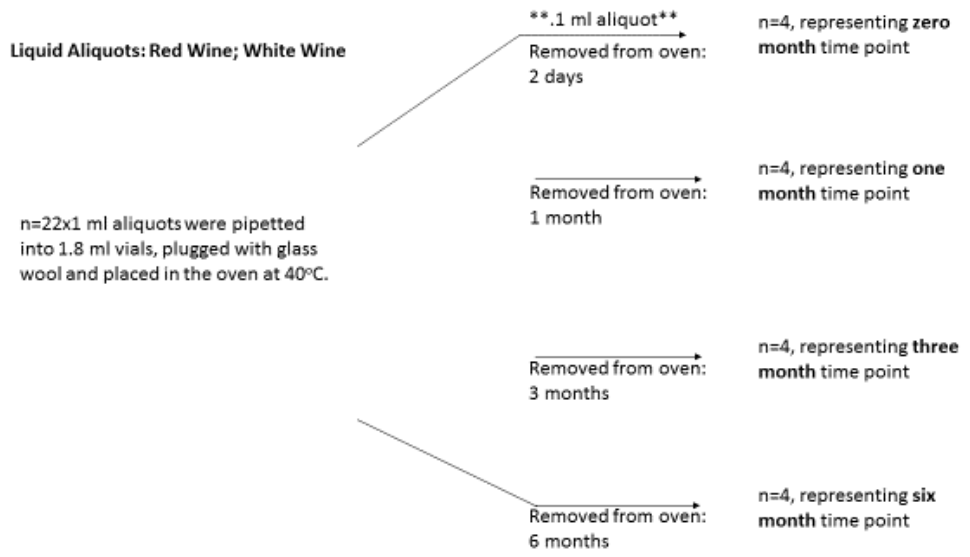


Figure 3.1. Preparation of the ageing experiment for liquid red wine and white wine aliquots.

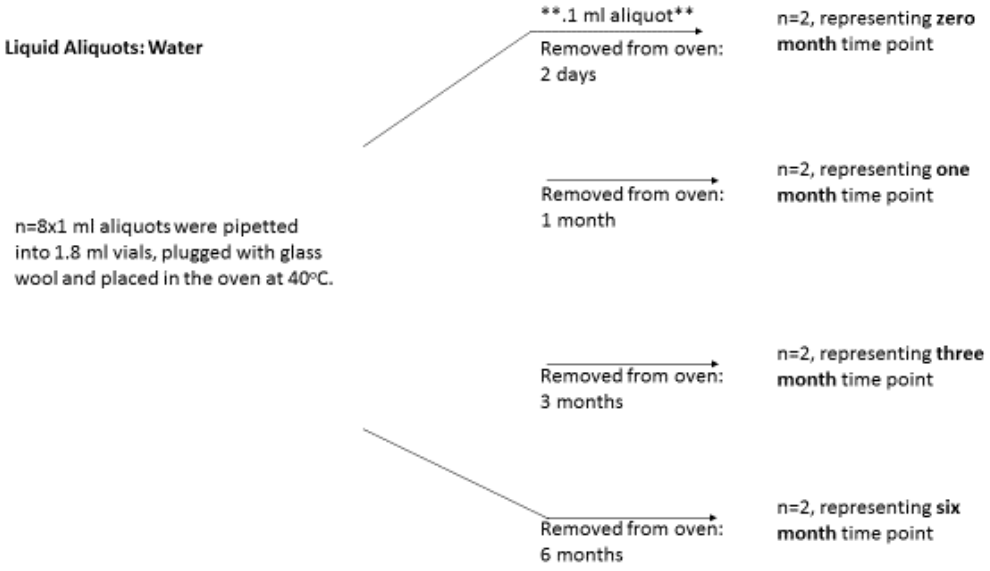


Figure 3.2. Preparation of the ageing experiment for liquid aliquots of water blank.

As stated in Chapter 2, for the wine/water permeated model sherds, approximately 350 ml of HPLC grade water were added to a 500 ml glass jar containing 12 randomly selected model pottery sherds (Figure 3.3). A low flow of nitrogen gas was added for 30 s in order to produce an inert atmosphere. The jar was covered tightly with a threaded cap, secured with parafilm, and allowed to sit at room temperature for seven days. The procedure was repeated for white wine and for red wine.

After the seven day period, the model sherds were removed from the liquid and allowed to air dry on methanol-cleaned glass surfaces. After drying overnight, tweezers were used to transfer 4 randomly chosen sherds to clean glass jars that were either empty, or contained 2 cm of dry sand or 2 cm of wet peat. Within the 'dry sand' jars, a further 2 cm of sand were added to cover the sherds. Within the 'wet peat' jars, a further 2 cm of wet peat were added to cover the sherds. The final labeled jars were: water/empty, water/sand, water/peat, red/empty, red/sand, red/peat, white/empty, white/sand, and white/peat. The different environments for this abiotic experiment imitated a dry sandy soil and a heavy, wet peat soil; the plain glass jars were utilised as baseline environments. All jars were placed in the ageing oven at 40° C. After six months, the samples were removed from the oven and any heavy debris from sand or peat was removed. The samples were wrapped in aluminum foil (methanol rinsed) and stored in the -80° C freezer until analysis.

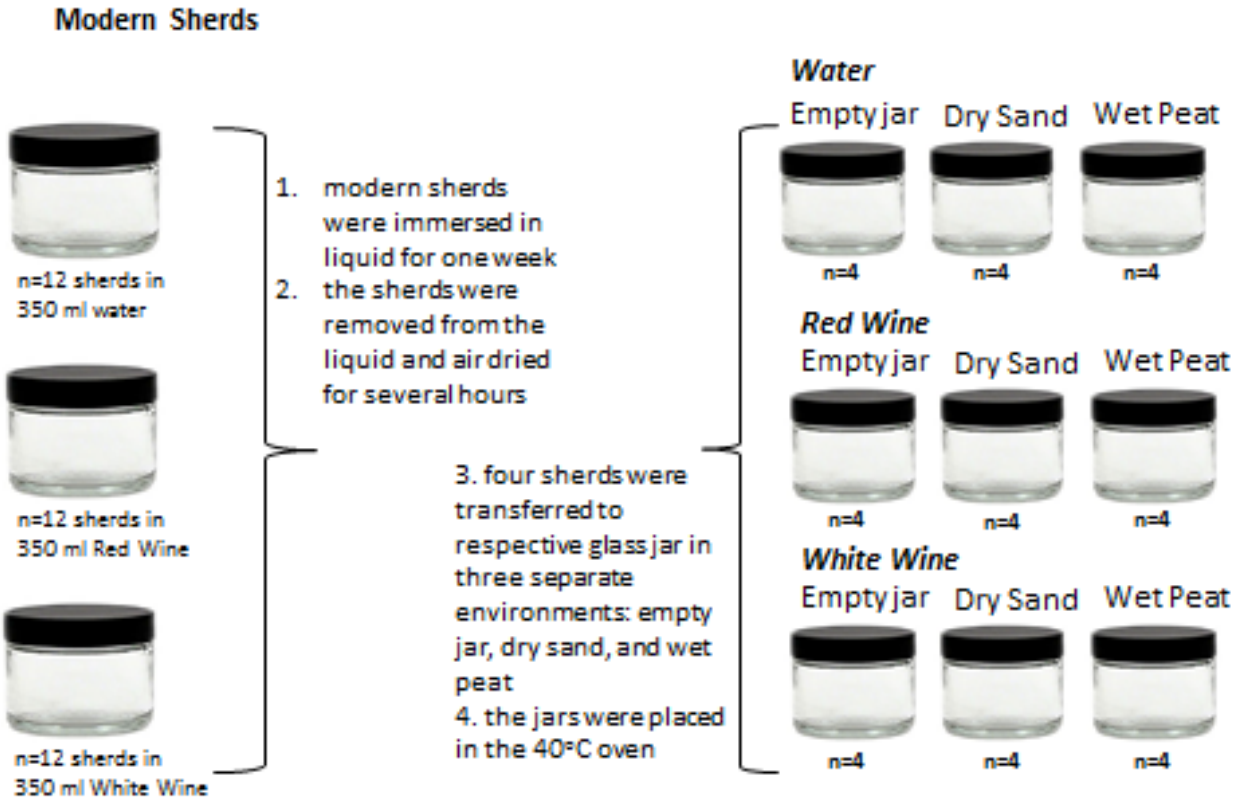


Figure 3.3. Preparation of the ageing experiment for the modern sherds permeated with water, red wine and white wine.

3.2 Analysis of Liquid Aged Wine/ Results and Discussion

All samples were run in triplicate in negative ion mode using a hybrid 7-Tesla Fourier transform ion cyclotron resonance mass spectrometer (LTQ-FT Ultra, Bremen, Germany) with a chip-based direct infusion nanoelectrospray ionisation assembly (Triversa, Advion Biosciences, Ithaca, NY). Quality control samples (QCs) were run with the samples and, unless otherwise noted, represent a mixture of all samples in that particular analysis. A full description of sample analysis is found in the Materials and Methods, Chapter 2. An explanation of the instrument and instrumental conditions can be found in the introduction, Chapter 1, as well as in the Materials and Methods, Chapter 2.

Unless otherwise noted, data collected from all samples were processed as follows. Red wine and white wine were processed as separately. The collected mass spectra were each 'binned' into windows of 100 *m/z*, whereby the edges of each window were 'stitched' using an in-house program, SIMstitch, written in the Matlab environment and more fully explained in Chapter 1.7.1 (Southam et al., 2007). The SNR was set at 100; replicate filtering was set at 2/3. An intragroup sample filter was based upon the number of samples in each group/total number of samples and determined as 50 %. Blanks were run in a scattered fashion throughout the analyses and were subtracted from the samples in order to remove solvent peaks as well as contamination from the extraction procedures. Missing values were imputed as described earlier and the final data matrix was normalised and g-log transformed. Univariate statistics were performed on the normalised matrix whereas the multivariate statistics were performed on the normalised and g-log transformed matrix.

3.2.1 LTQ-FT-ICR-MS Analysis of Zero-, One-, Three-, and Six-Month Ageing Time-Points

A small subset of samples was analysed at the end of the three-month time period in order to refine the analytical methods and monitor the wine's ageing characteristics. Sample vials were removed from the -80^{o} C freezer and allowed to reach room temperature. One millilitre of methanol was added to the glass vials, 100 µl added to the zero time point samples; the sides and the bottom of each vial were scraped with a glass pipette in order to disrupt the residue. Each sample was vortexed for 10 s and transferred to a 1.5 ml Eppendorf tube. Samples were centrifuged at 14,000 rpm for 5 min at 18^{o}C. The white wine samples were diluted 1 in10 in 10 mM ammonium acetate solution (80/20, methanol/water); the red wine samples were diluted 1 in 5 in 10 mM ammonium acetate solution.

The final liquid ageing study was performed on the four time points: zero, one, three, and six months of the red and white wine, with accompanying blanks. Samples were removed from the -80^{o} C freezer and prepared as above. Again, the sides and the bottom of the vials were scraped with a glass pipette in order to disrupt the residue. Each sample was vortexed for 10 s and transferred to a 1.5 ml Eppendorf tube and centrifuged at 14,000 rpm for 5 min at 18^{o} C. The samples analysed prior were removed from the -80^{o} C freezer, vortexed, and centrifuged, having undergone 2 freeze/thaw cycles. Based on the earlier analysis, the white wine samples were diluted 1 in 50 with 10 mM ammonium acetate solution in order to stabilise the electrospray current. The red wine samples were diluted 1 in 10 with 10 mM ammonium acetate. All samples were then vortexed for 10 seconds and centrifuged at 14,000 rpm for 5 min at 18^{o} C. Eight microlitres of each sample were pipetted into the well plates for analysis in triplicate. All samples and QCs were analysed from *m/z* 135-2000. The *m/z* range was extended to 2000 in order to identify any polymer formation occurring in either of the wine systems. However, upon examination of the data, there was very

little information found in the upper *m/z* range of the spectrum. Therefore, for statistical analyses of the data, *m/z* 800 was chosen as the upper mass limit. As stated earlier, in order to compare the results of this research with the published results of other examinations of archaeological wine, only the negative ion results were considered.

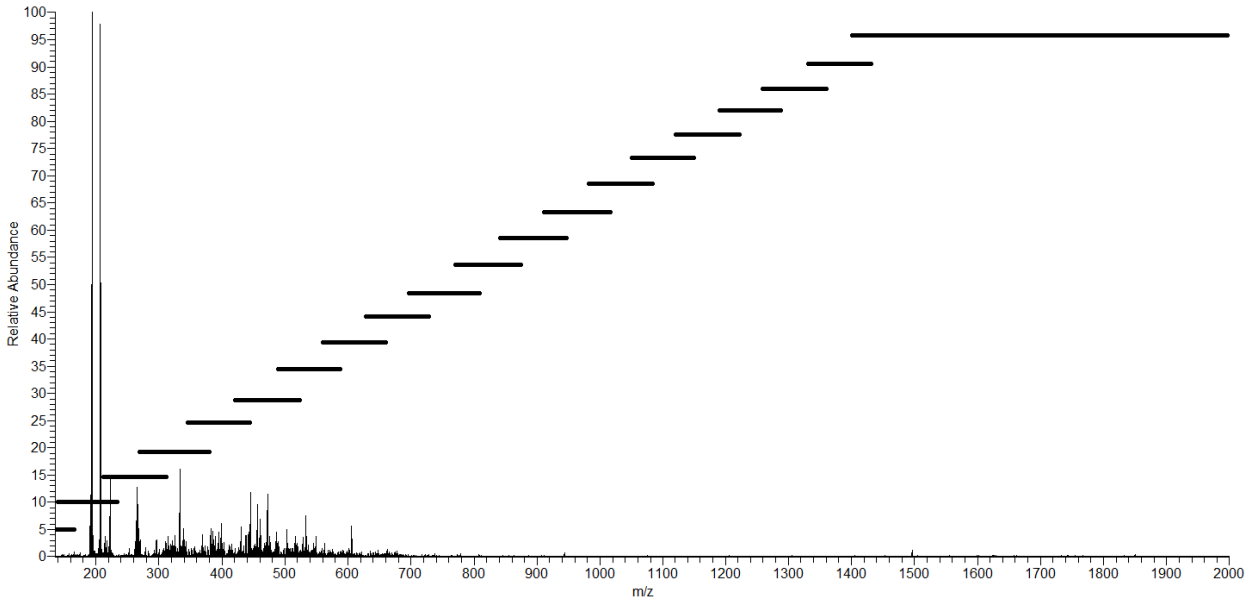


Figure 3.4 A typical negative polar spectrum collected from *m/z* 135-2000. The overlapping SIM windows are shown as horizontal lines, in stepwise fashion indicating the overlap of *m/z* 30.

Figure 3.4 depicts a typical negative polar mass spectrum from *m/z* 135-2000 collected with the protocols referenced in Tables 1 and 2. Prior to analysis, five separate tune files were collected over the following *m/z* ranges: 135-450, 420-730, 700-1010, 980-1030, and 1400-2000. The results were input to the file method and described more fully in Chapter 2.

Table 3.1 Conditions used for the collection of the liquid wine spectra in the LTQ-FT mass spectrometer.

Scan Mode	Wide SIM
AGC target	$1x10^6$
Resolution	100,000
Window acquisition time	.30 seconds
Acquisition Time	6.5 minutes

Table 3.2 Conditions used for the electrospray ionisation via the Triversa nanoelectrospray.

Sample Volume	5µl
Pressure	0.5psi
voltage	1.7kV
polarity	negative

3.2.2 Univariate Statistics of Liquid Aged Wine

An ANOVA test was applied in order to determine those peaks which significantly decreased or increased in peak intensity across all four time points for red wine and for white wine datasets. The results are found in Appendix Tables 3.1 and 3.2 and were sorted on the largest decrease in fold change (a descriptive change in intensity between the initial value and the final value) utilising zero-month time point as the control class, in conjunction with the adjusted p-value, indicative of significance. The largest decrease in fold change over six months indicated the greatest losses of metabolites from zero-month to the six-month time point, suggesting a loss of monomeric species

by (presumably) polymerisation. Appendix Tables 3.1 and 3.2 also contain putative identifications of the ions, the result of a MI-Pack search (described in Section 2.4.2).

The greatest loss over a six month period in both the red wine and the white wine was nominal mass 193, putatively identified as the acetate adduct of malic acid. Malic acid was one of the two main organic acids found in wine, tartaric being the other acid. Figures 3.5a and 3.5b represent the measurable drop off in signal from the initial time point and continuing throughout the remaining three time points for both malic acid and tartaric acid, putatively identified in the red wine aliquots. A t-test compared the three- and six-month time points for both the red wine and the white wine; the results indicated no significant change between the two time points suggesting a halt in the metabolic change. Figures 3.6 and 3.7 are mass spectra of the red wine samples at zero month time point and six month time point. Figures 3.8 and 3.9 are mass spectra of the white wine samples at zero month time point and six month time point.

Other losses over the six month period include several putatively identified flavonols from the red wine aliquot including quercitin glucoside and kaempferol. The loss of flavonols could be due to the formation of a larger polymeric network which then precipitated out of solution. A second explanation is copigmentation, a synergistic combination between anthocyanins and flavonols (or other nonpigmented phenolic compounds) that change the hue and color of the wine (Boulton, 2001). The greatest loss of ion intensity over a six month time frame for the white wine aliquot include the organic acids putatively identified as malic, citric, and tartaric, as well as sugar acids.

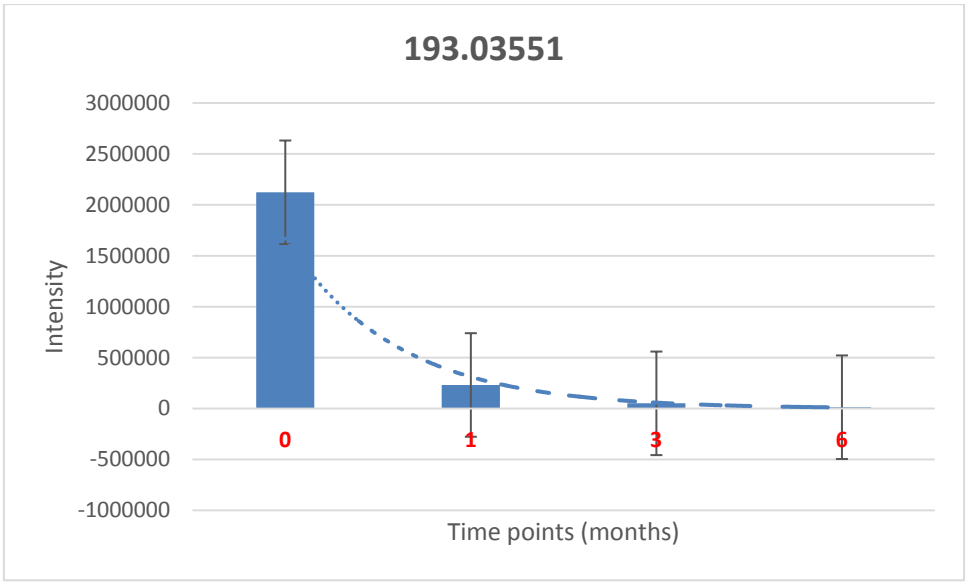
As explained in the introduction, the organic components of wines polymerise over time by forming large polymers between reactive anthocyanidin and tannin species with other monomeric species such as organic and phenolic acids. As these reactions occur, the larger polymeric molecules form

and precipitate out of solution. The methanol extraction primarily targets the monomeric species whose availability presumably decreased over time. It was assumed that a similar loss of metabolites (as monomers) occurred within the laboratory aged sherds. But rather than precipitate out of solution, these polymers have lodged within the clay interstices and are thereby preserved within the clay matrix. Through chemical attack and extraction, the original building blocks of wine may be released and analysed.

3.2.2.1 Rationalising the Loss of Tartaric Acid over a Six-Month Timeframe

The intensity of the peak with m/z 149, putatively identified as deprotonated tartaric acid, significantly decreased over the six-month time frame. However, there is a stable, low level of tartaric acid noticeable for the three- and six-month time points. This consistent low level may suggest the retainment of a tartrate salt, which is readily extracted with methanol/water. In a new wine, the precipitation of a potassium bitartrate salt is a common occurrence and the salt is often removed under controlled conditions by modern vintners (Dinsmore-Webb, 1974: 123; Berg and Keefer, 1958). As was mentioned earlier, tartaric acid identified in archaeological objects has often been attributed to the accumulation of a calcium tartrate salt, the substitution of calcium for two potassium cations due to the calcareous environment of the surrounding pottery (McGovern, 2003: 67). However, the formation of potassium tartrate in newly pressed wines, especially red wines, may be interrupted by the "complexation" of the tartaric acid with a phenolic compound (Balakion and Berg, 1968). A separate explanation for the loss of the acid is the condensation between the negatively charged acid and a nucleophilic site at C-4 flavan-3,4-ol forming an ester linkage. During alkaline hydrolysis, the ester bond is hydrolysed and the free acid is released.

(a)



(b)

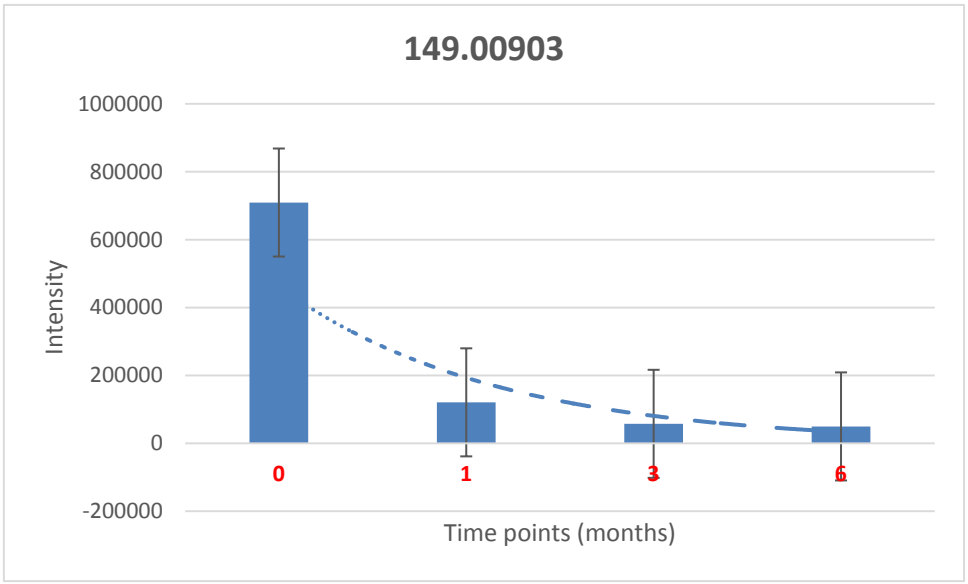


Figure 3.5. The graphical loss of two compounds over a six month time frame from the red wine aliquot; intensity vs time in months. The bars represent the mean of four replicates, whiskers show error. (a) The loss of the ion at m/z 193.03551 over a six month time frame. The ion was putatively identified as malic acid. (b) The loss of m/z 149.00903 over a six month time frame. The ion was putatively identified as tartaric acid.

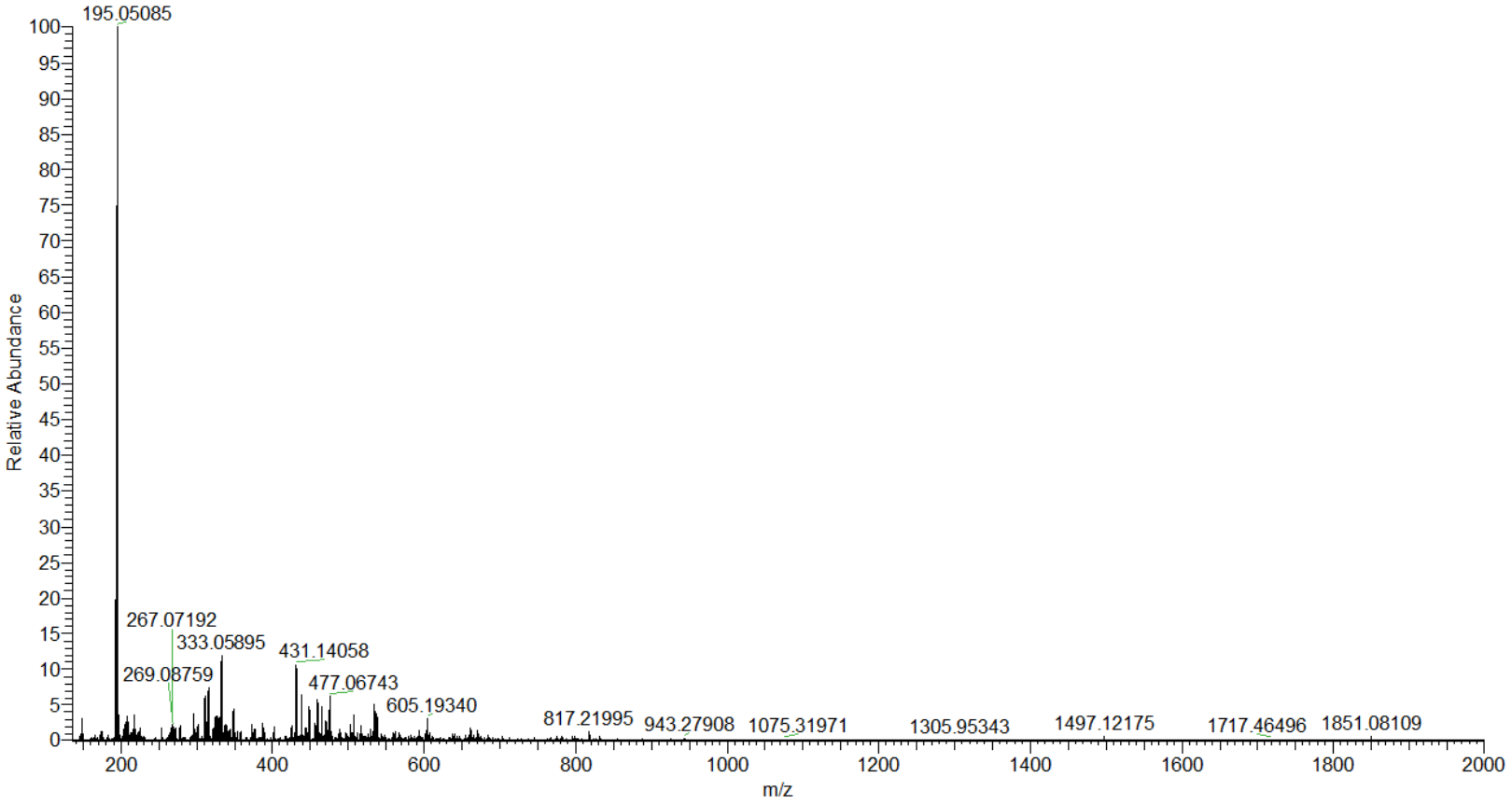


Figure 3.6. A negative ion mass spectrum of a liquid red wine sample at zero month time point.

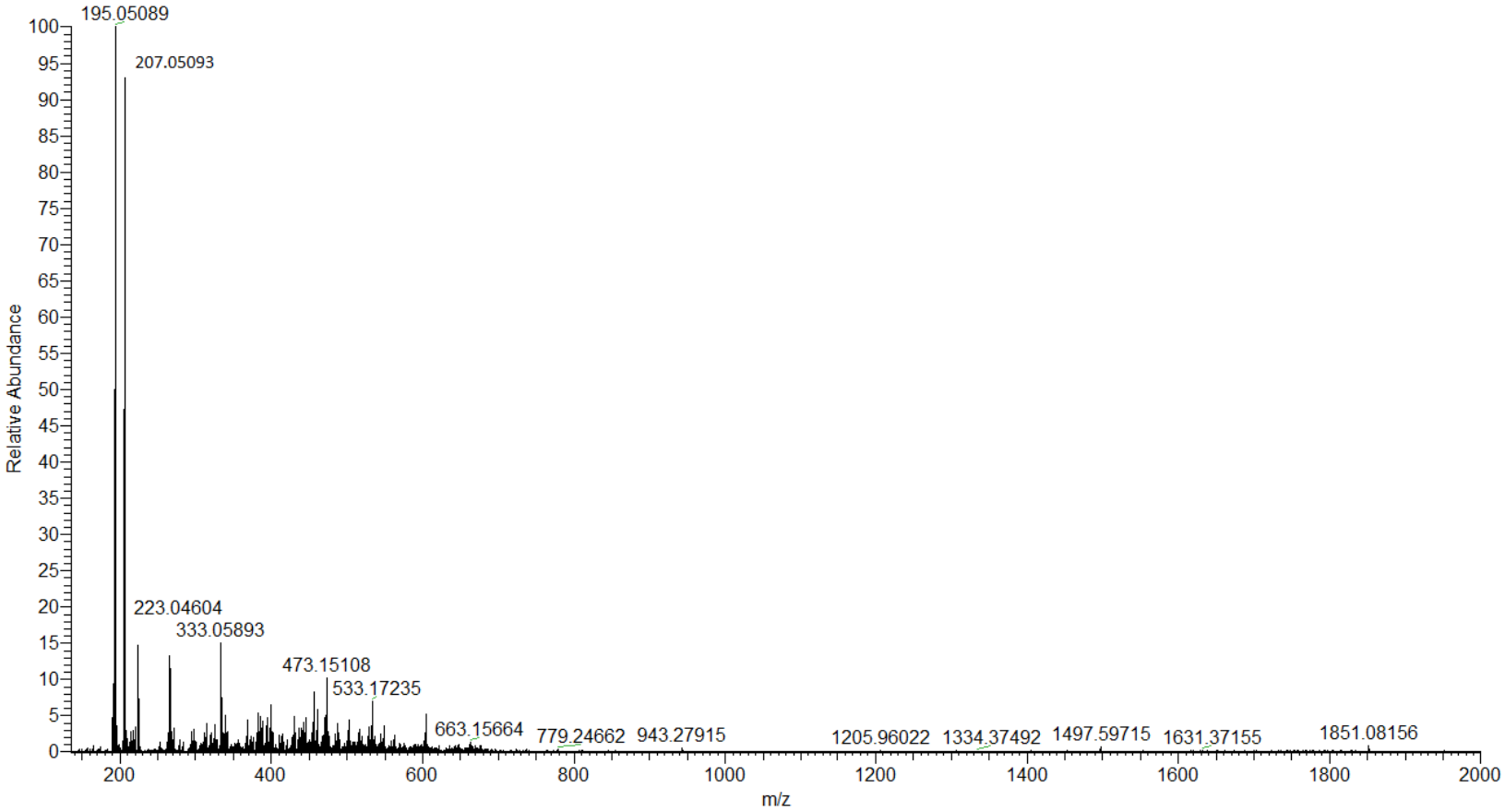


Figure 3.7. A negative ion mass spectrum of a liquid red wine sample at six month time point.

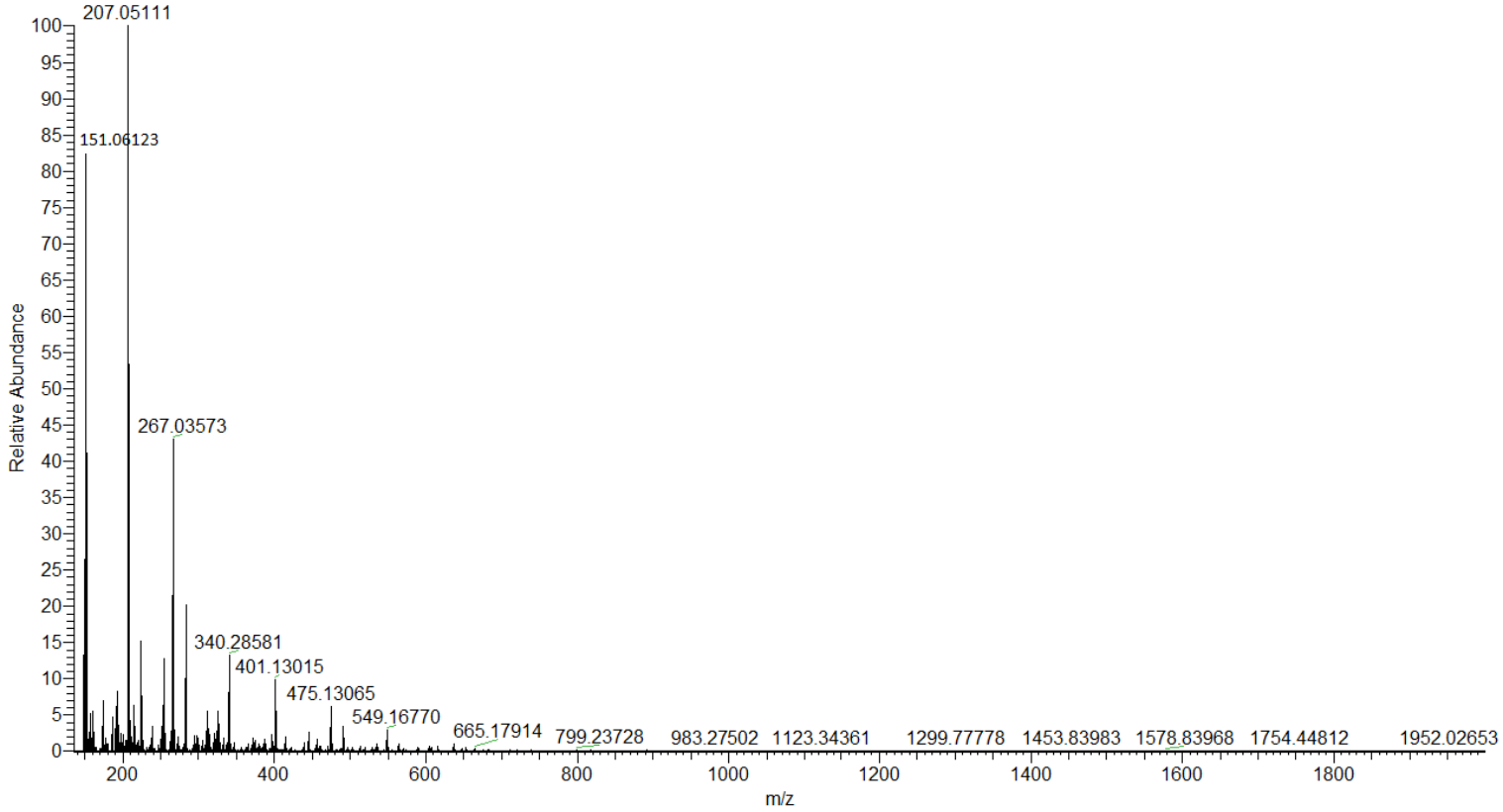


Figure 3.8. A negative ion mass spectrum of a liquid white wine sample at zero month time point.

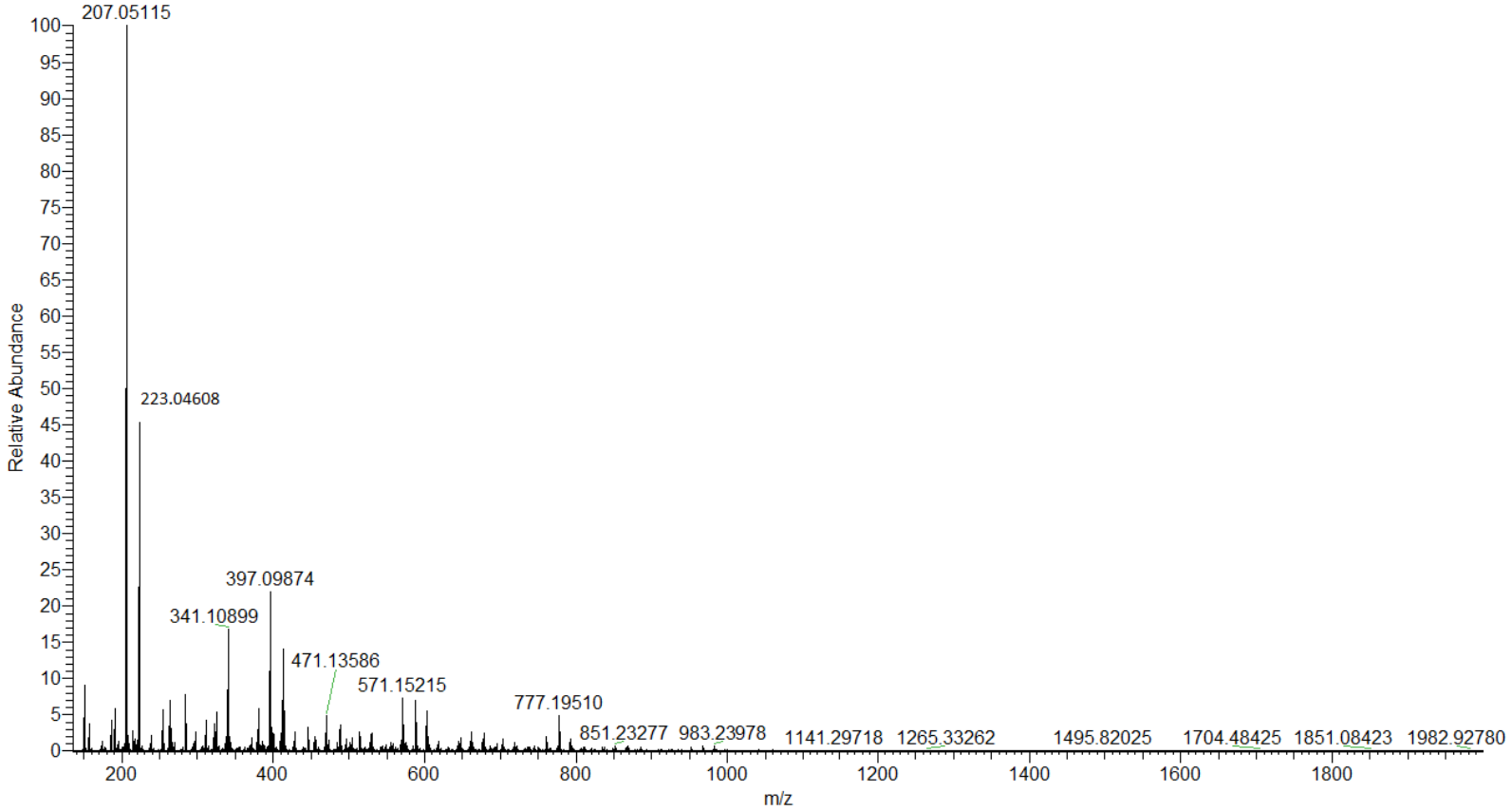


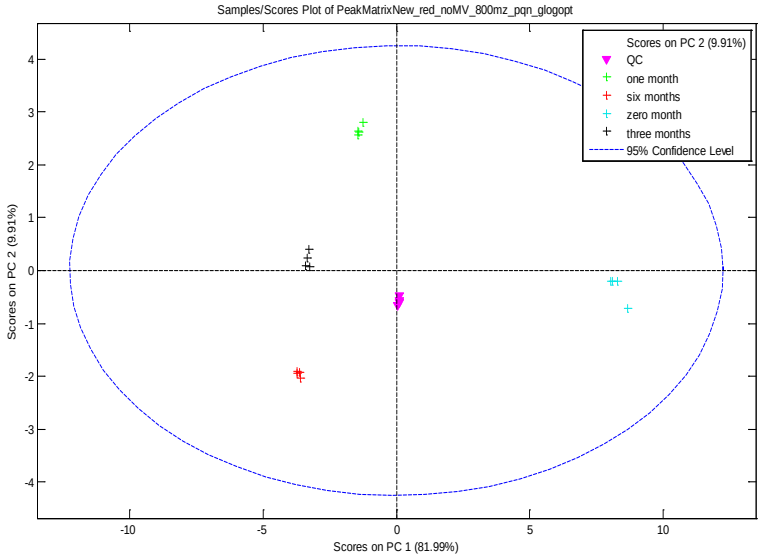
Figure 3.9. A negative ion mass spectrum of a liquid white wine sample at six month time point.

3.2.3 Multivariate Statistics of Liquid Aged Wine

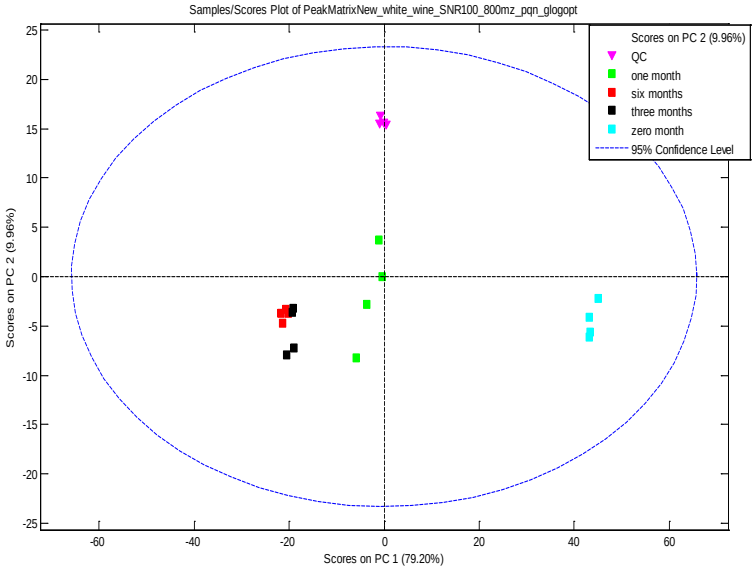
Multivariate statistics using principal components analysis (PCA) was applied to the finalized data matrices of all samples examined in this chapter. PCA was conducted using the PLS toolbox, from Eigenvector, in the Matlab environment.

3.2.3.1 Analysis of Zero-, One-, Three-, and Six-Month Ageing Time-points

Figure 3.10a shows the PCA scores plot for the red wine samples. The QCs in both the red wine analysis and the white wine analysis were clustered tightly together, confirming that the technical replication was relatively high with no perceived instrument drift over the whole analysis. In this scores plot, the greatest variability was along principal component 1(PC1). Zero-month was separated from the remaining three time points. The second greatest variability along PC2 separated the remaining three time points. Although statistically insignificant, a slight shift was obvious between three- and six-month time points. Figure 3.10b shows the corresponding PCA scores plot of the white wine samples. The greatest separation amongst the white wine sample was again along PC1, explaining 79.2% of the variation in the dataset. The three- and six-month aliquots vary little and appear to cluster together along PC1. The results suggest a six month ageing plateau whereby the metabolic change ceases.



(a)



(b)

Figure 3.10. PCA plots of red and white liquid aliquots showing an ageing plateau around six months, a time where the metabolic signature of the wine is no longer significantly changing. (a) A six month time point ageing study for red wine; although small, there is still a slight change between three months and six months. (b) A six month time point ageing study for white wine; the metabolic signature of three months and six months appeared to cluster.

3.3 Analysis of Permeated Modern Sherds

3.3.1 Sample Extraction

Appendix Table 3.3 lists the laboratory-aged sherds that were sampled and extracted. The extraction method used was described earlier in Section 2.2.2. Briefly, there were four sherds for each of the nine categories including: water/peat, water/sand, water/empty jar, white/peat, white/sand, white/empty jar, red/peat, red/sand, red/empty jar. Two samples of the sherds (ca. 100 mg) were taken with a tile cutter from separate areas of each sherd for a total of 72 samples. Samples were homogenised and extracted using a modified Bligh-Dyer extraction technique resulting in a polar and a non-polar extract (Bligh and Dyer, 1959). The remaining solids were allowed to dry overnight in a fume hood. After drying, 150 µl of 4 M KOH were added to each sample and heated for 10 min on a heating block at 50° C (Zsuga and Kiss, 1987). The samples were first cooled to room temperature and then acidified with 400 µl 2 N HCl, and then extracted with 450 µl ethyl acetate. The organic layer was transferred to a 1.8 ml champagne vial and blown down under a stream of nitrogen; the alkaline fusion aliquot (or KOH aliquot) was stored at -80° C.

At this point, the non-polar fraction was not considered for further analysis since the lipid fraction of wine was considered negligible. The polar aliquot and the alkaline fusion aliquot were considered for further mass spectrometric analysis.

3.3.2 FT-ICR-MS Analysis of Extracted Sherds

All samples were run in either triplicate or quadruplicate in negative ion mode using the LTQ-FT Ultra with direct infusion nanoelectrospray. QCs were run with the samples and, unless otherwise noted, represent a mixture of all samples in that particular analysis.

3.3.2.1 Analysis of the Polar Aliquots

The dried polar aliquots were reconstituted in 30 µl of 10 mM ammonium acetate for analysis in negative ion mode. Each sample was vortexed for 10 s and then centrifuged for 20 min at 14000 rpm at 18^{o} C. Five microlitres of each sample was pipetted into the well of a 384 well plate for quadruplicate analysis in negative ion mode from *m/z* 70-2000. The lower limit mass was shifted to *m/z* 70 in order to isolate and identify the majority of monomeric species.

3.3.2.2 Analysis of the Alkaline Fusion aliquot

Due to the high salt content produced from the alkaline fusion in the form of potassium chloride, the nanoelectrospray was highly unstable. A small sample set was analysed prior to determine the analytical protocols. The dried aliquots were reconstituted in 30 µl of 10 mM ammonium acetate. The electrospray from the water sherds was inconsistent, resulting in only 3 of 9 usable spectra. Therefore, in order to limit the disruption of the electrospray during the total analyses, it was decided to limit the number of water sherds used in the final run. The final analysis included seven samples from the following groups: water/sand, white/glass, white/sand, red/glass, and red/sand. In an attempt to form a more stable electrospray, two samples of the red glass were sacrificed in order to determine the amount

of negative ion solution used to reconstitute the samples. The goal was to achieve a more stable electrospray, yet maintain the ion signal strength. However, the signal strength dropped considerably when the sample was reconstituted in 50 µl of 10 mM ammonium acetate; therefore, 30 µl volume was maintained.

For the final analysis of the alkaline fusions aliquots, the samples were reconstituted in 30 µl of 10 mM ammonium acetate solution, vortexed for 10 s, and centrifuged for 6000 rpm for 20 min at 18^{o} C. QCs were prepared from an equal amount of all samples. Five microlitres of each sample were pipetted into the well plates for triplicate analysis in negative ion mode from *m/z* 70-800. The upper mass limit was chosen as *m/z* 800 based upon the successful release of monomeric species from the alkaline hydrolysis.

The peat samples were analysed separately from the glass and sand samples due to the overwhelming matrix effect of the wet peat. The final sample set included eight water/peat, seven red/peat and seven white/peat samples. All samples were prepared and analysed as above.

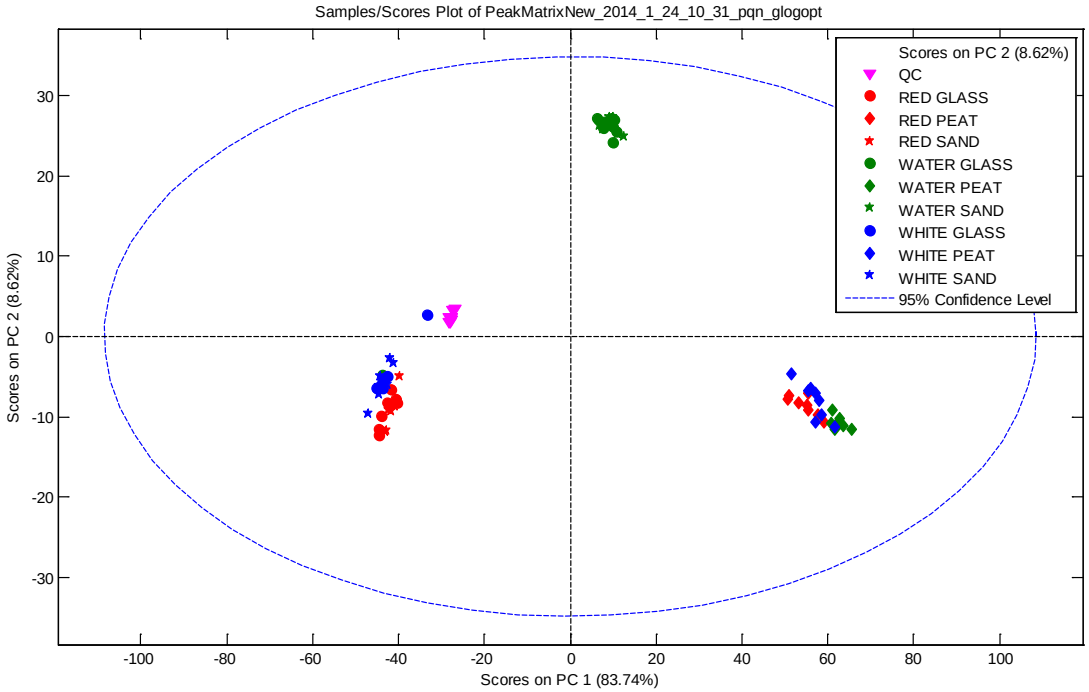
3.3.3 Multivariate Statistics of Wine Permeated Model Sherds

3.3.3.1 Analysis of Extracted Sherds: Polar Aliquot

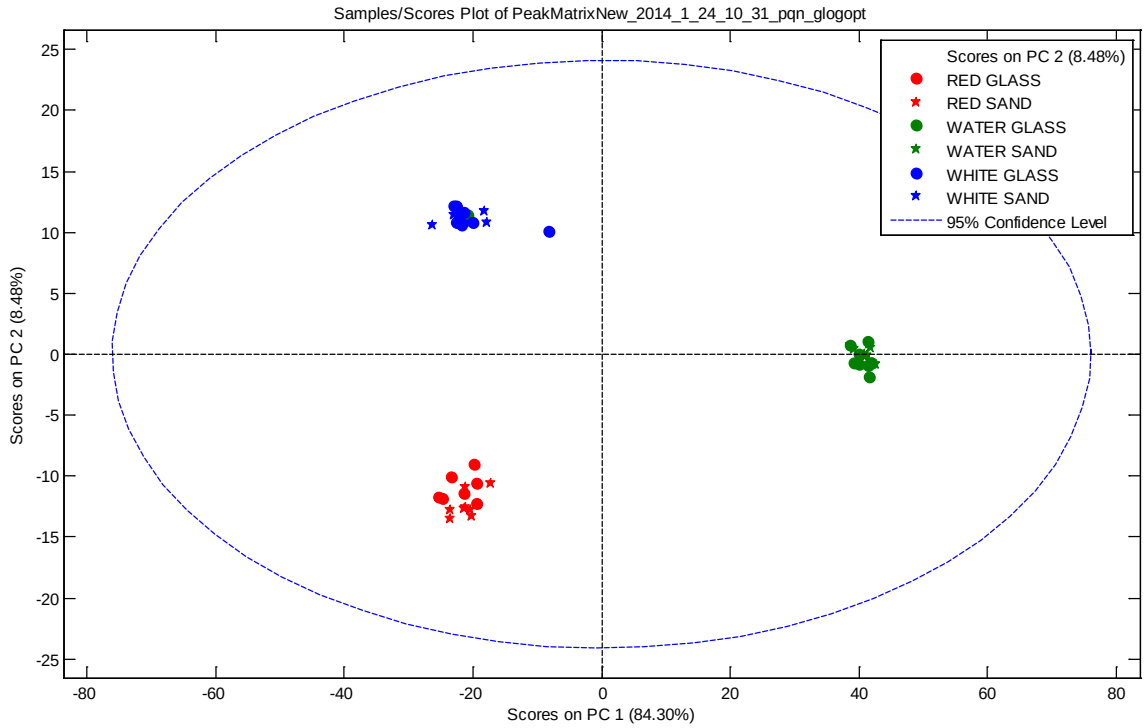
Briefly, the data was processed using the SIMstitch method using a minimum SNR of 100, from a *m/z* range from 70-2000, 2/4 replicate filtering, with a 30% sample filter. The final matrix processing included imputation of missing values, normalisation, and g-log transformation of the data.

The PCA scores plot of all samples analysed is presented in Figure 3.11a. The QCs are clustered together, indicating good technical replication with limited instrument drift throughout the run. The three remaining clusters are: 1. red/white/sand/glass, 2. water/sand/glass, and 3. water/white/red/peat. The matrix effects of the dry sand do not appear to be significant in effecting the metabolic signature of the red wine or the white wine. However, all of the peat samples, water, white wine, and red wine, were clustered together, which suggest an overwhelming matrix effect of the peat in conjunction with a lack of signature molecules for the red and white wine. The most likely reason for this is the incomplete polymerisation of the red wine and the white wine, probably due to sample preparation.

Figure 3.11b depicts the PCA plot with the peat samples removed. The greatest separation is along PC1 between the water and the wine samples; the red and white wine samples separate along PC2. The top 50 loadings (those ions which are most characteristic for each representative sample type) separating the red wine and the white wine for the polar metabolites are listed in Appendix Table 3.4. In terms of archaeological samples, it is highly likely that metabolites extractable in a methanol/water environment do not represent what has remained over millennia due to the potential prolonged exposure to water. The use of polar aliquots for an archaeological sample may be limited to the environment from which the material was unearthed (soil, sand, or clay), certainly a useful description for a full-scale archaeological excavation.


Samples/Scores Plot of PeakMatrixNew_2014_1_24_10_31_pqn_glogopt
Scores on PC 2 (8.62%)
QC
RED GLASS
RED PEAT
RED SAND
WATER GLASS
WATER PEAT
WATER SAND
WHITE GLASS
WHITE PEAT
WHITE SAND
95% Confidence Level
Scores on PC 2 (8.62%)
Scores on PC 1 (83.74%)
30
20
10
0
-10
-20
-30
-100
-80
-60
-40
-20
0
20
40
60
80
100

(a)



(b)

Figure 3.11. PCA scores plot of the polar aliquot of laboratory aged sherds. The samples analysed were white wine, red wine, and water in three separate environments, empty jar, dry sand, and wet peat. (a) The QCs are clustered tightly together indicative of good technical repeatability with limited instrumental drift. The peat samples are separated from other water and wine samples, indicative of overwhelming matrix effects. (b) PCA scores plot with QCs and peat samples removed. The separation between water and both types of wine samples is along PC1; the red wine and the white wine separate along PC2. There appears to be little difference between the environments of the glass jar and the dry sand, the separation was based upon the metabolic signatures of the wine only. The results suggest the successful extraction of polar metabolites from the sherds.

3.3.3.2 Analysis of Extracted Sherds: Alkaline Fusion Aliquot

Briefly, the data were processed using the SIMstitch method using a minimum SNR of 100, from a *m/z* range from 70-800, 2/3 replicate filtering, with a 20% sample filter. The final matrix processing included imputation of missing values, normalisation, and g-log transformation of the data. A comparison between the top 50 loadings from the polar aliquot and the top 50 loadings from the KOH aliquots are given in Appendix Table 3.5. The differences in the top loadings indicate the success of the alkaline fusion on the pottery sherds.

The nanoelectrospray stability during this run was not continuously maintained and 2 out of 5 QCs, 3 out of 7 water/sand samples, an extraction blank, and one of the red samples were lost during the run due to an under fill error caused by the electrospray dropping out. Figures 3.12a and b represent the PCA results of the remaining samples. As can be seen in Figure 3.12a, the remaining QCs were clustered tightly together confirming good technical repeatability with limited instrument drift. Figure 3.12b represents the PCA plot with the QCs removed. The three samples that fell out of the 95% confidence limit were considered outliers and removed from further data analysis: red glass 3B, white glass 4A, and white sand 2B. Table 6 in the Appendix lists the top 200 loadings for white wine and the top loadings for red wine, based upon the separation PC2.

The greatest separation between the wine samples and the water samples is along PC1, as seen in Figure 3.12b. The top 150 loadings of the wine samples, the masses that most significantly characterise wine from the water group, were searched against the KEGG database. The top 100 putatively identified masses were used to compile a targeted biomarker list for application to archaeological

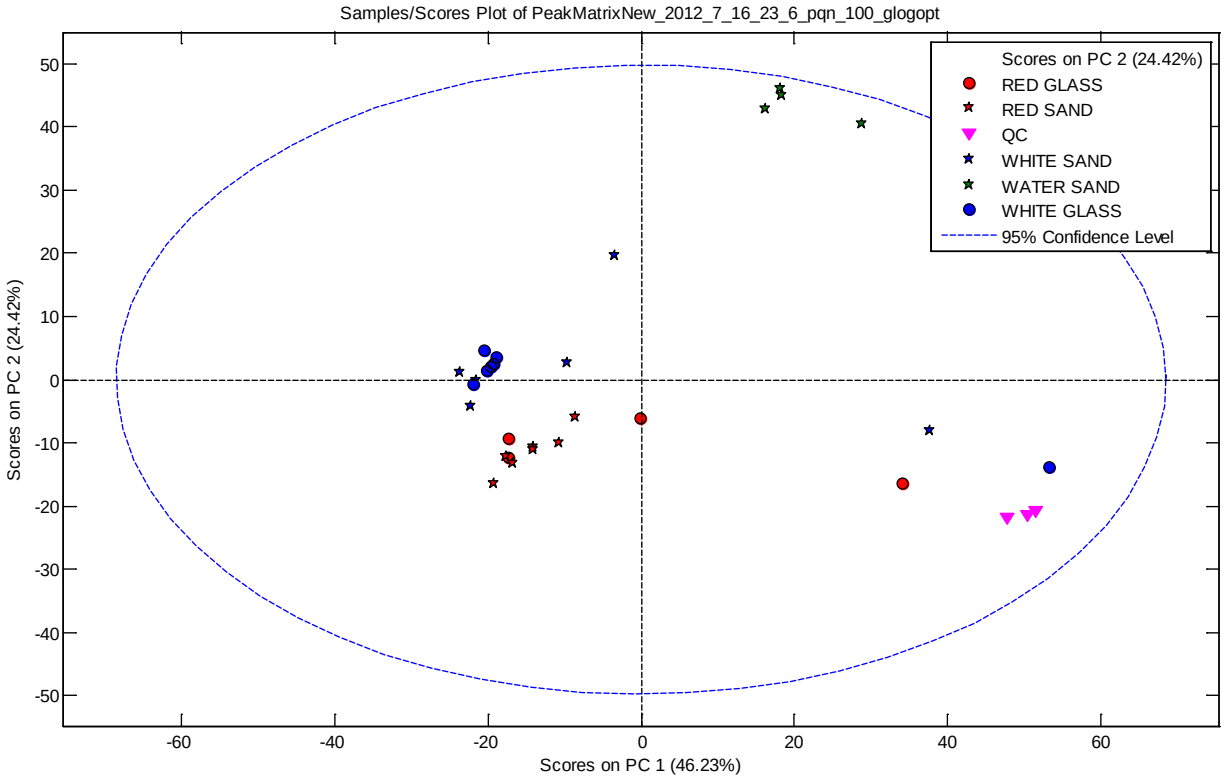
samples in an attempt to answer the question of presence/absence of aged wine remnants and are listed in Table 3.3. The complete list of the top 150 masses is listed in Appendix Table 3.7.

The information listed in Table 3.3 includes the suggested ion adduct, the empirical formula of the parent ion, the theoretical masses of the parent ion and of the adduct ion, and the mass error in ppm between the experimental mass and the theoretical mass. Putative identifications are listed and were based upon a search of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using MI-Pack, a script written in the Python environment (Weber and Viant, 2010). Putative metabolite identifications were chosen based upon a user defined parameter of 2 ppm mass error difference between the theoretical and experimental mass of proposed adduct formations in the negative ion mode. This mass error was based upon the weekly external calibration of the instrument. The adducts included: $[M-H]^-$, $[M+Hac-H]^-$, $[M+K-2H]^-$, $[M+Na-2H]^-$, $[M+Cl]^-$, and $[M+(37Cl)]^-$. As an example, an experimental peak identified at *m/z* 149.0090 was putatively identified as the deprotonated form of tartaric acid having a theoretical *m/z* of 149.0092 in the form $[M-H]^-$. In certain cases, compounds were identified by several different adduct forms and thus one putative ID could be associated with three separate ions.

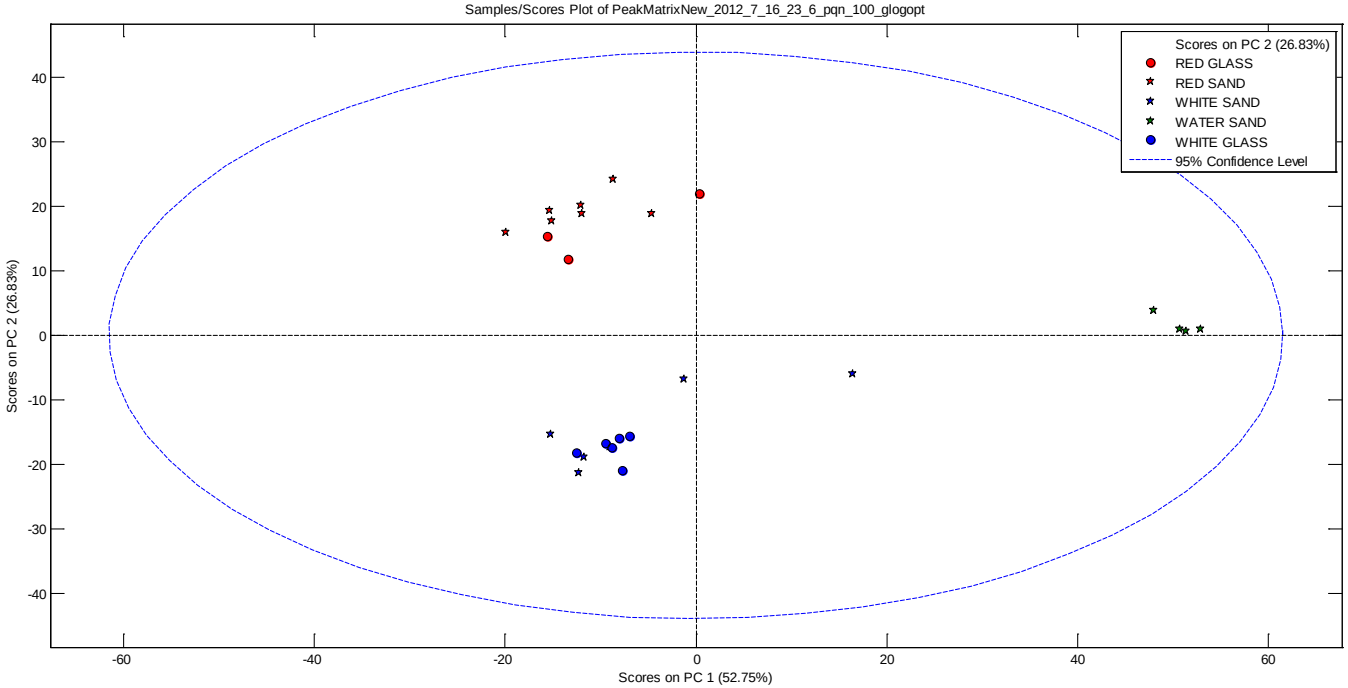
In nearly every case, the putative identifications offer several possible compounds based upon the experimentally determined mass. By comparing the resultant list from the KEGG results in Table 3.3 with the known components in wine described in the Introduction, Chapter 1, a putative list of compounds was compiled. Tartaric, malic, succinic, lactic, and citric acids are considered five of the major organic acids found in wine and their presence in the proposed biomarker list was expected. The presence of secondary organic acids on the list includes malonic acid, alpha-ketobutyric acid, as well as the sugar acids, including glucuronic acid. Phenolic acids are another major group of compounds in wine

and include the benzoic acids and the cinnamic acids. The benzoic acids are represented by vanillic acid, gallic acid, syringic acid and dihydroxybenzoic acid. The cinnamic acids are represented by coumaric acid, caffeic acid, and ferulic acid. Several of the remaining acids on the list are conjugated acids, either conjunctions of two separate acids, or possible artefacts of sample preparation including heating and/or methylation.

Under the action of alkaline fusion, ester bonds are hydrolysed releasing the acids for extraction and analysis. For the red wine sherds, the origin of the phenolic acids is mainly due to the breakdown products of the anthocyanidins including: syringic acid (malvidin), gallic acid (delphinidin), protocatechuic acid (cyanidin), vanillic acid (peonidin), and methylgallic acid (petunidin). The general breakdown of tannins such as catechin and gallocatechinn could release gallic acid and protocatechuic acid respectively. Free phenolic and organic acids esterified within a polymer are also released. In white wine, these include the following benzoic acids: protocatechuic, gallic and syringic acids (Tian et al., 2009; Betes-Saura et al., 1996). The organic acid, tartaric acid, could also originate from hydrolysis of the hydroxycinnamate tartrates (Figure 1.9), prevalent in both red and white wines. Since many of these acids are found in both red and white, it is risky to assign one acid as a biomarker for either red wine or white wine. A more conservative approach is to analyse a list of biomarkers to determine the absence/presence of wine. And comparative ratios of the chosen biomarkers found in aged wine may prove more reliable for the ultimate determination of red vs. white.


Samples/Scores Plot of PeakMatrixNew_2012_7_16_23_6_pqn_100_glogopt
Scores on PC 2 (24.42%)
RED GLASS
RED SAND
QC
WHITE SAND
WATER SAND
WHITE GLASS
95% Confidence Level
Scores on PC 2 (24.42%)
Scores on PC 1 (46.23%)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-40
-20
0
20
40
60

(a)



(b)

Figure 3.12. (a) The PCA scores plot of the alkaline fusion aliquot from the laboratory-aged sherds. The QCs are clustered together indicative of good technical repeatability with limited instrumental drift. (b) PCA scores plot of the alkaline fusion aliquot with QCs removed; three samples were also removed, having fallen outside of the 95% confidence limit: red glass 3B, white glass 4A, and white sand 2B. The wine samples separate from the water samples along PC1. Red wine and white wine samples separate along PC2 indicating distinct metabolic signature for both types of wine.

Table 3.3 The top putatively identified masses taken from the PCA presented in Figure 3.12b and sorted by PC1.

m/z	Relative intensity	PC1	Empirical formula (parent)	Empirical formula (peak)	Ion form	Theoretical mass (neutral) (Da)	Theoretical m/z (Da)	Mass error (ppm)	Putative annotation based on KEGG database	possible component in wine (after alkaline fusion)
149.00912	12839920.3	-0.102401503	$C_4H_6O_6$	$C_4H_6O_6$	$[M-H]^-$	150.01644	149.00916	-0.29	['(R,R)-Tartaric acid', '(S,S)-Tartaric acid', 'meso-Tartaric acid']	tartaric acid
175.06112	4971425.5	-0.094286393	$C_5H_8O_3$	$C_5H_8O_3$	$[M+Hac-H]^-$	116.04735	175.0612	-0.45	['2-Oxopentanoic acid', '3-Methyl-2-oxobutanoic acid', '3-Oxopentanoic acid', '5-Oxopentanoate']	oxopentanoic acid
175.06112	4971425.5	-0.094286393	$C_7H_{12}O_5$	$C_7H_{12}O_5$	$[M-H]^-$	176.06848	175.0612	-0.45	['(2R,3S)-3-Isopropylmalate', '(R)-2-(n-Propyl)-malate', '2-Propylmalate', '3-Propylmalate', 'alpha-Isopropylmalate']	isopropylmalic acid
133.01418	16494794.9	-0.085369204	$C_4H_6O_5$	$C_4H_6O_5$	$[M-H]^-$	134.02153	133.01425	-0.52	['(R)-Malate', '(S)-Malate', '3-Dehydro-L-threonate', 'Malate']	malic acid
147.02996	1233057	-0.075751737	$C_3H_4O_3$	$C_3H_4O_3$	$[M+Hac-H]^-$	88.016045	147.0299	0.42	['3-Hydroxypropenoate', '3-Oxopropanoate', 'Pyruvate']	pyruvic acid

147.02996	1233057	-0.075751737	$C_5H_8O_5$	$C_5H_8O_5$	$[M-H]^-$	148.03718	147.0299	0.42	['(R)-2-Hydroxyglutarate', '(R)-2-Methylmalate', '(S)-2-Hydroxyglutarate', '(S)-2-Methylmalate', '2-Dehydro-3-deoxy-D-xylonate', '2-Dehydro-3-deoxy-L-arabinonate', '2-Hydroxyglutarate', 'Citramalate', 'D-Arabinono-1,4-lactone', 'D-Xylono-1,4-lactone', 'D-Xylonolactone', 'D-erythro-3-Methylmalate', 'D-threo-3-Methylmalate', 'L-Arabinono-1,4-lactone', 'L-Arabinono-1,5-lactone', 'L-Xylono-1,4-lactone', 'L-threo-3-Methylmalate']	citramalic acid
153.01939	1799686.6	-0.070222273	$C_7H_6O_4$	$C_7H_6O_4$	$[M-H]^-$	154.02661	153.01933	0.37	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']	dihydroxybenzoic acid
191.01982	860206.9	-0.070063079	$C_4H_4O_5$	$C_4H_4O_5$	$[M+Hac-H]^-$	132.00588	191.01973	0.48	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']	oxaloacetic acid
191.01982	860206.9	-0.070063079	$C_6H_8O_7$	$C_6H_8O_7$	$[M-H]^-$	192.02701	191.01973	0.48	['(1R,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(1S,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(4R,5S)-4,5,6-Trihydroxy-2,3-dioxohexanoate', '2,5-Didehydro-D-gluconate', '2-Dehydro-3-deoxy-D-glucarate', '5-Dehydro-4-deoxy-D-glucarate', 'Carboxymethyloxysuccinate', 'Citrate', 'Isocitrate']	citric acid
147.06634	1883832.2	-0.068889369	$C_4H_8O_2$	$C_4H_8O_2$	$[M+Hac-H]^-$	88.05243	147.06628	0.38	['(R)-Acetoin', '1,4-Dioxane', '2-Methylpropanoate', 'Acetoin', 'Butanoic acid', 'Ethyl acetate']	butanoic acid
147.06634	1883832.2	-0.068889369	$C_6H_{12}O_4$	$C_6H_{12}O_4$	$[M-H]^-$	148.07356	147.06628	0.38	['(R)-2,3-Dihydroxy-3-methylpentanoate', '(R)-Mevalonate', '(R)-Pantoate', '(S)-Mevalonate', '2,3-Dihydroxy-3-methylpentanoate', '3,6-Dideoxy-L-galactose', 'Abequose']	mevalonic acid

133.05061	3301580.2	-0.06607991	$C_3H_6O_2$	$C_3H_6O_2$	$[M+Hac-H]^-$	74.03678	133.05063	-0.18	['(R)-Lactaldehyde', '(S)-Lactaldehyde', '3-Hydroxypropanal', 'Glycidol', 'Hydroxyacetone', 'Lactaldehyde', 'Methyl acetate', 'Propanoate']	
133.05061	3301580.2	-0.06607991	$C_5H_{10}O_4$	$C_5H_{10}O_4$	$[M-H]^-$	134.05791	133.05063	-0.18	['(R)-2,3-Dihydroxy-3-methylbutanoate', '1-Deoxy-D-xylulose', '2,3-Dihydroxy-3-methylbutanoate', '2-Deoxy-L-arabinose', '2-Deoxy-alpha-D-ribopyranose', 'Deoxyribose']	sugar acid
163.04002	1344736.2	-0.050834612	$C_9H_8O_3$	$C_9H_8O_3$	$[M-H]^-$	164.04735	163.04007	-0.3	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']	coumaric acid/phenylpyruvic acid
130.99865	298220	-0.049814628	$C_4H_4O_5$	$C_4H_4O_5$	$[M-H]^-$	132.00588	130.9986	0.39	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']	oxaloacetic acid
179.03508	313212.1	-0.048992668	$C_9H_8O_4$	$C_9H_8O_4$	$[M-H]^-$	180.04226	179.03498	0.54	['2-Hydroxy-3-(4-hydroxyphenyl)propenoate', '3-(4-Hydroxyphenyl)pyruvate', 'Aspirin', 'Caffeate', 'trans-2,3-Dihydroxycinnamate']	caffeic acid
211.02497	262104.9	-0.046164544	$C_9H_8O_6$	$C_9H_8O_6$	$[M-H]^-$	212.03209	211.02481	0.74	['2-Hydroxy-6-ketononatrienedioate', '3-(2-Carboxyethenyl)-cis,cis-muconate', '5-Carboxyvanillic acid']	carboxyvanillic acid
206.96793	191453.9	-0.045614948	$C_3H_7O_7P$	$C_3H_7O_7P$	$[M+Na-2H]^-$	185.99294	206.96761	1.54	['2-Phospho-D-glycerate', '3-Phospho-D-glycerate', '3-Phospho-DL-glycerate']	
144.03033	141336.5	-0.045401167	$C_5H_7NO_4$	$C_5H_7NO_4$	$[M-H]^-$	145.03751	144.03023	0.68	['2-Oxoglutaramate', '4-Oxoglutaramate']	2-keto-glutaramic acid
181.01434	180546.4	-0.04498824	$C_8H_6O_5$	$C_8H_6O_5$	$[M-H]^-$	182.02153	181.01425	0.5	['2-Hydroxyisophthalic acid', '3,5-Dihydroxyphenylglyoxylate', '4-Hydroxyphthalate', 'Stipitatate']	phthalic acid

195.03004	323515.6	-0.043538515	$C_9H_8O_5$	$C_9H_8O_5$	$[M-H]^-$	196.03718	195.0299	0.73	['3-(3,4-Dihydroxyphenyl)pyruvate']	dihydroxyphenylpyruvic acid
197.04542	6107928.3	-0.041884423	$C_7H_6O_3$	$C_7H_6O_3$	$[M+Hac-H]^-$	138.0317	197.04555	-0.65	['2-Hydroxy-5-methylquinone', '3,4-Dihydroxybenzaldehyde', '3-Hydroxybenzoate', '4-Hydroxybenzoate', 'Gentisate aldehyde', 'Salicylate', 'Sesamol']	hydroxybenzoic acid
197.04542	6107928.3	-0.041884423	$C_9H_{10}O_5$	$C_9H_{10}O_5$	$[M-H]^-$	198.05283	197.04555	-0.65	['3-(3,4-Dihydroxyphenyl)lactate', '3-Methoxy-4-hydroxymandelate', 'Syringic acid']	syringic acid
117.0193	2144058.9	-0.041306416	$C_4H_6O_4$	$C_4H_6O_4$	$[M-H]^-$	118.02661	117.01933	-0.29	['Methyl oxalate', 'Methylmalonate', 'Succinate']	succinic acid
103.00368	88796.1	-0.037181229	$C_3H_4O_4$	$C_3H_4O_4$	$[M-H]^-$	104.01096	103.00368	-0.03	['2-Hydroxy-3-oxopropanoate', 'Hydroxypyruvate', 'Malonate']	malonic acid
169.01433	182359	-0.036673686	$C_7H_6O_5$	$C_7H_6O_5$	$[M-H]^-$	170.02153	169.01425	0.48	['Gallate']	gallic acid
197.00934	78631	-0.035967678	$C_8H_6O_6$	$C_8H_6O_6$	$[M-H]^-$	198.01644	197.00916	0.9	['3,4-Dihydroxyphthalate', '4,5-Dihydroxyphthalate']	phthalic acid
199.01683	1125705.9	-0.03496134	$C_9H_8O_3$	$C_9H_8O_3$	$[M+Cl]^-$	164.04735	199.01675	0.42	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']	coumaric acid/phenylpyruvic acid
183.03001	115160.7	-0.033479499	$C_8H_8O_5$	$C_8H_8O_5$	$[M-H]^-$	184.03718	183.0299	0.61	['3,4-Dihydroxymandelate', '3-O-Methylgallate']	methyl gallic acid
201.01393	345164.1	-0.032280828	$C_9H_8O_3$	$C_9H_8O_3$	$[M+(37Cl)]^-$	164.04735	201.0138	0.66	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']	coumaric acid/phenylpyruvic acid

189.07696	334513.5	-0.031522479	$C_8H_{14}O_5$	$C_8H_{14}O_5$	$[M-H]^-$	190.08413	189.07685	0.59	['(R)-3-((R)-3-Hydroxybutanoyloxy)butanoate']	
205.03551	30085.2	-0.03117134	$C_5H_6O_5$	$C_5H_6O_5$	$[M+Hac-H]^-$	146.02153	205.03538	0.64	['2-Oxoglutarate', '5-Hydroxy-2,4-dioxopentanoate', 'Dehydro-D-arabinono-1,4-lactone', 'Methyloxaloacetate', 'Oxaloacetate 4-methyl ester']	oxoglutaric acid
205.03551	30085.2	-0.03117134	$C_7H_{10}O_7$	$C_7H_{10}O_7$	$[M-H]^-$	206.04266	205.03538	0.64	['(2S,3R)-3-Hydroxybutane-1,2,3-tricarboxylate', '(R)-2-Hydroxybutane-1,2,4-tricarboxylate', '2-Methylcitrate', 'Homoisocitrate']	methylcitric acid
161.04562	67109.1	-0.030741974	$C_4H_6O_3$	$C_4H_6O_3$	$[M+Hac-H]^-$	102.0317	161.04555	0.44	['(S)-Methylmalonate semialdehyde', '2-Methyl-3-oxopropanoate', '2-Oxobutanoate', 'Acetoacetate', 'Succinate semialdehyde']	alpha ketobutyric acid
161.04562	67109.1	-0.030741974	$C_6H_{10}O_5$	$C_6H_{10}O_5$	$[M-H]^-$	162.05283	161.04555	0.44	['(2R,3S)-2,3-Dimethylmalate', '(R)-2-Ethylmalate', '(R)-3,3-Dimethylmalate', '(S)-2-(Hydroxymethyl)glutarate', '1,5-Anhydro-D-fructose', '2-Dehydro-3-deoxy-D-fuconate', '2-Dehydro-3-deoxy-L-fuconate', '2-Dehydro-3-deoxy-L-rhamnonate', '2-Deoxy-scyllo-inososе', '2-Hydroxyadipate', '3,6-Anhydrogalactose', '3,6-Anhydroglucose', '3-Ethylmalate', '3-Hydroxy-3-methylglutarate', 'D-Fucono-1,4-lactone', 'Diethyl pyrocarbonate', 'L-Fucono-1,5-lactone', 'L-Rhamnono-1,4-lactone', 'Lichenin']	dimethyl malic acid/ ethyl malic acid/ sugar acids
145.01434	43325.5	-0.030439508	$C_5H_6O_5$	$C_5H_6O_5$	$[M-H]^-$	146.02153	145.01425	0.63	['2-Oxoglutarate', '5-Hydroxy-2,4-dioxopentanoate', 'Dehydro-D-arabinono-1,4-lactone', 'Methyloxaloacetate', 'Oxaloacetate 4-methyl ester']	oxoglutaric acid

181.05073	70728.7	-0.029838377	$C_7H_6O_2$	$C_7H_6O_2$	$[M+Hac-H]^-$	122.03678	181.05063	0.53	['3-Hydroxybenzaldehyde', '4-Hydroxybenzaldehyde', 'Benzoate', 'Salicylaldehyde', 'Tropolone']	benzoic acid
193.05078	194456.2	-0.029083865	$C_{10}H_{10}O_4$	$C_{10}H_{10}O_4$	$[M-H]^-$	194.05791	193.05063	0.76	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']	ferulic acid
129.01937	657155	-0.028954418	$C_5H_6O_4$	$C_5H_6O_4$	$[M-H]^-$	130.02661	129.01933	0.28	['(E)-Glutaconate', '2,5-Dioxopentanoate', '2-Methylmaleate', '4,5-Dioxopentanoate', 'Acetylpyruvate', 'Itaconate', 'Mesaconate']	metlymaleic acid
255.05092	130108.2	-0.028903558	$C_9H_8O_5$	$C_9H_8O_5$	$[M+Hac-H]^-$	196.03718	255.05103	-0.43	['3-(3,4-Dihydroxyphenyl)pyruvate']	dihydroxyphenylpyruvic acid
157.0507	47407.2	-0.02795424	$C_7H_{10}O_4$	$C_7H_{10}O_4$	$[M-H]^-$	158.05791	157.05063	0.42	['(1S,4S)-4-Hydroxy-3-oxocyclohexane-1-carboxylate', '2-Isopropylmaleate', '5-D-(5/6)-5-C-(Hydroxymethyl)-2,6-dihydroxycyclohex-2-en-1-one', 'Dimethyl citraconate']	isopropylmaleic acid
188.99615	94910.2	-0.027731671	$C_7H_6O_4$	$C_7H_6O_4$	$[M+Cl]^-$	154.02661	188.99601	0.73	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']	dihydroxybenzoic acid
213.04063	51947.8	-0.027101305	$C_7H_6O_4$	$C_7H_6O_4$	$[M+Hac-H]^-$	154.02661	213.04046	0.78	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']	dihydroxybenzoic acid

215.01744	167385.1	-0.026803166	$C_6H_{10}O_7$	$C_6H_{10}O_7$	$[M+Na-2H]^-$	194.04266	215.01732	0.54	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']	sugar acid
253.07166	211403.7	-0.026398465	$C_{10}H_{10}O_4$	$C_{10}H_{10}O_4$	$[M+Hac-H]^-$	194.05791	253.07176	-0.41	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']	ferulic acid
141.01942	49453.5	-0.025952037	$C_6H_6O_4$	$C_6H_6O_4$	$[M-H]^-$	142.02661	141.01933	0.61	['(S)-5-Oxo-2,5-dihydrofuran-2-acetate', '1,2,3,5-Tetrahydroxybenzene', '2,5-Dihydro-5-oxofuran-2-acetate', '2-Hydroxymuconate semialdehyde', '2-Oxo-2,3-dihydrofuran-5-acetate', 'Kojic acid', 'cis,cis-4-Hydroxymuconic semialdehyde', 'cis,cis-Muconate', 'cis,trans-Hexadienedioate']	kojic acid
167.03504	350068.8	-0.025797423	$C_8H_8O_4$	$C_8H_8O_4$	$[M-H]^-$	168.04226	167.03498	0.34	['(R)-4-Hydroxymandelate', '(S)-4-Hydroxymandelate', '1,2-Dihydrophthalic acid', '2,6-Dihydroxyphenylacetate', '2,6-Dimethoxybenzoquinone', '2-Hydroxy-6-oxoocta-2,4,7-trienoate', '3,4-Dihydroxymandelaldehyde', '3,4-Dihydroxyphenylacetate', '4-Hydroxymandelate', '4-Hydroxymethylsalicylate', '4-Hydroxypheoxyacetate', 'Homogentisate', 'Orsellinate', 'Vanillate']	vanillic acid

89.02438	57476.2	-0.024994887	$C_3H_6O_3$	$C_3H_6O_3$	$[M-H]^-$	90.031695	89.024419	-0.43	['(R)-Lactate', '(S)-Lactate', '3-Hydroxypropanoate', 'D-Glyceraldehyde', 'Glyceraldehyde', 'Glycerone', 'L-Glyceraldehyde', 'Lactate']	lactic acid

3.3.3.2.1. Alkaline Fusion Aliquot of the Peat Sherds

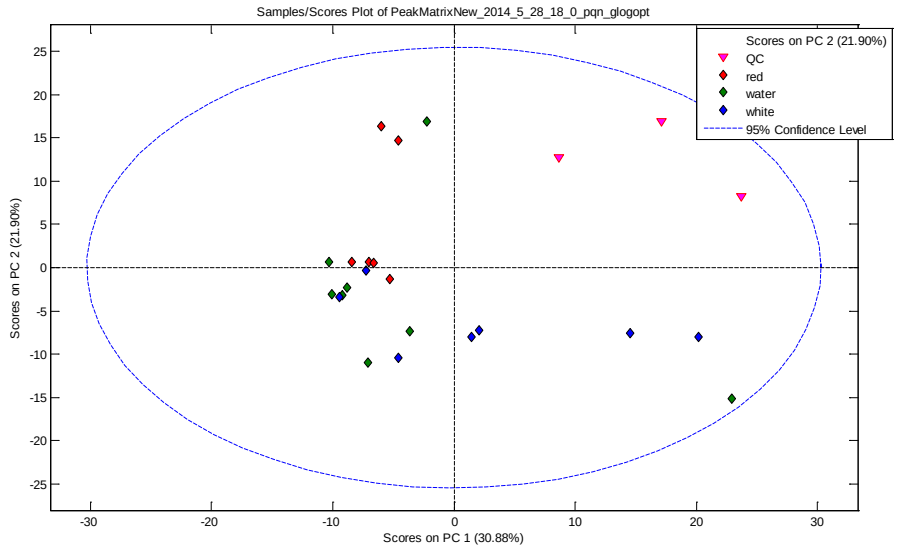
Due to the overwhelming matrix effect of the peat, the biomarker list chosen for the archaeological samples is listed in Table 3.3 and was developed from the results of the glass/sand matrix. However, a separate analysis of the peat samples was undertaken and may offer additional reference compounds for the analysis of objects from a heavy peat environment. Humic acid polymers often form in heavy peat soils; the application of alkaline hydrolysis to these polymers may release compounds that overlap with the proposed biomarkers for the aged wine.

3.3.3.2.1.1 Multivariate Statistics of Alkaline Fusion Peat Sherds

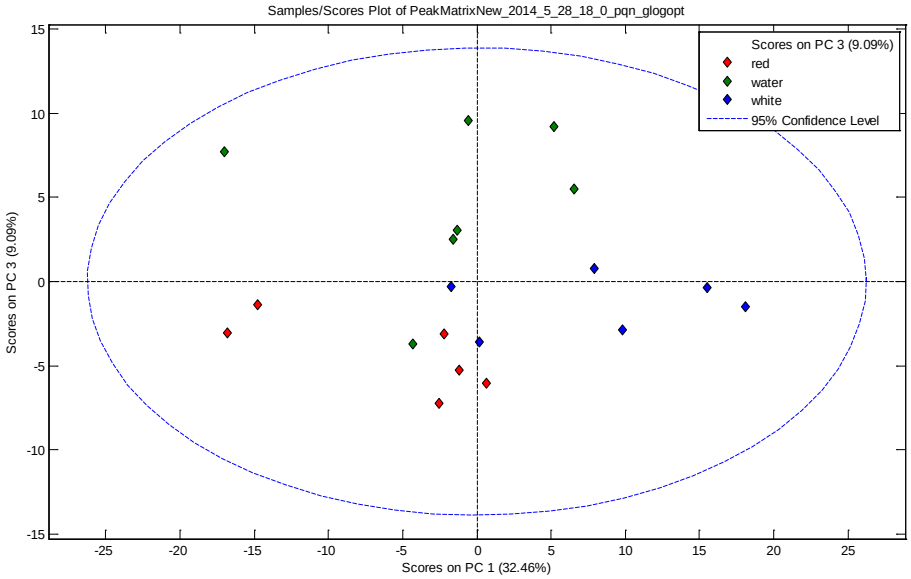
Figure 3.13a and 3.13b represent the PCA scores plots of the alkaline fusion results of the peat sherds immersed in water, red wine, and white wine and aged for six months. There is no obvious separation and univariate statistical analysis of the PC scores were then used to determine the significant differences between the three classes (water/red/white) along each principal component. Significance was determined between red and white wine along PC1 ($p=0.015$); significance was determined between water and wine along PC3 ($p=0.000369$). Table 3.4 represents the top 20 putatively annotated *m/z* values that distinguish wine from water in a heavy peat environment. The results were taken from Figure 3.13b and sorted by PC3.

From the results in Table 3.4, phenylpyruvic/coumaric acids were the only putatively annotated compounds also found in the glass/sand samples. Free 12-hydroxydodecanoic acid was identified in the volatile components of wine (Borea Carnacini et al., 1980). Methylbezaldehyde was also identified in

the volatile portion of wine (Flamini and Traldi, 2010: 236). The lack of effective signature molecules suggests an incomplete polymerisation of the wine. The reason for this may be due to the matrix effect of the peat or to the preparation of the sherds. The sherds should be re-prepared as described in Chapter 7 in order to determine if there is substantial overlap between the biomarkers determined from the aged wine and the possible biomarkers from the humic acid polymers.



(a)



(b)

Figure 3.13. The PCA scores plot of the alkaline fusion aliquot from the laboratory-aged sherds from the peat matrix only. (a) The QCs are not clustered together suggesting some drift during the analysis. Red3A was removed, having fallen outside of the 95% confidence limit. (b) PCA scores plot of the alkaline fusion aliquot with QCs removed, showing PC1vsPC3; two samples were also removed, having fallen outside of the 95% confidence limit: white 1A and water 2B. Univariate analysis revealed that PC1 represents a significant difference between red and white wine; PC3 represents a significant difference between water and both types of wine.

Table 3.4 The top twenty putatively identified masses taken from PCA seen in Figure 3.13b and sorted by PC3.

m/z	Relative intensity	PC3	Empirical formula (parent)	Empirical formula (peak)	Ion form	Theoretical mass (neutral) (Da)	Theoretical m/z (Da)	Mass error (ppm)	Putative annotation based on KEGG database	possible components in wine (after alkaline fusion)
590.44646	236712.5	-0.1687862		0					0	
618.47777	148087	-0.1562345		0					0	
634.47273	63584.6	-0.1249341		0					0	
119.05051	16281.1	-0.11175	$C_5H_{10}N$	$C_5H_{10}N$	$[M+Cl]^-$	84.081324	119.05073	-1.81	['1-Methylpyrrolinium']	
119.05051	16281.1		C_8H_8O	C_8H_8O	$[M-H]^-$	120.05752	119.05024	2.28	['2-Methylbenzaldehyde', '3-Methylbenzaldehyde', '4-Hydroxystyrene', 'Acetophenone', 'Phenylacetaldehyde', 'Styrene oxide', 'p-Tolualdehyde']	
215.1655	339387.2	-0.1113617	$C_{10}H_{20}O$	$C_{10}H_{20}O$	$[M+Hac-H]^-$	156.15142	215.16527	1.08	['(+)-Neomenthol', '(-)-Citronellol', '(-)-Menthol', 'Decanal', 'beta-Citronellol']	
215.1655	339387.2		$C_{12}H_{24}O_3$	$C_{12}H_{24}O_3$	$[M-H]^-$	216.17255	215.16527	1.08	['12-Hydroxydodecanoic acid']	12-Hydroxydodecanoic acid
144.04541	216030.9	-0.1066722	C_9H_7NO	C_9H_7NO	$[M-H]^-$	145.05276	144.04549	-0.54	['1(2H)-Isoquinolinone', '3-Methyleneoxindole', '8-Hydroxyquinoline', 'Indole-3-carboxaldehyde', 'Quinolin-2-ol', 'Quinolin-4-ol']	possible fungicide
205.01445	122744.4	-0.1006064	$C_{10}H_6O_5$	$C_{10}H_6O_5$	$[M-H]^-$	206.02153	205.01425	0.98	['Flaviolin']	
591.44997	76207.8	-0.0933017		0					0	
119.05017	202425.4	-0.0829639	$C_5H_{10}N$	$C_5H_{10}N$	$[M+Cl]^-$	84.081324	119.05073	-4.67	['1-Methylpyrrolinium']	

119.05017	202425.4		C_8H_8O	C_8H_8O	$[M-H]^-$	120.05752	119.05024	-0.58	['2-Methylbenzaldehyde', '3-Methylbenzaldehyde', '4-Hydroxystyrene', 'Acetophenone', 'Phenylacetaldehyde', 'Styrene oxide', 'p-Tolualdehyde']	methylbenzaldehyde
199.01694	138861.2	-0.0782624	$C_{10}H_{10}O_2$	$C_{10}H_{10}O_2$	$[M+K-2H]^-$	162.06808	199.01669	1.27	['(1S,2S)-1,2-Dihydronaphthalene-1,2-diol', '1,2-Dihydronaphthalene-1,2-diol', '4-Hydroxycinnamoylmethane', 'Isosafrole', 'Methyl cinnamate', 'Safrole', 'cis-1,2-Dihydronaphthalene-1,2-diol', 'p-Methoxycinnamaldehyde', 'trans-2-Phenylcyclopropanecarboxylic acid']	
199.01694	138861.2		$C_9H_8O_3$	$C_9H_8O_3$	$[M+Cl]^-$	164.04735	199.01675	0.97	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']	phenylpyrivic acid/coumaric acid
163.04019	289591.3	-0.0760137	$C_9H_8O_3$	$C_9H_8O_3$	$[M-H]^-$	164.04735	163.04007	0.74	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']	phenylpyrivic acid/coumaric acid
219.03014	163941	-0.0753632	$C_{11}H_8O_5$	$C_{11}H_8O_5$	$[M-H]^-$	220.03718	219.0299	1.1	['(3E)-4-(2-Carboxyphenyl)-2-oxobut-3-enoate', '(3Z)-4-(2-Carboxyphenyl)-2-oxobut-3-enoate', '3-[6-(Carboxymethylene)cyclohexa-2,4-dien-1-ylidene]-2-oxopropanate', 'Purpurogallin']	
138.03217	28433.3	-0.0726176	$C_3H_{10}NO_3P$	$C_3H_{10}NO_3P$	$[M-H]^-$	139.03983	138.03256	-2.79	['N-Monomethyl-2-aminoethylphosphonate']	

138.03217	28433.3		$C_4H_9NO_2$	$C_4H_9NO_2$	$[M+Cl]^-$	103.06333	138.03273	-4.06	['(R)-3-Amino-2-methylpropanoate', '(S)-2-Aminobutanoate', '2-Amino-2-methylpropanoate', '3-Aminoisobutyric acid', '4-Aminobutanoate', 'D-2-Aminobutyrate', 'HBA', 'L-3-Amino-isobutanoate', 'N,N-Dimethylglycine', 'N-Ethylglycine', 'N-Methyl-L-alanine', 'n-Propyl carbamate']	
138.03217	28433.3		$C_5H_{11}NO$	$C_5H_{11}NO$	$[M+K-2H]^-$	101.08406	138.03267	-3.63	['2-Methylpropanal O-methyloxime', '2-methylbutanal oxime', '3-Methylbutyraldehyde oxime', '4-Methylaminobutanal', '5-Aminopentanal', 'Pentanamide']	
138.03217	28433.3		C_8H_7N	C_8H_7N	$[M+Na-2H]^-$	117.05785	138.03252	-2.52	['Indole', 'Phenylacetonitrile']	
559.40093	207966	-0.0706236	$C_{32}H_{52}O_4$	$C_{32}H_{52}O_4$	$[M+Hac-H]^-$	500.38656	559.40041	0.92	['3beta-Hydroxylanostane-7,11-dione acetate']	
241.21756	1376803	-0.0700686	$C_{15}H_{30}O_2$	$C_{15}H_{30}O_2$	$[M-H]^-$	242.22458	241.2173	1.06	['12-Methyltetradecanoic acid', 'Pentadecanoic acid']	pentadecanoic acid
223.02509	112056.1	-0.0686461		0					0	
703.22247	92548.5	-0.0681196	$C_{32}H_{42}O_{16}$	$C_{32}H_{42}O_{16}$	$[M+Na-2H]^-$	682.24729	703.22196	0.73	['Bruceoside A']	
221.0094	60414	-0.0676636		0					0	
531.3697	480083.2	-0.06758	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$	$[M+Hac-H]^-$	472.35526	531.36911	1.1	['Alisol B', 'Alphitolic acid', 'Crataegolic acid', 'Echinocystic acid', 'Gratiogenin', 'Siaresinol', 'Sumaresinol']	
151.00361	97380.4	-0.0673335		0					0	

3.3.4 Metabolites Selected for Further Analysis

The "single peak search" within MI-Pack software was used to search against the KEGG database, revealing putatively annotated matches of the observed peaks with a defined empirical formula within a 2 ppm mass error between the theoretical mass and experimental mass. Since one empirical formula could have multiple hits within that 2 ppm window, there are several putative identifications. In all cases, an attempt was made to determine which putative identifications were also possible chemical compounds of wine and are listed in the final column of Table 3.3, *possible components in wine*. The predominant adducts for the chosen compounds were the deprotonated ion forms as well as the acetate adducts. Greater assurance was placed on compounds identified by multiple adducts, such as the two stable isotopes of chlorine: the more intense Cl^{35} (75.8%) and the lesser intense Cl^{37} (24.2%). In certain cases, identification utilising one chloride isotope only (Cl^{37}) appeared nonsensical and those putative annotations were not included in the final list.

In several cases, the KEGG compound list included isomeric components. Hydroxybenzoic acid includes: 2- (ortho), 3- (meta), and 4- (para) hydoxybenzoic acids. Dihydroxybenzoic acids includes six compounds: 2,3- (2-pyrocatechuic acid), 2,4- (β-resorcylic acid), 2,5- (gentisic acid), 2,6- (γ-resorcylic acid), 3,4- (protocatechuic acid), and 3,5-dihydroxybenzoic acid (α-resorcylic acid). Coumaric acid includes: ortho-(2-hydroxycinnamic acid), meta- (3-hydroxycinnamic acid), and para-coumaric acid (4-hydroxycinnamic acid). An attempt was made to choose the best representative compounds for the targeted list. In some cases, the availability of the standard was the limiting factor.

The effect of alkaline fusion on the wine polymer was discussed in Chapter 1.5. The effect on anthocyanidin species was the release of a carboxylic acid and its corresponding aldehyde. The initial approach for the targeted list was to focus on the acids determined from the alkaline fusion of the laboratory aged sherds; therefore, the putative identification for aldehydes was not utilised in developing the targeted method. Also, the known volatility of the corresponding aldehydes may result in inconsistent results (Flamini and Traldi, 2010: 236). In terms of the success of the chemical attack on the samples, the results were overwhelmingly monomeric species further confirming the success of the alkaline fusion on the glass/sand laboratory aged sherds.

3.3.4.1 Fragmentation Confirmation of Selected Analytes

An attempt was made to confirm the majority of masses putatively identified in Table 3.3. The remaining alkaline fusion samples were pooled and samples were nanoelectrosprayed into the LTQ for 25-50 scans in negative ion mode. The chosen parent ions were focused within a 1 Dalton window in the ion trap and fragmented with a collision energy of 35% NCE with a gas pressure of 0.5 psi. The MS^2 fragment ions were then collected in the ion trap. The fragmentation procedure including isolation, fragmentation, and collection all occurred in the ion trap. Figure 3.14 shows the fragmentation spectrum of the peak with nominal mass 149, putatively identified as tartaric acid. The acids have common losses which include loss of water (18), carbon monoxide (28), and carbon dioxide (44).

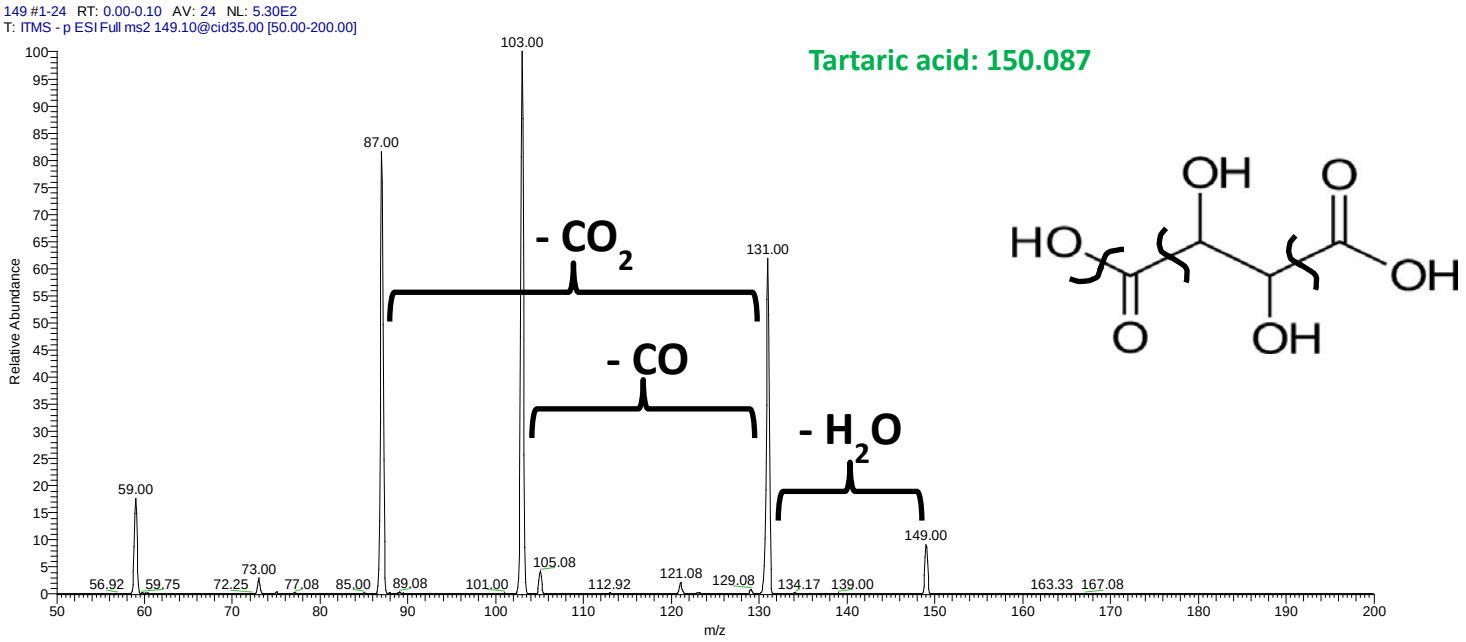


Figure 3.14. The product ion spectrum of the peak with m/z 149, putatively identified as the deprotonated form of tartaric acid. The common losses are identified in the spectrum.

Table 3.5 The putatively identified acids were fragmented in order to confirm their identity. All acids have common losses. ***large loss of 32 (O_2)

Putative identification	Parent ion (m/z)	-CH_3 (15)	-H_2O (18)	-CO (28)	-CO_2 (44)	-C_2H_6O (46)	-$C_2H_6O_2$ (62)	-NH_3 (17)	-C_2H_7NO (61)
Succinic acid	117	xxx	99	xxx	73	xxx	xxx	xxx	xxx
Alpha ketobutyric acid	101	xxx	73	xxx	57	xxx	xxx	xxx	xxx
Tartaric acid	149	xxx	131	103	87	xxx	xxx	xxx	xxx
Malic acid	133	xxx	115	xxx	89	87	71	xxx	xxx
Citric acid	191	xxx	173	xxx	xxx	xxx	129	xxx	xxx
Dihydroxybenzoic acid (possible mix with patulin)	***153***	***138***	***135***	***125***	***109***	***107***	***xxx***	***xxx***	***xxx***
Syringic acid	197	182	xxx	xxx	153	xxx	129	xxx	xxx
Isopropyl malic acid	175	xxx	157	xxx	131	129	113	xxx	xxx
Citramalic/mevalonic acid	147	xxx	129	xxx	xxx	101	85	xxx	xxx
Caffeic acid	179	164	xxx	151	135	xxx	xxx	xxx	xxx
Gallic acid	169	xxx	151	xxx	125	xxx	xxx	xxx	xxx
Coumaric acid	163	xxx	xxx	xxx	119	xxx	xxx	xxx	xxx
Vanillic acid	167	152	xxx	xxx	123	xxx	xxx	xxx	xxx
2-keto-glutaramic acid	144	xxx	126	xxx	100	xxx	xxx	127	83
Carboxyvanillic acid	211	196	193	183	167	xxx	xxx	xxx	xxx
Hydroxybenzoic acid	137	xxx	119	109	93	xxx	xxx	xxx	xxx
Citraconic/methyl maleic acid	129	114	111	101	85	xxx	xxx	xxx	xxx
***Methylcitric acid	205	xxx	187	xxx	161	159	xxx	xxx	xxx

3.4 Conclusions

An untargeted metabolomics approach was taken in order to discover a suite of biomarkers that are predictive of the presence of aged red and white wine, and which could then be applied to archaeological samples to identify ancient wine. A model wine system was aged in the laboratory over six months in the form of liquid aliquots and as wine-permeated sherds. All samples were analysed by direct infusion FT-ICR mass spectrometry and the resultant spectra then 'stitched' together using the SIMstitch program thus producing a list of *m/z* specific to each particular experiment. Multivariate and univariate statistical analyses were conducted on these mass spectral datasets to reveal any significant changes to the red and white wine as they age. Univariate statistics specifically monitored the loss of monomeric species as a presumed polymerisation occurred. The chemical fingerprints of the wine-permeated sherds were projected onto principal components scores plots which revealed the chemical similarities and differences between the samples, specifically the top PC loadings. In the case of the alkaline fusion aliquot, the top loadings were chosen for the next step in developing a targeted method for the analysis of archaeological samples.

The top loadings taken from the alkaline fusion aliquot (glass/sand) were searched against the KEGG database using the MI-Pack computer program. Putative identifications were based on comparison of theoretical mass values with experimental masses within a 2 ppm error. Nearly half of these ions were subsequently fragmented in an attempt to confirm their identities. Since many of these putatively identified compounds were acids, there were losses common to all of

them including loss of water, loss of carbon monoxide, and loss of carbon dioxide. Out of the 37 putatively identified acids, 20 of these acids were confirmed by MS/MS fragmentation.

It was decided that phthalic acids would not be pursued, since their origin was most probably from modern plasticizers. Also, sugar acids, such as glucuronic acid, were not pursued since the number of possible isomers for this type of compound was beyond the scope of this project. The low molecular weight of oxalic, butanoic, and lactic acid made these compounds impractical for the next phase of the research, developing a targeted LC-MS/MS method; therefore, these compounds were removed from further analysis. The remaining acids were selected for further studies and benefitted from being commercially available as standards.

In conclusion, using an untargeted high resolution mass spectrometry approach in a laboratory ageing situation, in conjunction with multivariate statistics, a suite of biomarkers was chosen based upon their characteristic significance in describing a particular sample. Those biomarkers were then used to prepare a targeted method to apply to archaeological samples and include the following acids: ketobutyric, ferulic, gentisic, 2,3-dihyhydroxybenzoic, syringic, p-coumaric, m-hydroxycinnamic, vanillic, isopropyl malic, succinic, malonic, malic, tartaric, citric, gallic, and caffeic.

4. Development of a Targeted Analytical Method for Application to Archaeological Samples

The objectives of this chapter were to build a targeted method based upon the biomarkers discovered during the research presented in Chapter 3, as well as to refine the extraction procedures in order to determine the presence/absence of aged, polymerised wine in an archaeological sample. It was presumed that wine, if still present, was present in minute amounts. Therefore, qualitative sensitivity was paramount in this analytical approach. DIMS was discounted for sample introduction and a more sensitive chromatographic approach was chosen. DIMS is an appropriate choice for a global determination for all materials in a sample matrix, however, direct infusion of all materials concurrently creates a situation whereby salts, sample matrix, and analytes of interest vie for ionisation. Ion suppression due to a sample's high salt content was also an issue with DIMS analyses.

Chromatography utilises separation and focusing of each component, which results in enhancement of detected signals. The detector chosen for this analysis was the triple quadrupole mass spectrometer, a highly sensitive instrument that utilises two mass analysers for the identification of precursor-product ions and their relative intensities. This instrument has been used previously for similar analyses (Barnard et al. 2010; Guasch-Jane et al., 2004; Stern et al., 2008). Combining an LC-triple quadrupole approach offered unambiguous identification of analytes based upon: 1. retention time from the analytical column, 2. identification of the precursor-product ions, and 3. the comparison of the product ion spectra of archaeological samples with those obtained from commercial standards.

4.1 Development of a Targeted LC-MS/MS method

Reversed phase liquid chromatography (RPLC) has long been considered the most popular choice for analysis in liquid chromatography. In a recent review of chromatographic analyses of acids in wine and grape juice, 18 of 33 chromatographic techniques chosen for the identification of organic acids were RPLC (Mato et al., 2005). The remaining techniques used were either ion exclusion or ion exchange chromatography. Introduced in 1950 (Howard and Martin, 1950), separation by RPLC is based upon distribution of an analyte between the non-polar stationary phase and the mobile phase. Packing material for most reversed phase columns contain silica with C18 chains bonded to its surface thus creating an environment for hydrophobic interactions. Hydrophobic components will elute off the column only when its partition coefficient becomes more favorable towards the mobile phase which consists of an increasing amount of organic solvent. A hydrophilic component will not bind to the C18 particles and will not be retained on the column, eluting at or close to the dead volume; therefore, the reversed phase column initially chosen for the targeted analysis of organic and phenolic acids was the Synergi-Hydro column 150 mm x 1 mm column with guard column. The Synergi-Hydro column attempts to create an environment for both hydrophobic and hydrophilic material by 'endcapping,' or chemically adhering a 'proprietary' polar compound to the end of the C18 chain, thereby creating an environment conducive to hydrophilic attachment.

The initial chromatographic method developed was based upon a published method for the elution of tartaric acid from a Synergi-Hydro column (Phenomenex Application Note: 14270). In this method, the mobile phase was 20 mM potassium phosphate. Since phosphates are powerful ionisation competitors and thus cannot be used in conjunction with a mass spectrometer detector, a mobile phase consisting of 10mM ammonium acetate in HPLC grade water was prepared. Initially, the HPLC was connected to the LTQ-FT-MS using the following conditions, Table 4.1.

Table 4.1 The LC-FT-ICR-MS conditions for the elution of tartaric acid from the Synergi-Hydro column.

Column	**Synergi Hydro RP 150mmx1mm**
Mobile Phase	**10mM ammonium acetate, pH = 6.8**
Flow Rate	**33ul/minute**
Detector	**LTQ-FT-Mass spectrometer**
Sheath Gas	**10 (psi)**
Auxillary Gas	**5 (arbitrary units)**
Spray Voltage	**-3.5 (kV)**
Capillary Temperature	**300°C**
Capillary Voltage	**-4.3 (kV)**

Ten milligrams of tartaric acid were weighed out and diluted in 1 ml of mobile phase and further diluted to 1:100. The dead time was monitored at approximately 3 min with the tartaric acid peak eluting at 3.80 min as seen in Figure 4.1. It was determined after several runs that the elution of the organic acid was too near the solvent front to maintain reproducibility and that reversed phase chromatography would not suit the purposes of this type of analysis of polar acids.

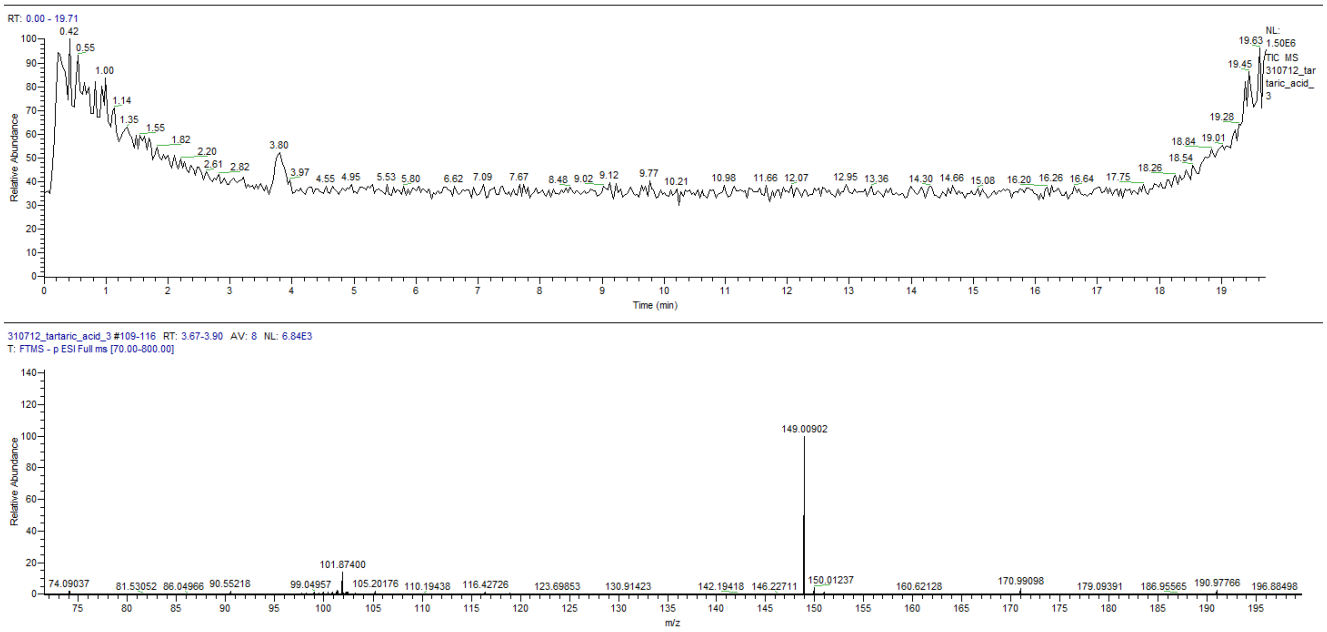


Figure 4.1. The elution of tartaric acid at *m/z*149 using a Synergi-Hydro column 150 mm x1 mm column with guard column. The acid eluted at 3.8 min, a time close to the solvent front.

To maintain the organic and phenolic acids on an LC column and create an environment for longer retention times, another approach was required. As discussed in Chapter 1, hydrophilic interaction liquid chromatography (HILIC) is an orthogonal approach to reversed phase liquid chromatography whereby hydrophilic compounds are retained on the column and elute off into the increasingly aqueous mobile phase. Although the physical properties behind the separation in a HILIC experiment are currently under debate and not completely agreed upon (Dinh et al., 2011; Buszewski and Noga, 2012), there are known mechanisms which are considered critical for a HILIC separation.

One of the major separation mechanisms is described as the partition of the analyte between the 'interior' water-rich layer (on the surface of the polar stationary phase) and the 'exterior' organic

rich mobile phase. Aqueous buffer salts introduced into the mobile phase will naturally migrate to this aqueous rich layer thus creating a larger volume due to displacement (Guo and Gaiki, 2005). The larger volume enhances the partitioning effect of a compound between the aqueous layer and the mainly organic mobile phase (Creek et al., 2011).

A second common mechanism is described as the electrostatic interaction of the analyte with the solid phase. Depending upon the protonated/deprotonated status, elution may become dictated by an anion/cation exchange mechanism between the analyte and the stationary phase (Dinh et al. 2011; Guo et al., 2007). Based upon the type of HILIC column chosen, ions from buffer salts may bind to a charged stationary phase thereby suppressing the overall charge and limiting electrostatic interactions (Karatapanis et al., 2011). Originally bare silica columns, materials used in HILIC chromatography now include amino, amide, cyano, diol, or cyclodextrin groups on either silica or polymeric substrates (Ikegami et al., 2008; Karatapanis et al.,2011).

In order to develop a solvent gradient for the mixture of chosen acids, stock solutions were prepared by weighing out a standard acid at 10 mg/1 ml of initial mobile phase. The final concentration was a diluted mixture based upon similar signals for all of the acids. The initial mobile phase concentrations were based on a published analysis of tartaric acid in fruit juice that used a Sequant 150x 2.1 mm (5 µm) HILIC column (Ehling and Cole, 2011).

Table 4.2 Elution profiles for the separation for phenolic and organic acids, (a) published method from Ehling and Cole, 2011, (b) initial profile on capillary ZIC-HILIC column, (c) later elution profile with a change in mobile phase A, a shallower gradient, and a lower flow rate.

Column	(a) ZIC-HILIC 150x 2.1 mm (5 µm) (*Ehling and Cole, 2011*)	(b) ZIC-HILIC 150mmx300 µm, 3.5 µm, 200Å	(c) ZIC-HILIC 150mmx300 µm, 3.5 µm, 200Å
Mobile Phase A	acetonitrile/water (90:10) containing 0.1% of ammonium acetate	acetonitrile/water (90:10) containing 100 mM ammonium acetate	acetonitrile
Mobile Phase B	water containing 0.1% of ammonium acetate	100 mM ammonium acetate	100 mM ammonium acetate
Temperature	30°C	30°C	30°C
Sample Volume	10µl	0.5/.8 µl	0.5/.8 µl
Step 1	0-20 min, 0-55% B at 0.2 mL/min	0-30 min, 0-50% B at 11 µL/min (initially at 4 µl/min)	1-90 min, 10-60% B at 7 µL/min
Step 2	20-25 min, 55% B at 0.2 mL/min	30-33 min, 50% B at 11 µL/min	90-105 min, 60% B at 7 µL/min
Step 3	25-38 min, 0% B at 0.6 mL/min	33-36 min, 10% B at 11 µL/min	105-115 min, 10% B at 7 µL/min
Step 4	38-40 min, 0% B at 0.2 mL/min	36-41 min, 10% B at 11 µL/min	115-135 min, 10% B at 7 µL/min

Early attempts to separate and resolve a mixture of standard acids on a capillary ZIC-HILIC 150 mm x 300 µm, 3.5 µm, 200Å column proved unsuccessful after multiple attempts at changing the flow rate, steepness of gradient, and mobile phase A (Table 4.2). However, during this time it was determined that increasing the concentration of mobile phase B (100 mM ammonium acetate) separated the acids into two classes: the early eluting phenolic acids and the later eluting organic acids. Later LC development of this initial mobile phase composition was based primarily on the increasing concentration of the strong eluting solvent, water.

Due to the difficulty in maintaining a constant pressure at capillary flow rates, a larger diameter analytical column was chosen for method development, an Agilent Zorbax column, 150 x 2.1 mm column and guard column. The stationary phase was bare silica that was 'endcapped' with proprietary compounds in order to deactivate the majority of the silanol groups. The pKa of the silanol group is 4.9 and therefore imparts a negative charge at pH 6.8, the pH of ammonium acetate (Agilent Zorbax column selection guide for HPLC, 2007). Unless attenuated by either the ammonium buffer cations or the water in the mobile phase (Hemström and Irgum, 2006), the anionic charged surface will repel the acidic analytes. If these conditions of deactivation are met (buffer salt and water), the chromatographic separation and elution of organic acids off the silica column would most likely be described by partition between the water-rich layer and the acetonitrile mobile phase with limited involvement of the silica solid phase.

In preparation for the next phase in method development, discussions with a colleague resulted in a ternary delivery system of solvents thereby maintaining a constant level of buffer salts throughout the

gradient (Dr. Ulf Sommer, personal communication). By maintaining this consistent concentration, the electrostatic effect between the buffer salts and the stationary phase did not change throughout the run resulting in a stable baseline.

The LC was connected to the mass spectrometer by a heated electrospray ionisation source (HESI). The conditions of the HESI source (voltage applied and nitrogen gas delivered) are critical in order to produce the charged droplets necessary for mass spectral detection. The conditions were chosen to increase the signal of several chosen ions and to maintain an excellent spray stability (no deviations beyond 2 %) when collected for two minutes. Ten microlitres of standard mix (gentisic, malic, and citric acids) were added to a sample well and 1 µl of the sample was injected onto the column in full injection mode.

Figure 4.2 shows a total ion chromatogram (TIC) with three extracted ion chromatograms (EIC) for gentisic, malic and citric acid. The first two acids are well-resolved. Citric acid, however, was problematic with poor focusing on the column. Two possible reasons for the broad peak of citric acid were: interference/chelation with iron in the stainless steel frits within the LC system (Alpert, 1990; Preinerstorfer et al., 2010) or that the pH of the mobile phase was below the compound's pKa resulting in a greater concentration of protonated species (vs deprotonated species). There were no obvious stainless steel components found on the liquid chromatograph, therefore, it was decided to raise the pH of the mobile phase to ensure full deprotonation (Aronson, 1983).

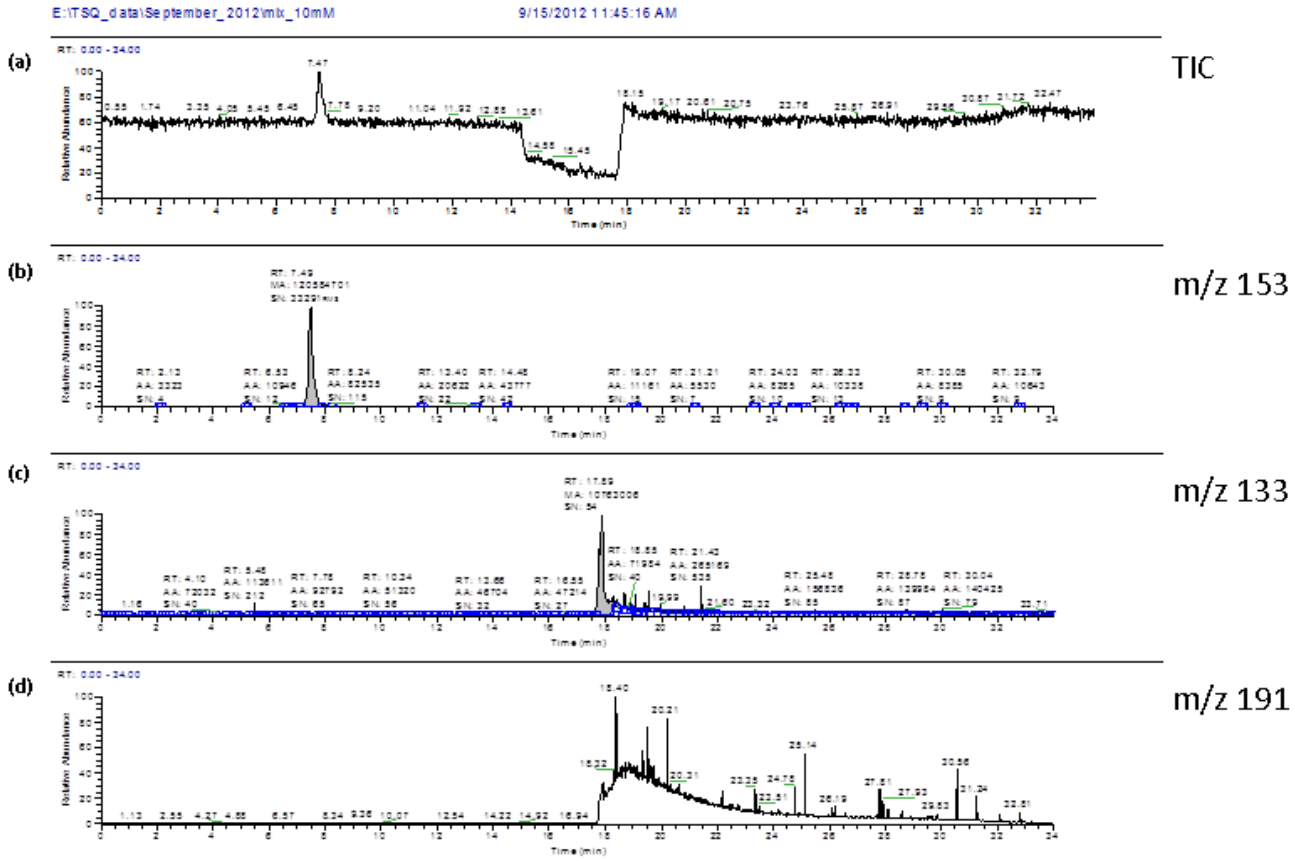


Figure 4.2. Gradient elution of a mixture of three acids on an Agilent Zorbax column 150 x 2.1 mm (a) full TIC (b) extracted ion chromatogram (EIC) of base peak 153 representing gentisic acid (c) EIC of base peak 133, representing malic acid and (d) EIC of base peak 191, representing citric acid.

4.1.1 pKa of chosen metabolites

The measurement of pKa is the pH at which the deprotonated and fully protonated concentrations of a weak acid are in equilibrium. At pH higher than the pKa, the concentrations of the deprotonated compounds are greater, at pH below the pKa, the fully protonated acid prevails. Polyprotic acids such as citric acid have several pKas depending upon the number of protons available for donations.

4.1.2 ZIC-pHILIC column

The maximum pH range of the Agilent Zorbax column is 1-8. Therefore, in order to increase the mobile phase above pH 8, a separate column was chosen, the ZIC-pHILIC 150 mm x 2.1 mm, 5 µm column from SeQuant. The polymeric stationary phase support is stable from pH 2-12. Attached to the polymer is a sulfoalkylbetaine, Figure 4.3. The quaternary ammonium and the sulfonic group are present in a 1:1 ratio for a total net charge of 0; however, due to the physical accessibility of the negative charge, and the physically hindered positive charge, the stationary phase yields a slightly negative charge (Jandera, 2011).

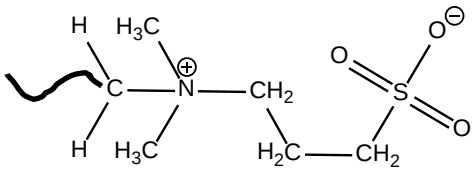


Figure 4.3. The zwitterionic stationary phase bound to the polymeric substrate on a ZIC-pHILIC column. The positively charged quarternary ammonium and the negatively charged sulfonate group together have a net charge of zero.

The sulfoalkylbetaine support provides a strong attraction for water resulting in a measurable layer of stagnant or slow moving water along the polymer layer (Jandera, 2011). Additional salts from the mobile phase are attracted to the cationic and anionic charges on the polymer support. The result is an attenuation of electronic attraction between solid support and analytes of interest as well as polymer swelling (Terayama et al., 2009). The increased polymer volume increases the volume of the aqueous layer which may increase the retention of the hydrophilic materials on the column (Melnikov et al., 2013).

4.1.3 Repeatability Study to Determine Retention Time Robustness

The gradient developed using the Zorbax column was applied to the ZIC-pHILIC column with several changes. The steepness of the gradient was lessened and the maximum percentage of water maintained at 50 % (v/v). Mobile phase B was prepared as 100 mM of ammonium acetate in 400 ml of HPLC grade water with the pH raised to 8.2 with the addition of a few drops of ammonium hydroxide. The pH was initially checked in the fume hood with a strip of pH paper and confirmed with a pH meter.

In Figure 4.4, two species were identified at *m/z* 191, eluting at 4 min and at 30 min. This suggests a dual citric acid species whereby the sharp peak eluting at 4 min probably represents an initially fully protonated species of citric acid partitioning into the acetonitrile and eluting early in the analysis. The species of interest eluting at 30 min is still broad and unfocused. Because there were inherent problems with the analysis of citric acid, the acid was not pursued in the analysis of the archaeological samples. The analysis of caffeic acid and gallic acid were also problematic; the acids were not properly focused on the column and therefore, were not pursued in the analysis of the archaeological samples. Although not

identified as a biomarker in Chapter 3, ascorbic acid was added to the mixture as an internal standard, to assess whether the retention times of the acids varied.

Table 4.3 Parameters of the two analytical ZIC-HILIC columns chosen for the analyses of the organic acids. The final column and parameters chosen for further analyses are for the SeQuant ZIC-pHILIC column.

Column	Agilent Zorbax column, 150 x2.1 mm	SeQuant ZIC-pHILIC column, 150 x2.1 mm, 5 µm
Mobile Phase A	Acetonitrile	Acetonitrile
Mobile Phase B	100 mM ammonium acetate, pH=6.8	100 mM ammonium acetate, pH = 8.2
Mobile Phase C	Water	Water
Temperature	30° C	30° C
Sample Volume	1 µl	1 µl
Step 1	Time: 0-1 min 10%B, 0%C	Time 0-7 min 10%B, 0%C
Step 2	Time: 1-15 min 10%B, 60%C	Time: 7-21 min 10%B, 50%C
Step 3	Time: 15-23 min 10% B, 60% C	Time: 21-29 min 10%B, 50%C
Step 4	Time: 23-25 min 10%B, 0% C	Time 29-33 min 10%B, 0%C
Step 5	Time: 25-35 min 10%B, 0%C	Time: 33-35 min 10%B, 0%C
Flow Rate	70µl/min (initially 100)	70µl/min
Detector	TSQ mass spectrometer	TSQ mass spectrometer
Electrospray voltage (eV)	-3000	-3600
Vaporization temperature, °C	0	0
Sheath gas (psi)	10	10
Auxiliary gas (arbitrary units)	0	0
Capillary temperature, °C	275	275
Detection	Full scan in Q1, m/z 40-300	Full scan in Q1, m/z 40-300

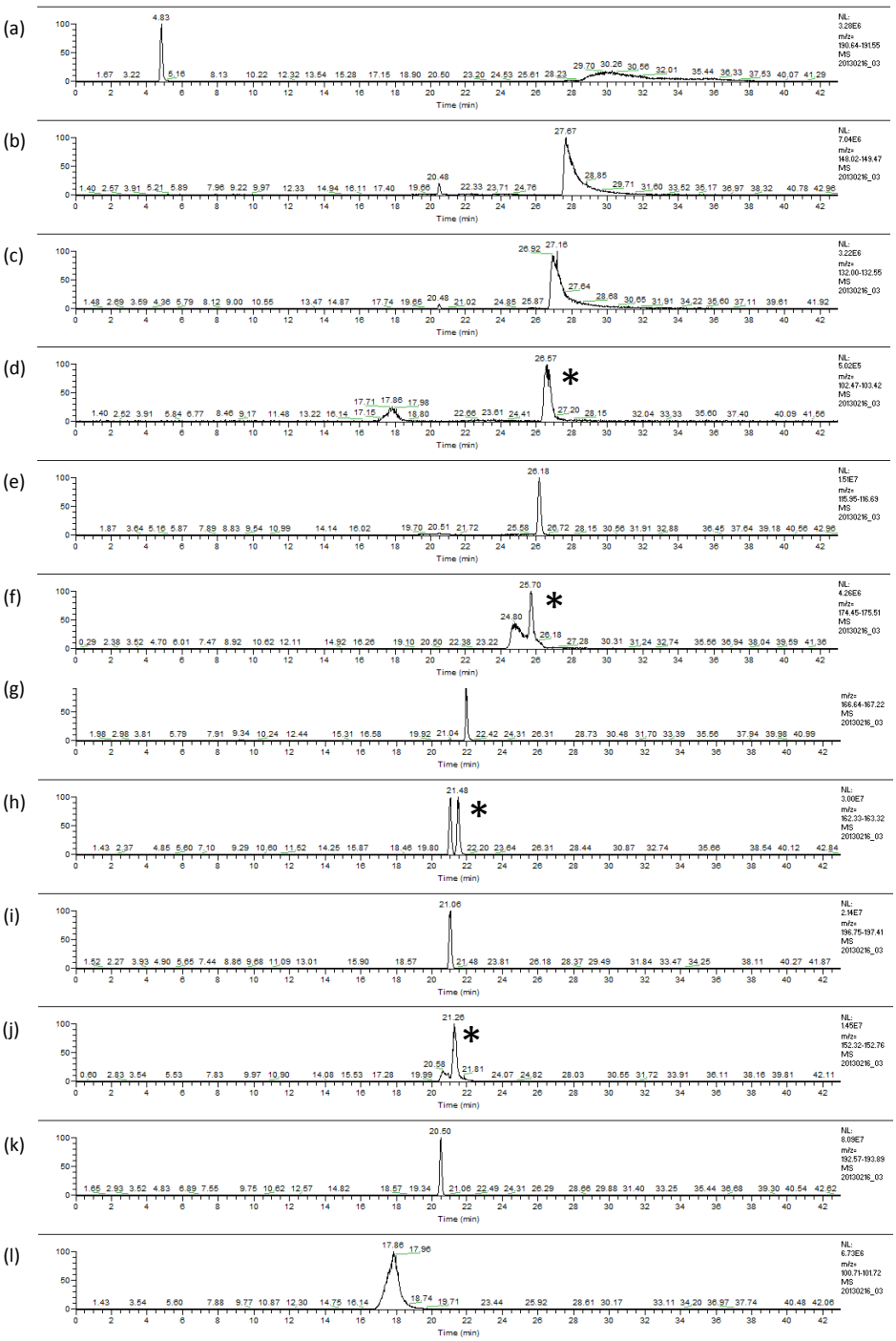


Figure 4.4. Extracted ion chromatograms of 15 acids analyzed in a retention time study (a)EIC of base peak m/z 191, citric acid (b) m/z 149, tartaric acid (c) m/z 133, malic acid (d) m/z 103, malonic acid (e) m/z 117, succinic acid (f) m/z 175, ascorbic and isopropyl malic acid (g) m/z 167, vanillic acid (h) m/z 163, p-coumaric acid and m-hydroxycinnamic acid (i) m/z 197, syringic acid (j) m/z 153, gentisic acid and 2,3-dihyhydroxybenzoic acid (k) m/z 193, ferulic acid (l) m/z 101, ketobutyric acid.

4.1.3.1 Relative Standard Deviation of Results

In order to determine the robustness of this method, a standard mix of acids was injected 31 times over the course of 36 hours. In order to develop a gradient run for the mixture of chosen acids, stock solutions were prepared by weighing out a standard acid at 10 mg/1 ml of initial mobile phase. The final concentration was a diluted mixture based upon similar signals for all of the acids. Stock solutions were diluted in 90/10 acetonitrile/100 mM ammonium acetate, pH 8.2, with the addition of 20 µl of potassium chloride (KCl, 25 mg/1 ml 100 mM ammonium acetate, pH 8.2). KCl, a byproduct of the alkaline fusion reaction was added to in order to gauge its effect on retention time reproducibility.

The RSD calculated from the retention times are shown in Figure 4.5. The results represent a high repeatability of the retention times of the standards over the course of several days. Blanks were run periodically during the 36 hour period in order to determine the extent of carryover from the column. There was small carryover from the dihydroxybenzoic and succinic acids. There was also a 'ghosting' effect in malic acid and tartaric acid; that is, there was carryover present in the blanks eluting one minute later than the elution of the standard acid. Because of this effect, the identification of malic and tartaric acid in the archaeological samples was dependent upon narrow retention time windows.

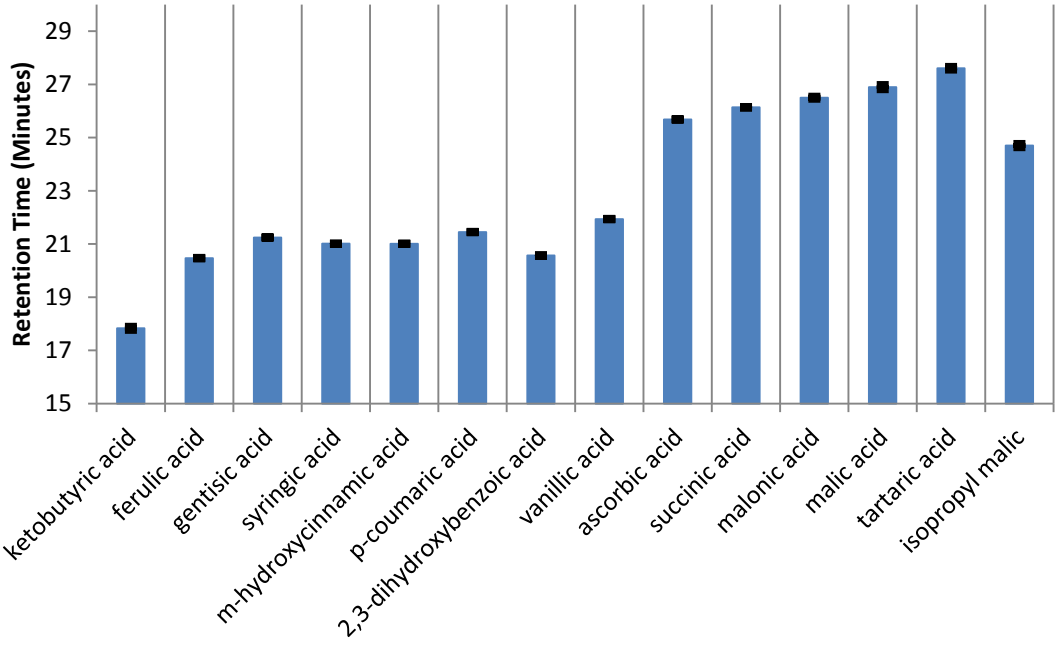


Figure 4.5. The RSD of retention time with error bars included derived from 31 consecutive injections of standard acid mixtures onto the ZIC-pHILIC LC MS/MS over 36 h. The y axis is the retention time in minutes.

4.1.4 Determination of Transitions for Chosen Metabolites

After the elution parameters were finalised, the precursor-product ion transitions were determined. The final targeted method is described in Chapter 2. Initially, the instrument was optimised for maximum signal and optimal electrospray stability. Each acid was then diluted in ACN/water (depending upon their elution from the column) and directly infused into the mass spectrometer. The automated procedures included the following steps:

1. The S-lens voltage was optimised for the particular parent ion.
2. The collision gas was stabilised in Q2 at 1.5mTorr.
3. The product ions were collected through an 8 step collision energy starting from 5 ev to 50 eV

The final output of relative intensity vs. optimised collision energy for the product ions of each acid standard are in the Appendix, Chapter 4, Figures 1a-1p. The transitions between the precursor ion and the most intense product ions were transferred to the targeted method. These transitions were limited to the elution time from the ZIC-pHILIC column, such that the final targeted method contained four segments: segment 1 from 0-17 min collected in full scan mode, segment 2 collected from 17-23 min in selected reaction monitoring (SRM) mode for 8 transitions of the early eluting acids, segment 3 from 23-33 min collected in SRM for the transitions of the later eluting acids, and segment 4 analysed at full scan from 33-43 min. The final identification of an acid in an archaeological sample was based on three criteria:

1. retention time
2. precursor-product ion transition
3. comparison between sample fragment intensities with standard fragment intensities

4.2 Extraction Procedures for Archaeological Samples

As introduced in Chapter 1, the original extraction method of 100 mg for laboratory sherds was increased to 1 g for archaeological samples due to the limited amount of analyte. The solvent volumes were also increased to take into account the larger sample. One hundred sixty microlitres of 4 M KOH were added to the laboratory sherds allowing enough liquid to cover the 100 mg (Zsuga and Kiss, 1987). This amount was diluted to 600 µl of 1 M KOH, enough material to cover 1 g of archaeological samples. The samples chosen to optimise the extraction procedures were the wine press and its preparatory layer, as this object was considered the 'positive control' for the remnants of ancient wine and recorded in Appendix, Chapter 4, Table 1. In an attempt to reproduce several previously reported standard

operating procedures (SOPs) and define the most efficient and sensitive extraction for the analysis of archaeological wine, several methodological approaches were tried on the samples (Pecci et al., 2013; Stern et al., 2008; Barnard et al., 2010; Guasch-Jane et al., 2004; McGovern et al., 2009).

4.2.1 Initial extraction and Analysis: Alkaline Fusion

In order to determine if the length of alkaline fusion was critical in breaking down the presumably aged and oxidised polymers, samples of the wine press and its preparatory layer were extracted using a modified SOP. Briefly, 1 g of sample was homogenised in a 7 ml Precellys homogenisation tube with 5.6 ml of methanol/water. The slurry was transferred to a 15 ml glass centrifuge tube to which was added 4 ml of chloroform and 2 ml of water. The tube was vortexed and centrifuged. The top polar layer was removed as was the bottom non-polar layer; these extracts were not analysed as this simple solvent extraction was assumed not to be sufficient for isolating polymerised material. The remaining solid was allowed to dry overnight in a fume hood. Six hundred microlitres of 1 M KOH were added to the solid powder to break down the polymer; the sample was then vortexed for 10 3-second bursts. A sample of the preparatory layer was then placed in a water bath at 50^{o} C for I h. Separate samples of the preparatory layer and wine press were placed in a water bath at 50^{o} C for I h followed by heating in a water bath in an oven at 50^{o} C overnight. Afterward, each sample was then acidified with 2.5 ml 2 N HCl in order to drop the pH below 3 (checked with pH paper). Three millilitres of ethyl acetate were then added to the centrifuge tube to extract the monomeric species and the tube vortexed and centrifuged at 500 rpm for 3 min. The top organic layer was then continuously added to a 1.8 ml champagne vial and dried down under a stream of nitrogen. The sample vials were capped and stored at -80^{o} C until analysis.

For analysis by LC/MS/MS, the samples were removed from the freezer and warmed to room temperature. Thirty microlitres of mobile phase A, 90/10 ACN/100mM ammonium acetate, were added to the sample vials. Ten microlitres of each sample were added to the sample well; 1 µl of sample was added to the LC column. All results are recorded in Appendix; Table 4.2. Figure 4.6 graphically represent the results based upon absence or presence of the acids. Five acids were identified in the prep layer following a 1 h KOH application. Three acids were identified in the preparatory layer after heating for 24 h in KOH; the extended period of time for the alkaline fusion did not increase extraction efficiency, therefore, the alkaline fusion portion of the extraction was maintained for 1 h.

4.2.2 Continuation of Extraction and Analysis Procedures

In order to further define the best extraction procedure, four samples were prepared for analysis: wine press, wine press spiked with syringic and p-coumaric acids, preparatory layer, and extraction blank. Samples were extracted as before (Appendix, Table 4.1). After the samples were acidified with 2.5 ml of 2 N HCl, a clean-up step was added utilising a C18 solid phase extraction (SPE) cartridge. At the acidic pH, the majority of acids were fully protonated and more likely to adhere to the hydrophobic C18 cartridge.

Briefly, a C18 100 mg bed SPE cartridge was conditioned with 1 ml of methanol followed by 1 ml of HPLC grade water. One millilitre of acidified sample was added to the cartridge and the remaining sample was stored in the cold room at 4^{o}C. The flow through was collected in a 1.5 ml Eppendorf tube. The material remaining on the SPE cartridge was eluted off with 3 x 1 ml using solvents increasing in non-polarity: water, methanol, and ethyl acetate. All three eluates were collected for MS analysis. The water and methanol samples were dried in the Speed Vac overnight. The ethyl acetate portion was immediately

transferred to glass vials and dried down with a stream of nitrogen. All dried samples were stored at -80^{o}C until analysis. The remaining samples stored in the cold room were extracted five days later with 1ml of ethyl acetate, vortexed and centrifuged. The top organic layer was transferred to a glass vial and dried down under nitrogen. The dried samples were stored at -80^{o} C until analysis.

For analysis by LC/MS/MS, samples representing the three eluates from the SPE cartridge, as well as the ethyl acetate liquid extraction were removed from the freezer and warmed to room temperature. Thirty microlitres of mobile phase 90/10, ACN/100 mM ammonium acetate were added to the sample vials. The vials were vortexed and centrifuged and 10 µl of sample were added to the sample well; 1 µl of sample was added to the LC column. All results are recorded in Appendix, Table 4.2.

Comparing the result of the extraction procedures, the methanol elution from the C18 SPE cartridge was moderately successful based on the six acids that were identified: syringic, vanillic, p-coumaric, succinic, and malonic acids, plus ferulic acid (wine press), and malic acid (preparatory layer), Figure 4.7. In contrast, Figure 4.8, eight acids were identified in the ethyl acetate liquid extraction of the preparatory layer: syringic, vanillic, p-coumaric, succinic, malonic, ferulic, malic, and tartaric acids. The results from the LC-MS/MS analysis of the preparatory layer/ethyl acetate extraction are seen in Figures 4.9 and 4.10. The supplementary data for this result is located in the Appendix, Chapter 4, Figure 2 (TIC), and Figure 3a-3h (EIC and product ions). Four of the identified compounds have multiple peaks within the chosen fragmentation window; the final identification was confirmed with the compounds' retention time and fragment intensities. There was contamination in both of the extraction blanks including succinic acid, as well as a low level of malonic acid. From these results, the most successful extraction procedure is the ethyl acetate liquid-liquid extraction that includes an extended period in acidic solution

suggesting a rate limiting step in the extraction procedure; that is, once the polymer is broken apart, a period of time is necessary for the liberated acids to migrate into the aqueous layer.

In an effort to duplicate this extraction procedure, a separate portion of the wine press was extracted and stored in the cold room for forty-eight hours at 4^{o} C in its acidified aqueous layer. The samples were then extracted and analysed; six acids were successfully identified from this analysis. From these results, it was decided that a period of time should elapse between the acidification and the final extraction with ethyl acetate. Rather than store the samples in the cold room, a slight rise in temperature was accomplished by leaving the samples at room temperature for 24 h.

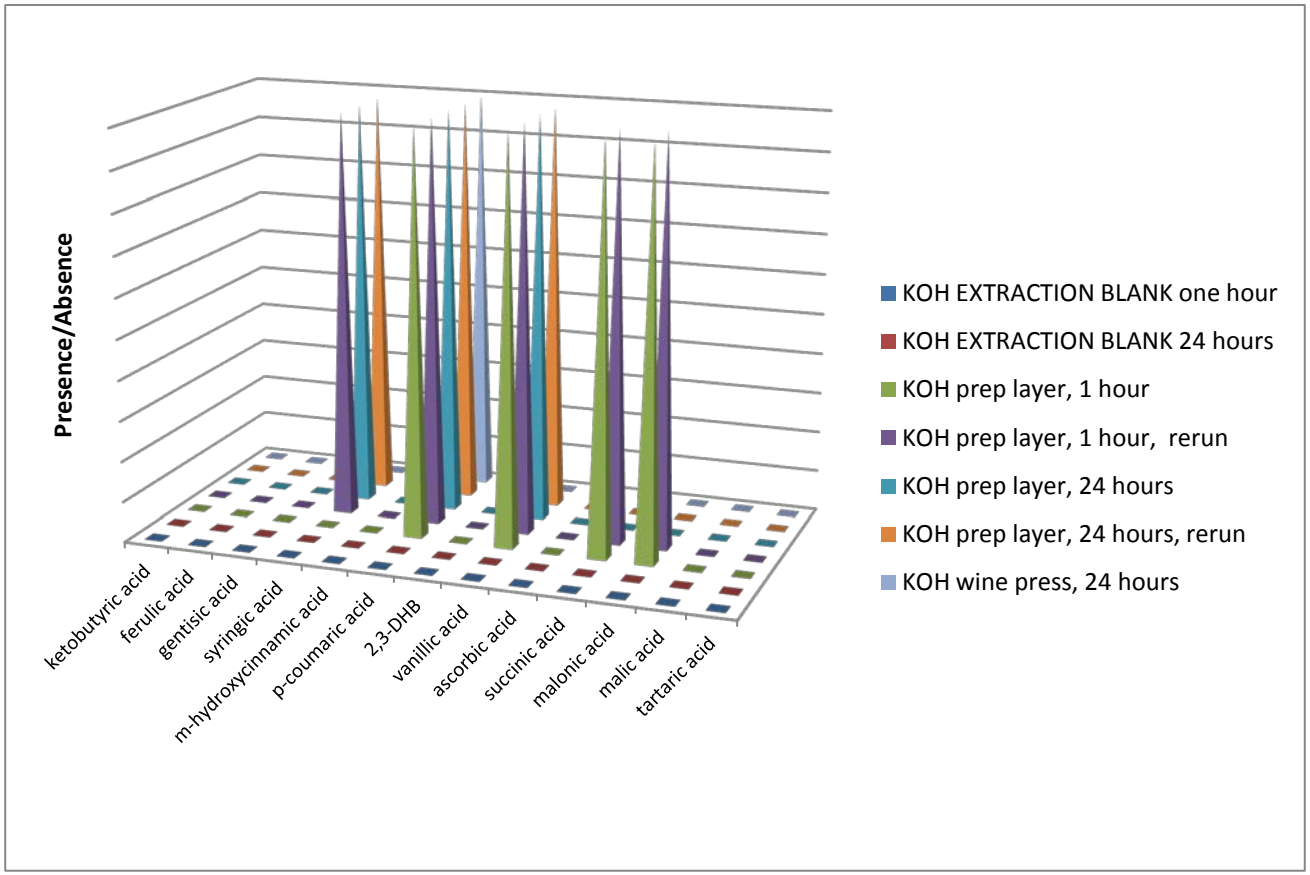


Figure 4.6. Presence or absence of acids from two separate extraction procedures that varied the length of time of the alkaline fusion for samples taken from the Sardinian wine press. The results indicate that alkaline fusion for 1 hour and 24 hours depolymerise the wine residues to the same extent.

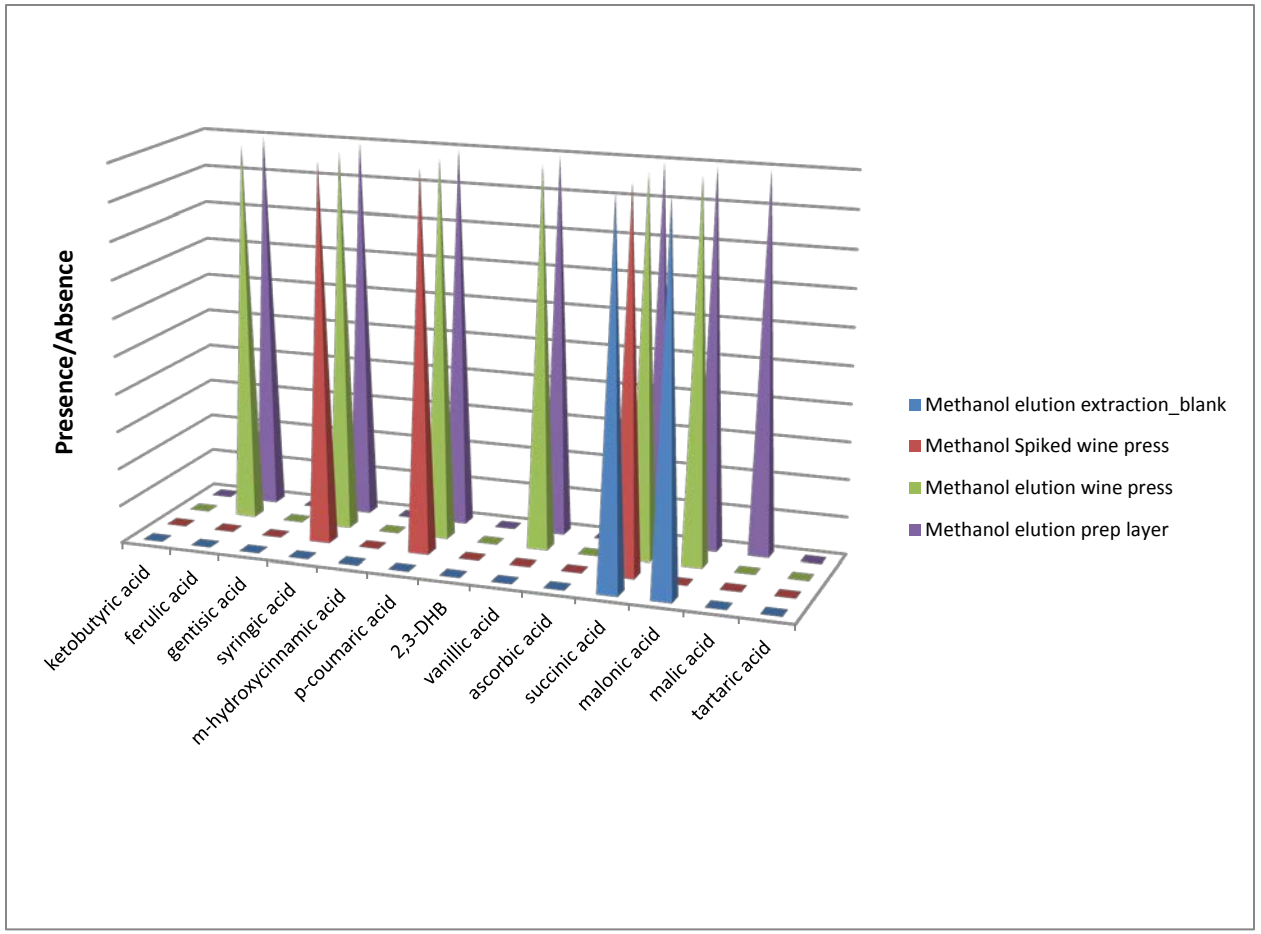


Figure 4.7. Presence or absence of acids from the methanol elution from the C18 SPE cartridge. Six acids were identified from the prep layer including: malic, vanillic, p-coumaric, syringic, and ferulic. Malonic acid was identified; however, a small amount of malonic acid was also identified in the two extraction blanks

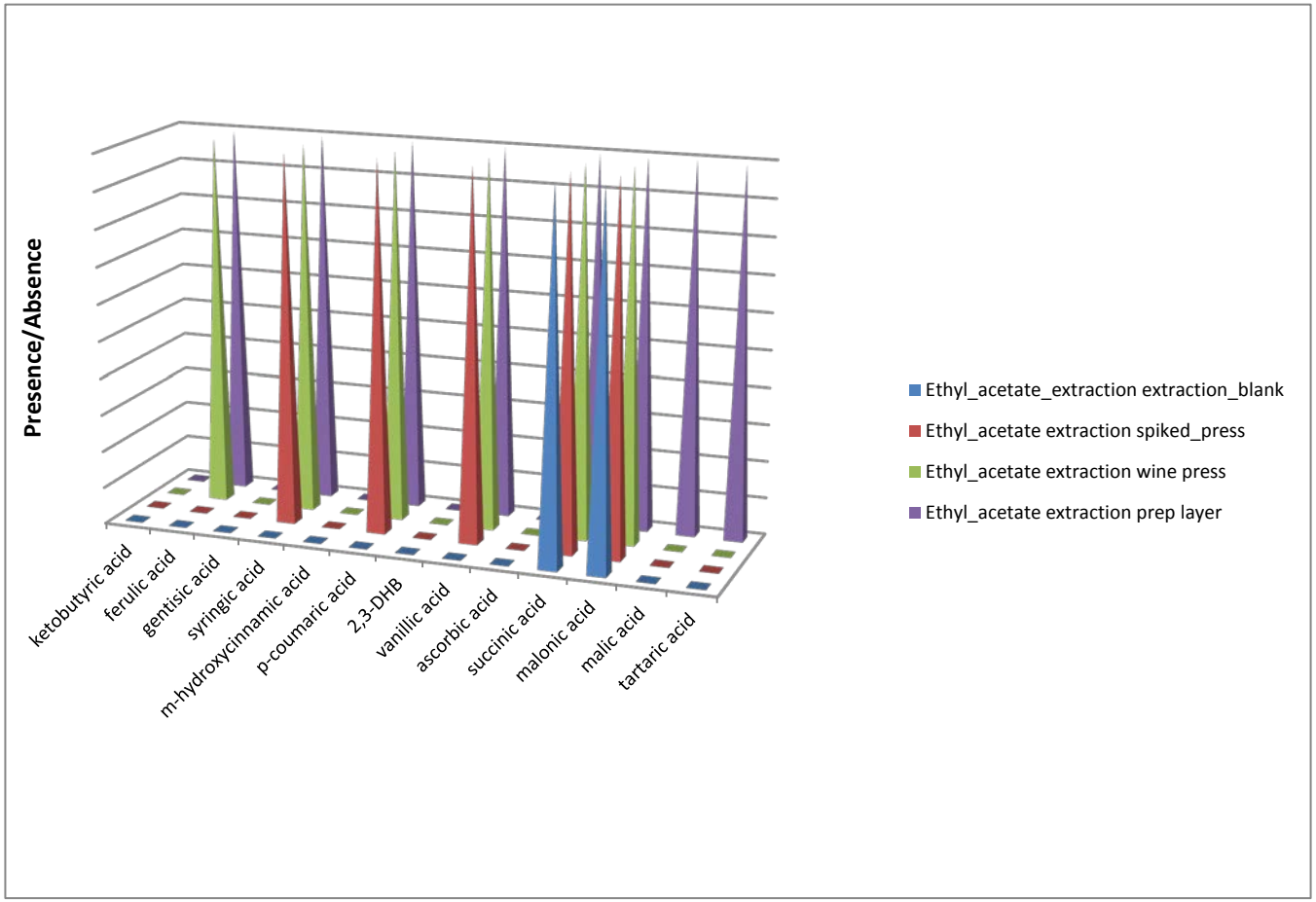


Figure 4.8. Presence or absence of acids from the ethyl acetate liquid extraction. Eight acids were identified, including: tartaric, malic, malonic, succinic, vanillic, p-coumaric, syringic, and ferulic acids. Low levels of succinic acid and malonic acid were also identified in the extraction blanks.

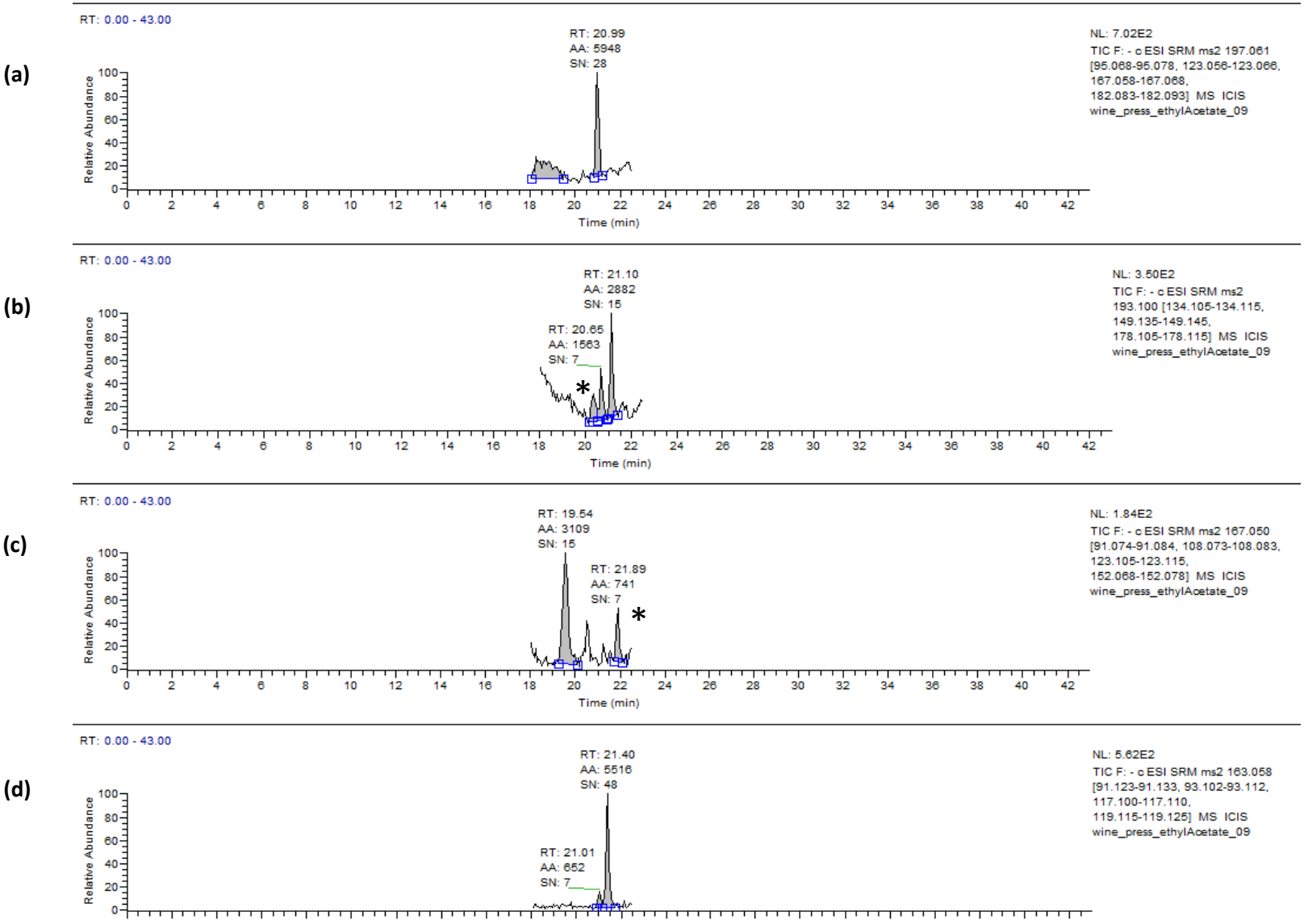


Figure 4.9. Extracted ion chromatograms of 4/8 acids identified in the preparatory layer of a Punic Sardinian wine press, (a) m/z 197, syringic acid (b) m/z 193, ferulic acid (c) m/z 167, vanillic acid and (d) m/z 163, p-coumaric acid. The retention times for ferulic and vanillic acids are highlighted with an asterisk.

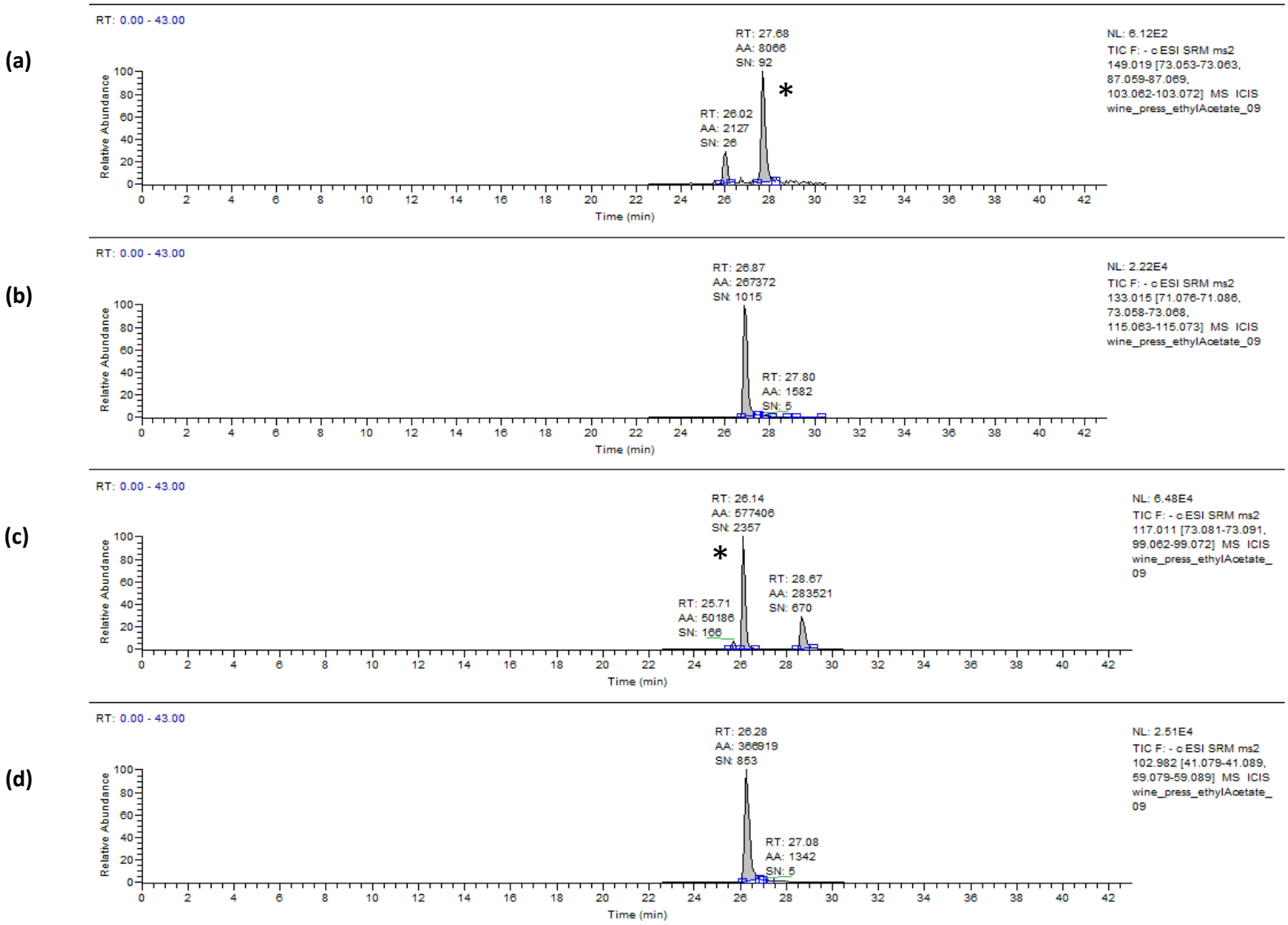


Figure 4.10. Extracted ion chromatograms of 4/8 acids identified in the preparatory layer of a Punic Sardinian wine press, (a) m/z 149, tartaric acid (b) m/z 133, malic acid (c) m/z 117, succinic acid and (d) m/z 103, malonic acid. The retention times for tartaric and succinic acids are highlighted with an asterisk.

4.3 Conclusions

Based upon the biomarkers determined from Chapter 3, a HILIC LC elution profile was successfully developed for the targeted analysis of fourteen phenolic and organic acids in an attempt to accurately describe the fingerprint of an aged, polymerised wine. These 14 acids are: ketobutyric, ferulic, gentisic, syringic, m-hydroxycinnamic, p-coumaric, 2,3-dihydroxybenzoic, vanillic, ascorbic, succinic, malonic, malic, tartaric, and isopropyl malic acid. Several extraction procedures in conjunction with the targeted LC/MS/MS method was applied to the Sardinian wine press and its preparatory layer, utilised as the archaeological 'positive control.' Eight acids were conclusively identified in the sample based upon each compound's retention time in combination with their fragmentation pattern. The most successful extraction procedure, based upon the number of acids identified, was the procedure which allowed the samples to sit for a period of time in an acidic medium prior to extraction with organic solvent. The final method was described in Chapter 2.2.1. This suggests a rate limiting step allowing for the depolymerised monomers to migrate into the aqueous environment. As described in Chapters 2 and 4, this new extraction method and targeted analytical approach was then applied to the analysis of transport amphorae and the wine press from the Sardinian archaeological site, as well as artifacts from the Roman British site of Vindolanda, in order to determine the presence of wine.

5. Applying a Targeted Approach to Archaeological Samples from Sardinia Italy

The objective of this chapter is to apply a targeted analytical approach that exploits the metabolomics-based discoveries from Chapter 3 to samples taken from the Punic farmstead, Trunce e' Molas, in Sardinia, Italy. This area has been actively growing grapes and preparing wine for several millennia. Samples include a double basin wine press as well as sherds representative of transport amphorae, identified by the archaeologists from the Terralba rural research project. This research will apply a predetermined suite of biomarkers that represent aged, polymerised wine to archaeological artifacts in order to describe the presence or absence of wine. In addition, the results will be compared with known standards in a further attempt to classify aged wine as more representative of white wine or red wine. A classification may assist archaeologists in their description of the objects excavated from this site.

5.1 Archaeological Sample Extraction

Table 5.1 in the Appendix represents a list of the archaeological sherds that were sampled and extracted from Trunce e' Molas in Sardinia, Italy. There were a total of 30 archaeological samples and one extraction blank. Sample identification was broken down as follows, Table 5.1.

Table 5.1 Identifications of the samples taken for analysis from the Punic farmstead, Trunce e' Molas, Sardinia, Italy.

Sample Identification	Fabric	Location	Samples analyzed	lab identifier
TM.003.3	cocciopesto /earthenware	Stone structure with plastered interior surfaces. Top earthenware preparatory layer.	5	wine press A
TM.027.3	limestone /earthenware	Limestone monolith	5	wine press B
TM.003.3	earthenware	Separated preparatory layer	5	preparatory layer
1.TM.044.1.3	B		1	neck
SAR.TM.67	B		1	neck
1.TM.028.1.33	B		1	neck
1.TM.060.1.15	B		1	neck
1.TM.042.1.7	B		1	neck
1.TM.033.1.14	B		1	side wall
SAR.TM.4	B		1	side wall
1.TM.034.1.20	B		1	side wall
1.TM.033.1.15	B		1	side wall
1.TM.060.1.45	B		1	side wall
1.TM.060.1.56	B		1	base
1.TM.035.1.1	B		1	base
1.TM.036.1.2	B		1	base
1.TM.028.1.31	B		1	base
1.TM.033.1.20	B		1	base

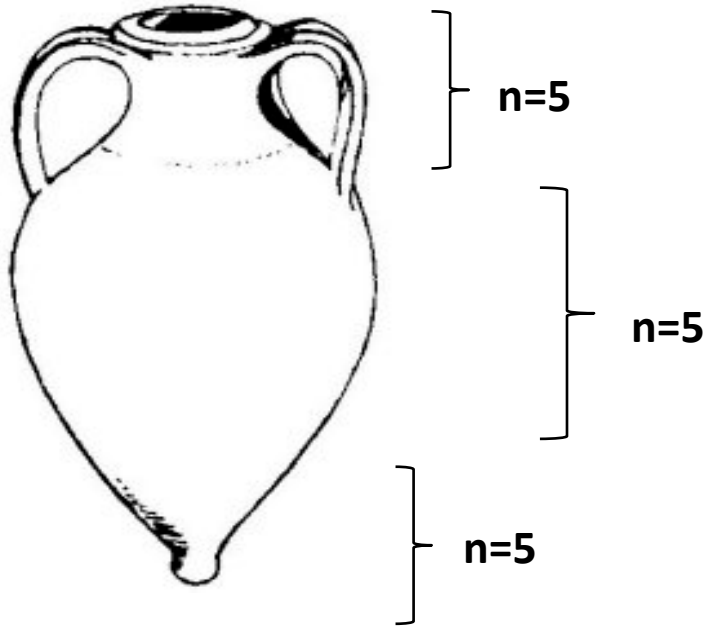


Figure 5.1. Diagram illustrating the sample locations of the 15 sherds taken from transport amphorae

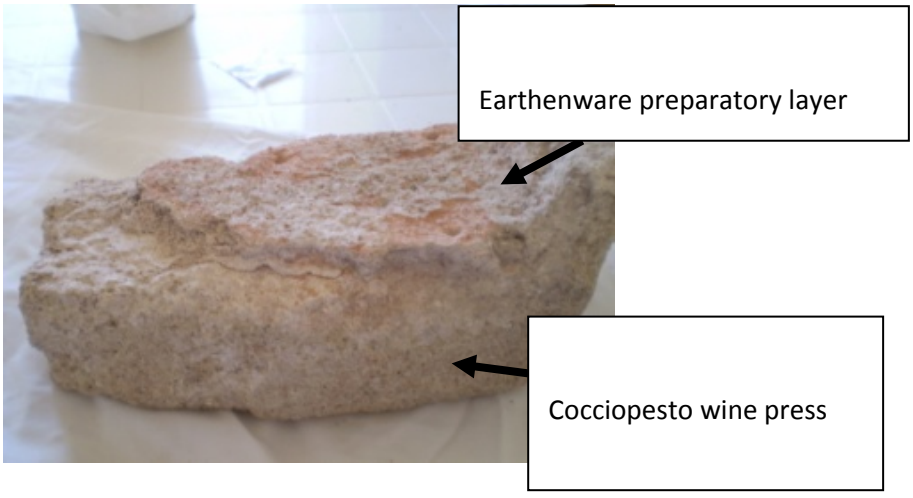


Figure 5.2. Photograph taken of the wine press TM.003.3 excavated from Trunce e' Molas, with its upper preparatory layer.

Thirty one samples were prepared for analysis: wine press (preparatory layer+mortar) n=10, preparatory layer only n=5, amphorae neck n=5, side wall of amphorae n=5, amphorae base n=5, and extraction blank n=1, Figures 5.1 and 5.2. The exact mass of each sample was recorded in the samples extraction log sheet located in the Appendix Table 5.1. As stated in the Chapter 1, the wine press and all sherds were excavated from Phase II stratigraphy by the archaeologists of the Trunc'e Molas rural research project and represent different amphorae. The distinction between neck, side wall, and base were categorised by the same archaeologists of the Terralba research project. Although there was mixing between Phase I and the upper portion of Phase II, the samples here examined were considered secure in the ancient timeline of $4^{th}$and 2^{nd} century B.C.E. habitation. Also, the wine press which was discovered in situ, was well below the topsoil and modern plough lines; abrasions from modern agricultural work were also absent from the press (person communication: P. van Dommelen).

The archaeological sample extraction method is described in Chapters 1 and 2. Briefly, one gram of sample from each of the sherds was removed with a tile cutter and placed in a 7 ml Precellys homogenisation tube. Samples were homogenised and extracted using a modified Bligh-Dyer biphasic extraction procedure resulting in a polar and a non-polar extract (Bligh and Dyer, 1959). The remaining solids were allowed to dry overnight in a fume hood. After drying, 600 µl of 1M KOH were added to the sample and heated for one hour at 50^{o}C in a water bath (Zsuga and Kiss, 1987). The samples were removed from the bath, cooled to room temperature, and then acidified to ~pH2 with 2.5 ml of 2N HCl. The pH was checked by strips of pH paper for randomly chosen samples. The samples vials were allowed to sit for 24 h at room temperature after which the samples were extracted with 3 ml of ethyl acetate. The samples were vortexed and centrifuged. The organic layer was then transferred to a 1.8

ml champagne vial with a glass pipette and blown down under a stream of nitrogen; the vials were refilled and dried down at least twice. All samples were capped and stored at -80^{o}C until analysis.

5.2 LC-MS/MS Analysis of the Archaeological Samples

The extracted samples were removed from the freezer and reconstituted in 30 µl of mobile phase 90%ACN/10% 100mM ammonium acetate, pH=8.2. The samples were vortexed for 10 s and then centrifuged for 20 min at 10^{o}C at 6000 rpm. Ten microlitres of sample were added to the 96 well plate and 1 µl of sample was injected onto the column for analysis. Sample 1.TM.042.1.7 from the neck was diluted 1:5 (v/v) due to its viscous nature.

Table 5.2 The absolute intensities of the acids identified by LC-MS/MS from the initial targeted analysis on the Sardinian wine press, preparatory layer and 15 sherds representing transport amphorae.

Sample Name	ketobutyric acid	ferulic acid	gentisic acid	syringic acid	p-coumaric acid	2,3-dihydroxybenzoic acid	vanillic acid	succinic acid	malonic acid	malic acid	tartaric acid	isopropyl malic acid
standard mixture	653221	1.00E+07	2.22E+09	5889038	123447807	683912170	815101	2476835	653221	4.00E+06	1712784	650250
blank	0	0	0	0	0	0	0	18325	3017	170716	41249	0
extraction blank	0	0	0	0	0	0	0	0	0	0	0	0
QC1	0	0	0	0	0	0	0	9519	0	77648	0	0
QC2	0	0	0	0	0	0	0	11896	0	56699	0	0
QC3	0	0	0	0	0	0	0	0	0	36223	0	0
SAR.TM.67	0	0	0	0	0	0	0	4242	0	21638	0	0
1.TM.028.1.33	0	0	0	0	0	0	0	5349	0	9840	0	0

Sample Name	ketobutyric acid	ferulic acid	gentisic acid	syringic acid	p-coumaric acid	2,3-dihydroxybenzoic acid	vanillic acid	succinic acid	malonic acid	malic acid	tartaric acid	isopropyl malic acid
1.TM.060.1.15	0	0	0	931	0	0	0	5165	0	5765	0	0
1.TM.042.1.7 (1:5)	0	0	0	0	0	0	0	36460	0	8033	0	0
1.TM.033.1.14	0	1276	0	1822	0	0	0	5605	0	4187	0	0
SAR.TM.4	0	2246	0	0	0	0	0	10481	0	7186	0	0
1.TM.033.1.15	0	1130	0	614	0	0	0	16276	0	7736	0	0
1.TM.060.1.45	0	956	0	1968	0	0	0	19654	4010	3397	0	0
1.TM.060.1.56	0	1532	0	842	0	0	0	100792	11109	2193	0	0
1.TM.035.1.1	0	1714	0	628	0	0	0	5286	0	0	0	0
1.TM.036.1.2	0	1469	0	0	0	0	0	4988	0	2039	0	0
1.TM.028.1.31	0	1123	0	1207	0	0	0	306697	4657	1892	0	0

Sample Name	ketobutyric acid	ferulic acid	gentisic acid	syringic acid	p-coumaric acid	2,3-dihydroxybenzoic acid	vanillic acid	succinic acid	malonic acid	malic acid	tartaric acid	isopropyl malic acid
1.TM.033.1.20	0	994	0	840	0	0	0	7257	0	2971	0	0
blank	0	1192	0	0	0	0	0	4535	0	2629	0	0
QC4	0	2043	0	1055	0	0	0	17350	4981	1492	0	0
QC5	0	1262	0	1012	0	0	0	18095	4656	2408	0	0
WP2_TM.003	0	2096	0	7197	8523954	0	5662	18897	0	1604	0	0
WP3_TM.003	0	5046	0	12103	12100482	0	8615	8246	1852	2055	0	0
WP4_TM.003	0	1770	0	3692	2085196	0	1922	80435	137095	0	0	0
WP5_TM.003	0	3464	0	11788	10440534	0	7335	229599	0	0	0	0
WP6_TM.027	0	0	0	4574	9070793	0	5121	7257	0	0	0	0
WP7_TM.027	0	0	0	0	0	0	0	4189	0	0	0	0

Sample Name	ketobutyric acid	ferulic acid	gentisic acid	syringic acid	p-coumaric acid	2,3-dihydroxybenzoic acid	vanillic acid	succinic acid	malonic acid	malic acid	tartaric acid	isopropyl malic acid
WP8_TM.027	0	597	0	4292	9242684	0	5186	3627	0	0	0	0
WP9_TM.027	0	0	0	5756	14367540	0	10146	4760	0	0	0	0
WP10_TM.027	0	0	0	3132	13425446	0	5393	3588	0	0	0	0
prep1	0	2558	0	19667	8379605	0	13461	225784	1764	0	0	0
prep2	0	3524	0	20855	9404386	0	15741	2635324	5035	0	0	0
prep3	0	4582	0	21594	5821124	0	14821	2565120	45942	0	0	0
prep4	0	3845	0	14496	25262847	0	19413	242615	1634	0	0	0
prep5	0	2693	0	9569	6032407	0	5045	33825	864	0	0	0
Standard mix (1:2)	5095282	9.00E+06	98161000	2432432	1.005E+09	NA	317863	306386	136119	0	0	0

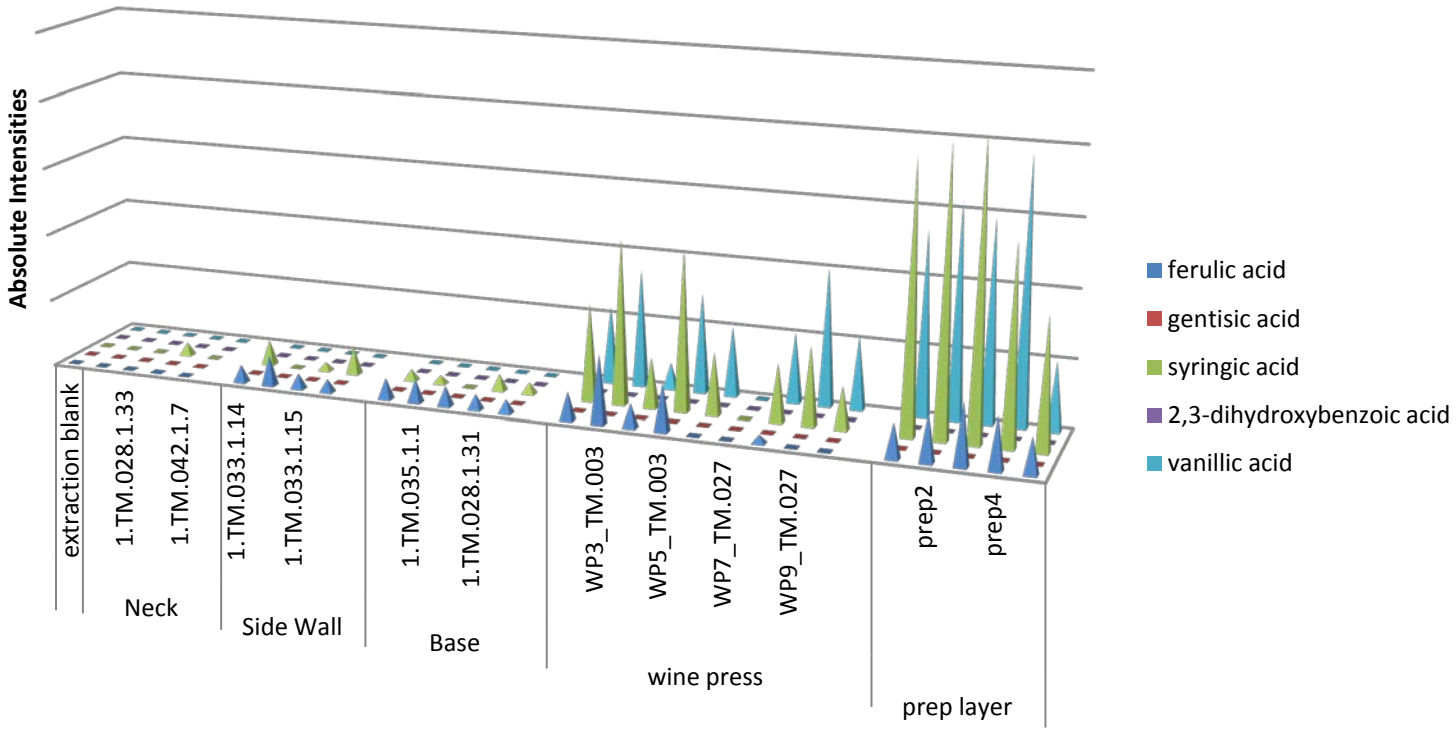


Figure 5.3. This diagram compares the absolute intensities of the phenolic acids identified in the targeted analysis of 15 samples from a wine press and 15 samples of transport amphorae excavated from a Punic farmhouse in Sardinia, Italy.

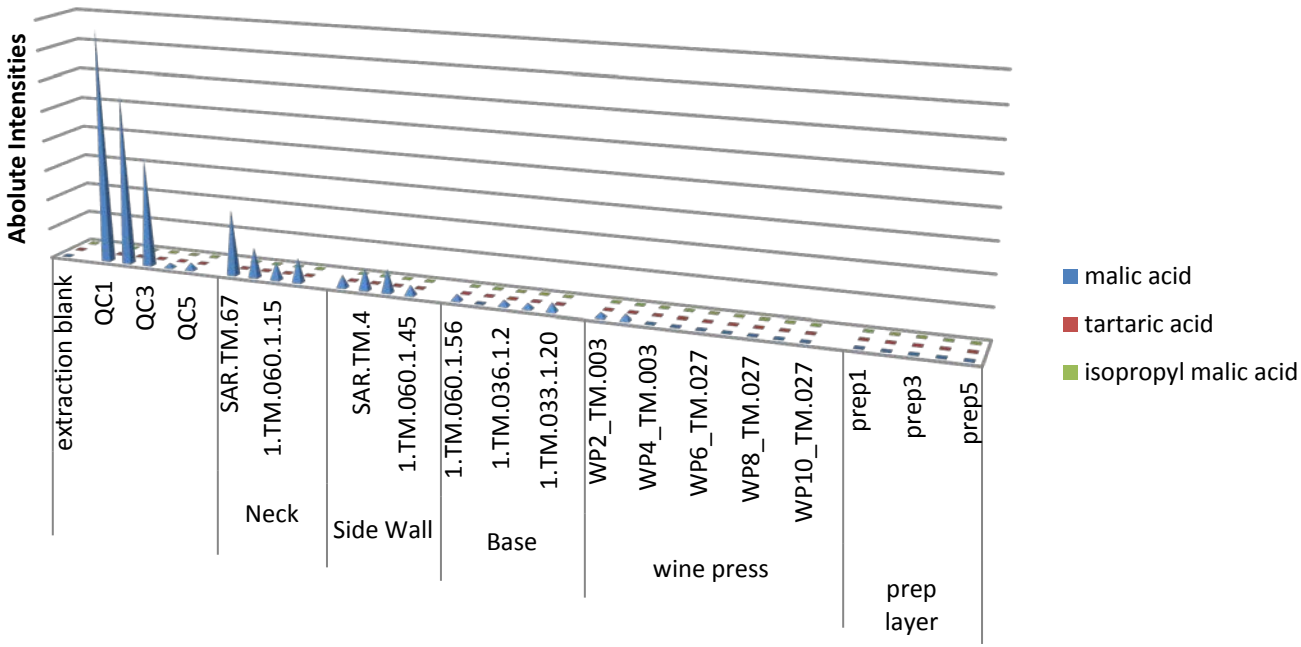


Figure 5.4. This diagram illustrates the fate of three organic acids during this analyses. There is an obvious decline in malic acid as the runs progress. The reason for this loss of intensity is described in the chapter.

5.2.1 Results of Targeted Analysis

Table 5.2 shows the results of the absolute intensities of the LC-MS/MS results of the targeted analysis of the wine press, its preparatory layer and 15 amphorae sherds. Seven acids out of fourteen from the biomarker list were identified in the group of thirty archaeological samples, these included malic, malonic, succinic, vanillic, p-coumaric, syringic, and ferulic. Integration of the peaks was performed automatically by Xcalibur software; in certain cases, manual integration was necessary to maintain baseline to baseline continuity. Figure 5.3 compares the absolute intensities of the phenolic acids identified in the targeted analysis of samples. The highest intensities were identified in the wine press and its preparatory layer; acids were virtually absent from the sherds representing the neck, except for syringic acid identified in sample 1.TM.060.1.15. Ferulic and syringic acids were identified in low levels in several of the side walls, as well as the bases. The increased intensity of these six acids: ferulic, syringic, vanillic, p-coumaric, succinic, and malonic, in the wine press and in the preparatory layer, strongly suggest the presence of an aged, polymerised wine. In contrast, due to the lack of consistent grouping of biomarkers within each sample in conjunction with the low intensity of acids identified, indicate an absence of wine in the amphorae sherds. This result highlights the precarious nature of using 1 or 2 biomarkers for the identification of an organic residue within an archaeological object.

5.2.1.1 Sample Carryover

There was some carryover from the original standard evident in the first blank analysed. However, the following analysis of the extraction blank was devoid of acids. Identified in the blank analysed halfway through the run was a low level of ferulic acid, succinic acid, and malic acid, indicative of some carryover

within the column. This result calls into question the identification of these three acids in the samples with intensities lower than the intensities identified in the blank. Succinic acid was a recurring contaminant during this analysis, and its conclusive identification in archaeological samples was based upon the absolute intensity exceeding the blank readings. Malic acid was identified in sixteen of the samples; however, the results of six samples are considered suspect based upon the results of the blank discussed earlier. There was also a noticeable decay in the malic acid intensity throughout the analysis. This trend is discussed in the next section.

5.2.1.2 Loss of Certain Metabolites

Figure 5.4 illustrates three of the organic acids examined in this targeted analysis: malic, tartaric, and isopropyl malic acid. The QCs, listed from 1-5, are also illustrated in this chart. Malic acid was identified in all five of the QC samples; however, there was a 10 fold drop in the intensity from QC1 (77648) to QC5 (2408). There was also a consistent drop in the intensity of malic acid throughout the analysis. A separate standard mixture was prepared and analysed at the end of the analyses and neither malic nor tartaric acid were identified in this final mixture (both were added to the standard mixture). It was hypothesised at the time that the binding efficiency of the guard column or the analytical column was increasing over the course of the total analyses and that malic acid with three hydroxyl groups and tartaric acid with four hydroxyl groups were the most affected by this increased binding capacity. The sample matrix, either the pottery matrix or the salt residue from the alkaline fusion, were both considered as possible culprits.

Upon dry down with nitrogen, there was a white sediment in the base of the sample vials. Initially, the identity of the sediment was thought to be KCl salt, a byproduct of the alkaline fusion. In order to

confirm the identity of this crystalline salt, two representative samples of the white powder (found on the bottom of nearly all the samples) were taken from a neck sherd and a sample of the wine press for analysis by x-ray diffraction (XRD). X-ray diffraction is an analytical technique developed out of Bragg's law ($n\lambda = 2d\sin\theta$) which describes a unique crystalline structure based upon its ability to reflect incident radiation. The samples were analysed by Jackie Deans of the School of Chemistry, University of Birmingham. The samples contained a remnant of solvent (90/10 (v/v), ACN/100mM ammonium acetate) and were dried overnight in a glassware drying oven. Once dried, the samples were prepared by mixing a small portion of the powder with mineral oil and applying to the end of a diffraction pattern free glass rod. Each sample was scanned separately for 26 min. The sample was scanned between 5-90^{o} with a 0.35 degree step. The results of the diffraction analyses are shown in Figures 5.5a and 5.5b and neither sample showed a distinct diffraction pattern; therefore, the white powder was not identified as a crystalline material, but rather an amorphous, poorly ordered material.

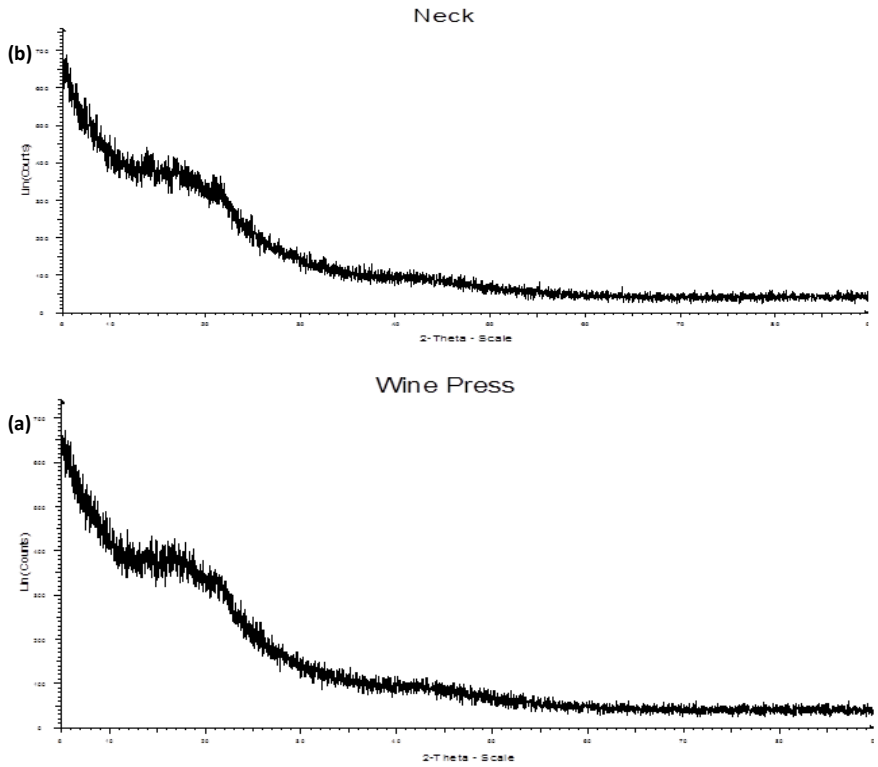


Figure 5.5. X-ray diffraction results of the white powder remaining after extraction of sherd (neck) and of a wine press sample. Each sample was scanned from 5 to 90°for 26 min at a 0.35 degree step. The results indicate a poorly ordered, amorphous material.

In order to gain more information on this white powder, two separate samples were taken from two sherds (neck, side wall) for elemental analysis by scanning electron microscopy with energy dispersive X-ray micro analysis (SEM/EDS). The two samples were adhered to an aluminum stub with double side carbon dots and then coated with carbon in order to provide a conductive surface. The samples were analysed by Paul Stanley at the Centre for Electron Microscopy in the School of Metallurgy and Materials, University of Birmingham. The surface of each sample was scanned manually across the surface to collect the elemental information. The elemental analyses of each sample were relatively

uniform across the surface of the sample and the graphs are shown in the Appendix, Figure 5.1**.** The common elements in the powder are aluminum and silicon, presumably leached out of the clay matrix during the alkaline fusion. Presumably the leaching of the silica/alumina matrix was chelating the most accessible compounds malic and tartaric acid (as well as isopropyl malic acid).

5.2.1.2.1 Pottery Science and the Bayer Method

Clay is produced from weathering of rocks and the elements most immune to such weathering are aluminium and silicon. Therefore, the main constituents of clay are silicon, surrounded by four oxygen atoms forming a tetrahedral, and aluminum, surrounded by six hydroxide (or oxygen in some cases) forming an octahedral. The electronic interaction amongst these two basic units form alternating sheets of silicate/aluminate/silicate separated by an interlayer often populated by other cations such as Fe, Mg, and K (Figure 5.6). Morphologically, these sheets appear as a lamellar-like structure and it is these sheets that provide the sliding movement of a clay. When water is added, the dipole attraction between the water molecules and the surface charged clays provide for ease of movement allowing for clays' most precious attribute: its plasticity (Rice, 1987: 41). This plasticity allows it to be molded into utilitarian forms and when heated and the added water is driven off, the form is maintained.

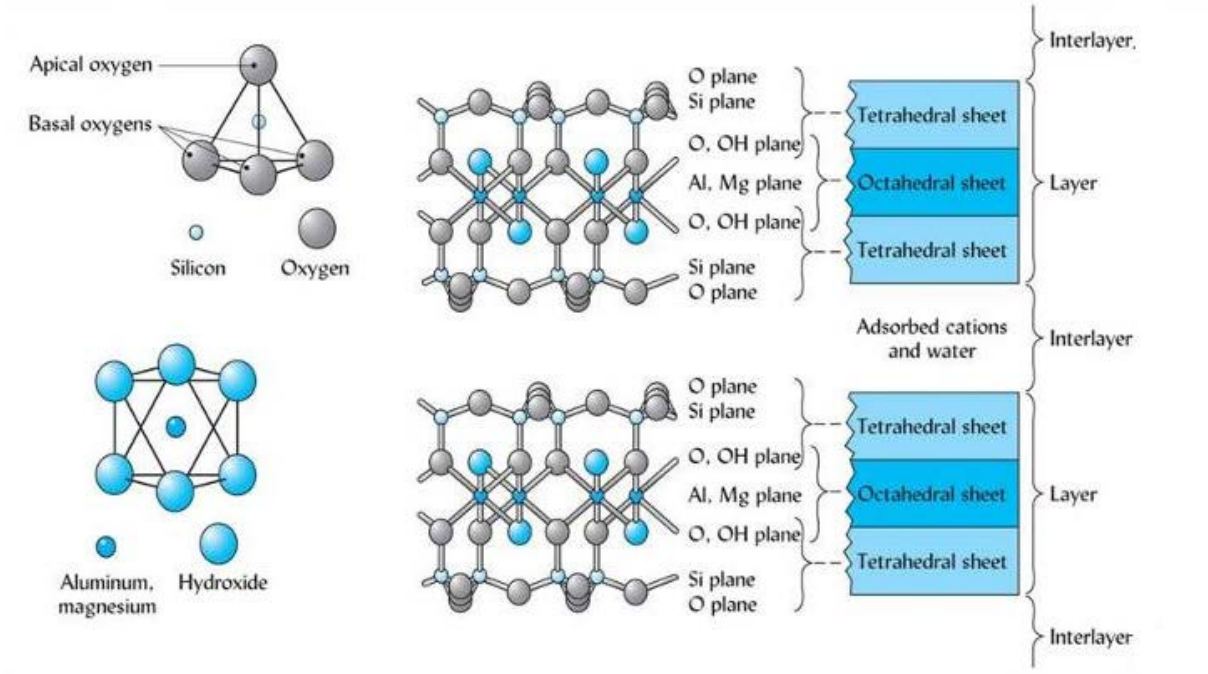


Figure 5.6. A diagram of the physical make-up of a clay body.
http://faculty.yc.edu/ycfaculty/ags105/week08/soil_colloids/soil_colloids_print.html

It is theorised that under strong alkaline solution, the aluminate (and silicate) building blocks leach out of the solution in a similar fashion to the preparation of aluminum ore from bauxite, the Bayer method (Loh et al., 2005, Caullett and Guth, 1989). The Bayer method was invented in 1887 for the purpose of extracting aluminates from ore at high pH followed by filtration, calcining and then collecting the product (Habashi, 2005). At alkaline pH, $Al(OH)_4^-$ is leached from aluminosilcates. As the pH is lowered, there is a transition in the oxidation state of aluminum from Al^4 to Al^6 forming $Al(H_2O)_6^{3+}$ the aluminate ion present in acidic medium (pH < 3) (Franks and Gan, 2007). It is theorised that this ion might have built up on the guard column during the successive runs and complexed with malic and tartaric acids.

$Al(H_2O)_6^{3+}$ is gelatinous rather than crystalline which explains the lack of diffraction pattern. The origin of the silicon in the elemental analysis may be due to silanol groups which although uncharged, could form hydrogen bonds with the aluminate complex.

In terms of the analysis of ancient wine, one of the more problematic biomarkers to identify is tartaric acid. Depending upon the published method, the absence of this compound is often attributed to the assumption that a tartrate salt will not survive archaeological time. However, it is more likely that the original tartaric acid, combining within the wine polymer and then released via alkaline hydrolysis has combined with an aluminate ion leached from the pottery matrix. The current extraction method applied in this thesis probably introduces more of the matrix effect by allowing the sample to sit in acidified solution for 24 h, yet any caustic solution applied to a pottery matrix will leach $Al(OH)_4^-$ which upon acidification is converted to $Al(H_2O)_6^{3+}$. Alkaline fusion is the most effective method to break down the wine polymer, therefore, a clean-up procedure must be added to the extraction step.

5.2.1.3 Strong Cation Exchange Solid Phase Extraction (SPE)

In order to capture the positively charged aluminate complex, an SPE step was added to the sample preparation method. The cartridge chosen was a negatively charged phenyl sulfonate cartridge, Figure 5.7.

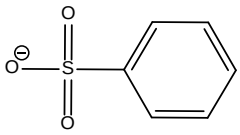


Figure 5.7. Phenyl sulfonate active group on the strong cation exchange SPE cartridge. At pH 2, the available negative charge binds the positively charged aluminate ion. The analytes of interest, either neutral or negatively charged at low pH will flow through the cartridge.

At acidic pH, the acids of interest are either fully protonated or negatively charged. In either case, the acids will not bind to the stationary phase and instead will flow through the SPE cartridge. In preparation for this added step, pottery samples were acidified for 24 h. After the allotted time, the SPE cartridges were conditioned per manufacturer recommendation with 1 ml methanol followed by 1 ml deionized water. The samples were centrifuged and the acidic aqueous layer was then added to the cartridge, the flow through captured and then extracted with ethyl acetate and dried down under a stream of nitrogen.

Prior to rerunning the samples, the guard column was regenerated with a wash of 0.5M NaCl for at least 30 column volumes. This was followed with a water rinse of 30 column volumes.

5.2.1.3.1 Retention Time Shift Post-SPE

A mixture of 9 acids was spiked into extracts of the wine press and then eluted through the SPE cartridge in order to determine if there was any retention time shift due to the procedure. The retention time of eight of the nine acids were shifted slightly. A major shift occurred with ferulic acid, its retention time shifted by nearly two min. The change in retention times are given below. However,

none of the retention time shifts were large enough to warrant a change in the time span of the MS/MS segments.

Table 5.3 The retention time for standard acids before and after sample clean-up with solid phase extraction.

identification	RT pre SPE	RT post SPE
ferulic acid	20.45	19.06
syringic acid	21.01	20.55
p-coumaric acid	21.46	21
vanillic acid	21.96	21.57
malonic acid	26.43	26.15
malic acid	26.8	27.11
tartaric acid	27.55	28
succinic acid	26.16	26.02
isopropylmalic acid	24.73	25.25

5.2.2 Reanalysis of Archaeological Samples

Following discovery of the aluminate ion interference with the organic acids, re-analysis of the entire sample set was undertaken. The thirty one samples were re-extracted: wine press (prep layer+mortar) n=10, wine press (prep layer) n=5, amphorae neck n=5, side wall of amphorae n=5, amphorae base n=5, and extraction blank n=1. The exact mass of each archaeological sample was recorded in the samples extraction log sheet located in the Appendix, Chapter 5, Table 2. The finalised standard operating procedure was described in Chapter 2. The samples were extracted as before with the additional SPE step. All samples were capped and stored at -80^{o}C until analysis.

As before, prior to analysis by LC-MS/MS, the samples were removed from the freezer and reconstituted in 30 µl of mobile phase 90% ACN/10% 100mM ammonium acetate, pH = 8.2. Based upon the results of the earlier analysis, two acids were dropped from the targeted list: 2,3-dihydroxybenzoic acid and ketobutyric acid.

Table 5.4 The absolute intensities of the LC-MS/MS targeted analysis of the Sardinian wine press, its preparatory layer, and fifteen sherds representing transport amphorae.

Sample name	ferulic acid	gentisic acid	syringic acid	p-coumaric acid	vanillic acid	succinic acid	malonic acid	malic acid	tartaric acid	isopropyl malic acid
QC1	3293	0	5426	8748	9408	667960	587254	0	0	0
QC2	3728	0	5772	8948	8621	702961	663624	0	0	0
blank	0	0	0	0	0	39382	47344	0	0	0
Ext_blnk_31	0	0	0	0	4337	39974	43318	0	0	0
WP5_TM.003	19855	0	14309	15642	9842	395223	743383	1390045	5885	182923
WP9_TM.027	9317	0	17258	26968	25393	934154	1077255	1338433	0	117354
1.TM.060.1.56	0	0	1894	6332	5001	92586	32532	0	0	0

prep11	8681	0	25264	16010	29954	1868501	1105105	749561	0	0
1.TM.033.1.14	1352	0	2735	3634	3401	34856	48847	0	0	0
WP4_TM.003	11607	0	8952	12413	9854	575125	545164	1232819	15952	619539
QC3	0	0	3808	5208	5762	406642	0	0	0	0
blank	0	0	0	0	0	35731	0	0	0	0
WP6_TM.027	6977	0	13972	39168	27748	1314494	1328163	0	0	0
prep15	7223	0	20221	30003	26246	1752745	1178465	0	0	0
WP1_TM.003	10323	0	16692	5963	8201	471943	757062	0	0	0
prep12	0	0	503	0	0	553122	1001212	0	0	0
SAR.TM.4	0	0	0	8364	0	150143	183042	224330	0	0

1.TM.033.1.15	0	0	0	0	0	58572	0	0	0	0
prep14	10348	0	16674	13496	16828	2308857	1224998	0	0	0
WP10_TM.027	6025	0	5604	11881	7187	929745	1097694	0	0	0
1.TM.060.1.15	0	0	2115	2099	2683	76572	0	0	0	0
1.TM.035.1.1	0	0	241	0	0	56492	0	0	0	0
1.TM.028.1.33	0	0	792	0	2040	58731	0	0	0	0
1.TM.028.1.31	0	0	856	0	2064	401987	0	0	0	0
SAR.TM.67	0	0	991	2209	2942	270373	0	0	0	0
QC 4	2750	0	4408	6250	5258	695976	587750	0	0	0
blank	0	0	0	0	1819	50906	0	0	0	0

1.TM.033.1.20	439	0	491	806	1806	53599	0	0	0	0
WP2_TM.003	13510	0	21394	8252	10774	803938	1307686	0	0	0
WP8_TM.027	6546	0	15500	33502	32132	1561604	1232583	683616	0	0
1.TM.042.1.7	0	0	249	549	0	68190	24710	0	0	0
1.TM.060.1.45	0	0	706	0	2603	44701	0	0	0	0
1.TM.034.1.20	0	0	456	0	2147	52046	0	0	0	0
1.TM.036.1.2	0	0	228	0	1385	63625	0	0	0	0
QC 5	3026	0	5258	8878	5962	762201	833825	0	0	0
blank	0	0	0	0	1124	49937	0	0	0	0
WP7_TM.027	2341	0	1905	0	4682	0	0	0	0	0

WP3_TM.003	0	0	407	0	0	79277	0	0	0	0
prep13	0	0	0	0	0	307473	0	0	0	0
1.TM.044.1.3	0	0	1742	0	0	98625	0	0	0	0

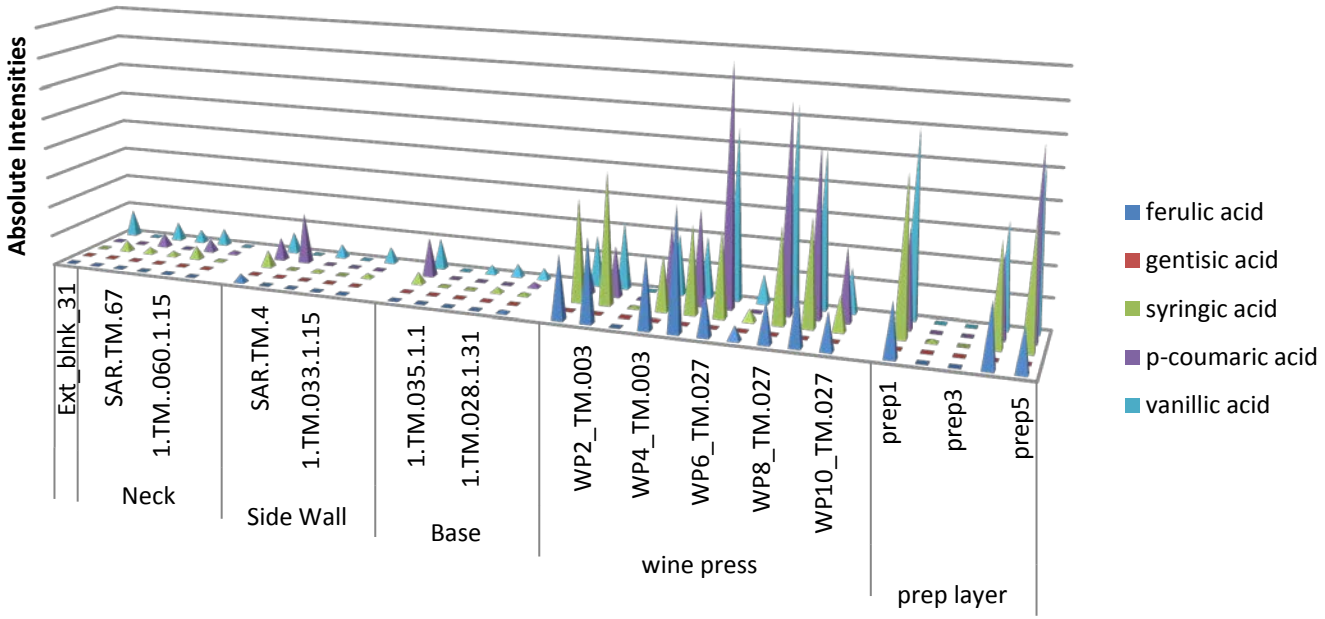


Figure 5.8. Graph illustrating the absolute intensities of the phenolic acids identified in the wine press and in the sherds from Trunc e Molas site, Sardinia, Italy. The greatest concentration of multiple biomarkers/sample is located in the wine press and in three of the prep layer samples. Prep layers 2 and 3 represent misinjections.

5.2.2.1.1 Sample Identification

Using the improved analytical method and based upon retention time, fragment ion transitions, and fragmentation intensities, two additional acids were identified in two samples of the wine press, bringing the total to nine acids detected. While a relative small increase, this should improve the specificity of the chemical fingerprint used to determine whether the archaeological sample was exposed to wine. Furthermore, between six and seven acids were identified in the majority of the remaining archaeological samples. Three acids were identified in the extraction blank: malonic, succinic, and vanillic acid. These levels identified in the extraction blank were considered baseline and any acid with an intensity below the extraction blanks intensity was considered not identified. For fragmentation intensities, absolute intensities were taken from each separate product ion and then relative intensities of each precursor product ions were determined; the relative intensities were then compared with the relative intensities of those same ions from the standards. In certain cases, inaccurate intensities could be explained by co-eluting peaks.

The results strongly corroborate the presence of wine in the press, particularly sample TM.003 and its prepatory layer. Figures 5.9-5.17 represent the extracted ion chromatograms and the transitions of the identified acids in the targeted analysis of sample 4 from the wine press, TM.003.3. Nine of the acids were identified in this sample and in sample 5 (TM.003.3). Eight of the acids were identified in wine press sample 9 (TM.027.3), and in prep layer 11. The majority of the remaining samples contained six acids: malonic, succinic, vanillic, syringic, p-coumaric, and ferulic acids. Comparisons of absolute intensities (Figure 5.8) show the greatest intensity and variety of acids were found in the wine press and its prep layer particularly when compared with the fifteen sherds taken from the transport amphorae.

This compares with the initial analysis of the archeological samples, however there was a difference in intensities. Intensities of the re-analysed samples were greater confirming the importance of the additional SPE step. Figure 5.18 compares the absolute intensities of four acids malonic, malic, vanillic, and syringic acids with the SPE step and without the SPE step. The intensities of malic and malonic acids are appreciably increased with the additional step of the SPE. For the phenolic acids, there was a general increase in intensity for the majority of samples although not as obvious most likely due to the fact that the phenolic acids have little interaction with the aluminate ion. However, there was at least one sample where the sample signal was lost suggesting a possible interaction between the phenolic acid and the ring structure on the SPE cartridge in the form of a pi-pi bond interaction.

The addition of an SPE step to the sample extraction protocol was done in order to capture a (possible and not yet confirmed) positively charged aluminate ion. However, by run 30 there was an increase in the column pressure suggesting a blockage of normal flow possibly caused by the accumulation of a compound adhering to the guard column. This affected the later sample runs particularly the final five analyses.

The results did not confirm the presence of wine in any of the sherds from the transport amphorae. For the results to suggest the presence of wine, a sample should contain a full biomarker complement including phenolic and organic acids. However, there was not even a complete list of phenolic acids identified in the sherds. Again, this highlights inconsistencies and spurious results that may occur when choosing only 1 or 2 biomarkers to identify archaeological residue. One theory for the lack of a biomarker signature is that these amphorae never held wine and instead were at the site in preparation for filling. Ancient texts on agriculture strongly recommend preparing amphorae by lining the interior

with pitch, to prevent seepage into the clay matrix (Columella, book 12: 197). Pitch was not identified on any of the interior surfaces of these sherds further suggesting that these amphorae were not ready to receive wine.

5.2.2.1.2 Insoluble or 'Bound' Lipids

It has been reported that insoluble (in chloroform), polymerised lipids were extracted under alkaline fusion; particularly prevalent were dicarboxylic acids (Evershed et al., 2002). Figure 5.19 is a typical output for the LC-MS/MS undertaken in this research. The early eluting peaks from 4.55-7.10 were tentatively identified as lipids, bound via ester bonds as a polymeric matrix and broken down under the alkaline attack. Tentative identifications were accomplished by comparing the experimental masses with the published masses in the LipidMaps database. The eluting peak at 4.55 minutes contains *m/z* 283 and 255, tentatively identified palmitic and stearic acids. This peak was also identified in the extraction blank and was considered a byproduct of the extraction procedure. The largest eluting peaks at retention time 5.6 and 6.03 include *m/z* 201 and 187 which were tentatively identified as decanedioic acid (sebacic) and nonanedioic acid (azelaic). Tentative identifications of other masses include: 3,12-dihydroxy-hexadecanoic acid (*m/z* 287), 2-hydroxydecandioc acid (*m/z* 217) , and 3-methyl-dodecanedioic acid (*m/z* 244). At this point, the origin of these fatty acids is unknown. One explanation for these acids is the degradation over an archaeological span of an unsaturated fatty acid by oxidation and dehydration, producing smaller remnants of the original fatty acid, as seen in Figure 5.20 (Regert et al., 1998). Therefore, the azelaic and sebacic may represent the degraded form of oleic acid.

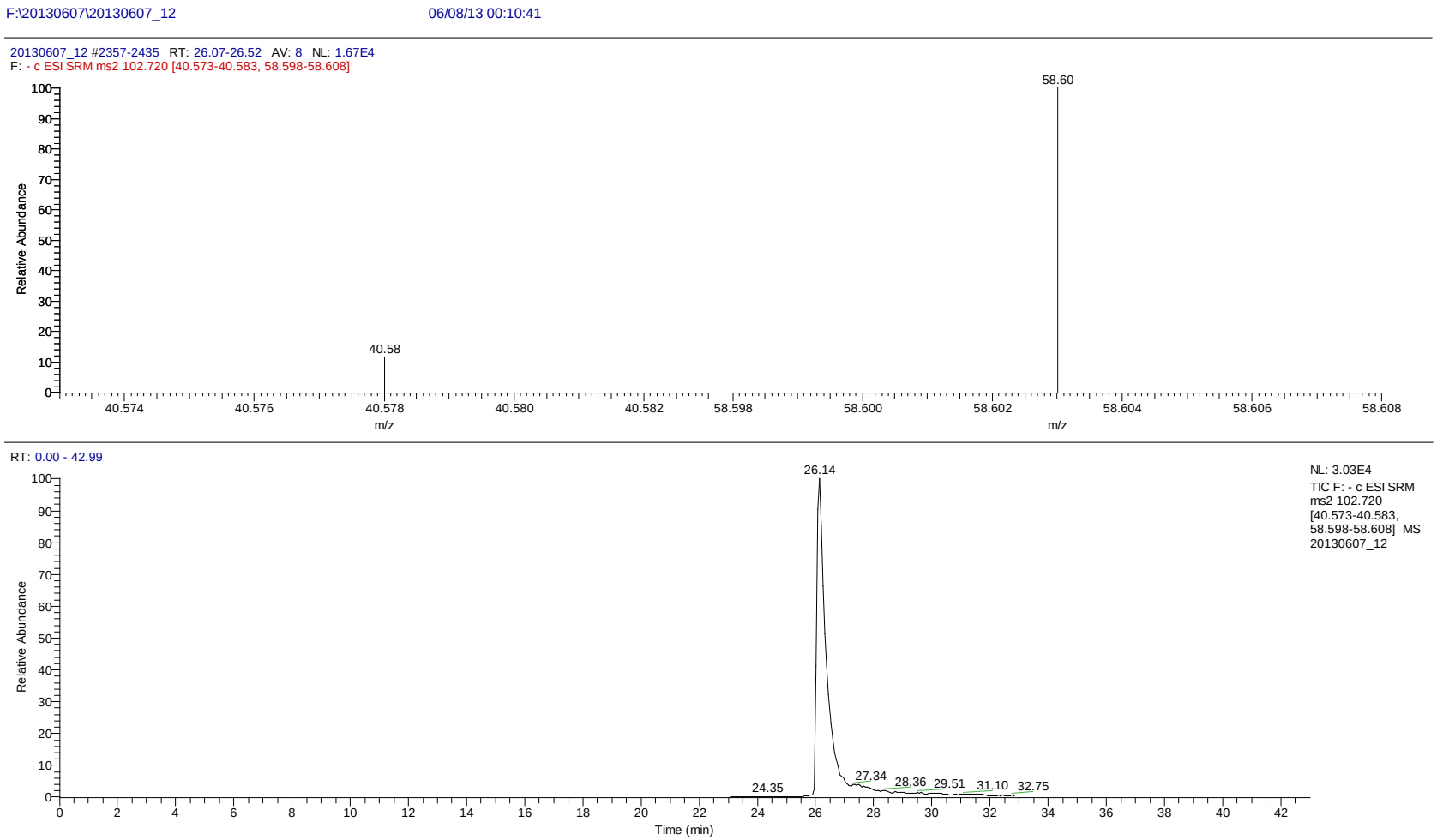


Figure 5.9. The identification of malonic acid from the Sardinian wine press based upon a) two product ions from *m/z* 103 and b) retention time (26.14).

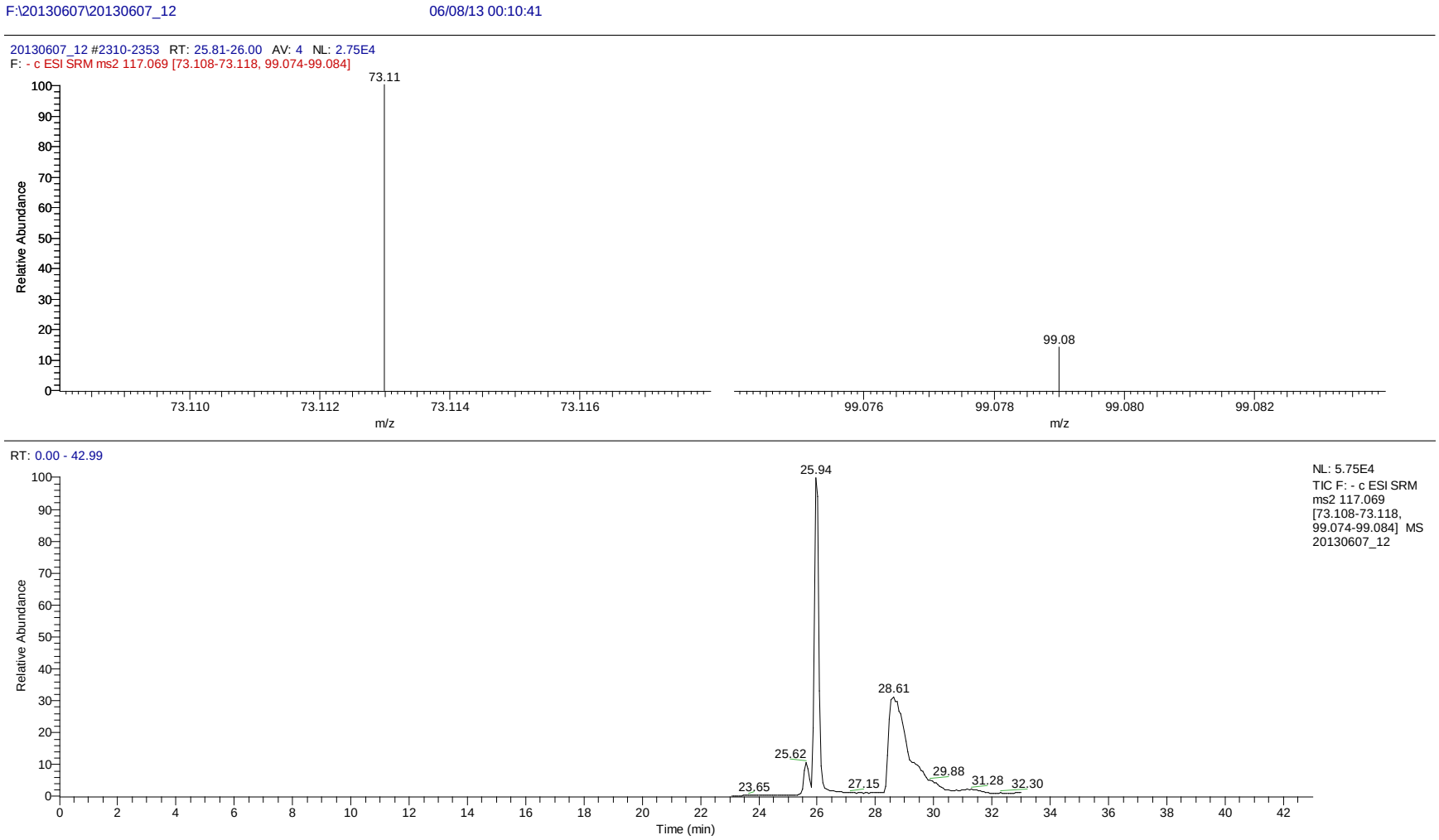


Figure 5.10. The identification of succinic acid from the Sardinian wine press based upon a) two product ions from *m/z* 117 and b) retention time (25.94).

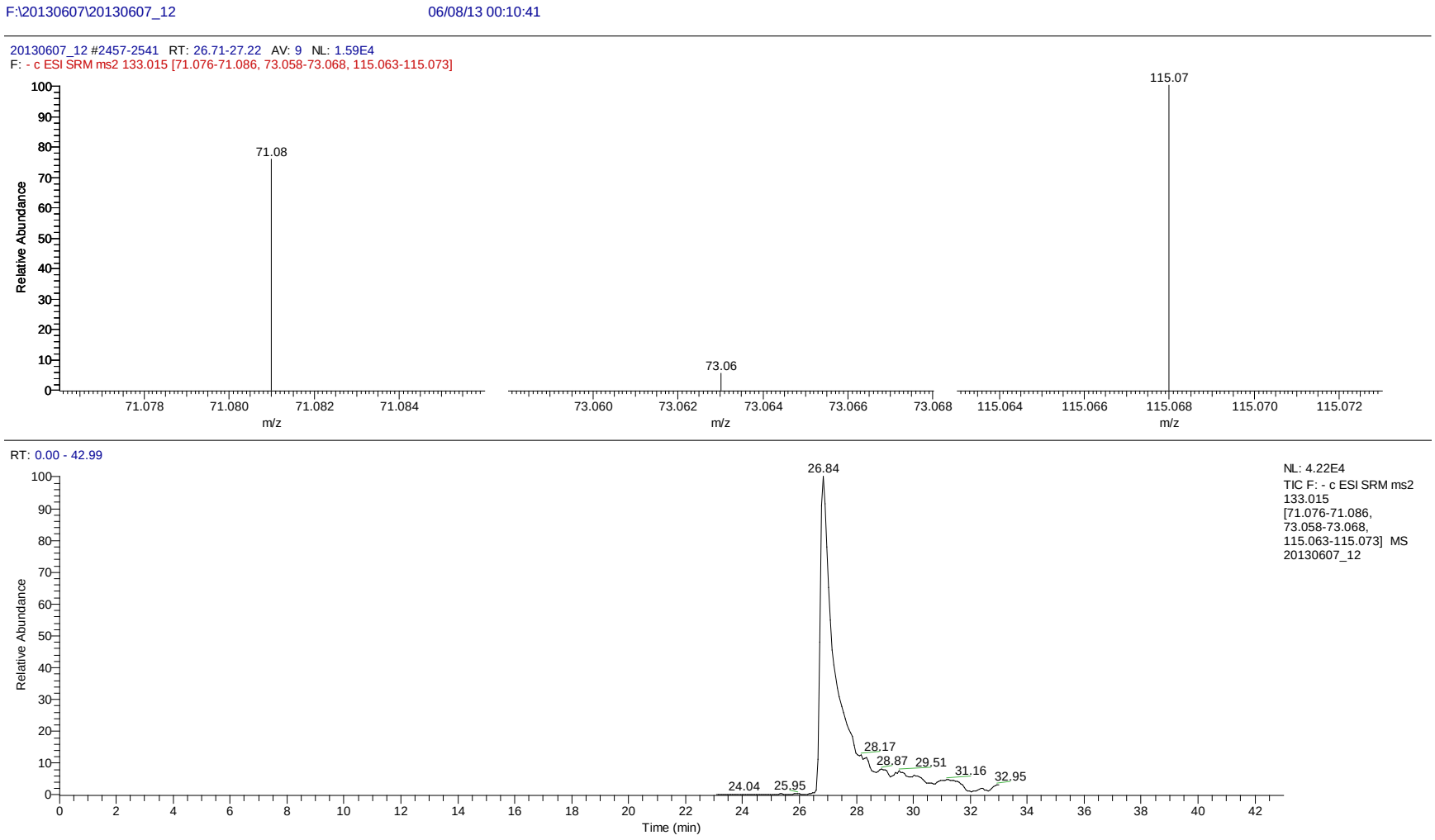


Figure 5.11. The identification of malic acid from the Sardinian wine press based upon a) three product ions from *m/z* 133 and b) retention time (26.84).

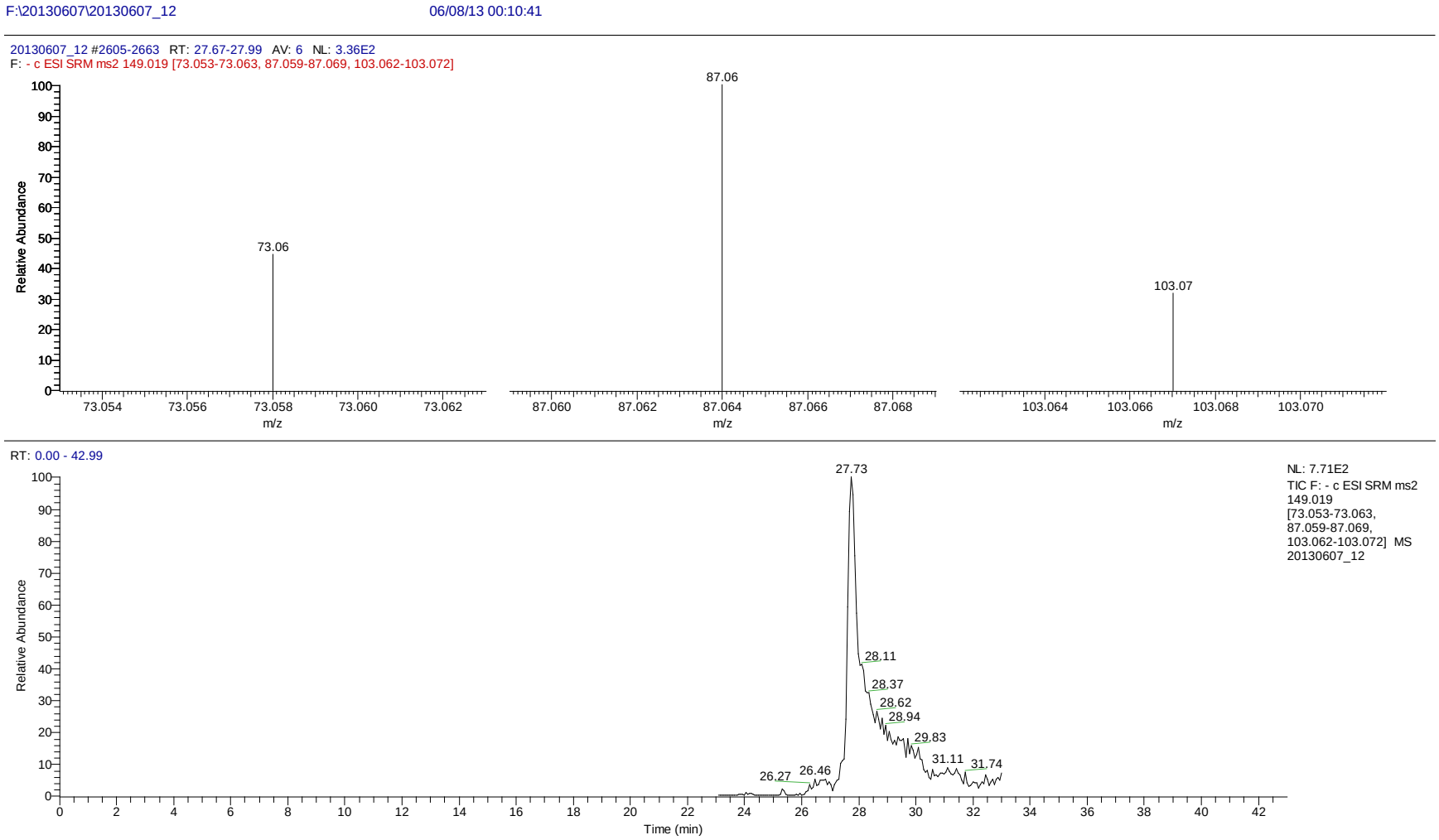


Figure 5.12. The identification of tartaric acid from the Sardinian wine press based upon a) three product ions from *m/z* 149 and b) retention time (27.73).

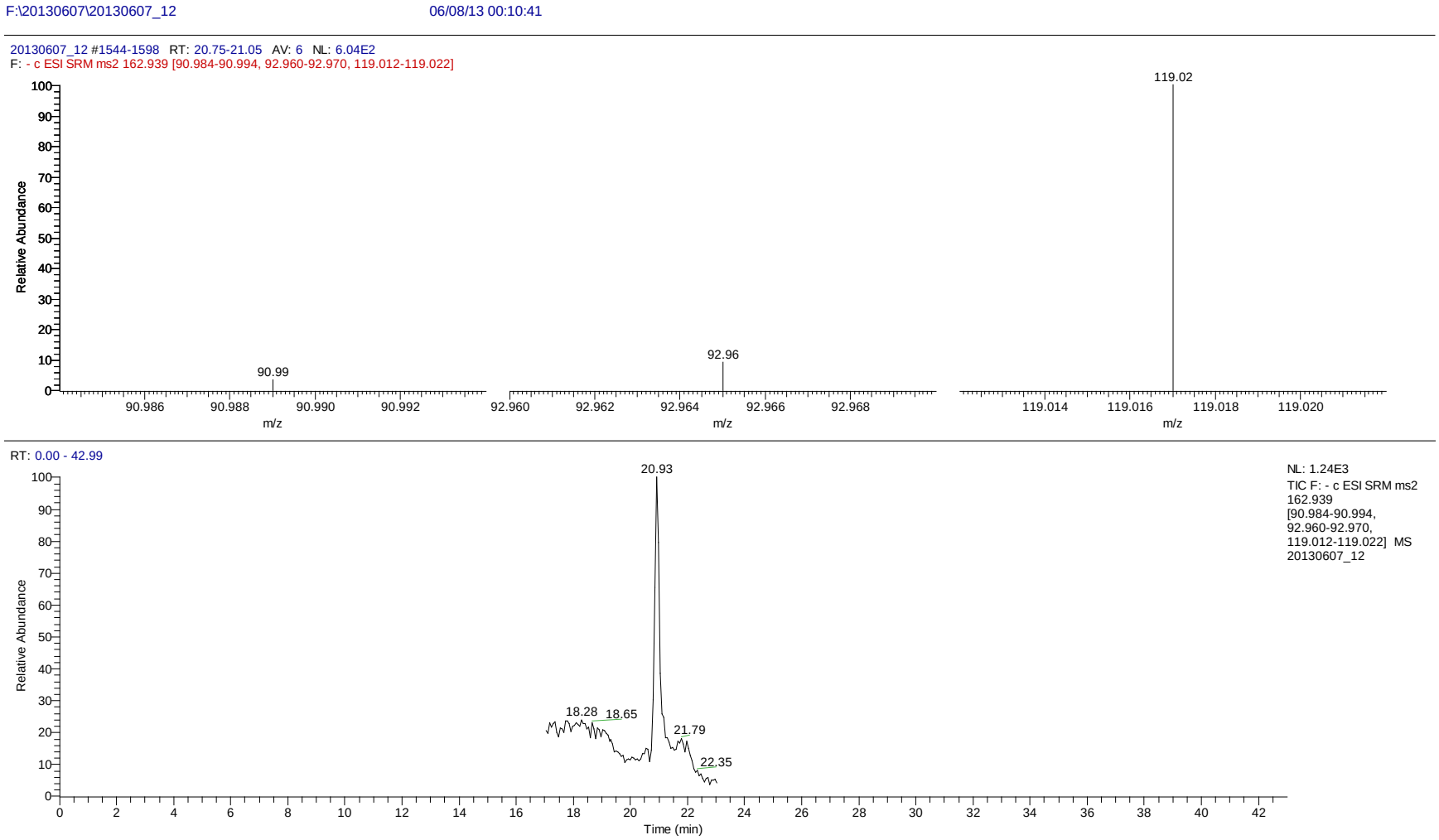


Figure 5.13. The identification of p-coumaric acid from the Sardinian wine press based upon a) three product ions from *m/z* 163 and b) retention time (20.93).

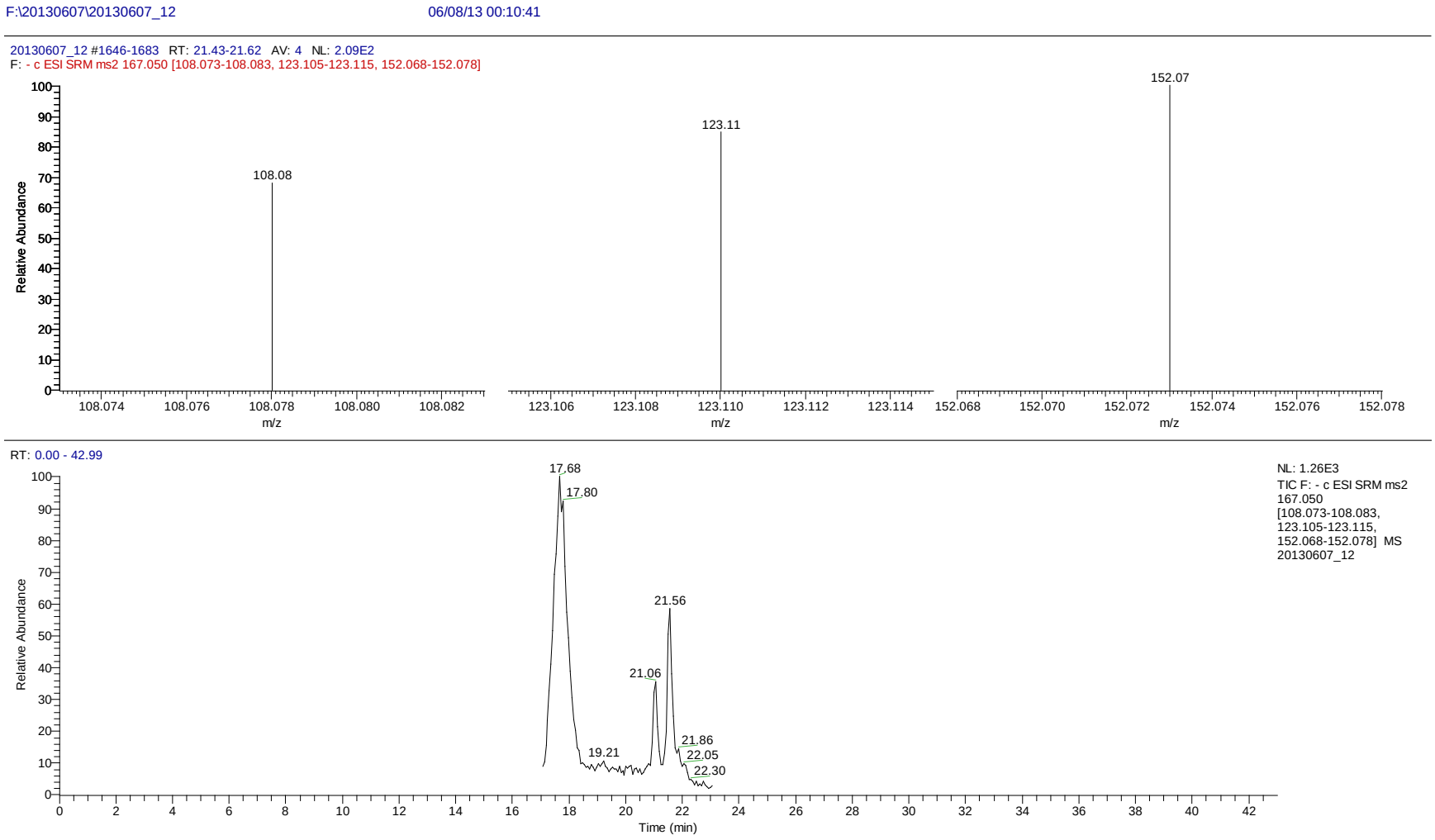


Figure 5.14. The identification of vanillic acid from the Sardinian wine press based upon a) three product ions from *m/z* 167 and b) retention time (21.56).

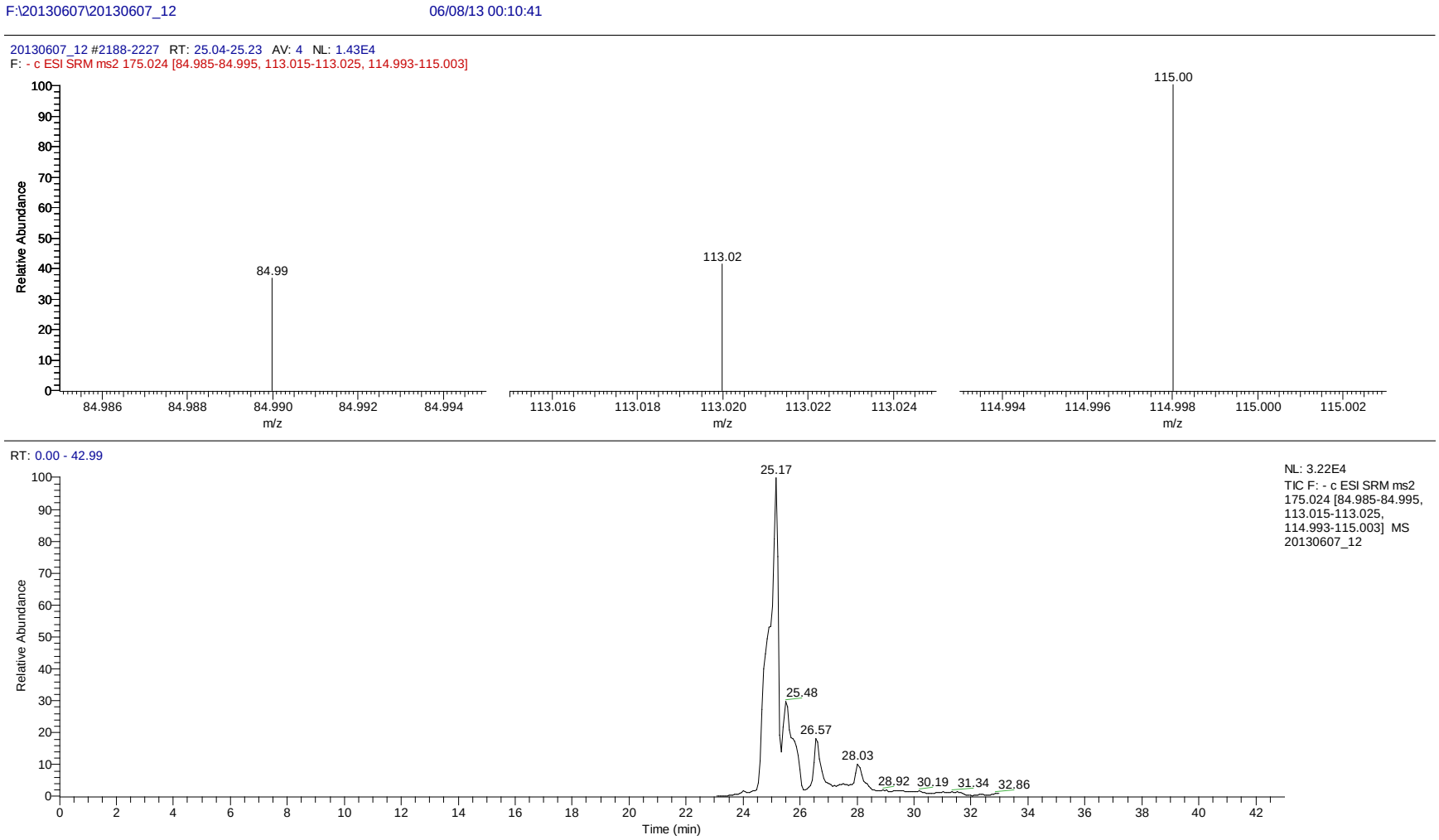


Figure 5.15. The identification of isopropyl malic acid from the Sardinian wine press based upon a) three product ions from *m/z* 175 and b) retention time (25.17).

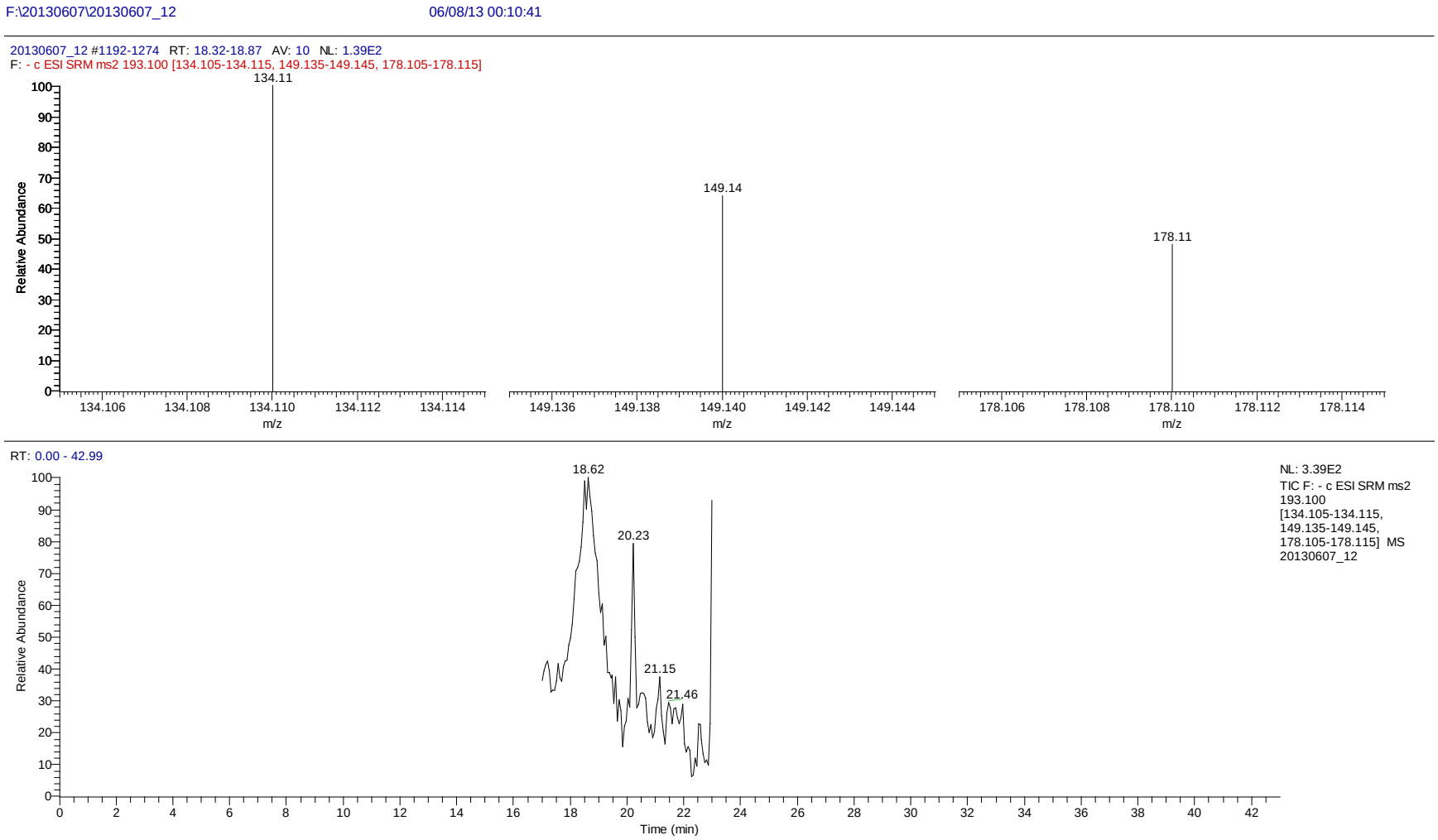


Figure 5.16. The identification of ferulic acid from the Sardinian wine press based upon a) three product ions from *m/z* 193 and b) retention time (18.62).

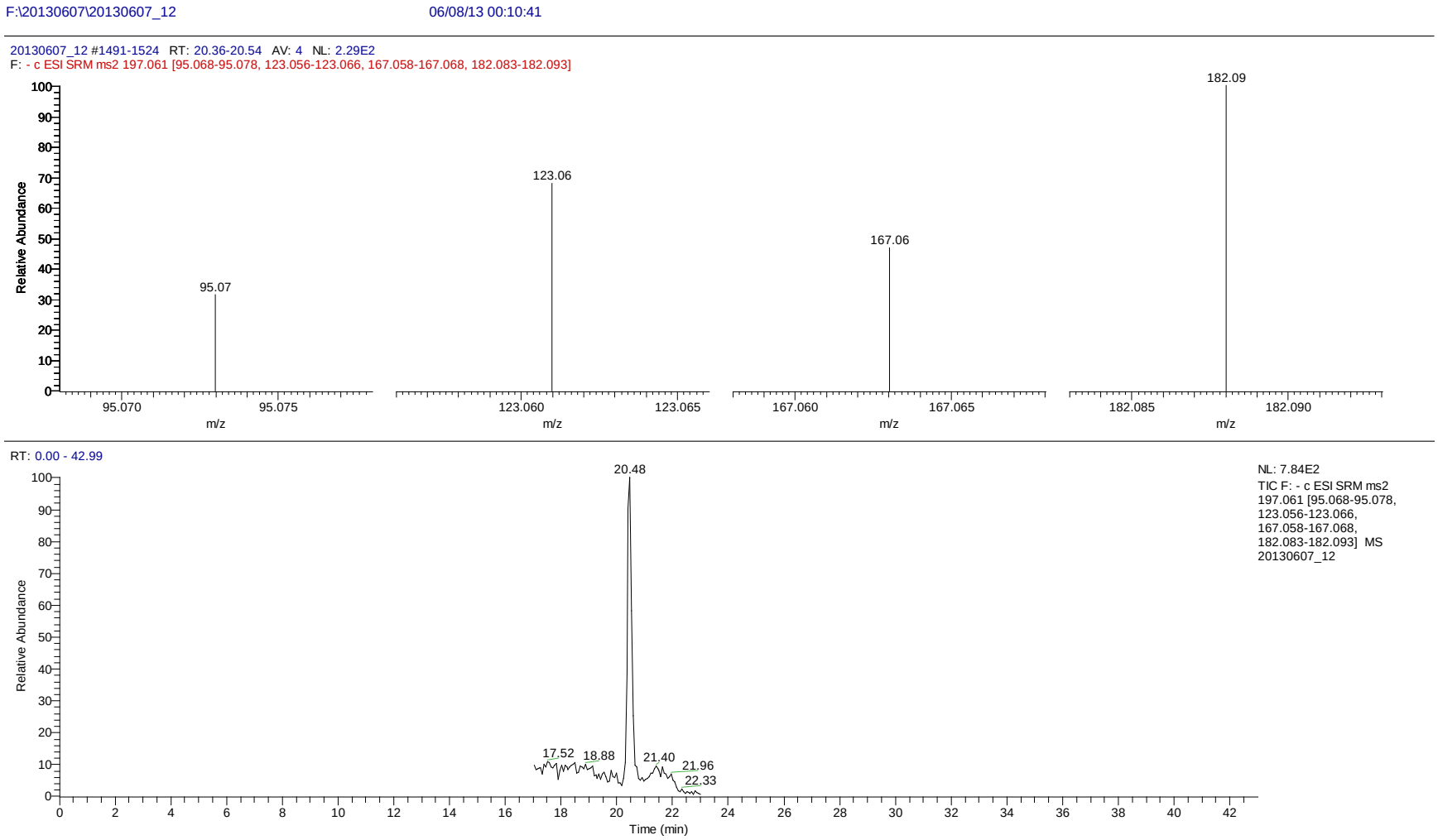


Figure 5.17. The identification of syringic acid from the Sardinian wine press based upon a) four product ions from *m/z* 197 and b) retention time (20.48).

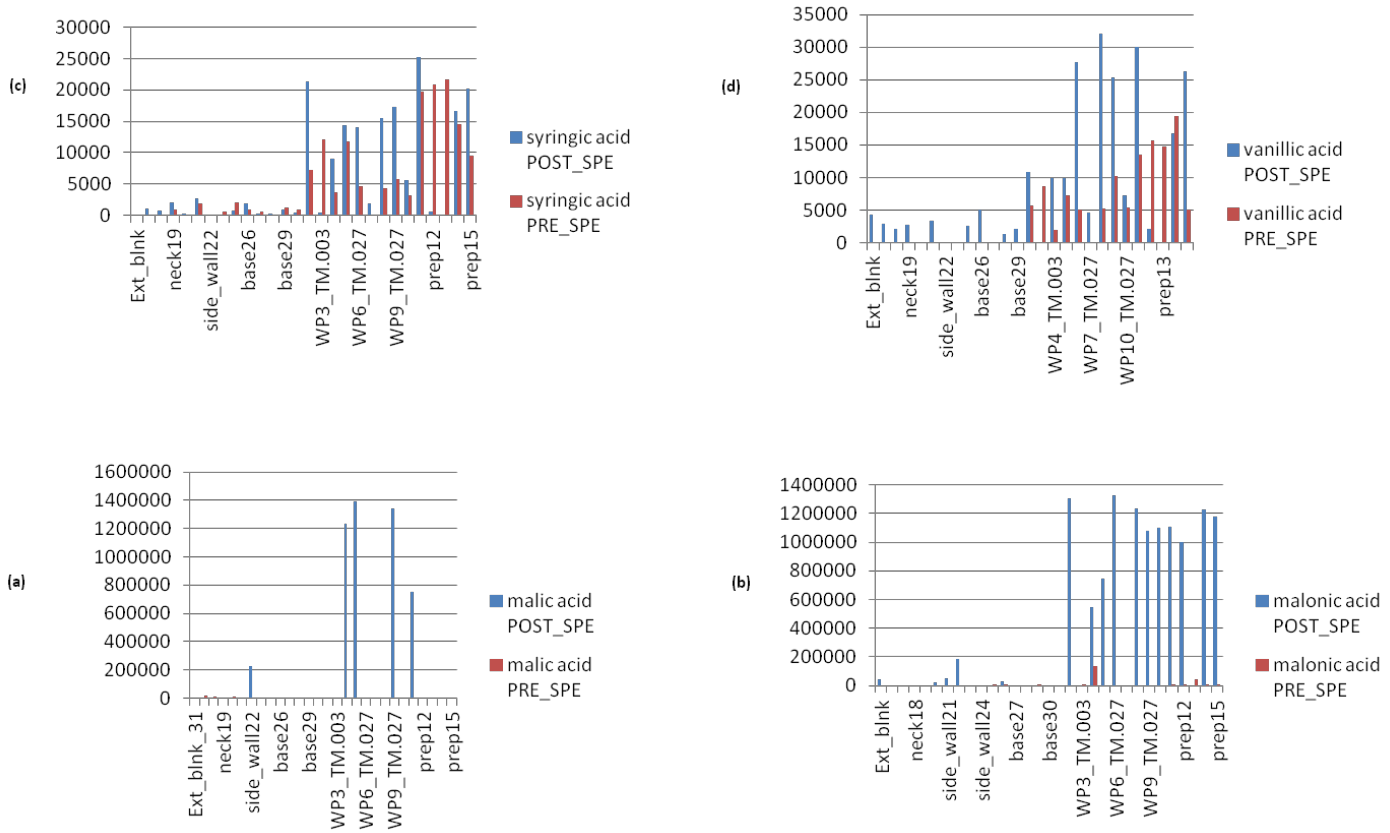


Figure 4.18. The graphs compare the absolute intensities from the LC results before and after SPE clean up on four acids: (a) malic acid (b) malonic acid (c) syringic acid and (d) vanillic acid, without SPE clean up and with SPE clean up. Overall, the absolute intensities are greater in the post SPE sample run. The phenolic acids, syringic and vanillic, are less affected by the SPE step.

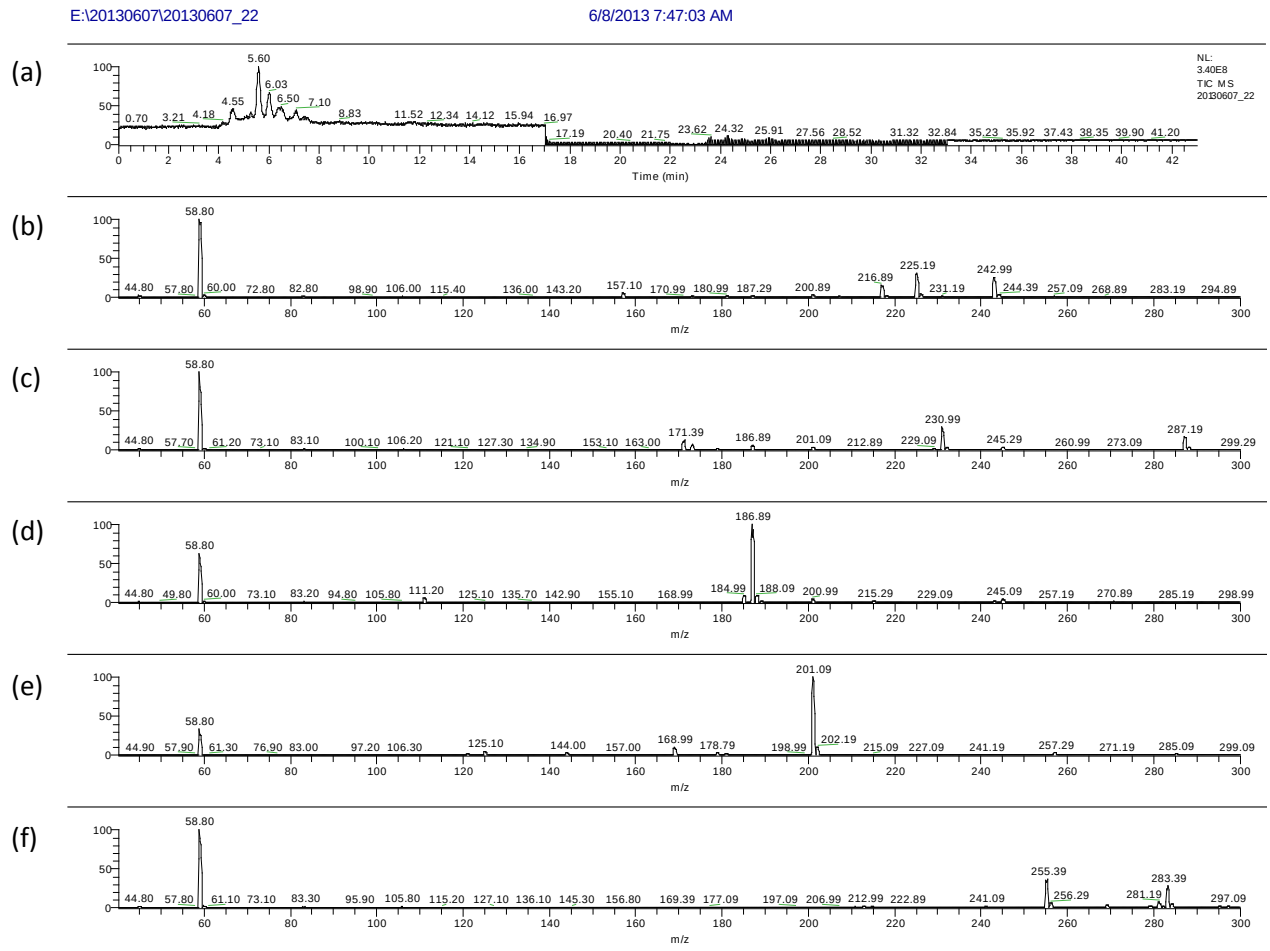


Figure 5.19. A representative total ion chromatogram from the LC-MS/MS analysis of the wine press and sherds taken from Trunce Molas, Sardinia, Italy. Early eluting peaks from 4.55-7.10 (a) represent insoluble lipid material released from the alkaline fusion. Tentative identifications include (masses rounded up to the nearest integer): 255: palmitic acid, 283: stearic acid, 201: sebacic acid, 187: azelaic acid, 287: 3,12-dihydroxy-hexadecanoic acid, 217: 2-hydroxydecandioc acid, and 243: 3-methyl-dodecanedioic acid.

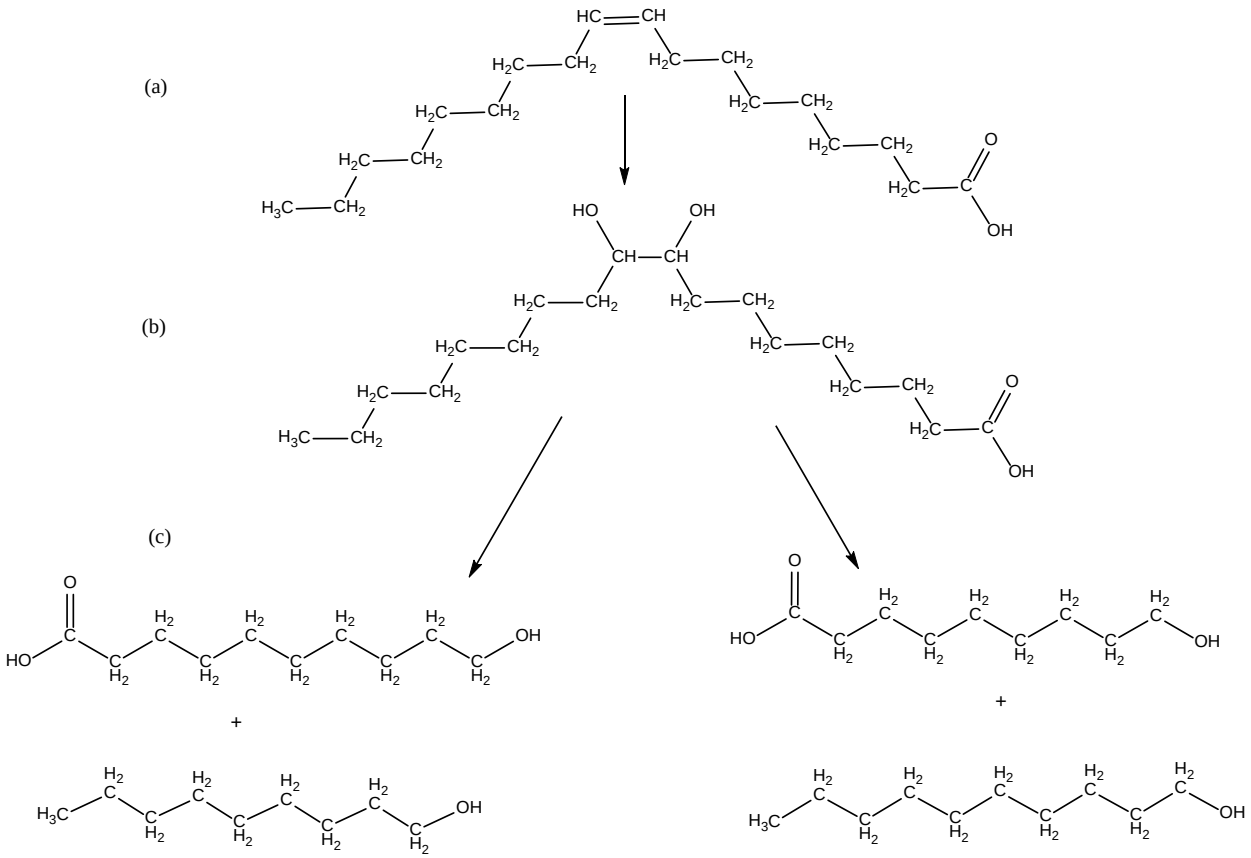


Figure 5.20. Illustration of suggested formation of unsaturated acids from (a) 9-octadecenoic acid (b) oxidation at the site of the double bond and (c) dehydration, reproduced from Regert et al., 1998.

5.2.2.1.3 Multivariate Analysis of Archaeological Samples

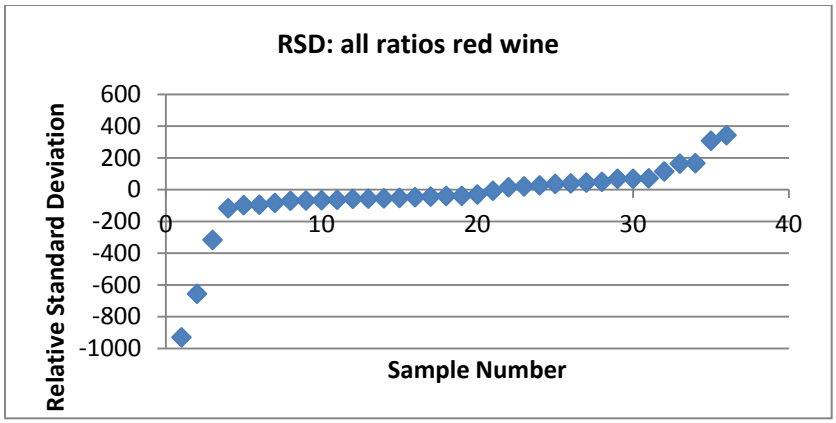
Utilising the multiple biomarkers identified in the wine press and its preparatory layer offers a chance to categorise the results as red wine or white wine by comparing ratios of the intensities of all nine acids identified in the samples. Ratios were logged (base 10) and the results are tabulated in Appendix, Chapter 5, Table 3. PCA was chosen as an unsupervised method of comparing the archaeological results with standard laboratory aged results. In order to have at least n=10 classification for the standard samples, ratios were prepared from the DIMS samples (alkaline fusion aliquot) from Chapter 3. Due to the inherent uncertainties in comparing two analytical platforms (high resolution DIMS with LC-triple quad analysis), several standard sherds were also analysed by LC-MS/MS as 'validation' samples. A relative standard deviation was calculated for the logged ratios from DIMS and LC-MS/MS of the standards only, in order to determine how variable the results were between the two platforms. An example is seen in Table 5.5.

Figure 5.21 illustrate the graphs of the RSD calculated from the logged ratios of red and white wine standard sherds analysed by two separate analytical platforms. 200 was identified as the cutoff point, based upon the start of a 'kneebend' in the graph, where the calculated RSD veers from the group. Only those ratios with the 200% cutoff were chosen: originally there were 36 ratios, 7 were removed, for a final total of 29 ratios used for classification.

Table 5.5 The table illustrates four representative logged ratios only from acids 103 (malonic), 117 (succinic), 133 (malic), and 149 (tartaric). Ten DIMS samples of the alkaline fusion aliquot of red wine were compared with 3 samples of the lab aged sherds analysed by LC-MS/MS. The standard deviation, mean, and relative standard deviation were calculated for the 13 samples, for all ratios examined.

		103:117	103:133	117:133	103:149
DIMS	**RG3A**	-1.47106	-1.50041	-2.5077	-1.48006
	RS3B	-1.45267	-1.38201	-2.03877	-1.85403
	RS2B	-1.5742	-1.69206	-2.43994	-2.10307
	RG4A	-1.28959	-1.46066	-2.25733	-1.57074
	RG1A	-1.84215	-1.642	-2.06484	-2.02399
	RS1A	-1.70278	-1.54046	-1.79581	-1.50232
	RS4A	-1.43297	-1.4787	-1.88438	-1.52619
	RS2A	-1.39828	-1.53215	-2.48595	-1.39064
	RS4B	-1.54929	-1.58637	-2.16048	-1.69141
	RS3A	-1.45338	-1.4845	-2.05726	-1.66818
LC-MS/MS	**red 1, 1:10**	-0.44752	-0.63316	-0.18565	-0.46627
	red 2, 1:10	-0.45113	-0.58501	-0.13388	-0.44493
	red 3, 1:10	-0.38493	-0.67971	-0.29478	-0.64963
	SD	0.497168	0.401757	0.888646	0.551778
	MEAN	-1.26538	-1.32286	-1.71591	-1.41319
	RSD	-39.29	-30.3703	-51.7887	-39.0449

(a)



(b)

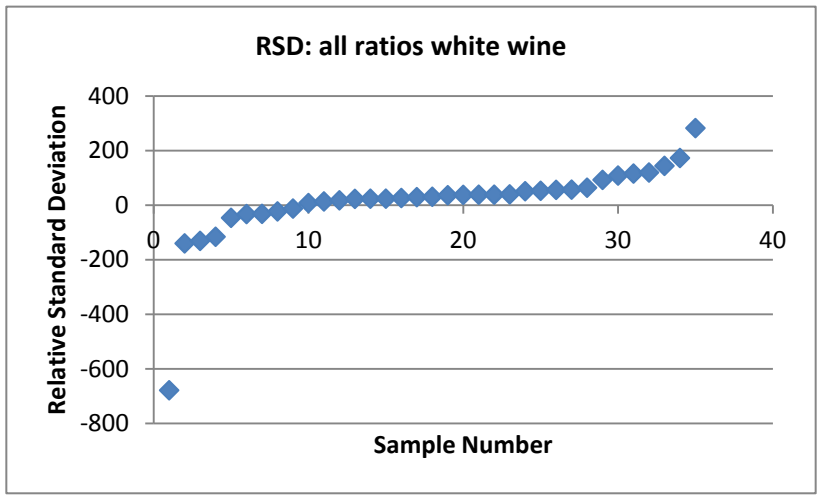


Figure 5.21. The relative standard deviation of the acids' logged ratios for the laboratory sherds (a) red and (b) white. 200 was chosen as the cutoff point for both red and white, based upon the start of a 'kneebend' in the graph at which point the ratios deviate from the group.

In preparation for multivariate analysis, all missing values in the wine press and the preparatory layer samples were assigned a value equal to half of the lowest intensity for each metabolite. This approach was applied to all acids. In the case of malonic, vanillic, and succinic acids, the missing values were half of the value in the extraction blank. Figure 5.22 represents a plot of the samples vs PC2 scores of laboratory aged sherds analysed by DIMS and LC/MS/MS, as well as the results from the wine press analysed by LC-MS/MS only. Preparatory layer 2 and 3, and wine press sample 3 were removed from this classification due to the lack of original features. The greatest separation along PC1 (60.57%) separated the samples based upon the analytical platform, DIMS vs. LC-MS/MS. The second greatest variation was identified by PC2 between the white DIMS and the red DIMS, with a smaller variation between the white and red LC samples. The archaeological samples from the wine press and the preparatory layer are most closely aligned with the white LC samples. A PCA scores test identified significant differences between all groups except the white LC and the archaeological samples; there was no significant difference between these two groups along PC2.

The multivariate analysis of the logged ratios of 9 acids suggests the biomarker fingerprint of the archaeological samples are more similar to laboratory aged white wine than to red wine. This very preliminary data suggests an application for classifying samples according to a suite of biomarkers, as opposed to a single marker as reported previously. The results of this classification support the archaeological descriptions of the wine press as a lower basin of a two tier wine press where the upper portion did not survive. Therefore, treading occurred on the upper platform. Whether the original grapes were red or white, the skins/seeds were not transferred to the lower vat, only the extracted juice resulting in a lower concentration of phenolic material from the grape must.

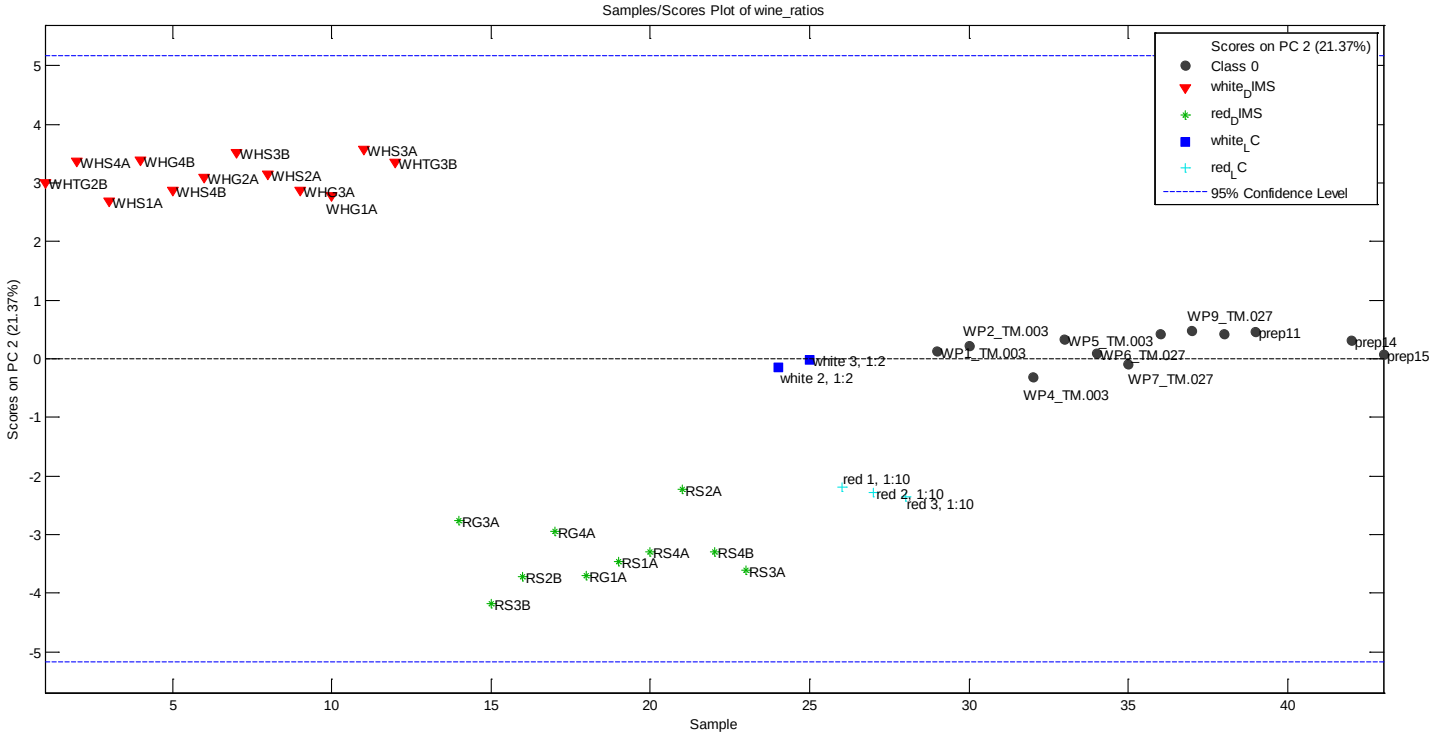


Figure 5.22. A plot of sample vs. PC2 scores taken from a PCA scores plot of laboratory aged sherds analysed by DIMS and LC/MS/MS, and an archaeological wine press analysed by LC/MS/MS. The plot aligns the archaeological samples more closely with LC-MS/MS analysis of laboratory aged white wine.

5.4 Conclusions

Taken together with Chapter 3 and 4, these results demonstrate for the first time the application of archaeological metabolomics to discover a suite of organic acid biomarkers and then their successful transfer to a targeted analytical method in an attempt to identify polymerised wine residues found in archaeological samples. The observations that the majority of the biomarkers were identified in the wine press and its preparatory layer strongly suggests the presence of an aged, polymerised wine consistent with the archaeological context. When compared with the biomarker fingerprint of the modern, laboratory aged wine sherds it was shown that the identified acids found in the wine press more closely aligned with white wine, which can be explained by the object's identification as a lowest tier basin. The presence of wine was not confirmed in the amphorae sherds. This result suggests that the amphorae may never have held wine and were instead at the site in preparation for filling and transport. The identity of 1 or 2 phenolic acids within several of the sherds highlights the fact that the use of 1 or 2 biomarkers for the identification of archaeological residue leads to spurious results and may ultimately produce incorrect conclusions about the objects usage within the site.

Also noted in this chapter was the detrimental effect of the matrix interference on several of the acids. The usage of alkaline solution with aluminum-silicate matrix (pottery) will ultimately leach out aluminum hydroxide. Upon acidification, it is hypothesised that the newly formed aluminate ion complexes with acids containing 3 or more hydroxyl groups, like malic acid and tartaric acid. The additional clean up step to remove the aluminate ion greatly increased the response of all acids, particularly the organic acids.

In order to characterise the remnants from an archaeological sample, it is critical to use multiple biomarkers for a responsible identification since an attempt to analyse liquid or food remnants in containers that were stored in the soil and were most likely reused multiple times results in a level of uncertainty. In the next chapter, these biomarkers will be applied to archaeological objects with considerable interference from food remnants.

6. Pilot Study: The Metabolomics Approach Applied to Archaeological Samples from Vindolanda, Northumberland, England

In Chapter 3 of this thesis, laboratory aged sherds doped with water and wine were aged in different environments in order to determine a biomarker signature for aged wine, and then apply those biomarkers to a targeted analysis of archaeological artifacts. In order to test the applicability of the identified biomarker list, two contrasting archaeological sites were chosen. Chapter 5 applied the targeted analysis of the biomarker list to a dry, sandy site at a Punic farmstead in Sardinia, Italy. Analysis of the winepress was highly indicative of wine remnants, whereas the application of the biomarker list to a group of amphora sherds was indeterminate. The second archaeological site chosen to test the biomarker list was Vindolanda, a Roman British garrison active from circa 85- 400 A.D.

6.1 Introduction

The environment in the Vindolanda settlement is a wet, anoxic environment with a heavy peat, organic rich soil. The site consists of transport amphorae, mortaria, and cooking pots from both military and civilian encampments. From the objects gathered in the early summer of 2012, two sample sets were analysed. Initially, a sample set consisting of transport amphorae were analysed for the remnants of wine utilising the targeted method developed in Chapter 4 and refined in Chapter 5. A second sample set was then analysed which included cooking pots and mortaria in order to identify the remnants of wine or vinegar used in food preparation. Since olive oil was a common ingredient in food preparation, several non-polar aliquots were also examined for the

presence of TAGs (Sheehan-Finn, 2012). Since the objects examined in this chapter represent multiple locations, the specific context information is given with each sample set listed in section 6.2 and for the remaining samples in the Appendix, Chapter 6.

Thirty two samples were extracted and analysed; the results of sixteen of those samples, as well as three soil samples are given here in the following sections. The remaining sample results are given in the Appendix, Chapter 6, Tables 5-14. Eight contexts represent area B, a civilian encampment active from 213-300A.D., and one context represents area A, a military fort originally constructed in 213 A.D., rebuilt in the 4th century. Samples were extracted using the method described in Chapter 2.2.3. The alkaline fusion aliquot, and in certain cases the non-polar aliquot, were analysed. The alkaline fusion aliquot was analysed by the targeted LC-MS/MS method developed in Chapter 4; the non-polar aliquot was analysed by a data dependent fragmentation method explained in Chapter 2.2.5.3.

The samples represent mortaria, amphorae, and cook pots that were excavated from several contexts within specific areas of the Vindolanda settlement by volunteers working under the direction of staff archaeologists from the Vindolanda Trust. Soil samples were also collected by volunteer excavators on the same day; one from a known context and one sample from an unknown context. In order to determine the presence or absence of wine in these samples, nine acids were targeted in the LC-MS/MS analysis. The nine acids chosen were those acids which were identified in the wine press from Chapter 5 and are considered highly indicative for the presence of wine: malic, iso-propyl malic, tartaric, succinic, vanillic, ferulic, p-coumaric, syringic, and malonic acids.

Samples were designated as in the following example: V12-30B/4, whereby V12 represents the place and year of excavation, Vindolanda, 2012, 30B represents the context, and /4 represents sample 4 taken from that particular context.

6.1.1 Targeted LC-MS/MS Analysis for Wine Residue

Prior to analysis by LC-MS/MS, the extracted samples were removed from the -80^{o}C freezer and reconstituted in 30 µl of mobile phase 90% ACN/10% 100mM ammonium acetate, pH = 8.2. The samples were vortexed for 10 s and then centrifuged for 20 min at 10^{o}C at 6000 rpm. Ten microlitres of each sample were added to a 96 well plate in a randomised order and 1 µl of sample was injected onto the column for analysis.

Samples were analysed in two batches over several days. The first day of sample runs experienced a substantial increase in column pressure which necessitated halting the run and changing the inline filter frit. The increased pressure shifted the retention times for several of the acids, as well as resulted in a drop in intensity for those acids. The samples that were affected are listed in the Appendix, Chapter 6, Tables 5-14 and their results are considered suspect; therefore, those samples were not used for the results of Chapter 6. As noted in Chapter 5, the elution time between 4-7 min yielded 'bound' lipids released after alkaline fusion. Putative identifications are given in the tables below based upon the integer mass and comparison with literature sources. For absolute confirmation of the lipids identity, a more targeted approach is necessary.

6.1.2 TAG analysis

As stated in the introduction, TAGs are lipid fractions composed of three fatty acyl chains esterified to a glycerol backbone. When known, the position of the acyl chains are designated from top to bottom, based on a Fisher projection as sn-1, sn-2, and sn-3, where sn= stereospecifically numbered; TAGs are identified by their acyl chains followed by the glycerol designation, triacyl-sn-glycerol, and are written as $C_{(\text{total number of acyl carbons: total number of double bonds})}$ (IUPAC-IUB Commission on Biochemical Nomenclature, 1977).

As described in Chapter 1, determination of TAG content in archaeological artifacts is useful to determine the origins of the lipid, e.g., plant vs. animal, ruminant vs. non-ruminant, ruminant dairy vs. ruminant adipose fat and is often undertaken with high temperature-gas chromatography-mass spectrometry (Evershed et al., 2002; Regert, 2011). In some cases, the identification of the acyl chain locations on the glycerol backbone will assist in the identification (Mottram et al., 1997; Lin and Arcinas, 2008). For samples analysed in this chapter, all samples were reconstituted in a lithium salt solution. The lithium cation-oxygen bond is stronger than that of other cations including sodium, potassium, as well as ammonium adducts, producing intense signals in the mass spectrometer down to the MS^3 fragmentation pattern (Adams and Gross, 1986). Analysis of lithiated TAGs has been successfully applied to the study of archaeological artifacts in earlier studies (Garnier et al., 2009; Mirabaud et al., 2007).

The approach discussed here combines the mass spectral fragmentation data with an algorithm written in the Python environment by Dr. Ralf Weber, University of Birmingham, which identified the acyl chains on a particular TAG. Briefly, using collisionally induced dissociation, samples were fragmented from 600-1000 *m/z* in a data dependent manner. The most intense ion from an initial

full scan is fragmented; the top three most intense ions from the resultant MS/MS fragmentation are then fragmented. In order to remove noise peaks and limit the number of falsely detected peaks, necessary criteria were added to the algorithm including: coverage within a certain number of scans (60%), ppm mass difference between the experimental mass and the theoretical mass stored within the Viant laboratory's empirical formulae library (3 ppm for FT-ICR-MS, 60 ppm for IT), and RSD of peak intensities across the different scans (<30%). The results of a data dependent collection were compared against a library of fatty acids determined from the expected fragmentation losses based on the published mechanism illustrated in the Appendix, Chapter 6, Figures 1 and 2 (Hsu and Turk, 2010).

As an example, Figure 6.1 represents the full scan mode of a lithiated TAG; the largest peak in the spectrum (*m/z* 867) was then fragmented. Figure 6.2 illustrates the MS^2 spectrum, the three resultant diacylglycerols (DAG) resulting from the fragmentation of *m/z* 867. There are two peaks associated with each DAG, one representing a loss of the neutral fatty acid from the original lithiated TAG, and the second peak resulting from the loss of the lithiated salt of the same neutral fatty acid. Figure 6.3 illustrates the MS^3 level of fragmentation of the DAG at *m/z* 611. The predominant peak at *m/z* 329 represents the loss of 18:0 as an α, β-unsaturated fatty acid. Its associated peak is *m/z* 289, the lithiated 18:1 acid; the two peaks are separated by a mass of 40, the dehydrated glycerol backbone.

In Hsu and Turk's 2010 paper, the fragmentation of *m/z* 867 yielded a peak at *m/z* 585 that was considerably smaller in intensity than either peak at *m/z* 611 or *m/z* 583. The rationale for the lower intensity was that the peak at *m/z* 585 was due to the loss of the fatty acyl chain at sn-2, the sterically hindered and energetically unfavorable position. Also, it was theorised that the highly labile α-hydrogen on the sn-2 acyl chain functions to easily eliminate either the sn-1 or the sn-3 acyl

chain. However, under the experimental conditions for this thesis, the majority of the MS^2 peaks are relatively similar in intensity, offering no obvious distinction between the acyl chain position on the glycerol backbone. The reason may be due to the multiple isomers formed within these TAGs, or the equal lability of any of the three α-hydrogens found on the three acyl chains.

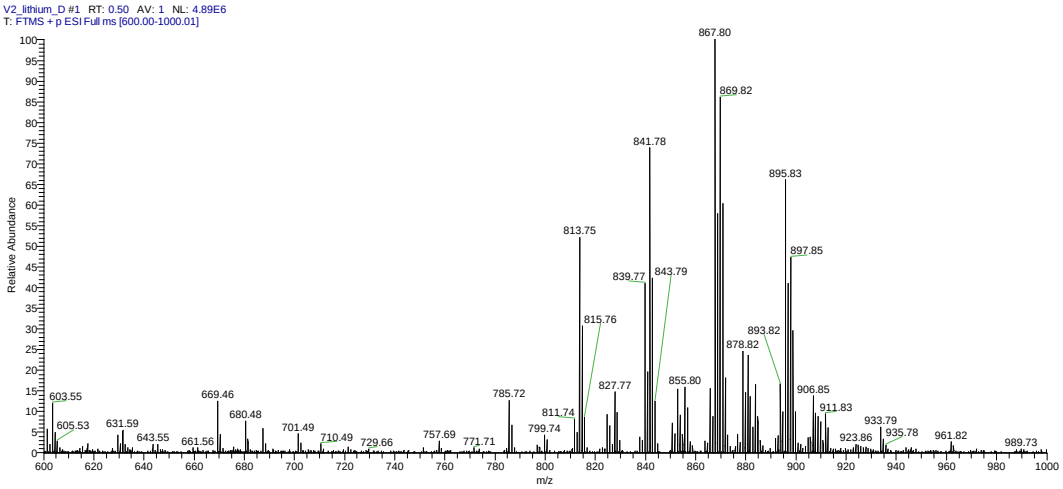


Figure 6.1. Direct infusion FT-ICR-MS of an archaeological sample, non-polar extract reconstituted in 2mM LiCl (1:4 CHCl3:MeOH).

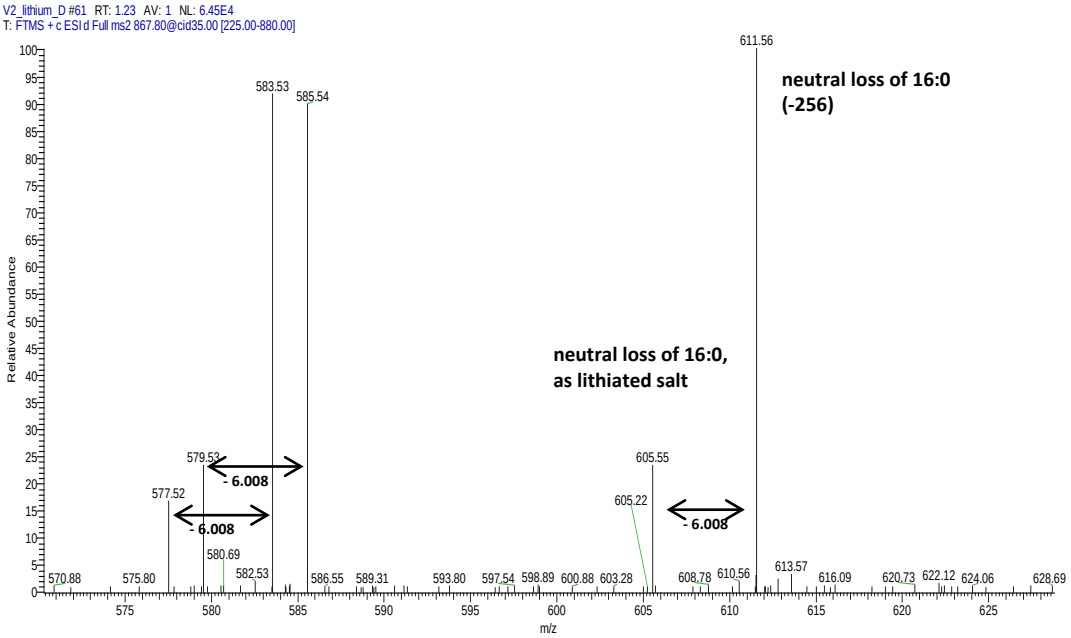


Figure 6.2. Direct infusion FT-ICR-MS^2 from the fragmentation of *m/z* 867. The results identify the loss of three fatty acids: 16:0 (*m/z* 611), 18:0 (*m/z* 583), and 18:1 (*m/z* 585).

867.8; MS2			
m/z	Intensity	Relative	explanation
611.55858	64517.6	100	neutral loss of FA (16:0)
583.52726	59139.9	91.66	neutral loss of FA (18:0)
585.54299	57895.9	89.74	neutral loss of FA (18:1)
605.55042	15031.8	23.3	neutral loss of FA (16:0); lithiated salt
579.53487	14971.2	23.2	neutral loss of FA (18:1); lithiated salt
577.51917	10770.7	16.69	neutral loss of FA (18:0); lithiated salt

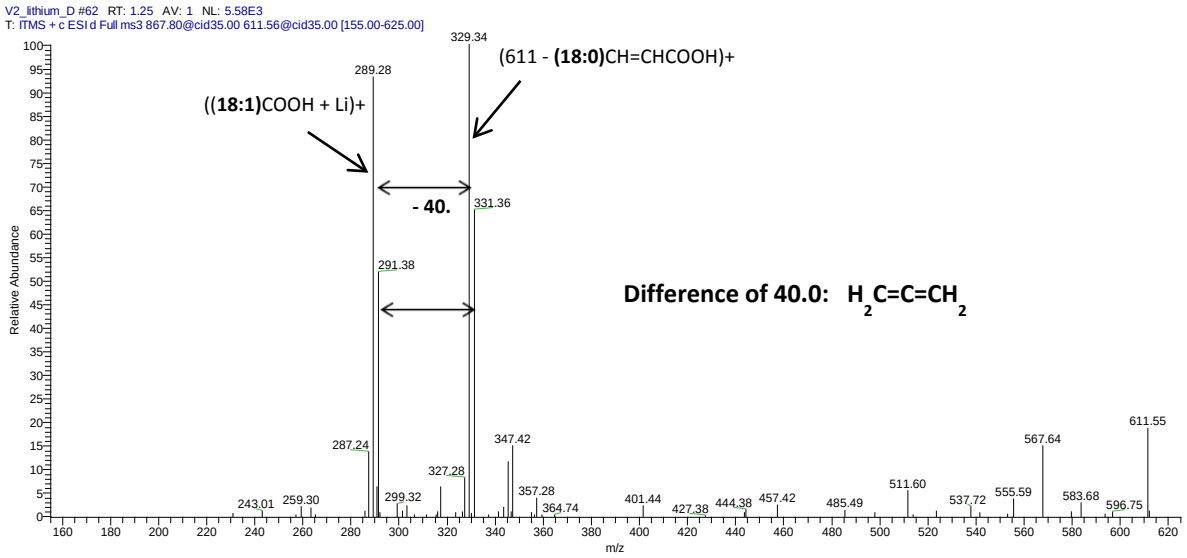


Figure 6.3. Direct infusion IT-MS^3 from the fragmentation of *m/z* 611, the two resulting fatty acyl chains are separated by 40, the dehydrated glycerol backbone.

611.5; MS3			
m/z	**Intensity**	**Relative**	**explanation**
329.33557	5577.5	100	loss of α, ß unsaturated 18:0
289.28076	5197.1	93.18	lithiated 18:1 associated with mz 329
331.35944	3625.1	64.99	loss of α, ß unsaturated 18:1
291.37537	2889.3	51.8	lithiated 18:0 associated with mz 331
611.55225	1040.3	18.65	base peak
347.41809	829.2	14.87	loss of the 18:1 as a ketene
287.24274	757.6	13.58	lithiated 18:2
345.30127	642.9	11.53	loss of the 18:0 as a ketene
327.27698	453.9	8.14	loss of 18:0 as fatty acid

6.1.2.1 TAG algorithm output

Table 6.1 illustrates the raw output from the algorithm. The TAG output includes the neutral fatty acid loss from MS^2, as well as the α, β-unsaturated fatty acid and the lithiated fatty acid losses from MS^3 that are linked by a difference in mass of 40 Da. Illustrated in Table 6.1, row 1 represents the neutral loss from the full scan to level 2, MS^2 ; the fatty acyl component was identified as 16:0, such as palmitic acid. Row 2 identified the loss from MS^2 to MS^3; the fatty acyl component was identified as 18:1, such as oleic acid. Row 3 links the *m/z* from row 2 (331) with a *m/z* separated by -40 (291). Row 4 identifies the third fatty acyl component (291) as 18:0, such as stearic acid.

Table 6.1 The TAG algorithm output from the data dependent scans identifying a neutral loss at MS^2 (level 2), as well as the α, β-unsaturated fatty acid and the lithiated fatty acid from MS^3.

m/z	m/z	Spectrum Header	Fatty acyl identification
867.7994493	611.5578521	['FTMS + p ESI Full ms [600.00-1000.00]', 'FTMS + c ESI d Full ms2 867.80@cid35.00 [225.00-880.00]']	[256.2402304, 'FA(16:0)', 'e', '1to2']
611.5578521	331.3710066	['FTMS + p ESI Full ms [600.00-1000.00]', 'FTMS + c ESI d Full ms2 867.80@cid35.00 [225.00-880.00]']	[280.2413285, 'FA(18:1)-2H', 'e', '2to3']
331.3710066	291.2958766	['FTMS + p ESI Full ms [600.00-1000.00]', 'FTMS + c ESI d Full ms2 867.80@cid35.00 [225.00-880.00]']	[40.0, 'glycerol-2[H2O]', 'i', 3]
	291.2958766	['ITMS + c ESI d Full ms3 867.80@cid35.00 611.56@cid35.00 [155.00-625.00]']	[291.2869869, '[FA(18:0)+Li]+', 'p', 3]

6.1.2.2 TAG analysis applied to aged lipid samples

Based upon literature sources for foodstuffs in Roman Britain several modern lipid samples were initially aged by sonicating a 6 g modern laboratory sherd in 50 ml of chloroform with 1 g of fat for 2 x 30 min; the fats examined were: goat cheese, sheep cheese, cows milk, olive oil, sardines, back bacon, and plain sherds (Dudd et al., 1998). The sherds were allowed to air dry and were then weighed over four days until the weight was maintained over a 24 hour period. On the fourth day, four sherds for each fat sample, plus four plain sherds were placed in chloroform rinsed glass vials and stored in an oven for three months at 40°C. Four sherds of each fat plus plain were wrapped in aluminum foil and stored at -80°C until analysis, as zero month time point. After three months, the sherds were removed from the oven, wrapped in foil and stored in the -80°C freezer until analysis. All samples were extracted using the extraction protocol described in Chapter 2.2.1 and analysed by the method described in Chapter 2.2.5.2. The results of the ruminant dairy samples are given in the Appendix, Chapter 6 tables 1, 2, and 3.

6.1.3 Foodstuff Analyses

In order to examine certain foodstuffs that may be rich in phenolic content and therefore overlap with the chosen wine biomarker list, seven foodstuffs common to Roman Britain were aged for 6 months at 40°C in preparation for analysis. Samples were extracted and analysed as described in Chapter 2 for laboratory aged standards analysed by DIMS-FT-ICR-MS. For data pre-processing, the collected mass spectra were each 'binned' into windows of 100 *m/z*, whereby the edges of each window were 'stitched' together. The signal to noise ratio was set at 100; replicate filtering was set at 12%. Missing values were imputed using an in-house code written in the Matlab environment, MVImpute version05 (Hrydziuszko and Viant, 2011). The final data matrix was normalised and then

g-log transformed in order to stabilise the technical variation across all peaks measured (Parsons et al., 2007).

Figure 6.4 illustrates the PCA scores plot of the alkaline aliquots of seven foodstuffs. The greatest separation is along PC1 between the (adulterated) olive oil and the remaining classes. There was a small separation between the spices (oregano, thyme, and basil) and honey, barley malt and spelt wheat. Table 4 in the Appendix, Chapter 6, lists the top 200 loadings of the samples, sorted by PC1 values. Three of the top loadings, 1, 7, and 13, were putatively identified as 2,3-dihydrobenzoic acid, syringic acid, and p-coumaric acid, respectively. Looking at each class individually, the *m/z* at 197.0455, putatively identified as syringic acid, is one of the most intense peaks in the oregano and in the basil samples. The *m/z* 153.0192 (putatively identified as 2,3-dihydrobenzoic acid) is also one of the most intense peaks in the mass spectra of all three spices. This suggests that the phenolic acid loadings are due in part to the contribution of the three classes of dried spices: basil, thyme, and oregano. These results also align with the results published on the phenolic acid content of spices determined after chemical hydrolysis (Herrmann and Nagel, 1989). Comparing these results to the results from the following archaeological contexts may suggest a secondary origin for phenolic acids, such as those found in the mortaria samples.

This small experiment highlights the commonplace nature of certain phenolic acids. For example, syringic acid, commonly used as the identifying compound for the remnants of red wine is also found in the alkaline fusion of common foodstuffs. Again, this highlights the dangers of utilising only 1 or 2 biomarkers to identify ancient wine.

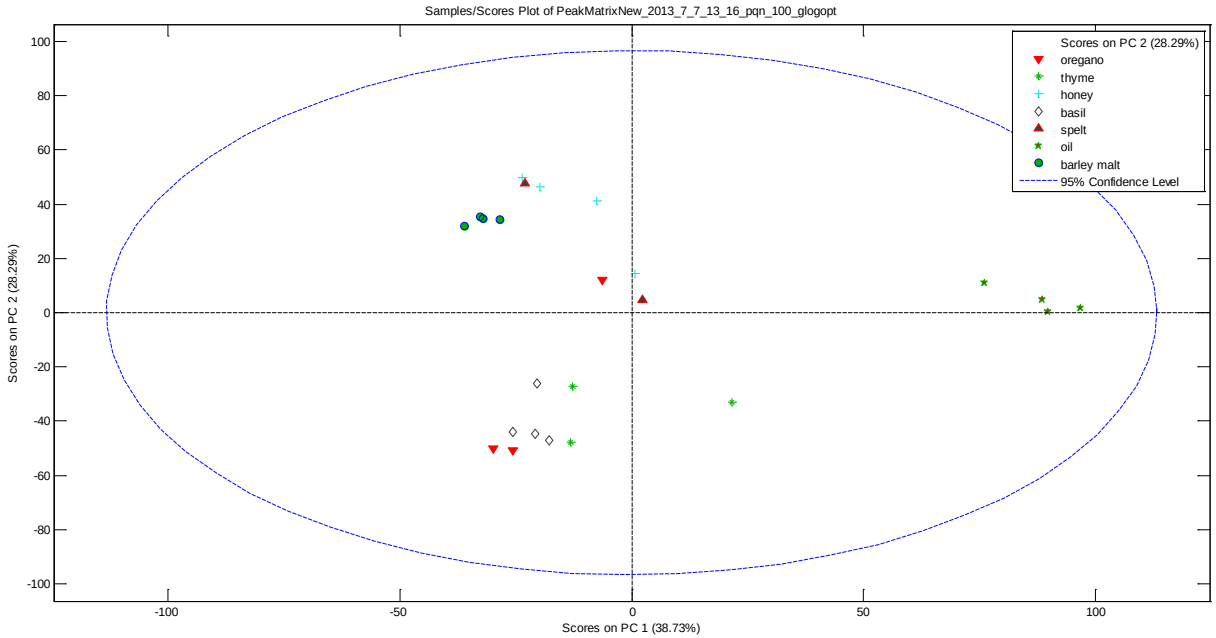


Figure 6.4. PCA scores plot comparing the KOH aliquot of aged foodstuffs including: oregano, thyme, basil, honey, spelt wheat, barley malt and olive oil. The separation of the spices (oregano, thyme, basil) is due to an overwhelming phenolic acid content.

6.2 Results: Area A

In the following sections, many of the contexts results are described in two tables. The first table contains the sample ID, sample type, sample weight, as well as the intensity of the nine acids identified from the targeted analysis. The final column suggests tentative identifications of bound lipids which were identified in the LC-MS/MS result. The second table illustrates the results from the TAG output and includes the acyl carbon number of each TAG identified as well as the number and identification of the TAGs associated with that designation. In certain cases, manual identification was included for descriptive purposes only; manual identification was utilised in cases where the samples did not pass the stringent rules put forth in the TAG output.

Area A represents a military stone fort built in A.D. 213, rebuilt in the 4th century, and consists of the centurion's apartment as well as soldiers' barracks. The samples collected from area A, having been excavated in 2011, were collected from the pottery conservation laboratory.

6.2.1 Context V11-VL-108A

This context represents the clay floor of the apartment where minimal animal bone was excavated. Several pieces of cookpot and amphora were gathered from this context. Targeted analysis of the acid components in sample 1, Table 6.2, identified three phenolic acids: syringic, vanillic, and p-coumaric acid. Ferulic acid was not identified in this analysis. Succinic acid was also identified. These results do not support the identification of wine within the cook pot. Levels of bound lipids were visible in this sample and were tentatively identified as hexadecanoic, octadecanoic, and octadecenoic acids. TAG analysis of the cookware identified $C_{50:1}$ (by manual identification). TAG

analysis of sample 7, cook pot, identified only one TAG $C_{52:1}$. Due to the limited TAG results, no conclusive origin of the lipid is possible.

Table 6.2 Context V11-VL-108A

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Cook pot V11-108A/1	1.02	ND	21166	ND	ND	1690	5187	ND	ND	6914	hexadecanoic, octadecanoic, and octadecenoic acids

	Acyl carbon number	Types of TAGs	Fatty acid constituents	Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
Cook pot, base V11-108A/8	ND	ND	ND	C50:1	1	16:0/16:0/18:1
Cook pot, base V11-108A/7	C52:1	1	18:1/18:0/16:0	ND	ND	ND

6.3 Analysis of samples: Area B

As stated in the Chapter 1 of this thesis, area B represents strip buildings used for civilian encampment located outside of the borders of the fort. The dates of habitation of the civilian encampment are *circa* 213-300 A.D., and are contemporaneous with the fort excavated in area A.

6.3.1 Context V11-VL-3B

This context was excavated from topsoil to the clay floor of one of the strip buildings, dated to after 213 A.D. based upon the coinage excavated from the area. These objects were excavated in 2011 and were housed in the pottery conservation laboratory.

Two sample classes were taken from this context, storage vessels and mortaria. Mortaria were tools used in meal preparation for grinding ingredients such as oil, wine, vinegar, spices, and plant and animal fats. They were often course grained, shallow bowls with thick side walls able to sustain the pressure of repetitive grinding (Alcock, 2001: 69). Lipid analyses on Iron Age mortaria sherds identified both animal as well as leafy plant products (Cramp, 2008; Cramp et al., 2011).

Sample 4 represents a mortarium base, gray/buff color. The results reported in Table 6.3 identified ferulic, syringic, p-coumaric, vanillic, succinic and malonic acids. Low levels of bound lipids were also tentatively identified. The identification of six out of the nine targeted acids is not highly indicative of wine, particularly in a multi-use environment. In this type of environment, the most highly indicative biomarker fingerprint for wine would be the identification of all nine acids.

Sample 6 was taken from a neck of a storage vessel. The results of the targeted analysis identified succinic and syringic acids, results not indicative of the presence of wine. The identification of syringic acid in both of these samples highlights the danger of a single biomarker approach for a complex foodstuff such as wine, particularly in an environment with other food contaminants. The need for a multi-biomarker approach is critical especially when there is overlap with several of the metabolites.

Table 6.3 Context V11-VL-3B

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Base of mortarium V11-3B/4	.978	83721	42630	ND	ND	13164	21446	ND	5520	7053	hexadecanoic, octadecanoic acids and unidentified at *m/z* 237
Neck of storage vessel V11-3B/6	1.01	ND	24004	ND	ND	ND	ND	ND	ND	2649	hexadecanoic, octadecanoic, and unidentified at *m/z* 237

6.3.2 Context V12-VL-30B

Context V12-30B is composed of the rubble found at the base of a separate context (V12-11B, backfill from an 1830s excavation). The date for this context is 213-270 A.D. Four samples were taken for analysis; the results of two of the samples are given below in Table 6.4.

The biomarker fingerprint for the base of a storage vessel sample 4, was not diagnostic for either wine or tentatively diagnostic for olive oil. In contrast, the biomarker fingerprint of sample 3 taken from the side of a cook pot, contained seven out of the nine targeted acids. The results suggest the presence of wine or vinegar, common ingredients in Roman British cooking (Alcock, 2001: 83). Translations from a surviving 2nd century A.D. cookbook written by the Roman writer Apicus, offer a unique perspective as to the recipes of that time and the ingredients suggest a palate that relies on heavily spiced, salty dishes with strong flavors. Current literary sources have collated several of the historic recipes and wine in its original form, its soured form (vinegar), sweetened with honey (caroenum), or as a cooked syrup (defructum), are common ingredients (Renfrew, 1985: 35-44). Several of the recipes are given in the Appendix, Chapter 6, Figure 3.

Concerning the lipid fraction, TAGs were not identified in the total lipid extract of sample 3. Low levels of hexadecanoic, octadecanoic acid as well as a reoccurring deprotonated mass at 237 were tentatively identified in the bound lipid fraction.

Table 6.4 Context V12-VL-30B

	Sample weight (g)	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Base of storage vessel V12-30B/4	1.04	ND	10963	45661	ND	2347	2189	ND	ND	1692	hexadecanoic, octadecanoic acids and unidentified *m/z* 237
Side of cook pot V12-30B/3	.994	88718	40630	161859	ND	9310	14019	ND	9716	11591	hexadecanoic, octadecanoic acids and unidentified *m/z* 237

No TAGs identified in VL-30B/3

6.3.3 Context V12-VL-21B

This context was excavated to the stone layer which sits above a Period V (circa 120-130A.D.) ditch. The context is secure and represents a civilian encampment from 213-270 A.D. Sample 2 represents the shoulder of a cookpot and the results are given in Table 6.5. The biomarker fingerprint suggests the presence of wine/vinegar as an ingredient with seven out of the nine targeted acids identified including: four phenolic acids, malonic, succinic, and malic acids. In the mass spectrum, the fragmentation profile of the ferulic acid peak was slightly askew, with a higher intensity of the 149 peak. This was explained by the surround co-eluting peaks whose fragmentation pattern was overwhelmingly *m/z* 149. The tentatively bound lipids included hexadecanoic, octadecanoic, and octadecenoic acids.

TAG analysis of the sample identified a narrow range of acyl carbons from C_{48}-C_{54}. The identification of odd number fatty acids (C_{15}, C_{17}, and C_{19}) represents a contribution from a ruminant animal, resulting from the fatty acid synthesis by the bacteria within the rumen (Christie, W.W., 1978). However, due to the TAG hydrolytic degradation, it is not possible to determine whether the fats originate from adipose tissue or from dairy products, such as milk or cheese.

Table 6.5 Context V12-VL-21B

	Sample weight (g)	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Shoulder of cook pot V12-21B/2	1.0	78981	82390	166147	ND	4792	2996	ND	1201	2450	hexadecanoic, octadecanoic, and octadecenoic acids

	Acyl carbon number	Types of TAGs	Fatty acid constituents								Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
Cook pot, shoulder VL-21B/2	C48:1	2	16/14/18:1	16/16/16:1							ND	ND	ND
	C50:1	8	16/15/19:1	16/16:1/18	16/16/18:1	18:1/14/18	18:1/15/19	18/14/20:1	18/16:1/18	18/15/19:1			
	C52:1	4	16/16/20:1	16/18/18:1	16/17/19:1	16/17:1/19							
	C52:2	3	18:1/16/18:1	18:1/18/16:1	16/18/18:2								

6.3.4 Context V12-VL-41B

The context is a layer of 'burnt laminate' suggesting the application of heat, either intentionally or an unintentional fire at some point in the past. The context was dated from 231-270 A.D. Sample V12-41B/1 was taken from the side of a thick walled amphora that appeared to be fabricated by joined rings or coils (personal communication: Dr. Helen Loney). The results of the targeted LC-MS/MS analysis listed in Table 6.6 identified three phenolic acids as well as succinic acid; therefore, the biomarker fingerprint of four out of the nine targeted acids does not suggest the presence of wine or vinegar. Tentatively identified within the bound lipid portion of the spectrum was octadecenoic acid, *m/z* 281. Its concentration overwhelms the spectrum and is obvious in Figure 6.5. A probable explanation for this *m/z* is oleic acid, indicative of olive oil. The identification of olive oil in an archaeological artifact is rare due to the degradation of its composite unsaturated fatty acids, commonly occurring over an archaeological time span due to microbial induced hydrolysis of the double bonds (Eglinton and Logan, 1991). The remarkable preservation of the (tentatively identified) unsaturated fatty acid reveals the capability of anoxic, waterlogged environments to slow the rate of decomposition. As well as the *m/z* 281, there are smaller contributions from *m/z* 171 (decanoic acid), *m/z* 255 (hexadecanoic acid), and *m/z* 295. The peak at *m/z* 295 may suggest any of the following compounds: a C_{19} cyclopropane fatty acid, originating from a bacterial lipid, a branched fatty acid, or an isoprenoid. This is conjecture, however, and targeted analysis is necessary for confirmatory evidence.

As stated above, the tentative identification of octadecenoic acid within the amphora suggests the presence of olive oil, a plant oil that is very high in oleic acid and whose TAG composition is predominantly comprised of trioleoylglycerol (OOO) (Rossell and Pritchard (ed.), 1991: 87; Kiritsakis

et al., 2002). Olive oil contains free phenolic acids, which are often used to classify geographical origin; however, the identification of syringic, ferulic, and p-coumaric acids (Table 6.6) probably originates from the alkaline fusion products of the phenolic backbones of oleuropein (Carrasco-Pancorbo et al., 2004). The identification of syringic acid in the alkaline fusion of an amphora sherd that most likely contained olive oil is especially concerning for those researchers who utilise a single biomarker approach for the identification of wine in amphora. As the main packaging unit for nearly two thousand years, amphora were consistently re-used to ship wine, olive oil, fish sauce, spices, as well as salted fish, resulting in an mixing of compounds (Twede, 2002; Foley et al., 2012).

The total lipid extract and lithiated TAG analysis of VL-41B/1 held limited information, with only three TAGs passing the algorithm filter, $C_{50:1}/C_{51:3}/C_{52:1}$. If in fact there was a fire in this context, the concentration of the total lipid extract appears to have been affected, whereas the olive oil was probably driven into the pottery sustaining its preservation.

Table 6.6 Context V12-VL-41B

	Sample weight (g)	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Side amphora V12-41B/1	.999	ND	6065	ND	ND	6970	ND	ND	4258	2045	octadecenoic acid (oleic acid)

	Acyl carbon number	Types of TAGs	Fatty acid constituents	Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
amphora	C50:1	1	16/16/18:1	ND	ND	ND
VL-41B/1	C51:3	1	19:3/16/16			
	C52:1	1	18:1/18/16			

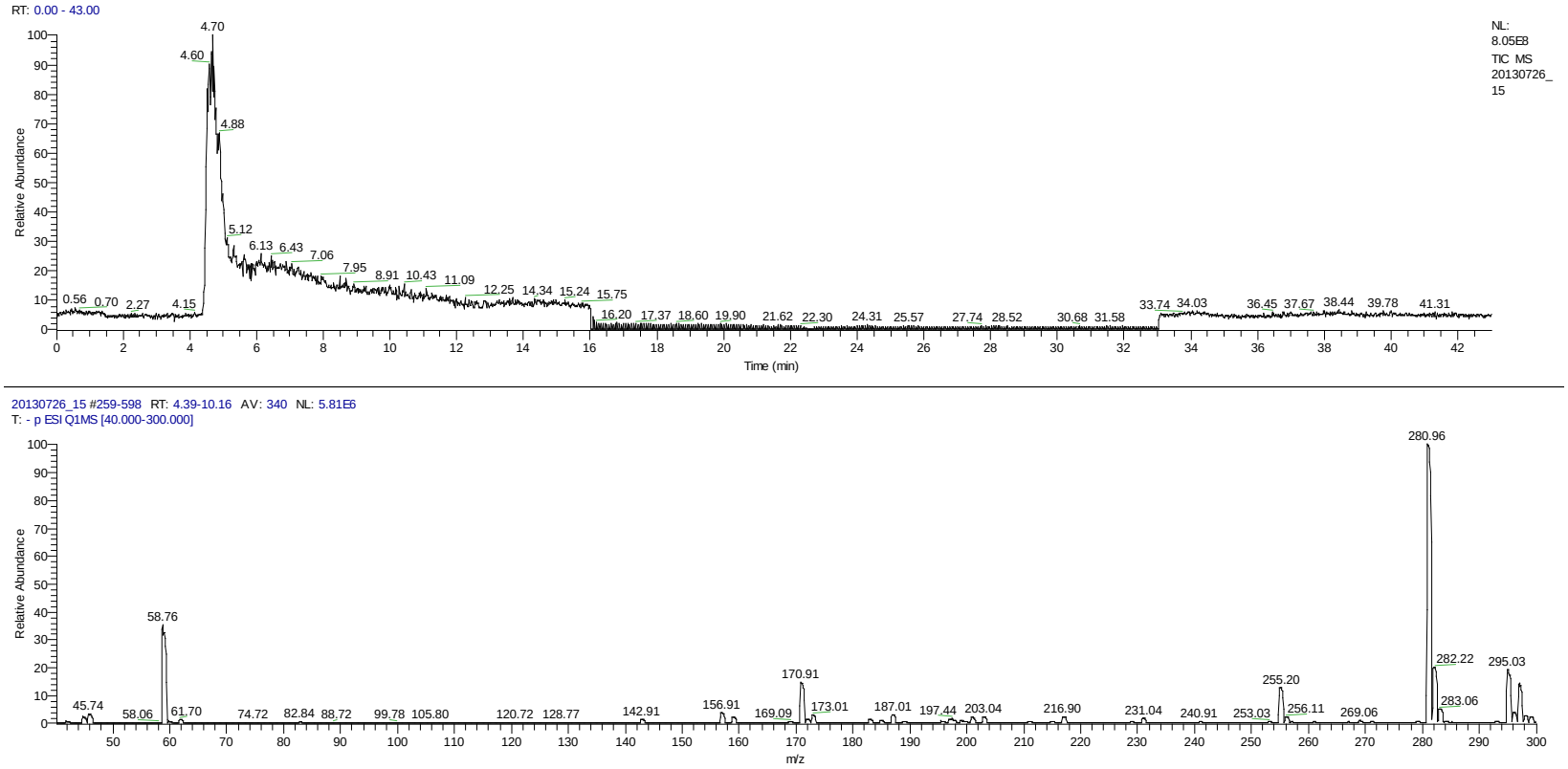


Figure 6.5. The TIC and extraction ion chromatogram of the LC-MS/MS analysis of sample V12-41B/1, a sherd from the side of an amphora. The spectrum is dominated by the elution at 4.7 minutes of a 'bound' lipid, tentatively identified as oleic acid based upon *m/z* 281.

6.3.5 Context V12-VL-47B

This context represents fill material located over an aqueduct consisting of rubble and clay over the supporting wall. The context is dated to the period 130-205 A.D. Five samples were taken from the area, four cook pots and one piece of domestic ware. Visually, the bulk of the samples have gray fabric, except for the visually dissimilar sample 2 representing the domestic ware (e.g., dinner plate), which consists of a red fabric (personal communication: Dr. Helen Loney).

Samples 3 and 5 are samples from a cook pot; sample 3 represents a side wall and sample 5 represents a rim of a cook pot. At this time, it has not yet been confirmed by an archaeologist whether the samples originate from the same object. However, their biomolecular fingerprints are very similar and strongly indicate the presence of wine or vinegar with the identification of eight of the nine targeted acids, Table 6.7. As was stated earlier, wine either as liquid, syrup, or vinegar, was consistently used in cooking. Comparing the two samples, the intensities are similar for five of the acids: malonic, succinic, malic, tartaric, and vanillic; however, the intensities of syringic, ferulic, and p-coumaric are considerably less in sample 3. One explanation for this drop in intensity is sample preparation or more specifically the interactions between the three phenolic acids in the sample with the ring on the phenyl sulfonate group, the active group on the strong cation exchange SPE cartridge. Upon re-examination of Figure 5.18 (c), there is a difference in the absolute intensities between the syringic acid, with and without SPE clean up. Compared with the gain in intensity for vanillic, malonic, and malic acids, the gain in intensity for the syringic acid after the SPE step is moderate, and at times, the intensity of syringic acid is slightly less after the clean-up procedure. Since all samples were collected as flow through without a wash step, these results suggest the

addition of a wash step is necessary. A dilute salt solution may work to break up any interaction between the ring structures yet not disrupt the aluminate ion binding to the sulfate group.

The biomolecular fingerprint of samples 1, 2, and 4, Table 6.7, were negative for wine (3/9 and 5/9 acids) and inconclusive for the identification of foodstuffs. The total lipid extract for sample 1 yielded no TAG information.

Table 6.7 Context V12-VL-47B

	Sample weight (g)	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Base of cook pot V12-47B/1	0.989	ND	9390	ND	ND	462	ND	ND	ND	128	hexadecanoic, octadecanoic acids
Domestic ware V12- 47B/2	0.998	70366	28865	ND	ND	1335	1935	ND	ND	3711	hexadecanoic, octadecanoic acids
Side of cook pot V12-47B/3	1.04	74806	41103	74220	1576	2857	10093	ND	4487	5205	hexadecanoic, octadecanoic acids
Shoulder of cook pot V12-47B/4	0.997	80400	46721	ND	ND	4644	7881	ND	ND	5788	hexadecanoic, octadecanoic acids, unidentified *m/z* 237
Rim of cook pot V12-47B/5	1.02	69913	43884	117159	1151	8757	11889	ND	12791	16994	hexadecanoic, octadecanoic acids, unidentified *m/z* 237

No TAGs identified in VL-47B/1

6.3.6 Context V12-VL-49B

Context 49B is a drainage ditch which contained animal bones, pottery, and cobble stones and dated to *circa* 213 A.D. The most informative sample taken from this context is sample 3, which represents the rim of a cook pot. The total lipid extract TAG analysis identified TAGs from C_{44}-$_{54:2}$, Table 6.8 and Figure 6.6. The odd number acyl carbons, C_{15}, C_{17}, and C_{19}, identify a ruminant animal, whereas the appearance of short acyl chains such as C_{14} and C_{12}, suggest a dairy origin. Cheese was a common foodstuff in Roman Britain and is found in several recipes (Alcock, 2001: 59-62; Renfrew, 1985: 36, 44). As a comparison, Appendix, Chapter 6, Table 2 illustrates the TAGs identified in a laboratory aged sample of cow's milk. The short chain fatty acids from C_4-C_{14} are evident in the standard. It has been theorised, that the shorter acyl chain are more readily hydrolysed and therefore do not remain over the period of an archaeological lifetime (Evershed, 2002).

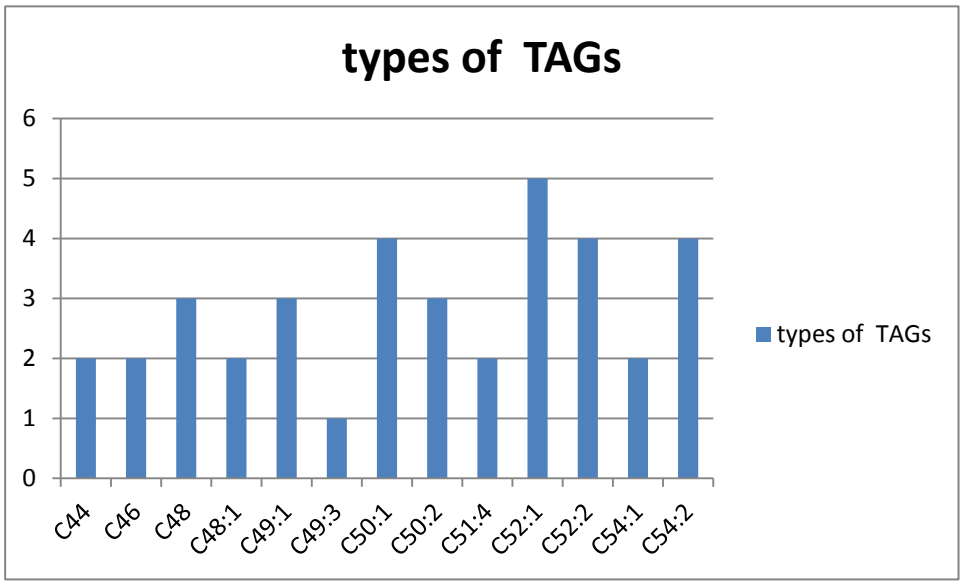


Figure 6.6. TAG acyl carbon distribution VL-49B/3.

Table 6.8 TAG results from the total lipid extract of VL-49B/3. The acyl carbon distribution ranges from C_{44}- $C_{54:2}$. The fatty acid constituents identify a ruminant animal; the presence of short acyl chains (C_{12} and C_{14}) suggest a dairy origin, rather than adipose fat.

	Acyl carbon number	Types of TAGs	Fatty acid constituents					Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
cook pot, rim	C44:0	2	141/14/16	16/12/16				ND	ND	ND
VL-49B/3	C46:0	2	16/16/14	16/12/18						
	C48:0	3	16/18/14	16/16/16	18/12/18					
	C48:1	2	18:1/14/16	16/16:1/16						
	C49:1	3	18:1/15/16	18:1/14/17	16/16/17:1					
	C49:3	1	18:3/15/16							
	C50:1	4	18:1/16/16	18:1/14/18	16/16:1/18	16/15/19:1				
	C50:2	3	18:1/14/18:1	18:1/16/16:1	18:2/16/16					
	C51:4	2	18:4/16/17	17:3/18:1/16						
	C52:1	5	16/16/20:1	16/17/19:1	16/18:1/18	18/16:1/18	18/15/19:1			
	C52:2	4	16/18/18:2	16/17/19:2	16/18:1/18:1	18:1/18/16:1				
	C54:1	2	18:1/18/18	18/16/20:1						
	C54:2	4	18/18:1/18:1	18/18/18:2	18/16/20:2	18:1/16/20:1				

6.3.7 Context V12-VL-51B

This context is a fabric of road which ran from the northwest- to the southeast, along the aqueduct next to the strip building. The date of the context is 213 A.D. Four samples were analysed, the results are given for three samples and are listed in Table 6.9. The biomolecular fingerprint of sample 1, an amphora base, was negative for the presence of wine or vinegar since only 1/9 of the acids were identified. The results of the targeted analysis identified only malonic acid; there was no evidence of bound lipids in the spectrum. The results of sample 4, a side wall of a mortarium, were negative for wine or vinegar content identifying only four of the nine targeted acids. The four acids that were identified included malonic, succinic, vanillic, and syringic. The origin of the acids is unknown at this time.

Sample 2 represents the upper portion of a cook pot. The biomolecular fingerprint of sample 2 suggests the presence of wine or vinegar with the identification of seven of the nine targeted acids. Neither tartaric acid nor iso-propyl malic acid was identified; however, the phenolic acids were identified, as well as malonic, succinic, and malic acids. Only one TAG was identified (manually) in this sample, $C_{51:3}$ which does not offer enough information for possible origin.

Table 6.9 Context V12-VL-51B, fabric of 3rd century road, circa 213 A.D.

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Base of amphora V12-51B/1	0.999	101361	ND	ND	ND	ND	ND	ND	ND	ND	ND
mortarium V12-51B/4	0.999	139670	78303	ND	ND	ND	6348	ND	ND	4535	ND
Shoulder of cook pot V12-51B/2	1.02	105195	62454	82802	ND	27466	21863	ND	22416	26192	hexadecanoic, octadecanoic acids, and unidentified *m/z* 237 and *m/z* 225

	Acyl carbon number	Types of TAGs	Fatty acid constituents	Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
cook pot, shoulder VL-51B/2	ND	ND	ND	C51:3	1	16:1/16:1/16:1
	Acyl carbon number	Types of TAGs	Fatty acid constituents	Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
cook pot, thin wall VL-51B/5	ND	ND	ND	ND	ND	ND

6.3.8 Context V12-VL-5B

Two samples were analysed from this context which is comprised of unstratified soil separating two other contexts, and dated to 353-358 A.D. The results for sample 2 are given below in Table 6.10. Sample 2 is a side wall from a mortarium, its biomolecular fingerprint is indeterminate for wine/vinegar since only six of the nine targeted acids were identified.

No TAGs were identified in sample 1, cook pot.

Table 6.10 Context V12-VL-5B, unstratified soil separating two other contexts and dated to 353-358 A.D.

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
mortarium V12-5B/2	.978	33129	27074	ND	ND	9629	10354	ND	7318	3774	ND

	Acyl carbon number	Types of TAGs	Fatty acid constituents	Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
cook pot, side VL-5B/1	ND	ND	ND	ND	ND	ND

6.4 Soil samples

Table 6.11 represents the results of the analysis of two soil samples collected on the same day by volunteer excavators. One of the soil samples was taken from context 52 which is comprised of yellowish clay that is bound on one side by rough cut stones and dated to 213 A.D; this sample was extracted and analysed twice. A separate soil sample was taken which did not have a context designation, yet was adhered to the base of an amphora or storage vessel.

Succinic acid was the only acid identified in the soil samples 1a and 1b taken from context 52. In contrast, targeted analysis of soil sample 2 from the unknown context identified seven out of the nine targeted acids. This sample was also devoid of 'bound' lipids. Rather than suggest the presence of wine, soil sample 2 may represent the monomeric species extracted from alkaline fusion applied to a humic polymer. The complexity of a soil sample cannot be underestimated, particularly a soil as rich in organic matter as the soil surrounding the Vindolanda settlement. The soil and humic substances (organic decomposition) encasing objects could include leaf litter, humic acid, and/or lignin polymers and the application of an alkaline material applied to any of these materials may release phenolic acids including benzoic and cinnamic acids (Vanholme et al., 2010; Burges et al., 1964; Rawlins et al., 2006; Bourbonniere and Meyers, 1983).

Concerning the organic acids, citric and malic acid have been identified in the rhizosphere, transported from a plants' roots into soil via the changing electrical gradient across cellular membranes in response to the plants nutritional needs (Rozycki and Strzelczyk, 1986). The symbiotic microbial population that cluster around the surface of the roots, however, metabolise those acid exudates, with some research suggesting 60% of the acid being transformed to CO_2, and

incorporating the remaining 40% leaving trace levels of the acids within the soil (Jones, 1998). Therefore, the intense malic acid peak is unexpected. Unfortunately, there is limited information concerning the alkaline hydrolysis of soils accompanying archaeological sherds and the current hypothesis is based upon the research carried out in this thesis. One important note is that the absence of acid signals (besides succinic acid) in soil samples 1a and 1b, versus the identification of seven acids in soil sample 2 highlights the immediate surroundings of soil samples and how quickly the soils' composition can change, probably due to polymeric networks, either wine polymers, lipid polymers, or humic acid polymers lodged within the soil preventing mass migration over long distances. Due to the unidentified context of this sample, a thorough explanation is unavailable; however, it should be taken as a cautionary tale for further investigations, particularly in the identification of a wine polymer whose biomarker list may overlap with several compounds found in humic substances. The actual nature of the humic substances is widely varied and dependent upon the organic decomposition from past lived fauna and flora. Therefore, future analyses of archaeological objects particularly collected in a heavy peat environment, should prepare and extract the adhered soil samples in the same fashion as the examined object.

Figure 6.7 compares six acids across soil sample 2 and five samples of the cooking pots representing four contexts: 52B, 47B, 21B, and 30B. The compared acids are: syringic, ferulic, vanillic, p-coumaric, tartaric and malonic. The difference in malonic acid is evident between the soil sample and the five pottery samples; malonic is considerably higher in the pottery samples than in the soil sample. Also, the level of syringic acid appears to be higher in the soil sample when compared with four of the five pottery samples. Figure 6.8 compares succinic acid and malic acid across the same samples. The intensities of these two acids are similar and suggest that, at least in a wet peat environment, these two acids occur relatively frequently and may not be descriptive for wine.

Table 6.11 Three soil samples were analysed by targeted LC-MS/MS for phenolic and organic acid content.

	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
Soil sample 1a Context 52: 1.0g	ND	8376	ND	ND	ND	ND	ND	ND	ND	hexadecanoic, octadecanoic acids
Soil sample 1b Context 52: .99g	ND	5455	ND	ND	ND	ND	ND	ND	ND	hexadecanoic, octadecanoic acids
Soil sample 2 Unknown context: .99g	13617	65743	116758	ND	4744	5216	ND	4151	28547	ND

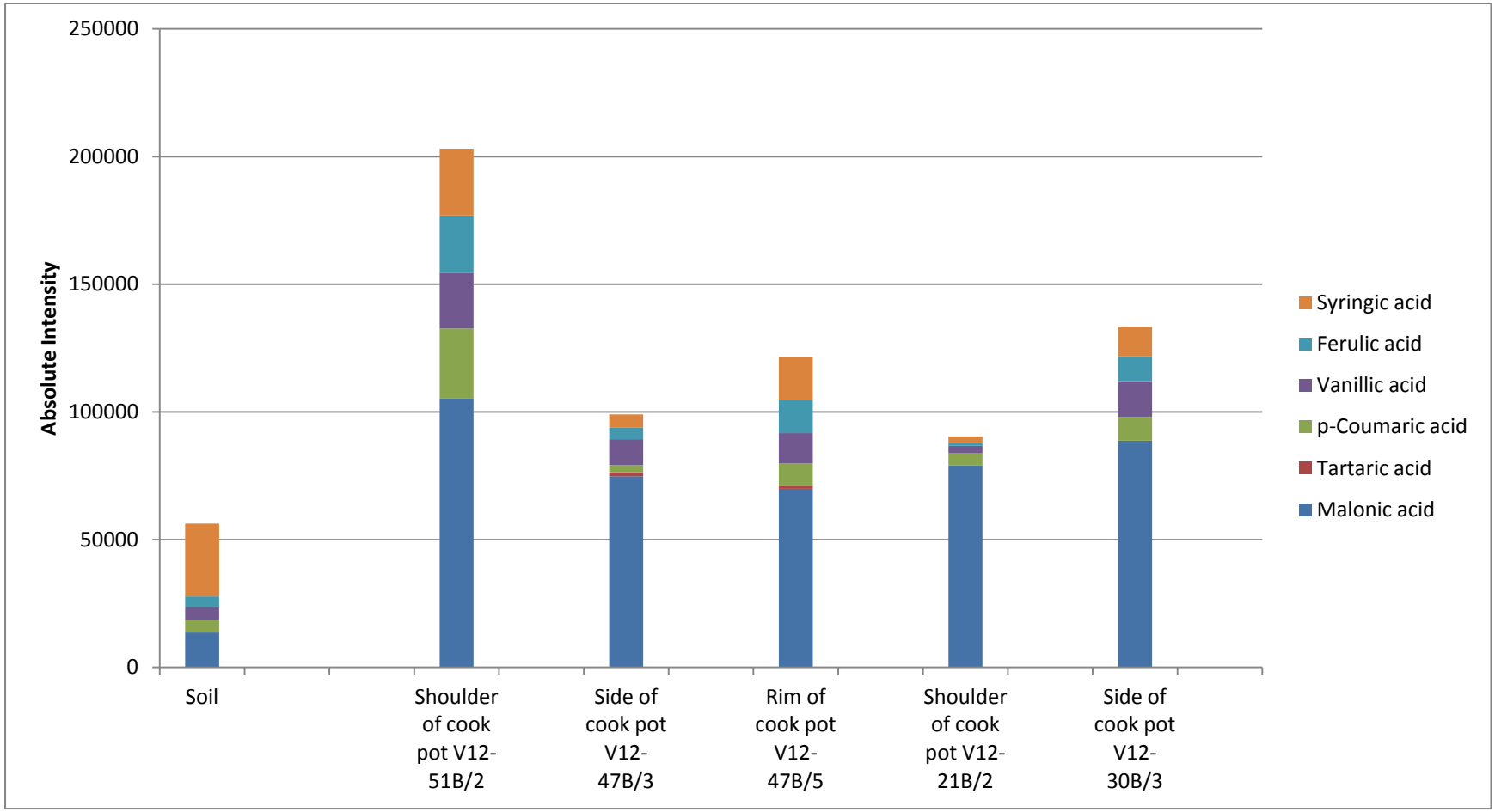


Figure 6.7. A comparison between soil sample 2 and five samples of cooking pots with the following acids: syringic, ferulic, vanillic, p-coumaric, tartaric, and malonic.

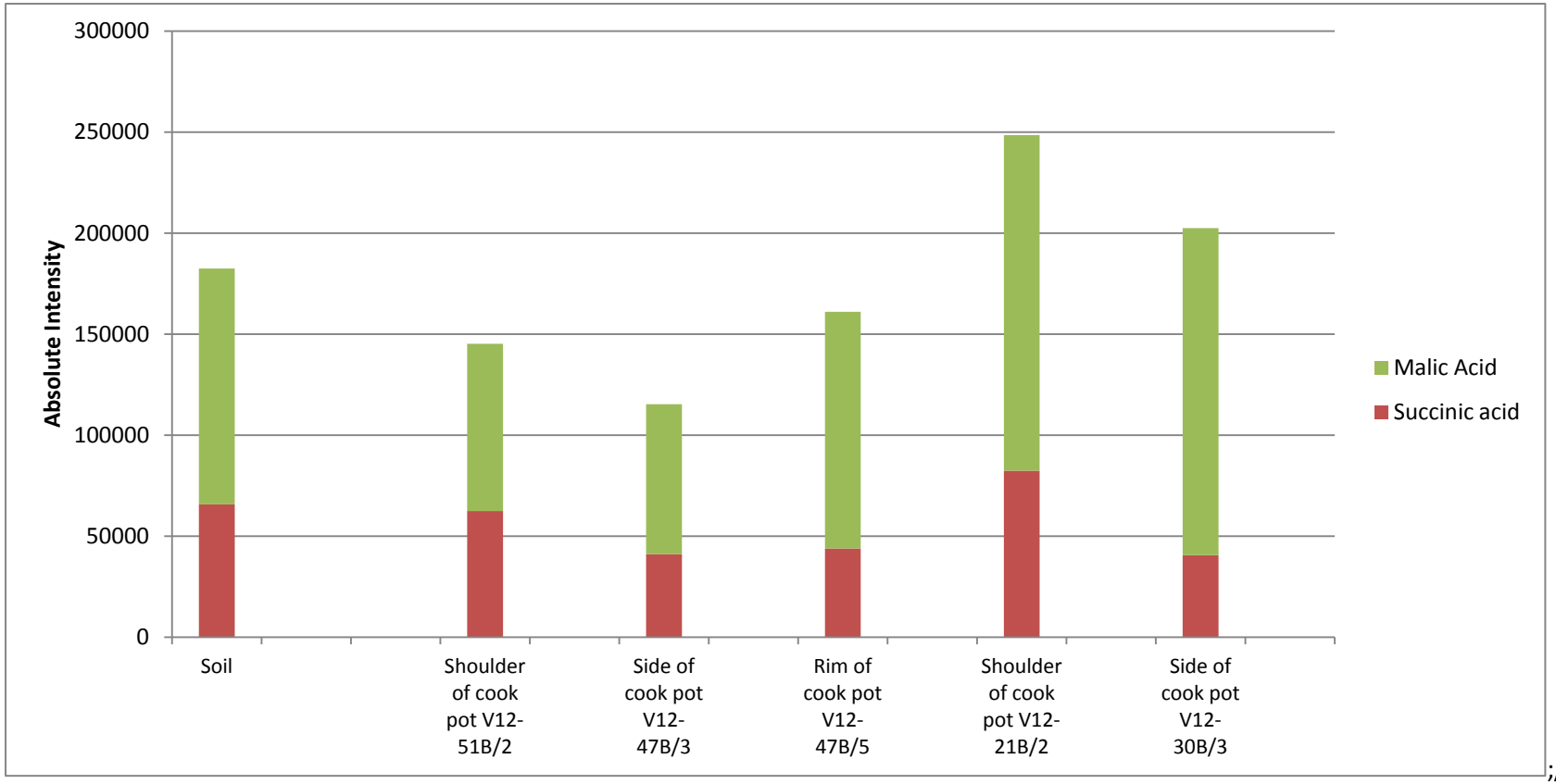


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Figure 6.8. A comparison between soil sample 2 and five samples of cooking pots with the following acids: succinic and malic.

6.5 Discussion

6.5.1 Cooking Pots

As stated earlier in the chapter, in order to determine the presence or absence of wine in these samples, nine acids were targeted in the LC-MS/MS analysis. The nine acids chosen were those acids identified in the wine press from Chapter 5 and are considered highly indicative for the presence of wine: malic, iso-propyl malic, tartaric, succinic, vanillic, ferulic, p-coumaric, syringic, and malonic acids. Two out of the eight samples of cooking pots had biomarker fingerprints that strongly indicated the presence of wine or vinegar since 8/9 targeted acids were identified. The two sherds V12-47B/3 and V12-47B/5 were taken from the same context and may represent the same cooking pot. Out of the remaining six analysed samples of the cooking pots, two of the sherds contained 7/9 targeted acids, suggesting the presence of wine and/or vinegar; one of these samples also contained TAGs indicative of ruminant animals. That cooking pots are rich in information is not unknown and it has been theorised in earlier investigations that the application of heat drives material into the pottery matrix (Heron and Evershed, 1993).

6.5.2 Mortaria

The three mortaria extracted had a similar biomarker fingerprint that included malonic, succinic, vanillic, and syringic acids, with two of the samples containing p-coumaric and ferulic acid. The results did not indicate the presence of wine or vinegar in the mortaria. Two of the samples had intense signals for vanillic acid suggesting a phenolic rich biomarker fingerprint left by the foodstuffs. A small aging study of foodstuffs identified phenolic rich spices; however, the fingerprint gathered

from the laboratory aging study does not correlate with the results from the mortaria. Therefore, more aging studies are required to identify a suite of biomarkers representative of Roman British foodstuffs.

6.5.3 Amphora

None of the amphora or the storage vessels indicated the presence of wine based upon the results of the targeted LC-MS/MS analysis. This is consistent with archaeological research in Vindolanda that reveals the main use for amphorae was to transport olive oil from the Iberian peninsula to the Vindolanda settlement (Sheehan-Finn, 2012). Wine delivered to the settlement was often transported through Gaul in wooden barrels (Symonds and Mason, (ed.) 2009). Based upon documentation in comparison with excavations records spanning the years 80-140 A.D., the percentage of amphora that transported wine (851) compared with the number of wooden barrels that transported wine (108,501) was 0.8% (Birley and Blake, 2005).

6.5.4 Soil

Two soil samples were analysed in this research. Succinic acid was the only acid identified in one sample; however, 7/9 targeted acids were identified in the second soil sample. The results suggest an overlap in the aged wine targeted acids and the alkaline fusion portion of an organic rich peat soil. Although the intensities of the acids identified in the soil varied when compared with the acids identified in the cook pots, the results highlight the possible effect of the archaeological environment on the chosen biomarkers. The results of the soil sample may reflect the breakdown of a humic acid polymer lodged within the peat matrix.

Concerning these results, it is theorised that an aged wine polymer may not move from the pottery and travel into the surrounding soil; similarly, it is also suggested that the polymeric materials found in humic rich soil may not travel distances through the soil causing interference with the archaeological pottery. However, at this time, this situation is only hypothetical and requires multiple testing of archaeological sherds and the adhered soil to answer the question of humic polymer/aged wine interaction, following the protocols of a previous study which examined lipids in the soil of surrounding pottery sherds (Heron et al., 1991).

6.6 Conclusions

The objectives put forth in the introduction to this thesis were to develop a diagnostic suite of biomarkers to apply to archaeological artifacts in order to determine the presence or absence of wine. The list of biomarkers developed in Chapters 3 and 4 was successfully applied to an ancient wine press from Sardinia, Italy. The biomarker list was then applied to samples excavated from Vindolanda, a wet anoxic environment with rich, organic soil. Out of the 32 samples analysed in Chapter 6, wine was strongly indicated in two of the samples: V12-47B/3 and V12-47B/5. The two sherds were taken from the same context, and eight of the acids were identified in each sample.

Comparisons between the levels of succinic acid and malic acid amongst a soil sample and five cook pots revealed that these two acids may not be diagnostic for wine under the scenario of this environment. In order to prove these acids uniqueness for wine within a peat rich environment, a successful laboratory aging study focusing on a wet peat environment should be undertaken in order to determine if these acids are still characteristic for laboratory aged wine.

7. Conclusions and Future Work

Four specific aims were identified in Chapter 1 of this thesis in an attempt to apply metabolomics to the analysis of ancient organic residue. The first aim set out in Chapter 3 was to age modern wine enriched sherds in a laboratory setting and then successfully extract those materials that represent the aged, polymerised wine. Once the materials were extracted, the samples were then analysed in a globally untargeted approach. Multi-variate statistics were applied to the final analysis and the top 150 loadings, most discerning for wine were chosen to prepare a targeted biomarker list.

In order to accomplish this aim, sample sherds were aged in several different environments in order to mimic archaeological environments: standard clean glass, dry sand, and wet peat. A successful metabolic signature for the aged wine was extracted from the clean jar and dry sand environments. However, there was no obvious signature from the wet peat. The reason may be that the wine never formed a polymeric network within the clay matrix, possibly due to the fact that water was continuously added to the jar in order to maintain moisture over the six month period. For future studies, a change in the methodology would be to age the wine permeated sherds for six months in glass jars and then introduce those sherds to the three specific environments (glass jar, dry sand, and wet peat) for further aging.

Concerning the liquid aging study and the identified six month plateau, the time point at which the metabolic signature had ceased to change, this plateau may be analogous to the initial polymerisation that occurs during grape treading, whereby the most reactive species are condensing to form larger polymers. There may be other plateaus which occur during the aging/polymerisation; however, due to time constraints, this thesis was limited to the identification of the first plateau. A recent article identified pinotin pigments forming after a year of aging indicating a continuation of

the aging/polymerisation process (Arapitsas et al., 2014). Whether the kinetics of polymerisation allows for a second plateau was untested in this initial research and should be pursued in further studies by monitoring the aging of liquid aliquots over at least a two year period.

The second aim of this research was to develop a sensitive targeted LC-MS/MS method based upon the biomarkers determined from Chapter 3 in order to detect trace level analytes. A HILIC method was successfully prepared for the trace analysis of thirteen acids for validation studies on objects excavated from two archaeological sites: ketobutyric, ferulic, gentisic, syringic, m-hydroxycinnamic, p-coumaric, 2,3-dihydroxybenzoic, vanillic, succinic, malonic, malic, tartaric, and isopropyl malic acid. Prior analyses utilised reversed phase liquid chromatography for the initial separation; however, using the orthogonal approach increased the capacity factor for the studied acids. The longer elution times allowed for more robust retention times and better reproducibility between samples and experiments. A short description of the acids on the biomarker list follows.

Gentisic acid and 2,3 dihydroxybenzoic acid were both chosen from the untargeted analysis discussed in chapter 3. However, the untargeted DIMS yielded only a *m/z* and a suggested molecular formula, $C_7H_6O_4$ with six possible choices for dihydroxybenzoic (DHB) acids. The final acids chosen were based upon literature searches on identified wine acids as well as on the final success of the LC elution profile. Neither 2,3-DHB or gentisic acid, identified as a wine acid, were identified in any of the archaeological samples. Another DHB found in wine is 3,4-DHB, protocatechuic acid. This acid is recommended as a replacement for the current acids on the biomarker list at *m/z* 153.

Meta-hydroxycinnamic and para-coumaric acids were two phenolic acids also chosen from a possible list of six isomers identified from one *m/z*. However, by comparing the fragmentation intensities between the standards and the archaeological results, p-coumaric was identified in the archaeological samples. Other phenolic acids include syringic, vanillic and ferulic acids. The initial

standard of ferulic acid analysed was trans-ferulic acid; however, the identification of ferulic acid in the archaeological samples revealed several extra peaks within the acid's retention time window which suggests an addition of cis-ferulic acid. It is unclear if the cis-ferulic acid is due to the aged wine polymer, or if it is an effect of extracting the samples in light. After SPE clean-up, however, the isomers appear to combine and elute in one larger peak.

Other acids include ketobutyric, malonic and succinic acids. Ketobutyric acid was not identified in the archaeological samples of Chapter 5 and was removed from the targeted biomarker list in Chapter 6. Malonic and succinic acids were consistently two of the most abundant acids identified in the archaeological samples.

Malic, tartaric, and isopropyl malic acid were the three organic acids used in this research to distinguish a list of acids identified in an archaeological sample from suggestive to highly indicative of wine. As stated in the introduction, earlier published results identified wine in archaeological amphora by the presence of tartaric acid as the sole biomarker due to its high concentration within grapes. However, this research has shown that identifying any foodstuff origin based upon only one or two biomarkers is risky and increases the chance of false positives. Since tartaric acid may also originate from microbial excretions, its identification should therefore be taken in combination with the remaining acids (Galkin et al., 1998).

The third aim of this thesis was to validate the targeted method on archaeological samples from a sandy soil environment of Sardinia, Italy, in an area with a rich viticulture history. For the first time, a metabolomics approach utilising a predetermined list of biomarkers was successfully applied to the analysis of an archaeological wine press, identifying nine of the thirteen targeted acids. The targeted analysis of amphorae sherds yielded indeterminate results since six or fewer of the acids were

identified, indicating the precarious nature of assigning only one or two biomarkers to the identification of ancient foodstuffs.

The fourth aim of this thesis was to apply that targeted method to the analysis of archaeological objects found in an environment with heavy food contamination in order to test the robustness of the biomarker list. As stated in Chapter 6, the original biomarker list containing thirteen acids was dropped to the nine acids identified in the Sardinian wine press; these nine acids are considered highly indicative for the presence of wine: malic, iso-propyl malic, tartaric, succinic, vanillic, ferulic, p-coumaric, syringic, and malonic acids. Two samples taken from cooking pots from the Vindolanda settlement yielded acid signatures highly indicative of wine based upon the identification of eight of the nine targeted acids. This result suggests a successful application to a multi-use environment and is the first time that the presence of wine was indicated in an ancient cook pot. In addition, the non-polar extracts were analysed for TAGs, and in several cases, combining the results of the alkaline fusion extracts with the results from the non-polar ones resulted in a highly descriptive story for the object and foodstuff origin.

The soil and environs of Vindolanda is a wet environment with an organic rich soil containing humic acid polymers. Based upon the initial analyses of a limited number of soil samples, there may be overlap between the targeted biomarker list for aged wine and humic polymers from peat-rich soils. This result highlights the importance of analysing the soil surrounding the archaeological objects. In order to test the theory that the humic acid polymers are immovable in the soil and therefore are not responsible for the biomarker fingerprint found in the excavated pottery sherds, a separate experiment should be carried out whereby in a peat rich environment, alkaline fusion is applied to the excavated pottery sherds and to the adhering soil for at least n=10, similar to a previous study on soil lipids (Heron et al., 1991).

As was identified in Chapter 5, applying an alkaline solution to pottery leaches out aluminate ions which may chelate the polyprotic acids such as malic and tartaric acids. The current method introduced a novel sample preparation step which removed the aluminate ion by cleaning up the sample on a strong cation exchange SPE column. In order to improve the method, the following procedures could be modified: decrease the 24 h time frame the samples spend in the acidified solvent prior to SPE clean-up, use a larger cartridge bed for SPE (500 mg instead of 100 mg), and introduce a wash step consisting of a dilute salt solution to break up any pi-pi interactions on the cartridge.

The metabolomics approach of global untargeted analysis in combination with statistical analysis resulted in a targeted biomarker list for the identification of aged wine residue that was successfully applied to two separate archaeological sites. This approach could also be applied to model sherds enriched with multiple foodstuffs, allowed to age over a prescribed time point. Using any of the recipes found from Roman texts, model sherds enriched with those representative foodstuffs and aged over a prescribed time could offer more specific biomarker lists which could add to the robustness in an environment with heavy food residues. The results of this type of analyses may yield biomarker information for objects such as mortaria, which although only a limited number were analysed, had fairly consistent phenolic fingerprints. It would not be surprising that repetitive grinding of phenolic rich foods would leave a fingerprint within the pottery matrix.

8. References

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9. Appendix

Chapter 3

Table 1 A table of m/z identified from the analysis of the red wine aliquots over four time points: zero, one, three, six months, which includes putative identifications. Univariate statistics were applied to this data; using the four time points, a fold change was determined using zero month as the reference time point, fold change 1 represents one month, fold change 2 represents three months, and fold change 3 represents the six month time point. The table is sorted on the smallest fold change 3 in conjunction with the smallest adjusted p-value, that is, the most significant peaks with the greatest change in intensities from zero month to six months.

M/Z	adjusted p-value	fold change 1	fold change 2	fold change 3	Empirical formula (parent)	Ion form	Theoretical mass (neutral) (Da)	Theoretical m/z (Da)	Mass error (ppm)	KEGG_COMPOUND
149.00847	8.30E-14	0.1315784	0.0150446	0.00379						0
193.03551	2.73E-09	0.1090107	0.0241104	0.006121	C10H8N2	[M+(37Cl)]-	156.068748	193.0351996	1.61	['3-Indoleacetonitrile']
193.03551	2.73E-09	0.1090107	0.0241104	0.006121	C4H6O5	[M+Hac-H]-	134.021525	193.0353786	0.68	['(R)-Malate', '(S)-Malate', '3-Dehydro-L-threonate', 'Malate']
193.03551	2.73E-09	0.1090107	0.0241104	0.006121	C6H10O7	[M-H]-	194.042655	193.0353786	0.68	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']
193.03525	2.73E-09	0.1090107	0.0241104	0.006121	C10H8N2	[M+(37Cl)]-	156.068748	193.0351996	0.26	['3-Indoleacetonitrile']
193.03525	2.73E-09	0.1090107	0.0241104	0.006121	C4H6O5	[M+Hac-H]-	134.021525	193.0353786	-0.67	['(R)-Malate', '(S)-Malate', '3-Dehydro-L-threonate', 'Malate']
193.03525	2.73E-09	0.1090107	0.0241104	0.006121	C6H10O7	[M-H]-	194.042655	193.0353786	-0.67	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']
349.1363	0	0.0470638	0.0076826	0.006479	C10H18N4O6	[M+Hac-H]-	290.122636	349.1364896	-0.54	['N-(L-Arginino)succinate']

349.1363	0	0.0470638	0.0076826	0.006479	C19H24N2OS	[M+Na-2H]-	328.160935	349.1356036	1.99	['Methotrimeprazine']
349.1363	0	0.0470638	0.0076826	0.006479	C24H24	[M+K-2H]-	312.1878	349.1364066	-0.31	['1,3,5-Triphenylcyclohexane', '2,4,6-Triphenyl-1-hexene']
194.03892	0	0.0898494	0.0234006	0.007464						0
193.03459	0.12046482	0.1090774	0.0232775	0.009106						0
321.09384	5.00E-15	0.0247784	0.0159402	0.010464						0
316.0439	5.16E-13	0.1790347	0.0196174	0.013673	C8H16NO10P	[M-H]-	317.051187	316.0439106	-0.03	['N-Glycolyl-D-mannosamine 6-phosphate']
350.12036	0	0.0711192	0.0197312	0.014881	C18H24O5S	[M-2H]-	352.134447	350.1198942	1.33	['Estradiol-17beta 3-sulfate']
387.0923	4.40E-14	0.0587978	0.0229128	0.0192						0
389.09357	3.00E-15	0.0530667	0.0266131	0.020181	C17H24N2O4S	[M+K-2H]-	352.14568	389.0942866	-1.84	['Mercaptoacetyl-Phe-Leu']
328.96802	9.00E-15	0.0887608	0.02963	0.021356						0
147.02928	3.75E-10	0.0397688	0.0166963	0.023502						0
427.04946	0	0.0835066	0.0351284	0.02537						0
327.05675	6.27E-12	0.086622	0.0351769	0.026424	C14H14N2O5	[M+(37Cl)]-	290.090273	327.0567246	0.08	['N2-Malonyl-D-tryptophan']
278.08791	8.81E-13	0.1522343	0.031381	0.029503	C14H15N3O	[M+(37Cl)]-	241.121512	278.0879636	-0.19	['4-(Dimethylamino)phenylazoxybenzene', 'CX-516']
278.08791	8.81E-13	0.1522343	0.031381	0.029503	C8H13NO6	[M+Hac-H]-	219.074289	278.0881426	-0.84	['N-Acetyl-D-mannosaminolactone', 'O-Succinyl-L-homoserine']
322.07786	9.16E-13	0.0674221	0.0402546	0.042062						0
264.07227	3.59E-13	0.2367747	0.0521316	0.043858						0
145.01417	3.00E-15	0.0796128	0.0497918	0.044018	C5H6O5	[M-H]-	146.021525	145.0142486	-0.54	['2-Oxoglutarate', '5-Hydroxy-2,4-dioxopentanoate', 'Dehydro-D-arabinono-1,4-lactone', 'Methyloxaloacetate', 'Oxaloacetate 4-methyl ester']
293.06828	8.11E-07	0.1143822	0.0732897	0.055831						0
149.00818	0.45950564	0.8392681	0.070108	0.056388	C3H4N4O2	[M+Na-2H]-	128.033426	149.0080946	0.57	['Ammelide']
535.15162	3.02E-13	0.0690184	0.0630969	0.059946						0
465.10413	7.46E-13	0.2926402	0.1042087	0.063117	C19H18O10	[M+Hac-H]-	406.09	465.1038536	0.59	['Lancerin']
465.10413	7.46E-13	0.2926402	0.1042087	0.063117	C21H22O12	[M-H]-	466.11113	465.1038536	0.59	['Plantagoside', 'Quercetin 7-O-glucoside']
149.00903	0	0.1701555	0.0809639	0.070141	C2H2O4	[M+Hac-H]-	89.99531	149.0091636	-0.9	['Oxalate']
149.00903	0	0.1701555	0.0809639	0.070141	C4H6O6	[M-H]-	150.01644	149.0091636	-0.9	['(R,R)-Tartaric acid', '(S,S)-Tartaric acid', 'meso-Tartaric acid']
321.02283	0	0.1384302	0.0809052	0.074236						0

329.06657	2.20E-14	0.1268503	0.1002789	0.087236	C15H10O5	[M+Hac-H]-	270.052825	329.0666786	-0.33	['2-Hydroxydaidzein', "3',4',7-Trihydroxyisoflavone", '3,6,4-Trihydroxyflavone', "4',6,7-Trihydroxyisoflavone", '5-Deoxykaempferol', 'Aloe-emodin', 'Apigenin', 'Baicalein', 'Emodin', 'Galangin', 'Genistein', 'Islandicin', 'Lucidin', 'Morindone', 'Norobtusifolin', 'Norwogonin', 'Purpurin 1-methyl ether', 'Sulphuretin']
329.06657	2.20E-14	0.1268503	0.1002789	0.087236	C17H14O7	[M-H]-	330.073955	329.0666786	-0.33	['(+)-Bisdechlorogeodin', '(-)-Bisdechlorogeodin', "3',4',5-Trihydroxy-3,7-dimethoxyflavone", 'Aflatoxin G2', 'Aurantio-obtusin', 'Cirsiliol', 'Hildecarpin', 'Tricin']
329.06657	2.20E-14	0.1268503	0.1002789	0.087236	C17H15O7	[M-2H]-	331.08178	329.0672272	-2	['Malvidin']
425.03382	0	0.1277376	0.095168	0.0935						0
173.00927	2.05E-12	0.1436403	0.1178257	0.096651	C4H2O4	[M+Hac-H]-	113.99531	173.0091636	0.61	['Acetylenedicarboxylate']
173.00927	2.05E-12	0.1436403	0.1178257	0.096651	C6H6O6	[M-H]-	174.01644	173.0091636	0.61	['Dehydroascorbate', 'cis-Aconitate', 'trans-Aconitate']
371.08311	1.10E-12	0.3653067	0.138132	0.107381						0
265.07154	2.20E-14	0.2903006	0.1467446	0.107418	C11H10O4	[M+Hac-H]-	206.05791	265.0717636	-0.84	['2-Hydroxy-3-methylbenzalpyruvate', '2-Hydroxy-8-methylchromene-2-carboxylate', 'Lathodoratin', 'Scoparone', 'cis-1,2-Dihydroxy-1,2-dihydro-8-carboxynaphthalene']
449.10911	2.01E-12	0.2993693	0.1664052	0.110737	C21H22O11	[M-H]-	450.116215	449.1089386	0.38	["2',3,4,4',6-Peptahydroxychalcone 4-O-glucoside", 'Astilbin', 'Neoastilbin', 'Neocarthamin']
539.14094	1.92E-12	0.2253417	0.1062973	0.114507						0
146.0458	2.80E-14	0.3609297	0.1505606	0.115217	C3H5NO2	[M+Hac-H]-	87.032029	146.0458826	-0.57	['2-Oxazolidinone', 'Dehydroalanine']
146.0458	2.80E-14	0.3609297	0.1505606	0.115217	C5H9NO4	[M-H]-	147.053159	146.0458826	-0.57	['2-Oxo-4-hydroxy-5-aminovalerate', 'D-Glutamate', 'DL-Glutamate', 'Isoglutamate', 'L-4-Hydroxyglutamate semialdehyde', 'L-Glutamate', 'L-threo-3-Methylaspartate', 'N-(Carboxymethyl)-D-alanine', 'N-Methyl-D-aspartic acid', 'O-Acetyl-L-serine']
671.1828	7.32E-13	0.1521108	0.1206806	0.119309						0
271.0246	0	0.3164202	0.1591549	0.120488						0
661.18321	5.40E-12	0.212673	0.1614287	0.121094						0
267.03556	1.49E-09	0.3541283	0.139664	0.126354						0
375.11443	3.81E-13	0.2938413	0.1424299	0.133768	C16H22N2O6	[M+(37Cl)]-	338.147788	375.1142396	0.51	['Aziridyl benzoquinone']

201.03825	1.16E-08	0.279144	0.194838	0.140132	C6H12O6	[M+Na-2H]-	180.06339	201.0380586	0.95	['2-Deoxy-D-gluconate', 'Aldohexose', 'D-Aldose', 'D-Allose', 'D-Altrose', 'D-Fructose', 'D-Fuconate', 'D-Galactose', 'D-Glucose', 'D-Gulose', 'D-Hamamelose', 'D-Hexose', 'D-Idose', 'D-Mannose', 'D-Psicose', 'D-Sorbose', 'D-Tagatose', 'D-Talose', 'Fructose(pyranose)', 'Ketose', 'L-Fructose', 'L-Fuconate', 'L-Galactose', 'L-Gulose', 'L-Rhamnonate', 'L-Sorbose', 'Sorbose', 'alpha-D-Galactose', 'alpha-D-Glucose', 'alpha-D-Mannose', 'alpha-L-Sorbopyranose', 'beta-D-Fructose', 'beta-D-Galactose', 'beta-D-Glucose', 'beta-D-Hamamelopyranose', 'beta-D-Mannose', 'muco-Inositol', 'myo-Inositol', 'scyllo-Inositol']
189.05773	2.73E-11	0.2725396	0.1981769	0.14099						0
477.06757	1.81E-11	0.2689602	0.1891489	0.142129						0
214.04888	2.20E-14	0.3898431	0.2413598	0.14343	C10H11NO3	[M+Na-2H]-	193.073894	214.0485626	1.48	['(3-Arylcarbonyl)-alanine', '3-Carbamoyl-2-phenylpropionaldehyde', '4-Hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one', '5,6-Dihydroxy-3-methyl-2-oxo-1,2,5,6-tetrahydroquinoline', 'Cichorine', 'Gentioflavine', 'Phenylacetylglycine']
214.04888	2.20E-14	0.3898431	0.2413598	0.14343	C3H10NO4P	[M+Hac-H]-	155.034747	214.0486006	1.31	['D-1-Aminopropan-2-ol O-phosphate', 'L-1-Aminopropan-2-ol O-phosphate', 'N-Methylethanolamine phosphate']
214.04888	2.20E-14	0.3898431	0.2413598	0.14343	C5H14NO6P	[M-H]-	215.055877	214.0486006	1.31	['sn-glycero-3-Phosphoethanolamine']
214.04888	2.20E-14	0.3898431	0.2413598	0.14343	C6H13NO5	[M+Cl]-	179.079374	214.0487756	0.49	['1-Amino-1-deoxy-scyllo-inositol', 'D-Galactosamine', 'D-Glucosamine', 'D-Mannosamine', 'Kanosamine', 'Nojirimycin', 'beta-D-Glucosamine', 'neo-Inosamine-2']
214.04888	2.20E-14	0.3898431	0.2413598	0.14343	C7H15NO2S	[M+(37Cl)]-	177.082351	214.0488026	0.36	['Dihomomethionine']
214.04888	2.20E-14	0.3898431	0.2413598	0.14343	C7H15NO4	[M+K-2H]-	177.100109	214.0487156	0.77	['Anthopleurine']
478.07088	4.18E-12	0.2753949	0.1944882	0.146733						0
285.04023	8.30E-14	0.3493056	0.203257	0.146914	C15H10O6	[M-H]-	286.04774	285.0404636	-0.82	['2-Hydroxygenistein', '6-Demethoxycapillarisin', 'Aureusidin', 'Citreorosein', 'Datiscetin', 'Fisetin', 'Isoscutellarein', 'Kaempferol', 'Luteolin', 'Maritimetin', 'Orobol', 'Scutellarein']

325.09278	5.26E-13	0.385688	0.2130926	0.147888	C15H18O8	[M-H]-	326.10017	325.0928936	-0.35	['4-O-beta-D-Glucosyl-4-hydroxycinnamate', '4-O-beta-D-Glucosyl-cis-p-coumarate', 'Bilobalide', 'cis-beta-D-Glucosyl-2-hydroxycinnamate', 'p-Coumaroyl-D-glucose', 'trans-beta-D-Glucosyl-2-hydroxycinnamate']
325.09278	5.26E-13	0.385688	0.2130926	0.147888	C19H16N2O	[M+(37Cl)]-	288.126263	325.0927146	0.2	['Difenpiramide']
205.05088	3.56E-12	0.3377731	0.2048182	0.154257	C11H10O4	[M-H]-	206.05791	205.0506336	1.2	['2-Hydroxy-3-methylbenzalpyruvate', '2-Hydroxy-8-methylchromene-2-carboxylate', 'Lathodoratin', 'Scoparone', 'cis-1,2-Dihydroxy-1,2-dihydro-8-carboxynaphthalene']
205.05088	3.56E-12	0.3377731	0.2048182	0.154257	C9H6O2	[M+Hac-H]-	146.03678	205.0506336	1.2	['Coumarin']
150.01241	2.20E-14	0.3531449	0.1830736	0.154631						0
507.11481	2.30E-11	0.3747309	0.2424242	0.162779	C21H20O11	[M+Hac-H]-	448.100565	507.1144186	0.77	['Astragalin', 'Aureusidin 6-O-glucoside', 'Carthamone', 'Fisetin 8-C-glucoside', 'Isoorientin', 'Kaempferol 3-O-beta-D-galactoside', 'Luteolin 7-O-beta-D-glucoside', 'Orientin', 'Plantaginin', 'Quercitrin']
214.99649	1.61E-06	0.4742	0.2587974	0.164592	C2H5O6P	[M+Hac-H]-	155.982378	214.9962316	1.2	['2-Phosphoglycolate']
214.99649	1.61E-06	0.4742	0.2587974	0.164592	C4H9O8P	[M-H]-	216.003508	214.9962316	1.2	['3-Phospho-D-erythronate', '4-Phospho-D-erythronate']
214.99649	1.61E-06	0.4742	0.2587974	0.164592	C6H10O6	[M+K-2H]-	178.04774	214.9963466	0.67	['2,4,6/3,5-Pentahydroxycyclohexanone', '2-Dehydro-3-deoxy-D-galactonate', '2-Dehydro-3-deoxy-D-gluconate', '2-Dehydro-D-glucose', '2-Deoxy-5-keto-D-gluconic acid', '3-Dehydro-2-deoxy-D-gluconate', '3-Keto-beta-D-galactose', '5-Dehydro-2-deoxy-D-gluconate', '5-Dehydro-D-fructose', '5-Deoxy glucuronic acid', 'D-Galactono-1,4-lactone', 'D-Galactono-1,5-lactone', 'D-Glucono-1,4-lactone', 'D-Glucono-1,5-lactone', 'D-galacto-Hexodialdose', 'Gulono-1,4-lactone', 'Hexose-1,5-lactone', 'L-Galactono-1,4-lactone', 'L-Gulono-1,4-lactone', 'myo-Inosose-5']
151.00357	2.32E-11	0.3585314	0.2279074	0.172366						0
173.10452	1.82E-10	0.3732101	0.2357923	0.178295	C6H14N4O2	[M-H]-	174.111676	173.1043996	0.7	['Amino acid(Arg-)', 'D-Arginine', 'L-Arginine']
148.03317	0	0.2078304	0.147258	0.18146						0
387.07804	3.81E-13	0.260909	0.1899433	0.192606	C16H20N2O5S	[M+Cl]-	352.109295	387.0786966	-1.7	['Benzylpenicilloic acid']
273.0016	4.01E-06	0.6481906	0.2919672	0.195089	C4H7O8P	[M+Hac-H]-	213.987858	273.0017116	-0.41	['2-Oxo-3-hydroxy-4-phosphobutanoate']

273.0016	4.01E-06	0.6481906	0.2919672	0.195089	C6H11O10P	[M-H]-	274.008988	273.0017116	-0.41	['1-Phospho-alpha-D-galacturonate', '3-Dehydro-L-gulonate 6-phosphate', '6-Phospho-2-dehydro-D-gluconate', 'D-Glucuronate 1-phosphate']
515.22071	1.92E-12	0.3816218	0.2696937	0.209189	C34H30N4	[M+Na-2H]-	494.247046	515.2217146	-1.95	['UCL 1684']
147.02979	3.37E-13	0.2477154	0.1829392	0.212782	C3H4O3	[M+Hac-H]-	88.016045	147.0298986	-0.74	['3-Hydroxypropenoate', '3-Oxopropanoate', 'Pyruvate']
147.02979	3.37E-13	0.2477154	0.1829392	0.212782	C5H8O5	[M-H]-	148.037175	147.0298986	-0.74	['(R)-2-Hydroxyglutarate', '(R)-2-Methylmalate', '(S)-2-Hydroxyglutarate', '(S)-2-Methylmalate', '2-Dehydro-3-deoxy-D-xylonate', '2-Dehydro-3-deoxy-L-arabinonate', '2-Hydroxyglutarate', 'Citramalate', 'D-Arabinono-1,4-lactone', 'D-Xylono-1,4-lactone', 'D-Xylonolactone', 'D-erythro-3-Methylmalate', 'D-threo-3-Methylmalate', 'L-Arabinono-1,4-lactone', 'L-Arabinono-1,5-lactone', 'L-Xylono-1,4-lactone', 'L-threo-3-Methylmalate']
311.06188	1.06E-10	0.6773053	0.2473923	0.216064						0
329.10878	1.92E-10	0.2896865	0.1852571	0.216801						0
312.06524	9.86E-11	0.7095464	0.2538063	0.216911						0
384.96649	2.80E-14	0.5007189	0.2910484	0.222273						0
325.04114	1.70E-08	0.5731017	0.2791467	0.222945						0
475.13037	2.41E-12	0.3853396	0.2556664	0.226803						0
316.09652	2.60E-09	0.6020491	0.2853716	0.228						0
292.10352	6.06E-13	0.2935021	0.255741	0.234716	C11H19NO8	[M-H]-	293.111069	292.1037926	-0.93	['N-Acetylmuramate']
209.03056	2.20E-14	0.1693487	0.1832235	0.239391	C4H6O6	[M+Hac-H]-	150.01644	209.0302936	1.27	['(R,R)-Tartaric acid', '(S,S)-Tartaric acid', 'meso-Tartaric acid']
209.03056	2.20E-14	0.1693487	0.1832235	0.239391	C6H10O8	[M-H]-	210.03757	209.0302936	1.27	['Glucarate']
456.17233	4.73E-12	0.4710542	0.3346852	0.242794						0
537.16722	1.38E-13	0.2921718	0.2355661	0.243085						0
293.10281	9.62E-09	0.7850553	0.3984659	0.244741	C13H14O4	[M+Hac-H]-	234.08921	293.1030636	-0.87	['1-Acetoxychavicol acetate', 'Coixinden B']
293.10281	9.62E-09	0.7850553	0.3984659	0.244741	C15H18O6	[M-H]-	294.11034	293.1030636	-0.87	['Tutin']
329.0724	9.69E-10	0.3280667	0.2220302	0.251395						0
224.04177	2.07E-12	0.4731896	0.3541985	0.252767	C5H11NOS2	[M+Hac-H]-	165.028208	224.0420616	-1.3	['4-Methylthiobutylthiohydroximate']
475.21842	2.55E-11	0.5255358	0.2845786	0.256265						0
344.07522	2.20E-14	0.21198	0.2884878	0.260398	C11H19NO9	[M+Cl]-	309.105984	344.0753856	-0.48	['N-Acetylneuraminate', 'O-Acetylneuraminic acid']
487.14576	1.16E-09	0.3921999	0.3333083	0.262443						0
533.1358	4.01E-12	0.4278908	0.3314699	0.266055						0

260.07736	5.60E-11	0.9000296	0.3623426	0.266392	C10H15NO7	[M-H]-	261.084854	260.0775776	-0.84	['Hymexazol O-glucoside', 'Hymexazol N-glucoside']
260.07736	5.60E-11	0.9000296	0.3623426	0.266392	C14H13N3	[M+(37Cl)]-	223.110947	260.0773986	-0.15	['Mepanipyrim']
313.07752	8.76E-10	0.5628097	0.2762627	0.266728	C7H16N8O4	[M+K-2H]-	276.129452	313.0780586	-1.72	['Trimethylenetetraurea']
315.09315	2.58E-09	0.6182748	0.2888085	0.268638	C14H18N2O4	[M+(37Cl)]-	278.126658	315.0931096	0.13	['Oxadixyl', 'alpha-Ribazole']
459.1356	6.27E-12	0.4374709	0.3097557	0.269108						0
439.08591	2.52E-13	0.4148144	0.3119318	0.273358						0
145.02942	1.26E-09	0.4025725	0.2996906	0.289749	C9H6O2	[M-H]-	146.03678	145.0295036	-0.58	['Coumarin']
327.09311	1.48E-07	0.6676362	0.3603943	0.291584	C9H16O9	[M+Hac-H]-	268.079435	327.0932886	-0.55	['2(alpha-D-Mannosyl)-D-glycerate']
349.01775	1.30E-10	0.3011965	0.304668	0.293827						0
439.07717	1.01E-11	0.4797876	0.3794624	0.303106						0
367.02843	2.04E-09	0.4564197	0.2829375	0.306401						0
210.02611	6.70E-10	0.5602934	0.4275785	0.308395						0
299.0983	7.14E-09	0.9093184	0.4644473	0.310729	C14H18N2O3	[M+(37Cl)]-	262.131743	299.0981946	0.35	['Methohexital', 'Physovenine']
224.95746	5.48E-12	0.5261936	0.344552	0.316431						0
340.97655	1.38E-13	0.5153677	0.3534763	0.320481						0
206.95535	6.26E-09	0.5260582	0.4428468	0.326538	C3H6O6S	[M+(37Cl)]-	169.988512	206.9549636	1.87	['(2R)-3-Sulfolactate', '(R)-2-O-Sulfolactate', '(S)-2-O-Sulfolactate', '(S)-3-Sulfolactate', '3-Sulfolactate', 'Glycerone sulfate']
343.08805	4.92E-12	0.4509893	0.2986489	0.327658	C15H18N2O5	[M+(37Cl)]-	306.121573	343.0880246	0.07	['Z-Gly-Pro']
431.14052	2.34E-13	0.5721897	0.3520579	0.331072						0
175.06133	6.77E-08	0.5192425	0.3936447	0.333649	C5H8O3	[M+Hac-H]-	116.047345	175.0611986	0.75	['2-Oxopentanoic acid', '3-Methyl-2-oxobutanoic acid', '3-Oxopentanoic acid', '5-Oxopentanoate']
175.06133	6.77E-08	0.5192425	0.3936447	0.333649	C7H12O5	[M-H]-	176.068475	175.0611986	0.75	['(2R,3S)-3-Isopropylmalate', '(R)-2-(n-Propyl)-malate', '2-Propylmalate', '3-Propylmalate', 'alpha-Isopropylmalate']
195.04925	3.80E-11	0.2445641	0.2146929	0.333874						0
253.05636	1.11E-11	0.696028	0.4450446	0.341549	C6H10O7	[M+Hac-H]-	194.042655	253.0565086	-0.59	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']
607.15187	2.76E-08	0.6563634	0.4272613	0.343646						0
465.14614	0.00144975	0.5844204	0.302111	0.350071						0
422.9224	1.61E-10	0.6941811	0.4202825	0.350521						0
168.99941	2.75E-09	0.3472652	0.3705017	0.350589						0

203.01997	3.53E-08	0.6126981	0.4734441	0.354093	C7H8O7	[M-H]-	204.027005	203.0197286	1.19	['Oxaloglutarate']
152.0114	4.44E-06	0.5497884	0.4220416	0.35586						0
529.11784	1.48E-10	0.5643161	0.3988349	0.356263						0
593.19349	4.40E-11	0.4668984	0.3702708	0.363427						0
193.02419	1.97E-05	0.7346498	0.4868844	0.366508	C11H8O	[M+(37Cl)]-	156.057515	193.0239666	1.16	['1-Naphthaldehyde', '2-Naphthaldehyde']
537.06298	3.41E-09	0.5734473	0.4611645	0.372423						0
295.06701	5.09E-10	0.7547813	0.4239837	0.37326						0
245.00673	3.04E-05	0.6392776	0.4628263	0.379509	C3H7O7P	[M+Hac-H]-	185.992943	245.0067966	-0.27	['2-Phospho-D-glycerate', '3-Phospho-D-glycerate', '3-Phospho-DL-glycerate']
245.00673	3.04E-05	0.6392776	0.4628263	0.379509	C6H10O8	[M+Cl]-	210.03757	245.0069716	-0.99	['Glucarate']
245.00673	3.04E-05	0.6392776	0.4628263	0.379509	C7H12O5S	[M+(37Cl)]-	208.040547	245.0069986	-1.1	['2-(2-Methylthio)ethylmalic acid', '3-(2-Methylthio)ethylmalic acid']
208.97096	5.34E-07	0.531239	0.4312958	0.381597						0
197.0555	0	0.3916328	0.3419088	0.382883						0
252.96041	1.44E-06	0.5565313	0.4809974	0.383241						0
587.12356	2.76E-08	0.3867935	0.3859943	0.388177						0
217.01221	4.18E-10	0.7520005	0.4177919	0.390371	C6H12O4S	[M+(37Cl)]-	180.045632	217.0120836	0.58	['5-Methylthio-D-ribose']
217.01221	4.18E-10	0.7520005	0.4177919	0.390371	C6H12O6	[M+K-2H]-	180.06339	217.0119966	0.98	['2-Deoxy-D-gluconate', 'Aldohexose', 'D-Aldose', 'D-Allose', 'D-Altrose', 'D-Fructose', 'D-Fuconate', 'D-Galactose', 'D-Glucose', 'D-Gulose', 'D-Hamamelose', 'D-Hexose', 'D-Idose', 'D-Mannose', 'D-Psicose', 'D-Sorbose', 'D-Tagatose', 'D-Talose', 'Fructose(pyranose)', 'Ketose', 'L-Fructose', 'L-Fuconate', 'L-Galactose', 'L-Gulose', 'L-Rhamnonate', 'L-Sorbose', 'Sorbose', 'alpha-D-Galactose', 'alpha-D-Glucose', 'alpha-D-Mannose', 'alpha-L-Sorbopyranose', 'beta-D-Fructose', 'beta-D-Galactose', 'beta-D-Glucose', 'beta-D-Hamamelopyranose', 'beta-D-Mannose', 'muco-Inositol', 'myo-Inositol', 'scyllo-Inositol']
217.01221	4.18E-10	0.7520005	0.4177919	0.390371	C9H8O5	[M+Na-2H]-	196.037175	217.0118436	1.69	['3-(3,4-Dihydroxyphenyl)pyruvate']
409.07524	2.68E-09	0.6037053	0.4767959	0.393868						0
475.19727	0.00669331	0.5471717	0.468483	0.399269	C23H28O7	[M+Hac-H]-	416.183505	475.1973586	-0.19	['Erioflorin methacrylate']
475.19727	0.00669331	0.5471717	0.468483	0.399269	C25H32O9	[M-H]-	476.204635	475.1973586	-0.19	['2-Methoxyestrone 3-glucuronide']
193.01448	5.68E-07	0.7531526	0.73541	0.400316						0
373.09873	1.57E-10	0.6495727	0.3878782	0.401437						0
470.15153	7.93E-11	0.5459139	0.4192783	0.404812						0
219.05128	5.04E-11	1.1371003	0.5687679	0.405348	C12H10N2	[M+(37Cl)]-	182.084398	219.0508496	1.96	['Azobenzene', 'Harman']

219.05128	5.04E-11	1.1371003	0.5687679	0.405348	C6H8O5	[M+Hac-H]-	160.037175	219.0510286	1.15	['2-Formylglutarate', '2-Oxoadipate', '3-Oxoadipate', '3D-(3,5/4)-Trihydroxycyclohexane-1,2-dione', 'D-2,3-Diketo 4-deoxy-epi-inositol']
219.05128	5.04E-11	1.1371003	0.5687679	0.405348	C8H12O7	[M-H]-	220.058305	219.0510286	1.15	['(R)-(Homo)2-citrate', '1-Hydroxypentane-1,2,5-tricarboxylate']
369.06747	3.57E-09	1.0115992	0.5088755	0.419746	C12H18O13	[M-H]-	370.074745	369.0674686	0	['1,2-beta-D-Glucuronosyl-D-glucuronate', 'Digalacturonate']
369.06747	3.57E-09	1.0115992	0.5088755	0.419746	C16H18N2O4S	[M+Cl]-	334.09873	369.0681316	-1.79	['Penicillin G']
491.17705	1.82E-08	0.6139424	0.4146412	0.42592	C17H34N4O10	[M+K-2H]-	454.227496	491.1761026	1.93	['Ribostamycin', 'Xylostasin']
491.17705	1.82E-08	0.6139424	0.4146412	0.42592	C19H28O11	[M+Hac-H]-	432.163165	491.1770186	0.06	['Zizybeoside I']
491.17705	1.82E-08	0.6139424	0.4146412	0.42592	C25H30N2O6	[M+(37Cl)]-	454.210388	491.1768396	0.43	['beta-Funaltrexamine']
196.05455	5.00E-15	0.4229404	0.3874496	0.430849						0
196.05371	0	0.4229405	0.0954447	0.430849	C10H11NO	[M+Cl]-	161.084064	196.0534656	1.25	['Boschniakine', 'Indole-3-ethanol']
196.05371	0	0.4229405	0.0954447	0.430849	C11H13N	[M+K-2H]-	159.104799	196.0534056	1.55	['Pargyline']
196.05371	0	0.4229405	0.0954447	0.430849	C13H10O2	[M-2H]-	198.06808	196.0535272	0.93	['1,2-Dihydroxyfluorene', '3,4-Dihydroxyfluorene', '4-Hydroxybenzophenone', 'Capillarin', 'Dehydrosafynol']
212.98937	2.92E-10	0.5866853	0.5476482	0.433125						0
275.01718	2.57E-05	0.7985902	0.5232248	0.439891	C4H9O8P	[M+Hac-H]-	216.003508	275.0173616	-0.66	['3-Phospho-D-erythronate', '4-Phospho-D-erythronate']
275.01718	2.57E-05	0.7985902	0.5232248	0.439891	C6H13O10P	[M-H]-	276.024638	275.0173616	-0.66	['2-Carboxy-D-arabinitol 1-phosphate', '6-Phospho-D-gluconate']
275.01718	2.57E-05	0.7985902	0.5232248	0.439891	C8H14O8	[M+K-2H]-	238.06887	275.0174766	-1.08	['3-Deoxy-D-manno-octulosonate']
351.03339	2.77E-08	0.6460223	0.4617414	0.442043						0
149.04545	1.22E-11	0.3099331	0.2872372	0.445354	C3H6O3	[M+Hac-H]-	90.031695	149.0455486	-0.66	['(R)-Lactate', '(S)-Lactate', '3-Hydroxypropanoate', 'D-Glyceraldehyde', 'Glyceraldehyde', 'Glycerone', 'L-Glyceraldehyde', 'Lactate']
149.04545	1.22E-11	0.3099331	0.2872372	0.445354	C5H10O5	[M-H]-	150.052825	149.0455486	-0.66	['D-Apiose', 'D-Arabinose', 'D-Lyxose', 'D-Ribose', 'D-Ribulose', 'D-Xylose', 'D-Xylulose', 'L-Arabinofuranose', 'L-Arabinose', 'L-Lyxose', 'L-Ribulose', 'L-Xylose', 'L-Xylulose', 'Ribulose', 'Xylose', 'alpha-D-Lyxose', 'alpha-D-Ribulose', 'alpha-L-Arabinose', 'beta-D-Apiose', 'beta-D-Ribofuranose', 'beta-D-Ribopyranose', 'beta-D-Xylose', 'beta-L-Arabinose']

212.03332	5.23E-08	0.6441335	0.621294	0.448378	C10H9NO3	[M+Na-2H]-	191.058244	212.0329126	1.92	['5,6-Dihydroxy-3-methyl-2-oxo-1,2-dihydroquinoline', '5-Hydroxyindoleacetate', '5-Phenyl-1,3-oxazinane-2,4-dione']
212.03332	5.23E-08	0.6441335	0.621294	0.448378	C3H8NO4P	[M+Hac-H]-	153.019097	212.0329506	1.74	['Fosamine']
212.03332	5.23E-08	0.6441335	0.621294	0.448378	C6H11NO5	[M+Cl]-	177.063724	212.0331256	0.92	['3-Keto-scyllo-inosamine', '4-Hydroxy-4-methylglutamate']
212.03332	5.23E-08	0.6441335	0.621294	0.448378	C7H13NO4	[M+K-2H]-	175.084459	212.0330656	1.2	['Calystegin B2']
212.03332	5.23E-08	0.6441335	0.621294	0.448378	C9H10O6	[M-2H]-	214.04774	212.0331872	0.63	['2-Hydroxy-6-oxonona-2,4-diene-1,9-dioate']
665.21446	8.78E-09	0.5583893	0.4501172	0.453145	C24H42O21	[M-H]-	666.221865	665.2145886	-0.19	['1,3-alpha-D-Mannosyl-1,2-alpha-D-mannosyl-1,2-alpha-D-mannosyl-D-mannose', 'Cellotetraose', 'Glycogen', 'Isolychnose', 'Lychnose', 'Maltotetraose', 'Stachyose', 'alpha-D-Galactosyl-(1-6)-alpha-D-galactosyl-(1-6)-beta-D-fructosyl-(2-1)-alpha-D-glucoside']
270.04672	8.34E-10	0.8059132	0.6088738	0.455271						0
317.10878	2.17E-07	0.6176856	0.4482826	0.455382	C18H20N2S	[M+Na-2H]-	296.13472	317.1093886	-1.92	['Methdilazine']
391.10931	1.92E-11	0.3035513	0.3293264	0.455431	C13H21N2O4PS	[M+Hac-H]-	332.095968	391.1098216	-1.31	['Butamifos', 'O-Ethyl-O-(5-methyl-4-nitrophenyl)(1-methylpropyl)phosphoramidothioate']
391.10931	1.92E-11	0.3035513	0.3293264	0.455431	C16H22N2O7	[M+(37Cl)]-	354.142703	391.1091546	0.4	['Dinocton 6']
391.10931	1.92E-11	0.3035513	0.3293264	0.455431	C17H26N2O4S	[M+K-2H]-	354.16133	391.1099366	-1.6	['Sultopride']
663.15659	9.52E-08	0.7729028	0.5854414	0.461372						0
348.92176	4.07E-09	0.7136336	0.5673939	0.464179						0
211.01008	4.73E-12	0.5046896	0.4851478	0.464927	C4H8O2S2	[M+Hac-H]-	151.996574	211.0104276	-1.65	['3-Mercapto-2-mercaptomethylpropanoate', '4,5-cis-Dihydroxy-1,2-dithiacyclohexane', 'Oxidized dithiothreitol']
447.13564	9.59E-09	0.5641487	0.4920347	0.465324	C20H30N2O5S	[M+K-2H]-	410.187545	447.1361516	-1.14	['Benfuracarb']
245.01517	6.42E-10	0.7267123	0.5308309	0.472251						0
161.04565	6.20E-07	0.8777122	0.7706932	0.472652	C4H6O3	[M+Hac-H]-	102.031695	161.0455486	0.63	['(S)-Methylmalonate semialdehyde', '2-Methyl-3-oxopropanoate', '2-Oxobutanoate', 'Acetoacetate', 'Succinate semialdehyde']

161.04565	6.20E-07	0.8777122	0.7706932	0.472652	C6H10O5	[M-H]-	162.052825	161.0455486	0.63	['(2R,3S)-2,3-Dimethylmalate', '(R)-2-Ethylmalate', '(R)-3,3-Dimethylmalate', '(S)-2-(Hydroxymethyl)glutarate', '1,5-Anhydro-D-fructose', '2-Dehydro-3-deoxy-D-fuconate', '2-Dehydro-3-deoxy-L-fuconate', '2-Dehydro-3-deoxy-L-rhamnonate', '2-Deoxy-scyllo-inososе', '2-Hydroxyadipate', '3,6-Anhydrogalactose', '3,6-Anhydroglucose', '3-Ethylmalate', '3-Hydroxy-3-methylglutarate', 'D-Fucono-1,4-lactone', 'Diethyl pyrocarbonate', 'L-Fucono-1,5-lactone', 'L-Rhamnono-1,4-lactone', 'Lichenin']
355.0669	6.75E-11	0.5687875	0.539913	0.477677	C13H12O8	[M+Hac-H]-	296.05322	355.0670736	-0.49	['Phaseolic acid']
326.96093	2.01E-11	0.8606344	0.5608697	0.483441						0
443.19235	8.03E-09	0.6073158	0.5391161	0.48371						0
195.05088	7.00E-15	0.4970207	0.445317	0.490658	C10H10N2	[M+(37Cl)]-	158.084398	195.0508496	0.16	['1,5-Naphthalenediamine', 'Nicotyrine']
195.05088	7.00E-15	0.4970207	0.445317	0.490658	C4H8O5	[M+Hac-H]-	136.037175	195.0510286	-0.76	['Threonate']
195.05088	7.00E-15	0.4970207	0.445317	0.490658	C6H12O7	[M-H]-	196.058305	195.0510286	-0.76	['2-Carboxy-D-arabinitol', 'D-Altronate', 'D-Gluconic acid', 'D-Mannonate']
310.96601	1.80E-08	0.922486	0.6498982	0.492097	C6H10O10S	[M+(37Cl)]-	273.999472	310.9659236	0.28	['L-Iduronate 2-sulfate']
393.04403	3.86E-08	0.6641472	0.4616755	0.501341	C15H16O11	[M+Na-2H]-	372.069265	393.0439336	0.25	['2-O-Caffeoylglucarate']
294.0828	2.05E-10	1.1432976	0.7965046	0.503097	C8H13NO7	[M+Hac-H]-	235.069204	294.0830576	-0.88	['N-Acetylgalactosaminate', 'N-Glycolyl-D-mannosaminolactone']
379.02841	3.72E-09	0.5571035	0.4780298	0.508254						0
324.09347	1.84E-05	1.0562043	0.6449995	0.510103	C11H19NO10	[M-H]-	325.100899	324.0936226	-0.47	['N-Glycoloyl-neuraminate']
324.09347	1.84E-05	1.0562043	0.6449995	0.510103	C15H17N3O3	[M+(37Cl)]-	287.126992	324.0934436	0.08	['SR95531']
338.10915	3.24E-06	0.9803515	0.6289546	0.515461	C16H19N3O3	[M+(37Cl)]-	301.142642	338.1090936	0.17	['Febrifugine', 'Isofebrifugine']
273.01003	1.83E-08	0.7188113	0.588413	0.51746						0
513.08652	7.79E-11	0.6123679	0.5315235	0.525245						0
209.06696	3.00E-15	0.4672038	0.4600067	0.526261	C5H10O5	[M+Hac-H]-	150.052825	209.0666786	1.35	['D-Apiose', 'D-Arabinose', 'D-Lyxose', 'D-Ribose', 'D-Ribulose', 'D-Xylose', 'D-Xylulose', 'L-Arabinofuranose', 'L-Arabinose', 'L-Lyxose', 'L-Ribulose', 'L-Xylose', 'L-Xylulose', 'Ribulose', 'Xylose', 'alpha-D-Lyxose', 'alpha-D-Ribulose', 'alpha-L-Arabinose', 'beta-D-Apiose', 'beta-D-Ribofuranose', 'beta-D-Ribopyranose', 'beta-D-Xylose', 'beta-L-Arabinose']
209.06696	3.00E-15	0.4672038	0.4600067	0.526261	C7H14O7	[M-H]-	210.073955	209.0666786	1.35	['Sedoheptulose', 'alpha-D-Mannoheptulopyranose', 'beta-D-Sedoheptulopyranose']

445.208	2.96E-07	0.6891546	0.6165431	0.527769						0
194.94682	0.00013296	0.7407897	0.7130936	0.53079						0
377.13007	1.49E-09	0.5346965	0.4547914	0.533645	C13H23N2O3PS	[M+Hac-H]-	318.116703	377.1305566	-1.29	['Tebupirimfos']
377.13007	1.49E-09	0.5346965	0.4547914	0.533645	C16H26N2O4S	[M+Cl]-	342.16133	377.1307316	-1.75	['Penicillin K']
325.07752	1.59E-07	0.7425966	0.564222	0.543721						0
195.05285	8.79E-08	0.572757	0.4372166	0.544333						0
461.13007	1.43E-07	0.6244438	0.5951622	0.548495						0
214.89208	4.90E-07	0.8120964	0.6357129	0.54898	H4O7P2	[M+K-2H]-	177.943231	214.8918376	1.13	['Diphosphate']
527.10215	1.92E-11	0.4235609	0.4954678	0.563015						0
675.17542	5.80E-06	0.5187501	0.5226454	0.580023						0
737.23617	2.98E-05	0.5684854	0.5880734	0.582512						0
217.00253	0.00183037	0.7962293	0.922499	0.593591	C7H6N2O4	[M+Cl]-	182.032758	217.0021596	1.71	['2,4-Dinitrotoluene', '2,6-Dinitrotoluene', '3,5-Dinitrotoluene', 'Nitrofurylacrylamide']
217.00253	0.00183037	0.7962293	0.922499	0.593591	C8H8N2O3	[M+K-2H]-	180.053493	217.0020996	1.98	['Isonicotinylglycine', 'Nicotinurate']
195.05241	3.53E-11	0.5803249	0.4787904	0.594097	C5H4N4O	[M+Hac-H]-	136.038511	195.0523646	0.23	['Hypoxanthine']
195.05241	3.53E-11	0.5803249	0.4787904	0.594097	C7H8N4O3	[M-H]-	196.059641	195.0523646	0.23	['1,7-Dimethyluric acid', '3,7-Dimethyluric acid']
337.07748	6.53E-13	0.7228047	0.6649034	0.597697	C12H18O11	[M-H]-	338.084915	337.0776386	-0.47	['L-Ascorbic acid-2-glucoside']
337.07748	6.53E-13	0.7228047	0.6649034	0.597697	C16H16N2O4	[M+(37Cl)]-	300.111008	337.0774596	0.06	['5-Nitro-2-(3-phenylpropylamino)benzoic acid', 'Desmedipham', 'Phenmedipham']
199.01005	7.03E-08	0.6273519	0.5078039	0.597884	C5H12O4S2	[M-H]-	200.017704	199.0104276	-1.9	['(R)-2-Hydroxypropyl-CoM', '(S)-2-Hydroxypropyl-CoM']
153.01922	2.68E-09	0.7265566	0.6551012	0.610829	C7H6O4	[M-H]-	154.02661	153.0193336	-0.74	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']
330.98369	1.10E-07	0.5755378	0.5774092	0.625129						0
165.04061	1.24E-08	0.3633088	0.4059095	0.626453	C3H6O4	[M+Hac-H]-	106.02661	165.0404636	0.89	['D-Glycerate']
165.04061	1.24E-08	0.3633088	0.4059095	0.626453	C5H10O6	[M-H]-	166.04774	165.0404636	0.89	['D-Ribonate', 'D-Xylonate', 'L-Arabinonate']
445.12005	1.78E-07	0.7989763	0.7363941	0.62941	C20H28N2O5S	[M+K-2H]-	408.171895	445.1205016	-1.01	['Tamsulosin']
445.12005	1.78E-07	0.7989763	0.7363941	0.62941	C23H24N2O4S	[M+Na-2H]-	424.14568	445.1203486	-0.67	['Eprosartan']
153.05561	1.96E-07	0.6990111	0.715614	0.632227	C6H6O	[M+Hac-H]-	94.041865	153.0557186	-0.71	['Arene oxide', 'Phenol']
153.05561	1.96E-07	0.6990111	0.715614	0.632227	C8H10O3	[M-H]-	154.062995	153.0557186	-0.71	['2,6-Dimethoxyphenol', '4-Hydroxy-3-methoxy-benzenemethanol']
403.10928	0.00022601	0.7527866	0.4895915	0.637497						0
405.12496	1.14E-07	0.7235801	0.6191933	0.639371						0
401.09369	1.88E-07	0.9598867	0.6885419	0.639894						0
237.06143	7.17E-11	0.5666956	0.5986669	0.64181	C12H12N2O	[M+(37Cl)]-	200.094963	237.0614146	0.06	['4,4-Diaminodiphenyl ether', 'Harmalol']

237.06143	7.17E-11	0.5666956	0.5986669	0.64181	C6H10O6	[M+Hac-H]-	178.04774	237.0615936	-0.69	['2,4,6/3,5-Pentahydroxycyclohexanone', '2-Dehydro-3-deoxy-D-galactonate', '2-Dehydro-3-deoxy-D-gluconate', '2-Dehydro-D-glucose', '2-Deoxy-5-keto-D-gluconic acid', '3-Dehydro-2-deoxy-D-gluconate', '3-Keto-beta-D-galactose', '5-Dehydro-2-deoxy-D-gluconate', '5-Dehydro-D-fructose', '5-Deoxy glucuronic acid', 'D-Galactono-1,4-lactone', 'D-Galactono-1,5-lactone', 'D-Glucono-1,4-lactone', 'D-Glucono-1,5-lactone', 'D-galacto-Hexodialdose', 'Gulono-1,4-lactone', 'Hexose-1,5-lactone', 'L-Galactono-1,4-lactone', 'L-Gulono-1,4-lactone', 'myo-Inosose-5']
237.06143	7.17E-11	0.5666956	0.5986669	0.64181	C8H14O8	[M-H]-	238.06887	237.0615936	-0.69	['3-Deoxy-D-manno-octulosonate']
257.00672	4.24E-07	1.2958549	0.9201223	0.650848	C11H8O6	[M+Na-2H]-	236.03209	257.0067586	-0.15	['2-Carboxy-2-hydroxy-8-carboxychromene', '2-Hydroxy-3-carboxybenzalpyruvate']
257.00672	4.24E-07	1.2958549	0.9201223	0.650848	C4H7O7P	[M+Hac-H]-	197.992943	257.0067966	-0.3	['Phosphoenol-4-deoxy-3-tetrulosonate']
257.00672	4.24E-07	1.2958549	0.9201223	0.650848	C6H11O9P	[M-H]-	258.014073	257.0067966	-0.3	['2-Dehydro-3-deoxy-6-phospho-D-gluconate', '2-Dehydro-3-deoxy-D-galactonate 6-phosphate', '2-Deoxy-5-keto-D-gluconic acid 6-phosphate', '6-Phospho-5-dehydro-2-deoxy-D-gluconate', 'D-Glucono-1,5-lactone 6-phosphate']
257.00672	4.24E-07	1.2958549	0.9201223	0.650848	C8H12O7	[M+K-2H]-	220.058305	257.0069116	-0.75	['(R)-(Homo)2-citrate', '1-Hydroxypentane-1,2,5-tricarboxylate']
256.02272	2.47E-08	0.8633354	0.6618869	0.65132	C11H9NO5	[M+Na-2H]-	235.048074	256.0227426	-0.09	['3,4-Dihydro-7-methoxy-2-methylene-3-oxo-2H-1,4-benzoxazine-5-carboxylic acid']
256.02272	2.47E-08	0.8633354	0.6618869	0.65132	C8H13NO6	[M+K-2H]-	219.074289	256.0228956	-0.69	['N-Acetyl-D-mannosaminolactone', 'O-Succinyl-L-homoserine']
606.19681	4.84E-08	0.6722764	0.7017095	0.65223	C30H35NO11	[M+Na-2H]-	585.221014	606.1956826	1.86	['epsilon-Rhodomycin T']
317.05467	9.89E-11	0.8820438	0.6932609	0.652948	C12H14N2O6	[M+Cl]-	282.085188	317.0545896	0.25	['Dinoseb acetate']
317.05467	9.89E-11	0.8820438	0.6932609	0.652948	C16H12N2O4	[M+Na-2H]-	296.079708	317.0543766	0.93	['3,3-Dimethoxybenzidine-4,4-diisocyanate']
505.20813	0.00073329	0.750391	0.6625687	0.658016	C24H30O8	[M+Hac-H]-	446.19407	505.2079236	0.41	['Estrone glucuronide', 'Yangambin']
505.20813	0.00073329	0.750391	0.6625687	0.658016	C26H34O10	[M-H]-	506.2152	505.2079236	0.41	['Limonoate']
333.05897	3.66E-11	0.7599722	0.6696672	0.658688	C17H14NO4	[M+(37Cl)]-	296.092284	333.0587356	0.7	['2,3,9,10-Tetrahydroxyberberine']
333.05897	3.66E-11	0.7599722	0.6696672	0.658688	C7H15O9P	[M+Hac-H]-	274.045373	333.0592266	-0.77	['1-Deoxy-D-altro-heptulose 7-phosphate']
333.05897	3.66E-11	0.7599722	0.6696672	0.658688	C9H19O11P	[M-H]-	334.066503	333.0592266	-0.77	['2-(alpha-D-Galactosyl)-sn-glycerol 3-phosphate', '2-(beta-D-Glucosyl)-sn-glycerol 3-phosphate', "alpha-D-Galactosyl-(1,1')-sn-glycerol 3-phosphate", 'sn-glycero-3-Phospho-1-inositol']

334.06244	1.92E-11	0.785739	0.6669554	0.659607	C12H15N3O6	[M+(37Cl)]-	297.096087	334.0625386	-0.3	['Musk xylene']
308.09861	2.60E-09	0.9275886	0.6489633	0.662561	C11H19NO9	[M-H]-	309.105984	308.0987076	-0.32	['N-Acetylneuraminate', 'O-Acetylneuraminic acid']
429.06512	3.27E-10	0.4298733	0.485579	0.663043						0
323.03847	1.94E-10	0.6472829	0.5793439	0.669212						0
269.08758	1.62E-07	0.9578489	0.7123637	0.675424	C13H16N2O2	[M+(37Cl)]-	232.121178	269.0876296	-0.18	['Aminoglutethimide', 'Melatonin']
269.08758	1.62E-07	0.9578489	0.7123637	0.675424	C7H14O7	[M+Hac-H]-	210.073955	269.0878086	-0.85	['Sedoheptulose', 'alpha-D-Mannoheptulopyranose', 'beta-D-Sedoheptulopyranose']
517.12002	0.0003364	0.7834451	0.8329271	0.69354						0
518.12344	0.00485222	0.7791812	0.7104766	0.697284						0
224.00538	0.01093812	0.6600771	0.6581408	0.703178	C9H7N3S	[M+Cl]-	189.036069	224.0054706	-0.4	['Tricyclazole']
205.03559	1.01E-06	0.8743662	0.7089508	0.710468	C5H6O5	[M+Hac-H]-	146.021525	205.0353786	1.03	['2-Oxoglutarate', '5-Hydroxy-2,4-dioxopentanoate', 'Dehydro-D-arabinono-1,4-lactone', 'Methyloxaloacetate', 'Oxaloacetate 4-methyl ester']
205.03559	1.01E-06	0.8743662	0.7089508	0.710468	C7H10O7	[M-H]-	206.042655	205.0353786	1.03	['(2S,3R)-3-Hydroxybutane-1,2,3-tricarboxylate', '(R)-2-Hydroxybutane-1,2,4-tricarboxylate', '2-Methylcitrate', 'Homoisocitrate']
245.04303	1.76E-06	0.8515053	0.7554842	0.712671	C11H12O5	[M+Na-2H]-	224.068475	245.0431436	-0.46	['Sinapate']
245.04303	1.76E-06	0.8515053	0.7554842	0.712671	C6H15O8P	[M-H]-	246.050458	245.0431816	-0.62	['Glycerophosphoglycerol']
245.04303	1.76E-06	0.8515053	0.7554842	0.712671	C7H14O7	[M+Cl]-	210.073955	245.0433566	-1.33	['Sedoheptulose', 'alpha-D-Mannoheptulopyranose', 'beta-D-Sedoheptulopyranose']
315.12955	3.69E-06	0.9917881	0.6712976	0.716674						0
601.13889	6.51E-09	0.7139133	0.7063692	0.719651						0
268.94688	0.02927171	1.0342467	0.8159588	0.733822						0
216.00585	0.00806021	0.6833554	1.0015149	0.744351						0
357.10371	2.85E-10	0.6460287	0.6819763	0.755495						0
221.0306	1.03E-10	1.514048	0.9663753	0.755694	C5H6O6	[M+Hac-H]-	162.01644	221.0302936	1.39	['4-Hydroxy-2-oxoglutarate', 'D-4-Hydroxy-2-oxoglutarate']
461.16637	0.00010206	0.7535825	0.8116996	0.757433	C24H28N2O5	[M+(37Cl)]-	424.199823	461.1662746	0.21	['Benazepril']
209.09348	3.24E-09	1.9581177	1.3310131	0.761755	C10H14N2O3	[M-H]-	210.100443	209.0931666	1.5	['2,6-Dihydroxypseudooxynicotine', 'Aprobarbital']
209.09348	3.24E-09	1.9581177	1.3310131	0.761755	C8H10N2O	[M+Hac-H]-	150.079313	209.0931666	1.5	['N-Methylanthranilamide']
217.03566	0.00303073	1.0980489	0.8153223	0.766704	C6H6O5	[M+Hac-H]-	158.021525	217.0353786	1.3	['2-Hydroxymuconate', '2-Maleylacetate', '3-Hydroxy-cis,cis-muconate', '4-Methylene-2-oxoglutarate', 'gamma-Oxalocrotonate']

605.19342	2.02E-05	0.7774883	0.8135175	0.770257						0
563.18298	0.00026707	0.9249743	0.7921744	0.770863	C18H32O16	[M+Hac-H]-	504.16904	563.1828936	0.15	['1F-beta-D-Fructosylsucrose', '6-alpha-Maltosylglucose', '6F-alpha-D-Galactosylsucrose', 'Cellotriose', 'D-Gal alpha 1->6D-Gal alpha 1->6D-Glucose', 'Galactomannan', 'Isomaltotriose', 'Maltotriose', 'Melezitose', 'Panose', 'Raffinose', 'Umbelliferose', 'beta-D-Fructofuranosyl O-beta-D-glucopyranosyl-(1-6)-alpha-D-glucopyranoside']
342.97422	0.01693952	0.9873538	1.035809	0.774457	C9H11N2O8P	[M+K-2H]-	306.025306	342.9739126	0.9	["2',3-Cyclic UMP"]
251.07712	0.01071764	1.2745136	0.9861017	0.77455	C13H14N2O	[M+(37Cl)]-	214.110613	251.0770646	0.22	['Harmaline']
251.07712	0.01071764	1.2745136	0.9861017	0.77455	C7H12O6	[M+Hac-H]-	192.06339	251.0772436	-0.49	['2D-5-O-Methyl-2,3,5/4,6-pentahydroxycyclohexanone', 'Quinate', 'Valiolone']
210.0411	3.47E-10	1.9695227	1.0548326	0.791598	C7H5NO3	[M+Hac-H]-	151.026944	210.0407976	1.44	['6-Imino-5-oxocyclohexa-1,3-dienecarboxylate']
210.0411	3.47E-10	1.9695227	1.0548326	0.791598	C9H9NO5	[M-H]-	211.048074	210.0407976	1.44	['5-(2-Formylethyl)-4,6-dihydroxypicolinate', 'Betalamic acid', 'DIMBOA']
152.99572	4.23E-06	0.7694445	0.8265903	0.792313	C3H7O5P	[M-H]-	154.003113	152.9958366	-0.76	['Glycerol 1,2-cyclic phosphate', 'Hydroxyacetone phosphate', 'Propanoyl phosphate']
152.99572	4.23E-06	0.7694445	0.8265903	0.792313	C4H6O4	[M+Cl]-	118.02661	152.9960116	-1.91	['Methyl oxalate', 'Methylmalonate', 'Succinate']
152.99572	4.23E-06	0.7694445	0.8265903	0.792313	C5H8O3	[M+K-2H]-	116.047345	152.9959516	-1.51	['2-Oxopentanoic acid', '3-Methyl-2-oxobutanoic acid', '3-Oxopentanoic acid', '5-Oxopentanoate']
310.11426	7.35E-07	1.0094389	0.7094194	0.795665	C15H19N3O2	[M+(37Cl)]-	273.147727	310.1141786	0.26	['N-Desmethylzolmitriptan']
310.11426	7.35E-07	1.0094389	0.7094194	0.795665	C9H17NO7	[M+Hac-H]-	251.100504	310.1143576	-0.31	['Muramic acid']
154.9751	3.08E-05	1.0407251	0.9465319	0.802833	C2H5O6P	[M-H]-	155.982378	154.9751016	-0.01	['2-Phosphoglycolate']
154.9751	3.08E-05	1.0407251	0.9465319	0.802833	C3H4O5	[M+Cl]-	120.005875	154.9752766	-1.14	['Hydroxymalonate']
154.9751	3.08E-05	1.0407251	0.9465319	0.802833	C4H6O4	[M+K-2H]-	118.02661	154.9752166	-0.75	['Methyl oxalate', 'Methylmalonate', 'Succinate']
152.95945	4.43E-06	0.8716356	0.8408769	0.803023	C3H2O5	[M+Cl]-	117.990225	152.9596266	-1.15	['Oxomalonate']
152.95945	4.43E-06	0.8716356	0.8408769	0.803023	C4H4O4	[M+K-2H]-	116.01096	152.9595666	-0.76	['Formylpyruvate', 'Fumarate', 'Maleic acid']
398.13041	0.00489583	1.3180826	0.7692107	0.805601						0
489.1462	7.43E-09	0.4196573	0.6723242	0.81228						0
151.02603	7.04E-07	0.8978715	0.8281472	0.824827	C5H4N4O2	[M-H]-	152.033426	151.0261496	-0.79	['Alloxanthine', 'Xanthine']
195.04875	6.08E-05	0.6236576	0.5474836	0.827591						0
603.1779	1.21E-07	0.4801917	0.7331303	0.838032						0
271.10321	2.73E-05	1.0601982	0.7937968	0.841613	C13H18N2O2	[M+(37Cl)]-	234.136828	271.1032796	-0.26	['Lenacil', 'p-Coumaroylputrescine']

271.10321	2.73E-05	1.0601982	0.7937968	0.841613	C7H16O7	[M+Hac-H]-	212.089605	271.1034586	-0.92	['Perseitol', 'Volemitol']
463.32768	0.49219606	0.9146847	1.0825694	0.848701						0
388.99778	1.73E-06	0.7626183	0.7696675	0.85262						0
215.00499	0.04544988	0.9250156	1.0660222	0.868341	C3H8O3S2	[M+Hac-H]-	155.991489	215.0053426	-1.64	['2-(Methylthio)ethanesulfonate']
387.11431	3.20E-05	1.1642861	0.8388888	0.876146	C17H24N2O4S	[M+Cl]-	352.14568	387.1150816	-1.99	['Mercaptoacetyl-Phe-Leu']
445.18686	0.45754487	0.7507914	1.0936735	0.900807	C22H26O6	[M+Hac-H]-	386.17294	445.1867936	0.15	['(+)-Eudesmin', 'Burseran']
445.18686	0.45754487	0.7507914	1.0936735	0.900807	C24H30O8	[M-H]-	446.19407	445.1867936	0.15	['Estrone glucuronide', 'Yangambin']
224.05672	3.36E-08	1.5943842	1.0123749	0.916325	C10H11NO5	[M-H]-	225.063724	224.0564476	1.22	['2-Amino-2-deoxyisochorismate', '4-Amino-4-deoxychorismate']
224.05672	3.36E-08	1.5943842	1.0123749	0.916325	C8H7NO3	[M+Hac-H]-	165.042594	224.0564476	1.22	['3,4-Dihydroxymandelonitrile', '4-Nitroacetophenone', '4-Pyridoxolactone', '5-Pyridoxolactone', 'Formylanthranilate', 'HBOA']
417.12494	0.00558841	1.0683865	0.89838	0.918479	C18H26N2O5S	[M+Cl]-	382.156245	417.1256466	-1.69	['Furathiocarb']
475.16681	0.34839962	0.972892	0.9598871	0.921405						0
388.11774	0.13887748	1.0989958	0.8676693	0.925065	C19H21N5O2	[M+K-2H]-	351.169525	388.1181316	-1.01	['Pirenzepine']
272.90563	0.03504469	1.164847	0.9726916	0.94008						0
469.0965	0.00035833	0.7699898	0.9243663	0.942129						0
647.14429	0.02487663	1.3287418	1.0693405	0.943864	C34H30N2O9	[M+K-2H]-	610.195133	647.1437396	0.85	['Atalanine']
201.02576	0.18876585	1.0835349	1.0919296	0.947852	C8H10N2S	[M+Cl]-	166.05647	201.0258716	-0.56	['Ethionamide']
416.98428	0.8492882	1.015241	0.9777597	0.950534						0
472.16733	0.01149644	1.4006218	1.1706685	0.954364	C21H31N3O5S	[M+Cl]-	437.198444	472.1678456	-1.09	['FMLP']
577.14134	0.0024369	0.8391029	1.1019944	0.965724						0
459.17202	0.26364783	0.9163207	0.9501999	0.969943						0
200.0567	3.76E-10	1.7606944	1.2592771	0.975589	C6H7NO3	[M+Hac-H]-	141.042594	200.0564476	1.26	['2-Aminomuconate semialdehyde', '6-Oxo-1,4,5,6-tetrahydronicotinate', 'Gentianaine']
517.14096	0.68986816	0.9324054	1.0185801	0.990394						0
313.11388	0.01942606	1.2527742	0.998339	0.994632	C9H18O8	[M+Hac-H]-	254.10017	313.1140236	-0.46	['2-(beta-D-Glucosyl)-sn-glycerol', '3-beta-D-Galactosyl-sn-glycerol']

401.13	0.10417652	1.0856536	1.0801216	1.001097	C12H22O11	[M+Hac-H]-	342.116215	401.1300686	-0.17	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']
311.09825	9.93E-09	1.518312	1.0126555	1.002915	C11H20O10	[M-H]-	312.10565	311.0983736	-0.4	['6-O-(beta-D-Xylopyranosyl)-beta-D-glucopyranose', '6-O-beta-D-Xylopyranosyl-D-glucose', 'Arabino-galactose', 'Vicianose']
311.09825	9.93E-09	1.518312	1.0126555	1.002915	C7H16N8O4	[M+Cl]-	276.129452	311.0988536	-1.94	['Trimethylenetetraurea']
297.08266	0.03789948	1.20141	0.9868918	1.021982	C14H16N2O3	[M+(37Cl)]-	260.116093	297.0825446	0.39	['Maculosin']
297.08266	0.03789948	1.20141	0.9868918	1.021982	C8H14O8	[M+Hac-H]-	238.06887	297.0827236	-0.21	['3-Deoxy-D-manno-octulosonate']
503.16175	0.05218903	1.283564	1.1582157	1.02389	C18H32O16	[M-H]-	504.16904	503.1617636	-0.03	['1F-beta-D-Fructosylsucrose', '6-alpha-Maltosylglucose', '6F-alpha-D-Galactosylsucrose', 'Cellotriose', 'D-Gal alpha 1->6D-Gal alpha 1->6D-Glucose', 'Galactomannan', 'Isomaltotriose', 'Maltotriose', 'Melezitose', 'Panose', 'Raffinose', 'Umbelliferose', 'beta-D-Fructofuranosyl O-beta-D-glucopyranosyl-(1-6)-alpha-D-glucopyranoside']
266.9397	0.61460998	1.0504721	0.9426151	1.038006						0
199.03795	0.64110124	0.9557178	1.0026336	1.040477	C10H10O3	[M+Na-2H]-	178.062995	199.0376636	1.44	['1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene', '3-Acetyl-6-methoxybenzaldehyde', 'Coniferyl aldehyde', 'Vermelone']
199.03795	0.64110124	0.9557178	1.0026336	1.040477	C3H9O4P	[M+Hac-H]-	140.023848	199.0377016	1.25	['2-Hydroxypropylphosphonate']

199.03795	0.64110124	0.9557178	1.0026336	1.040477	C6H12O5	[M+Cl]-	164.068475	199.0378766	0.37	['(+)-Quercitol', '(-)-Viburnitol', '1,5-Anhydro-D-glucitol', '1,5-Anhydro-D-mannitol', '2-Deoxy-D-galactose', '2-Deoxy-D-glucose', '6-Deoxy-D-galactose', '6-Deoxy-D-glucose', '6-Deoxy-L-galactose', 'D-Rhamnose', 'L-Fuculose', 'L-Rhamnofuranose', 'L-Rhamnose', 'L-Rhamnulose', 'alpha-D-Quinovopyranose', 'alpha-L-Rhamnose', 'beta-D-Fucose', 'beta-L-Rhamnose']
199.03795	0.64110124	0.9557178	1.0026336	1.040477	C7H14O2S	[M+(37Cl)]-	162.071452	199.0379036	0.23	['7-Mercaptoheptanoic acid']
199.03795	0.64110124	0.9557178	1.0026336	1.040477	C7H14O4	[M+K-2H]-	162.08921	199.0378166	0.67	['beta-Cymaropyranose', 'beta-L-Oleandropyranose']
505.17759	0.97515814	1.0029903	0.9963443	1.044953						0
519.15676	0.32298745	1.0433581	1.0920165	1.048378						0
323.06186	6.79E-05	0.6143976	0.8321323	1.050635						0
263.96788	0.41478987	1.0945957	0.9677855	1.058849	C5H10NO7P	[M+K-2H]-	227.019492	263.9680986	-0.83	['L-Glutamyl 1-phosphate', 'L-Glutamyl 5-phosphate', 'alpha-D-Glutamyl phosphate']
621.18837	0.08257576	0.8644057	0.8812629	1.061483						0
306.08298	1.72E-07	1.5379363	1.0634273	1.066674	C15H15N3O2	[M+(37Cl)]-	269.116427	306.0828786	0.33	['Disperse Yellow 3', 'Methyl red']
306.08298	1.72E-07	1.5379363	1.0634273	1.066674	C9H13NO7	[M+Hac-H]-	247.069204	306.0830576	-0.25	['N-Succinyl-L-glutamate']
341.07243	0.0201002	1.0374981	0.9518966	1.077556						0
487.13046	0.01301508	0.7701902	0.9755343	1.080991						0
341.10879	2.64E-05	1.071709	1.1837597	1.101231	C10H18O9	[M+Hac-H]-	282.095085	341.1089386	-0.44	['Xylobiose']
341.10879	2.64E-05	1.071709	1.1837597	1.101231	C12H22O11	[M-H]-	342.116215	341.1089386	-0.44	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']
399.07804	2.36E-09	2.0735073	1.2200164	1.118709	C14H17N2O4PS	[M+Hac-H]-	340.064668	399.0785216	-1.21	['Pyridafenthion']
521.17243	0.01721419	0.8776961	0.9999763	1.120026						0

473.11469	0.19656423	1.411616	1.2397144	1.128522						0
477.14613	0.27640069	0.9638726	0.9364469	1.13233						0
599.12333	2.44E-05	0.8754605	1.0083366	1.139792						0
192.02328	1.14E-08	2.2321375	1.6375073	1.142043						0
661.15987	0.00147709	1.2275803	1.145108	1.152819						0
383.08309	1.04E-09	2.577215	1.3772077	1.157212	C17H18N2O6	[M+(37Cl)]-	346.116488	383.0829396	0.39	['Miraxanthin-V', 'Nifedipine']
647.20385	0.00090076	0.8307284	0.8952926	1.176263	C33H34N6O6	[M+K-2H]-	610.253984	647.2025906	1.95	['Candesartan cilexetil']
345.08261	0.00763764	0.978572	1.0938536	1.226003	C18H16N2O3	[M+(37Cl)]-	308.116093	345.0825446	0.19	['Citrus Red No.2']
335.1571	0	3.9919919	2.6130833	1.270028						0
471.13555	4.41E-05	1.0004019	1.1966979	1.276521						0
355.08805	6.53E-07	1.4448639	1.180762	1.281174						0
412.14603	4.73E-12	2.5661531	1.7232769	1.302525	C22H26N2O4S	[M-2H]-	414.16133	412.1467772	-1.81	['Diltiazem']
591.17792	1.06E-06	1.0409529	1.3408306	1.303731						0
449.15129	0.00205325	0.9799767	1.2029758	1.305144						0
251.11484	4.27E-08	2.9365328	1.6132863	1.323062						0
597.08416	0.00031838	1.2277685	1.2987713	1.356039						0
262.09301	0.01034049	1.1711377	1.0475362	1.361226	C14H15N3	[M+(37Cl)]-	225.126597	262.0930486	-0.15	['Cyprodinil', 'ortho-Aminoazotoluene', 'para-(Dimethylamino)azobenzene']
262.09301	0.01034049	1.1711377	1.0475362	1.361226	C8H13NO5	[M+Hac-H]-	203.079374	262.0932276	-0.83	['N2-Acetyl-L-aminoadipate']
221.06693	9.78E-09	1.0199648	1.2554341	1.400382	C12H12N2	[M+(37Cl)]-	184.100048	221.0664996	1.95	['2,4-Diphenyldiamine', 'Benzidine', 'Diquat', 'Withasomnine']
221.06693	9.78E-09	1.0199648	1.2554341	1.400382	C6H10O5	[M+Hac-H]-	162.052825	221.0666786	1.14	['(2R,3S)-2,3-Dimethylmalate', '(R)-2-Ethylmalate', '(R)-3,3-Dimethylmalate', '(S)-2-(Hydroxymethyl)glutarate', '1,5-Anhydro-D-fructose', '2-Dehydro-3-deoxy-D-fuconate', '2-Dehydro-3-deoxy-L-fuconate', '2-Dehydro-3-deoxy-L-rhamnonate', '2-Deoxy-scyllo-inososе', '2-Hydroxyadipate', '3,6-Anhydrogalactose', '3,6-Anhydroglucose', '3-Ethylmalate', '3-Hydroxy-3-methylglutarate', 'D-Fucono-1,4-lactone', 'Diethyl pyrocarbonate', 'L-Fucono-1,5-lactone', 'L-Rhamnono-1,4-lactone', 'Lichenin']
221.06693	9.78E-09	1.0199648	1.2554341	1.400382	C8H14O7	[M-H]-	222.073955	221.0666786	1.14	['6-Acetyl-D-glucose']
152.0352	1.64E-11	2.0538587	1.7262199	1.401555	C7H7NO3	[M-H]-	153.042594	152.0353176	-0.77	['1-Carbapen-2-em-3-carboxylic acid', '2-Nitroanisole', '3-Amino-4-hydroxybenzoic acid', '3-Hydroxy-2-methylpyridine-5-carboxylate', '3-Hydroxyanthranilate', '4-Aminosalicylate', '4-Nitroanisole', 'AHBA', 'Salicylhydroxamic acid', 'o-Hydroxylaminobenzoate']

388.06509	0.03643281	1.4359156	1.3769155	1.403039	C11H20NO12P	[M-H]-	389.072317	388.0650406	0.13	['N-Acetylneuraminate 9-phosphate']
388.06509	0.03643281	1.4359156	1.3769155	1.403039	C13H21NO10	[M+K-2H]-	351.116549	388.0651556	-0.17	['N-Acetyl-4-O-acetylneuraminate', 'N-Acetyl-7-O-acetylneuraminate', 'N-Acetyl-9-O-acetylneuraminate']
223.10912	4.84E-10	2.0625306	1.1433096	1.424848	C9H12N2O	[M+Hac-H]-	164.094963	223.1088166	1.36	['Fenuron']
210.07746	1.88E-07	1.8867485	1.7806103	1.444953	C10H13NO4	[M-H]-	211.084459	210.0771826	1.32	['Enicoflavine', 'Methyldopa anhydrous']
210.07746	1.88E-07	1.8867485	1.7806103	1.444953	C8H9NO2	[M+Hac-H]-	151.063329	210.0771826	1.32	['(E)-4-Hydroxyphenylacetaldehyde-oxime', '(R)-Mandelamide', '(Z)-4-Hydroxyphenylacetaldehyde-oxime', '2-Amino-3-methylbenzoate', '2-Descarboxy-cyclo-dopa', 'Acetaminophen', 'Dopamine quinone', 'L-Phenylglycine', 'N-(Acetyloxy)benzenamine', 'N-Methyl-4-aminobenzoate', 'N-Methylanthranilate']
403.14566	2.72E-08	2.0390648	1.3553072	1.446327	C12H24O11	[M+Hac-H]-	344.131865	403.1457186	-0.15	['Clusianose', 'Melibiitol']
343.12444	9.31E-06	1.5187133	1.499538	1.452153	C12H24O11	[M-H]-	344.131865	343.1245886	-0.43	['Clusianose', 'Melibiitol']
343.12444	9.31E-06	1.5187133	1.499538	1.452153	C20H22N2S	[M+Na-2H]-	322.15037	343.1250386	-1.74	['Mequitazine']
197.04576	0.0003572	1.289618	1.4587064	1.459957	C7H14NO3	[M+K-2H]-	160.097369	197.0459756	-1.09	['3-Dehydrocarnitine']
197.04576	0.0003572	1.289618	1.4587064	1.459957	C7H6O3	[M+Hac-H]-	138.031695	197.0455486	1.07	['2-Hydroxy-5-methylquinone', '3,4-Dihydroxybenzaldehyde', '3-Hydroxybenzoate', '4-Hydroxybenzoate', 'Gentisate aldehyde', 'Salicylate', 'Sesamol']
197.04576	0.0003572	1.289618	1.4587064	1.459957	C9H10O5	[M-H]-	198.052825	197.0455486	1.07	['3-(3,4-Dihydroxyphenyl)lactate', '3-Methoxy-4-hydroxymandelate', 'Syringic acid']
446.31246	0.57723831	1.3973134	2.0250206	1.466737						0
265.09267	5.96E-05	1.1027568	1.2751198	1.629895						0
443.17721	2.20E-07	1.4212885	1.649314	1.635068	C17H26N8O5	[M+Na-2H]-	422.202617	443.1772856	-0.17	['Blasticidin S']
463.14555	3.87E-07	1.2273393	1.3925406	1.640055	C17H24O11	[M+Hac-H]-	404.131865	463.1457186	-0.36	['Gardenoside', 'Scandoside methyl ester']
438.16186	3.94E-10	3.6756697	2.7180226	1.653591						0
371.11945	3.03E-07	1.4700412	1.6568416	1.699604	C11H20O10	[M+Hac-H]-	312.10565	371.1195036	-0.14	['6-O-(beta-D-Xylopyranosyl)-beta-D-glucopyranose', '6-O-beta-D-Xylopyranosyl-D-glucose', 'Arabino-galactose', 'Vicianose']
513.12293	4.62E-06	1.0744056	1.6170062	1.719623						0
378.10416	3.00E-15	9.1166602	2.7338447	1.724065	C14H21NO11	[M-H]-	379.111464	378.1041876	-0.07	['3-(4-Deoxy-beta-D-gluc-4-enuronosyl)-N-acetyl-D-glucosamine', '4-Deoxy-beta-D-gluc-4-enuronosyl-(1,3)-N-acetyl-D-galactosamine']
382.13544	2.04E-08	1.773105	1.6014548	1.728624	C14H25NO11	[M-H]-	383.142764	382.1354876	-0.12	['Lacto-N-biose', 'N-Acetyllactosamine', 'beta-D-Galactosyl-(1->3)-N-acetyl-D-galactosamine']
382.13544	2.04E-08	1.773105	1.6014548	1.728624	C18H23N3O4	[M+(37Cl)]-	345.168857	382.1353086	0.34	['Pefurazoate']

147.06619	0.0029495	1.4189799	1.6491337	1.729317	C4H8O2	[M+Hac-H]-	88.05243	147.0662836	-0.64	['(R)-Acetoin', '1,4-Dioxane', '2-Methylpropanoate', 'Acetoin', 'Butanoic acid', 'Ethyl acetate']
147.06619	0.0029495	1.4189799	1.6491337	1.729317	C6H12O4	[M-H]-	148.07356	147.0662836	-0.64	['(R)-2,3-Dihydroxy-3-methylpentanoate', '(R)-Mevalonate', '(R)-Pantoate', '(S)-Mevalonate', '2,3-Dihydroxy-3-methylpentanoate', '3,6-Dideoxy-L-galactose', 'Abequose']
531.19307	2.73E-07	1.3096052	1.6127909	1.732604						0
267.10832	0.10758083	1.3488933	1.5092538	1.773126	C14H18N2O	[M+(37Cl)]-	230.141913	267.1083646	-0.17	['Camoensine']
212.05673	5.60E-11	1.8444209	1.8460338	1.796894	C7H7NO3	[M+Hac-H]-	153.042594	212.0564476	1.33	['1-Carbapen-2-em-3-carboxylic acid', '2-Nitroanisole', '3-Amino-4-hydroxybenzoic acid', '3-Hydroxy-2-methylpyridine-5-carboxylate', '3-Hydroxyanthranilate', '4-Aminosalicylate', '4-Nitroanisole', 'AHBA', 'Salicylhydroxamic acid', 'o-Hydroxylaminobenzoate']
212.05673	5.60E-11	1.8444209	1.8460338	1.796894	C9H11NO5	[M-H]-	213.063724	212.0564476	1.33	['N,N-Dihydroxy-L-tyrosine']
307.06697	4.83E-08	2.1565645	1.7126419	1.800215	C12H16NO7	[M+Na-2H]-	286.092679	307.0673476	-1.23	['N-Glucosylnicotinate']
307.06697	4.83E-08	2.1565645	1.7126419	1.800215	C15H14N2O3	[M+(37Cl)]-	270.100443	307.0668946	0.25	['Dihydroxycarbazepine']
669.1052	0.0009287	1.6873112	1.555145	1.804639						0
309.08264	3.74E-07	2.3785596	2.0986587	1.823537						0
525.0864	2.32E-10	1.0460035	1.6517741	1.825637						0
577.19863	4.00E-08	1.3968763	1.7670732	1.837293						0
323.09824	2.51E-06	1.4043843	1.6783233	1.851573	C12H20O10	[M-H]-	324.10565	323.0983736	-0.41	["Bis-D-fructose 2',1:2,1-dianhydride", "D-Fructofuranose 1,2':2,3-dianhydride"]
323.09824	2.51E-06	1.4043843	1.6783233	1.851573	C16H18N2O3	[M+(37Cl)]-	286.131743	323.0981946	0.14	['Cromakalim', 'Difenoxuron', 'Levcromakalim', 'Pilosine']
323.09824	2.51E-06	1.4043843	1.6783233	1.851573	C8H24N4O3P2	[M+(37Cl)]-	286.132367	323.0988186	-1.79	['Schradan']
407.05968	1.78E-08	1.7074064	1.8490362	1.858836	C16H18O11	[M+Na-2H]-	386.084915	407.0595836	0.24	['O-Feruloylgalactarate']
407.05968	1.78E-08	1.7074064	1.8490362	1.858836	C9H17O12P	[M+Hac-H]-	348.045768	407.0596216	0.14	['2-(alpha-D-Mannosyl)-3-phosphoglycerate', '2-O-(6-Phospho-alpha-mannosyl)-D-glycerate']
212.09313	0.00029309	1.8727739	1.880618	1.865334	C10H15NO4	[M-H]-	213.100109	212.0928326	1.4	['Kainic acid', 'N-(3-Oxohexanoyl)homoserine lactone', 'alpha-Allokainic acid']
212.09313	0.00029309	1.8727739	1.880618	1.865334	C8H11NO2	[M+Hac-H]-	153.078979	212.0928326	1.4	['1-(4-Hydroxyphenyl)-2-aminoethanol', 'Dopamine', 'Vanillylamine']
269.12529	7.98E-09	2.4763026	2.13995	1.874463						0
677.21465	4.61E-10	1.2683692	1.8861452	1.890582						0
247.04577	0.00013765	1.5782354	1.4470844	1.90651	C13H10N2O	[M+(37Cl)]-	210.079313	247.0457646	0.02	['2-Aminoacridone']

247.04577	0.00013765	1.5782354	1.4470844	1.90651	C7H8O6	[M+Hac-H]-	188.03209	247.0459436	-0.7	['(E)-2-(Methoxycarbonylmethyl)butenedioate', '(E)-3-(Methoxycarbonyl)pent-2-enedioate', '(Z)-But-1-ene-1,2,4-tricarboxylate', '(Z)-But-2-ene-1,2,3-tricarboxylate', 'trans-Homoaconitate']
151.06109	3.03E-07	1.3707113	1.9608998	1.91479	C3H8O3	[M+Hac-H]-	92.047345	151.0611986	-0.72	['Glycerol']
151.06109	3.03E-07	1.3707113	1.9608998	1.91479	C5H12O5	[M-H]-	152.068475	151.0611986	-0.72	['D-Apiitol', 'L-Arabitol', 'Ribitol', 'Xylitol']
333.02285	1.97E-10	1.0893804	1.6382619	1.959041	C12H15N2O3PS	[M+Cl]-	298.054103	333.0235046	-1.97	['Phoxim', 'Quinalphos']
333.02285	1.97E-10	1.0893804	1.6382619	1.959041	C6H11O10P	[M+Hac-H]-	274.008988	333.0228416	0.03	['1-Phospho-alpha-D-galacturonate', '3-Dehydro-L-gulonate 6-phosphate', '6-Phospho-2-dehydro-D-gluconate', 'D-Glucuronate 1-phosphate']
395.05971	4.92E-06	1.7397759	1.6814715	1.969053	C14H19N2O7P	[M+(37Cl)]-	358.092991	395.0594426	0.68	['N1-(5-Phospho-alpha-D-ribosyl)-5,6-dimethylbenzimidazole']
561.16717	7.93E-06	1.0262043	1.4743198	1.987671	C18H30O16	[M+Hac-H]-	502.15339	561.1672436	-0.13	['alpha-L-Rhamnopyranosyl-(1->2)-beta-D-galactopyranosyl-(1->2)-beta-D-glucuronopyranoside']
207.08768	3.31E-09	1.2723167	2.0001001	2.004061	C6H12O4	[M+Hac-H]-	148.07356	207.0874136	1.29	['(R)-2,3-Dihydroxy-3-methylpentanoate', '(R)-Mevalonate', '(R)-Pantoate', '(S)-Mevalonate', '2,3-Dihydroxy-3-methylpentanoate', '3,6-Dideoxy-L-galactose', 'Abequose']
481.09658	0.00158748	1.8948495	2.2241491	2.015567	C12H23O14P	[M+Hac-H]-	422.082548	481.0964016	0.37	['6-Phospho-beta-D-glucosyl-(1,4)-D-glucose', 'Lactose 6-phosphate', 'Maltose 6-phosphate', 'Sucrose 6-phosphate', 'alpha,alpha-Trehalose 6-phosphate', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside 6F-phosphate']
267.0719	1.29E-08	2.0563167	1.908346	2.01582	C9H16O9	[M-H]-	268.079435	267.0721586	-0.97	['2(alpha-D-Mannosyl)-D-glycerate']
203.05641	1.84E-10	1.8362368	2.172464	2.033347	C6H8O4	[M+Hac-H]-	144.04226	203.0561136	1.46	['2,3-Dimethylmaleate', '2-Methyleneglutarate', 'Methylitaconate', 'Triacetate']
203.05641	1.84E-10	1.8362368	2.172464	2.033347	C8H12O6	[M-H]-	204.06339	203.0561136	1.46	['3-o-Ethyl-L-ascorbic acid']
413.09384	0.00023455	2.853221	2.4425751	2.067906						0
268.07526	4.03E-09	2.1858686	1.9932921	2.075941						0
272.07734	4.09E-06	2.4971553	2.5479332	2.116802	C12H15N2O4	[M+Na-2H]-	251.103183	272.0778516	-1.88	['3-Oxohexobarbital']
272.07734	4.09E-06	2.4971553	2.5479332	2.116802	C9H11NO5	[M+Hac-H]-	213.063724	272.0775776	-0.87	['N,N-Dihydroxy-L-tyrosine']
549.16732	3.69E-08	2.0681477	2.1367664	2.129861						0

541.11776	1.41E-09	1.5353098	1.9941368	2.146672	C18H32O16	[M+K-2H]-	504.16904	541.1176466	0.21	['1F-beta-D-Fructosylsucrose', '6-alpha-Maltosylglucose', '6F-alpha-D-Galactosylsucrose', 'Cellotriose', 'D-Gal alpha 1->6D-Gal alpha 1->6D-Glucose', 'Galactomannan', 'Isomaltotriose', 'Maltotriose', 'Melezitose', 'Panose', 'Raffinose', 'Umbelliferose', 'beta-D-Fructofuranosyl O-beta-D-glucopyranosyl-(1-6)-alpha-D-glucopyranoside']
515.1619	1.35E-09	1.133866	1.8141361	2.218174						0
387.15071	0.00068105	1.7513409	1.8472211	2.251688						0
277.05629	5.56E-07	1.8160409	2.2337566	2.263406	C11H14NO6	[M+Na-2H]-	256.082114	277.0567826	-1.78	['Nicotinate D-ribonucleoside']
154.01447	7.61E-10	1.3246129	1.8501949	2.332328	C6H5NO4	[M-H]-	155.021859	154.0145826	-0.73	['2,6-Dihydroxynicotinate', '4-Nitrocatechol']
208.09825	1.70E-10	3.2286185	2.8991357	2.33911	C11H15NO3	[M-H]-	209.105194	208.0979176	1.6	['Anhalamine', 'Propoxur', 'Tyr-OEt', 'p-Lactophenetide']
208.09825	1.70E-10	3.2286185	2.8991357	2.33911	C9H11NO	[M+Hac-H]-	149.084064	208.0979176	1.6	['3-Phenylpropionaldoxim', 'D-Cathinone']
533.17238	7.48E-09	1.8621381	2.3805793	2.391576						0
511.10736	2.99E-10	1.7846116	2.2804387	2.4165						0
213.02579	5.44E-10	1.4608246	2.3079008	2.435811	C4H10O2S2	[M+Hac-H]-	154.012224	213.0260776	-1.35	['Dithioerythritol', 'Dithiothreitol']
429.1249	1.64E-11	1.5582525	1.9730319	2.439144						0
441.06537	2.77E-08	1.393014	2.0746617	2.440903						0
400.99776	9.15E-13	2.2376289	2.5501557	2.445803						0
349.05409	1.48E-07	2.8888772	2.2251783	2.451883	C11H20O10	[M+K-2H]-	312.10565	349.0542566	-0.48	['6-O-(beta-D-Xylopyranosyl)-beta-D-glucopyranose', '6-O-beta-D-Xylopyranosyl-D-glucose', 'Arabino-galactose', 'Vicianose']
349.05409	1.48E-07	2.8888772	2.2251783	2.451883	C14H16O9	[M+Na-2H]-	328.079435	349.0541036	-0.04	['2-Succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate', 'Bergenin']
349.05409	1.48E-07	2.8888772	2.2251783	2.451883	C7H15O10P	[M+Hac-H]-	290.040288	349.0541416	-0.15	['D-glycero-D-manno-Heptose 1-phosphate', 'D-glycero-D-manno-Heptose 7-phosphate', 'Sedoheptulose 1-phosphate', 'Sedoheptulose 7-phosphate']
534.17616	9.04E-09	1.996231	2.5228577	2.488739						0
519.05264	1.44E-05	1.6437678	2.550501	2.517986						0
517.17751	3.93E-09	1.4911424	2.2928153	2.546192						0
336.0935	7.75E-10	2.3136231	2.3302293	2.546633						0
467.14068	8.85E-10	1.0857067	2.1548589	2.574758						0
468.17233	8.37E-09	2.0344133	2.6607498	2.625511	C16H27NO11	[M+Hac-H]-	409.158414	468.1722676	0.13	['Linustatin']
328.08848	0.00333953	0.7314206	3.2645005	2.625638						0
543.1568	2.70E-08	0.9632301	1.7431449	2.666492						0

214.07238	1.43E-10	2.0024729	2.5473006	2.685338	C7H9NO3	[M+Hac-H]-	155.058244	214.0720976	1.32	['(3S,5S)-Carbapenam-3-carboxylic acid']
214.07238	1.43E-10	2.0024729	2.5473006	2.685338	C9H13NO5	[M-H]-	215.079374	214.0720976	1.32	['Succinylproline']
485.15129	2.34E-09	3.3119098	3.0212735	2.692304						0
392.11985	1.80E-07	2.6529722	2.7752758	2.777083						0
541.17748	3.18E-09	1.9802531	3.5750077	2.884382						0
457.11992	1.93E-09	2.2188947	2.9631352	2.896844	C20H24N2O8	[M+(37Cl)]-	420.153268	457.1197196	0.44	['2-N,6-N-Bis(2,3-dihydroxy-N-benzoyl)-L-serine']
467.08096	9.33E-11	2.3324016	2.7318179	2.899764						0
351.09314	1.58E-06	1.9982095	2.0825511	2.915146						0
253.09278	1.12E-05	2.564232	3.3736173	2.927675	C13H16N2O	[M+(37Cl)]-	216.126263	253.0927146	0.26	['Girgensonine', 'Tetrahydroharmine']
253.09278	1.12E-05	2.564232	3.3736173	2.927675	C7H14O6	[M+Hac-H]-	194.07904	253.0928936	-0.45	['(-)-Quebrachitol', '1-O-Methyl-myo-inositol', '3-O-Methyl-myo-inositol', '4-O-Methyl-myo-inositol', '5-O-Methyl-myo-inositol', '6-O-Methyl-myo-inositol', 'D-Pinitol', 'Methyl beta-D-galactoside', 'O-Methyl-scyllo-inositol']
253.09278	1.12E-05	2.564232	3.3736173	2.927675	C9H18O8	[M-H]-	254.10017	253.0928936	-0.45	['2-(beta-D-Glucosyl)-sn-glycerol', '3-beta-D-Galactosyl-sn-glycerol']
453.06527	3.66E-12	1.7743048	2.6935394	2.948445						0
305.02793	2.36E-09	2.7283129	3.1057682	2.948923	C9H16O9	[M+K-2H]-	268.079435	305.0280416	-0.37	['2(alpha-D-Mannosyl)-D-glycerate']
535.04747	1.06E-09	1.9830727	2.7997677	3.021548						0
340.09648	1.04E-09	2.5537685	2.6293846	3.028684	C17H15N5O	[M+Cl]-	305.12766	340.0970616	-1.71	['Zaleplon']
339.09311	7.69E-11	2.6889599	2.7452994	3.102891	C12H20O11	[M-H]-	340.100565	339.0932886	-0.53	['3-Ketolactose', '3-Ketosucrose', 'Cellobiono-1,5-lactone']
531.1567	7.30E-10	2.2380584	2.9356673	3.104888						0
527.16189	1.52E-06	1.8217221	2.5630108	3.168129						0
336.12988	2.09E-05	2.4797513	2.6852022	3.206049	C13H23NO9	[M-H]-	337.137284	336.1300076	-0.38	['Streptobiosamine']
513.1463	1.94E-10	0.8874944	2.3866428	3.221929						0
293.08754	3.21E-13	2.6808233	3.3887599	3.250388	C11H18O9	[M-H]-	294.095085	293.0878086	-0.92	['Tuliposide B']
293.08754	3.21E-13	2.6808233	3.3887599	3.250388	C15H16N2O2	[M+(37Cl)]-	256.121178	293.0876296	-0.31	['Ancymidol']
293.08754	3.21E-13	2.6808233	3.3887599	3.250388	C9H14O7	[M+Hac-H]-	234.073955	293.0878086	-0.92	['(R)-(Homo)3-citrate', '1-Hydroxyhexane-1,2,6-tricarboxylate']
454.15669	1.96E-09	3.0728973	3.5000202	3.263628	C21H27N3O6	[M+(37Cl)]-	417.189987	454.1564386	0.55	['Casimiroedine']
445.15642	2.66E-10	1.8409979	3.013828	3.275494	C20H30N2O5S	[M+Cl]-	410.187545	445.1569466	-1.18	['Benfuracarb']
223.04466	1.14E-06	1.2389683	3.0135906	3.308369						0
385.13516	2.32E-10	3.5944554	3.6322963	3.324913	C12H22O10	[M+Hac-H]-	326.1213	385.1351536	0.02	['2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose', 'Robinobiose', 'Rutinose']
385.13516	2.32E-10	3.5944554	3.6322963	3.324913	C18H24N2O5	[M+(37Cl)]-	348.168523	385.1349746	0.48	['Enalaprilate', 'Funebrine']
384.12285	1.07E-07	1.5472581	2.7685024	3.330142						0
547.18796	1.14E-07	1.8781706	2.8428449	3.352564						0

475.03454	1.22E-07	3.1271099	3.7228951	3.491515						0
347.0469	1.40E-13	2.3468745	3.5464483	3.578353	C13H16N2O5S	[M+Cl]-	312.077995	347.0473966	-1.43	['Epithienamycin B', 'Epithienamycin D']
452.14103	9.52E-11	3.8248025	4.1553498	3.610093						0
415.14568	2.62E-10	2.1150372	3.1898242	3.682884						0
335.0385	1.22E-11	3.0827858	3.4219048	3.757381	C6H13O10P	[M+Hac-H]-	276.024638	335.0384916	0.03	['2-Carboxy-D-arabinitol 1-phosphate', '6-Phospho-D-gluconate']
293.05118	1.20E-11	4.2632068	4.0511461	3.787062						0
461.15116	1.39E-08	2.47009	3.1928509	3.797204						0
191.0197	6.69E-12	10.989506	6.4675903	3.799667	C4H4O5	[M+Hac-H]-	132.005875	191.0197286	-0.15	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']
191.0197	6.69E-12	10.989506	6.4675903	3.799667	C6H8O7	[M-H]-	192.027005	191.0197286	-0.15	['(1R,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(1S,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(4R,5S)-4,5,6-Trihydroxy-2,3-dioxohexanoate', '2,5-Didehydro-D-gluconate', '2-Dehydro-3-deoxy-D-glucarate', '5-Dehydro-4-deoxy-D-glucarate', 'Carboxymethyloxysuccinate', 'Citrate', 'Isocitrate']
474.15463	1.30E-10	1.4102167	3.002475	3.885623						0
447.33288	0.00027997	3.1878961	5.3802663	3.895351						0
400.98934	1.08E-11	3.9512866	4.1585047	3.919364						0
397.09874	4.60E-12	1.973103	2.8698226	3.932107	C12H18O11	[M+Hac-H]-	338.084915	397.0987686	-0.07	['L-Ascorbic acid-2-glucoside']
397.09874	4.60E-12	1.973103	2.8698226	3.932107	C18H20N2O6	[M+(37Cl)]-	360.132138	397.0985896	0.38	['3-Methoxytyramine-betaxanthin', 'Nitrendipine']
397.09874	4.60E-12	1.973103	2.8698226	3.932107	C19H24N2O3S	[M+K-2H]-	360.150765	397.0993716	-1.59	['LY395153']
373.13511	5.04E-11	2.7544142	3.4958803	3.961131						0
545.17245	2.77E-10	1.227723	2.8300321	3.97262						0
487.1669	1.24E-09	2.0400498	3.7584996	4.078818						0
443.14075	7.07E-11	2.2849398	3.9855075	4.129649	C14H24O12	[M+Hac-H]-	384.12678	443.1406336	0.26	['Acetyl-maltose']
443.14075	7.07E-11	2.2849398	3.9855075	4.129649	C20H26N2O7	[M+(37Cl)]-	406.174003	443.1404546	0.67	['Myxochlin A']
443.14075	7.07E-11	2.2849398	3.9855075	4.129649	C20H28N2O5S	[M+Cl]-	408.171895	443.1412966	-1.23	['Tamsulosin']
473.15117	4.32E-11	1.5520364	3.1689033	4.159902						0
539.10222	7.93E-10	2.8657084	4.0723665	4.202114	C18H30O16	[M+K-2H]-	502.15339	539.1019966	0.41	['alpha-L-Rhamnopyranosyl-(1->2)-beta-D-galactopyranosyl-(1->2)-beta-D-glucuronopyranoside']
319.04353	2.72E-12	3.658652	4.1866656	4.218418	C10H18O9	[M+K-2H]-	282.095085	319.0436916	-0.51	['Xylobiose']
319.04353	2.72E-12	3.658652	4.1866656	4.218418	C13H14O8	[M+Na-2H]-	298.06887	319.0435386	-0.03	['D-Prephenyllactate']

319.04353	2.72E-12	3.658652	4.1866656	4.218418	C6H13O9P	[M+Hac-H]-	260.029723	319.0435766	-0.15	['(3S,4R)-Ketose 1-phosphate', '1D-myo-Inositol 3-phosphate', 'Aldohexose 6-phosphate', 'D-Allose 6-phosphate', 'D-Allulose 6-phosphate', 'D-Fructose 1-phosphate', 'D-Fructose 6-phosphate', 'D-Galactose 1-phosphate', 'D-Galactose 6-phosphate', 'D-Glucose 1-phosphate', 'D-Glucose 6-phosphate', 'D-Hamamelose 2(1)-phosphate', 'D-Hexose 6-phosphate', 'D-Mannose 1-phosphate', 'D-Mannose 6-phosphate', 'D-Tagatose 6-phosphate', 'D-arabino-Hex-3-ulose 6-phosphate', 'Fructose 1-phosphate(pyranose)', 'Hexose 1-phosphate', 'Inositol 1-phosphate', 'L-Galactose 1-phosphate', 'L-Gulose 1-phosphate', 'L-Tagatose 6-phosphate', 'L-myo-Inositol 5-phosphate', 'Sorbose 1-phosphate', 'alpha-D-Galactose 1-phosphate', 'alpha-D-Glucose 3-phosphate', 'alpha-D-Glucose 6-phosphate', 'alpha-D-Hexose 1-phosphate', 'alpha-D-Hexose 6-phosphate', 'beta-D-Fructose 2-phosphate', 'beta-D-Fructose 6-phosphate', 'beta-D-Glucose 1-phosphate', 'beta-D-Glucose 6-phosphate', 'myo-Inositol 4-phosphate']
469.15626	1.81E-11	2.4123072	3.8251926	4.224103						0
224.0496	1.89E-11	2.0733941	3.4173083	4.24793						0
509.09173	8.48E-13	1.5762087	3.7008657	4.265369						0
529.14108	2.20E-07	2.1867706	3.4903216	4.31744						0
205.07207	7.78E-08	3.3003648	4.4402087	4.32231	C6H10O4	[M+Hac-H]-	146.05791	205.0717636	1.49	['(R)-3-Hydroxy-3-methyl-2-oxopentanoate', '(R)-4-Dehydropantoate', '(S)-2-Aceto-2-hydroxybutanoate', '2-Aceto-2-hydroxybutanoate', '2-Dehydropantoate', '3-Hydroxy-3-methyl-2-oxopentanoic acid', '3-Hydroxy-5-oxohexanoate', '4-Hydroxy-2-oxohexanoic acid', 'Adipate', 'Mevaldate']
462.15483	9.42E-08	2.583375	3.3275961	4.325948						0
589.19867	3.03E-08	1.8103313	3.3728601	4.456761						0
413.13	5.27E-11	4.2175306	4.4788266	4.471917						0
483.11216	2.80E-07	3.2598389	4.0644544	4.481632						0
339.12949	2.55E-06	2.4059455	3.9326141	4.494842	C13H24O10	[M-H]-	340.13695	339.1296736	-0.54	['Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside']

441.12505	3.57E-10	2.6334666	4.0778814	4.532993	C20H24N2O7	[M+(37Cl)]-	404.158353	441.1248046	0.56	['Myxochelin A']
421.07528	1.04E-11	2.1936123	4.0857677	4.533534	C12H23O14P	[M-H]-	422.082548	421.0752716	0.02	['6-Phospho-beta-D-glucosyl-(1,4)-D-glucose', 'Lactose 6-phosphate', 'Maltose 6-phosphate', 'Sucrose 6-phosphate', 'alpha,alpha-Trehalose 6-phosphate', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside 6F-phosphate']
421.07528	1.04E-11	2.1936123	4.0857677	4.533534	C14H24O12	[M+K-2H]-	384.12678	421.0753866	-0.25	['Acetyl-maltose']
415.10928	1.18E-10	2.7089319	3.6646195	4.643629						0
353.10879	1.33E-10	2.6055777	4.2951352	4.66696	C11H18O9	[M+Hac-H]-	294.095085	353.1089386	-0.42	['Tuliposide B']
417.02061	5.63E-08	2.781381	3.3414251	4.673092						0
207.04913	1.81E-08	2.2139015	3.5047331	4.740241						0
325.11389	2.30E-06	2.9753195	4.4236702	4.902078	C12H22O10	[M-H]-	326.1213	325.1140236	-0.41	['2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose', 'Robinobiose', 'Rutinose']
325.11389	2.30E-06	2.9753195	4.4236702	4.902078	C16H20N2O3	[M+(37Cl)]-	288.147393	325.1138446	0.14	['Methyl 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-p-toluate', 'Methyl 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-m-toluate']
375.03352	6.37E-12	3.9828739	4.4704587	4.913412	C12H18O11	[M+K-2H]-	338.084915	375.0335216	0	['L-Ascorbic acid-2-glucoside']
375.03352	6.37E-12	3.9828739	4.4704587	4.913412	C14H17N2O4PS	[M+Cl]-	340.064668	375.0340696	-1.47	['Pyridafenthion']
375.03352	6.37E-12	3.9828739	4.4704587	4.913412	C15H14O10	[M+Na-2H]-	354.0587	375.0333686	0.4	['2-Caffeoylisocitrate']
337.11387	2.44E-06	3.0975974	4.4181921	4.987821	C11H18O8	[M+Hac-H]-	278.10017	337.1140236	-0.46	['Tuliposide A']
337.11387	2.44E-06	3.0975974	4.4181921	4.987821	C17H20N2O3	[M+(37Cl)]-	300.147393	337.1138446	0.08	['Bifenazate']
400.11776	5.27E-11	3.7647008	4.1045607	5.031193						0
191.01885	8.30E-14	78.900447	46.434825	5.038264	C5H6N4O3	[M+Na-2H]-	170.043991	191.0186596	1	['5-Ureido-4-imidazole carboxylate']
427.10926	5.20E-07	4.2059415	4.3800524	5.04602						0
386.10212	1.03E-11	4.4689105	4.2979373	5.152308						0
383.11943	1.46E-08	2.446913	4.419375	5.174557	C12H20O10	[M+Hac-H]-	324.10565	383.1195036	-0.19	["Bis-D-fructose 2',1:2,1-dianhydride", "D-Fructofuranose 1,2':2,3-dianhydride"]
383.11943	1.46E-08	2.446913	4.419375	5.174557	C14H24O12	[M-H]-	384.12678	383.1195036	-0.19	['Acetyl-maltose']
342.98365	5.65E-11	4.5011677	5.3427734	5.288079						0
385.00288	7.00E-15	3.4128219	5.0582649	5.397598						0
374.04946	1.64E-12	3.4432618	4.3059785	5.427069						0
441.16142	4.00E-14	1.9092537	3.7776368	5.472952						0
425.07021	4.18E-13	3.0542767	4.6732769	5.502602						0
265.05625	1.21E-12	3.7761599	4.1872425	5.55954	C7H10O7	[M+Hac-H]-	206.042655	265.0565086	-0.98	['(2S,3R)-3-Hydroxybutane-1,2,3-tricarboxylate', '(R)-2-Hydroxybutane-1,2,4-tricarboxylate', '2-Methylcitrate', 'Homoisocitrate']
265.05625	1.21E-12	3.7761599	4.1872425	5.55954	C9H16N4S2	[M+Na-2H]-	244.08164	265.0563086	-0.22	['Metiamide']

575.18313	2.30E-11	3.0755518	5.2656521	5.616734						0
385.09872	2.62E-11	4.8068869	4.7064377	5.636592						0
261.06137	3.45E-07	3.5717731	4.932399	5.758635						0
399.11433	1.04E-10	4.2011967	4.727204	5.764864	C12H20O11	[M+Hac-H]-	340.100565	399.1144186	-0.22	['3-Ketolactose', '3-Ketosucrose', 'Cellobiono-1,5-lactone']
399.11433	1.04E-10	4.2011967	4.727204	5.764864	C19H26N2O3S	[M+K-2H]-	362.166415	399.1150216	-1.73	['DU 122290']
223.04612	1.33E-12	2.6681982	4.6163182	5.765654	C11H10N2O	[M+(37Cl)]-	186.079313	223.0457646	1.59	['Credazine', 'Deoxyvasicinone']
223.04612	1.33E-12	2.6681982	4.6163182	5.765654	C5H8O6	[M+Hac-H]-	164.03209	223.0459436	0.79	['2-Dehydro-D-xylonate']
379.06471	3.81E-13	3.2647186	4.9133998	5.794453	C12H22O11	[M+K-2H]-	342.116215	379.0648216	-0.29	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']
266.05964	1.03E-11	3.9255859	4.2796763	5.840599						0
390.13344	3.85E-09	3.7589046	3.8146016	5.880415						0
424.14607	7.32E-13	5.5127403	6.0223677	5.920582						0
520.31284	0.04122018	3.1424856	7.3491811	5.992662						0
209.0556	2.20E-14	4.4884595	5.8024095	6.318664	C12H12O	[M+(37Cl)]-	172.088815	209.0552666	1.59	['Capillone']
410.13045	1.14E-11	3.527985	4.9790131	6.374387	C13H21NO10	[M+Hac-H]-	351.116549	410.1304026	0.12	['N-Acetyl-4-O-acetylneuraminate', 'N-Acetyl-7-O-acetylneuraminate', 'N-Acetyl-9-O-acetylneuraminate']
391.06473	1.30E-10	5.0653109	6.7670898	6.582032	C16H18O10	[M+Na-2H]-	370.09	391.0646686	0.16	['Fraxin']
208.05464	6.62E-13	3.9877925	5.44874	6.790505						0
389.12999	8.46E-12	4.4942104	5.3223434	6.887534	C17H26N2O4S	[M+Cl]-	354.16133	389.1307316	-1.91	['Sultopride']
482.15157	6.14E-06	5.0081626	6.9659244	6.995297						0
369.10382	8.30E-14	3.619161	4.972374	7.281638						0
263.07701	6.29E-09	2.8046349	6.3091821	7.297703	C14H14N2O	[M+(37Cl)]-	226.110613	263.0770646	-0.21	['Metyrapone']
263.07701	6.29E-09	2.8046349	6.3091821	7.297703	C8H12O6	[M+Hac-H]-	204.06339	263.0772436	-0.89	['3-o-Ethyl-L-ascorbic acid']
559.18811	1.84E-10	2.9053877	6.573124	7.365639						0

321.0826	3.18E-07	4.7487993	6.9876311	7.528341	C16H16N2O3	[M+(37Cl)]-	284.116093	321.0825446	0.17	['Metominostrobin']
351.15199	4.17E-13	5.5493446	8.4484312	7.944238						0
397.13509	2.66E-07	4.0239173	7.5415192	7.96425	C19H26N2O3S	[M+Cl]-	362.166415	397.1358166	-1.83	['DU 122290']
345.02282	3.81E-13	3.2764926	6.9503998	8.258422						0
381.10381	2.59E-13	2.7078004	5.2806353	8.366795						0
342.11931	1.80E-14	10.791161	9.7813507	8.565065						0
458.15976	9.54E-09	2.4369165	5.5648031	8.635193						0
437.07037	1.30E-14	4.26284	7.7518897	8.735145						0
427.14567	2.04E-07	2.1576393	4.7519551	8.815182						0
469.11975	1.27E-11	3.3654411	7.3528669	9.158153						0
355.12441	1.92E-11	1.7517238	4.7785364	9.405106						0
320.09856	4.65E-08	5.6995241	8.2086562	9.481797	C10H15NO7	[M+Hac-H]-	261.084854	320.0987076	-0.46	['Hymexazol O-glucoside', 'Hymexazol N-glucoside']
466.15666	2.47E-10	4.7789002	7.7821343	9.490838						0
207.05271	2.20E-14	5.5565958	8.5239201	10.15512						0
457.15626	1.14E-11	3.1909662	7.2016084	10.17518						0
326.98882	5.35E-13	7.0839207	10.490157	10.3027	C7H15O10P	[M+K-2H]-	290.040288	326.9888946	-0.23	['D-glycero-D-manno-Heptose 1-phosphate', 'D-glycero-D-manno-Heptose 7-phosphate', 'Sedoheptulose 1-phosphate', 'Sedoheptulose 7-phosphate']
451.08598	5.00E-15	5.8994555	9.5491668	10.36302						0
471.17194	7.57E-13	3.4207196	7.1570119	10.4715						0
453.10164	5.24E-13	4.3988393	9.867378	11.2169						0
359.03843	9.15E-13	6.2603968	10.267949	11.95718						0
358.05443	2.52E-13	5.5208847	9.586293	12.21416						0
425.13003	1.08E-11	9.7268731	12.59722	12.40165	C20H24N2O6	[M+(37Cl)]-	388.163438	425.1298896	0.33	['Nisoldipine']
455.14071	1.33E-12	3.8480884	9.624797	12.40364	C21H26N2O7	[M+(37Cl)]-	418.174003	455.1404546	0.56	['Nimodipine']
405.08029	5.00E-15	10.845743	14.601419	13.39942						0
529.17759	4.90E-12	2.5174434	8.1351353	13.8723						0

207.05096	2.80E-14	7.3718725	11.20727	13.87457	C5H8O5	[M+Hac-H]-	148.037175	207.0510286	-0.33	['(R)-2-Hydroxyglutarate', '(R)-2-Methylmalate', '(S)-2-Hydroxyglutarate', '(S)-2-Methylmalate', '2-Dehydro-3-deoxy-D-xylonate', '2-Dehydro-3-deoxy-L-arabinonate', '2-Hydroxyglutarate', 'Citramalate', 'D-Arabinono-1,4-lactone', 'D-Xylono-1,4-lactone', 'D-Xylonolactone', 'D-erythro-3-Methylmalate', 'D-threo-3-Methylmalate', 'L-Arabinono-1,4-lactone', 'L-Arabinono-1,5-lactone', 'L-Xylono-1,4-lactone', 'L-threo-3-Methylmalate']
279.07193	9.74E-13	6.498242	12.717197	15.25485	C8H12O7	[M+Hac-H]-	220.058305	279.0721586	-0.82	['(R)-(Homo)2-citrate', '1-Hydroxypentane-1,2,5-tricarboxylate']
411.11438	2.66E-12	8.3099995	14.870155	17.82251						0
394.13546	9.05E-10	4.1087288	9.6447409	19.1882						0
396.12283	3.30E-09	5.387287	13.906043	23.19137	C15H22N6O5S	[M-2H]-	398.137241	396.1226882	0.36	['S-Adenosyl-L-methionine']
399.1507	5.06E-09	2.798875	11.774758	24.01297	C13H24O10	[M+Hac-H]-	340.13695	399.1508036	-0.26	['Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside']
365.10894	6.52E-11	5.4811247	16.860221	28.96936	C18H22N2O2S	[M+Cl]-	330.1402	365.1096016	-1.81	['Pyributicarb']
365.10894	6.52E-11	5.4811247	16.860221	28.96936	C19H24N2OS	[M+K-2H]-	328.160935	365.1095416	-1.65	['Methotrimeprazine']
192.05968	1.88E-09	14.926316	35.449695	31.99183						0
453.12509	1.45E-09	8.0064399	20.573735	32.87536						0
395.11944	8.15E-10	6.9488762	20.350613	34.46331	C19H24N2O3S	[M+Cl]-	360.150765	395.1201666	-1.84	['LY395153']
208.05363	1.08E-10	6.9985704	10.95796	55.56405	C11H11NO	[M+Cl]-	173.084064	208.0534656	0.79	['1,3-Dimethyl-8-isoquinolinol', 'Pyroquilon']
208.05363	1.08E-10	6.9985704	10.95796	55.56405	C14H10O2	[M-2H]-	210.06808	208.0535272	0.49	['1,2-Anthracenediol', '9,10-Dihydroxyanthracene', 'Phenanthrene-3,4-diol']
191.05527	7.78E-08	31.535158	66.471808	57.64901	C6H10N4O2	[M+Na-2H]-	170.080376	191.0550446	1.18	['N-Isopropylammelide']
191.05623	4.01E-09	72.724748	144.77501	113.8161	C5H8O4	[M+Hac-H]-	132.04226	191.0561136	0.61	['(4S)-4,5-Dihydroxypentan-2,3-dione', '(S)-2-Acetolactate', '2-(Hydroxymethyl)-4-oxobutanoate', '2-Acetolactate', '3-Hydroxy-3-methyl-2-oxobutanoic acid', '4-Hydroxy-2-oxopentanoate', 'Deoxyribonolactone', 'Glutarate']
191.05623	4.01E-09	72.724748	144.77501	113.8161	C7H12O6	[M-H]-	192.06339	191.0561136	0.61	['2D-5-O-Methyl-2,3,5/4,6-pentahydroxycyclohexanone', 'Quinate', 'Valiolone']

Table 2 A table of m/z identified from the analysis of the white wine aliquots over four time points: zero, one, three, six months, which includes putative identifications. Univariate statistics were applied to this data; using the four time points, a fold change was determined using zero month as the reference time point, fold change 1 represents one month, fold change 2 represents three months, and fold change 3 represents the six month time point. The table is sorted on the smallest fold change 3 in conjunction with the smallest adjusted p-value, the peaks changing most significantly between zero month and six months, decreasing over time to increasing over time.

M/Z	adjusted p-value	fold change 1	fold change 2	fold change 3	Empirical formula (parent)	Ion form	Theoretical mass (neutral) (Da)	Theoretical m/z (Da)	Mass error (ppm)	KEGG_COMPOUND
193.03569	2.37E-11	0.012760957	0.003183353	0.002184046	C4H6O5	[M+Hac-H]-	134.021525	193.0353786	1.61	['(R)-Malate', '(S)-Malate', '3-Dehydro-L-threonate', 'Malate']
193.03569	2.37E-11	0.012760957	0.003183353	0.002184046	C6H10O7	[M-H]-	194.042655	193.0353786	1.61	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']
529.03705	1.81E-10	0.024171317	0.003751459	0.00293527						0
268.03919	7.87E-11	0.0160741	0.005888001	0.005709178						0
251.04101	1.66E-12	0.044394299	0.007859212	0.005711511	C6H8O7	[M+Hac-H]-	192.027005	251.0408586	0.6	['(1R,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(1S,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(4R,5S)-4,5,6-Trihydroxy-2,3-dioxohexanoate', '2,5-Didehydro-D-gluconate', '2-Dehydro-3-deoxy-D-glucarate', '5-Dehydro-4-deoxy-D-glucarate', 'Carboxymethyloxysuccinate', 'Citrate', 'Isocitrate']
251.04101	1.66E-12	0.044394299	0.007859212	0.005711511	C8H14N4S2	[M+Na-2H]-	230.06599	251.0406586	1.4	['Thiaburimamide']
267.03583	1.61E-10	0.017506945	0.006788292	0.006251924						0
513.05935	9.19E-11	0.032665725	0.00850576	0.007227499						0
491.12608	3.49E-09	0.034628872	0.008805512	0.007302791						0
327.0572	2.17E-10	0.027169891	0.008208649	0.007678674	C14H14N2O5	[M+(37Cl)]-	290.090273	327.0567246	1.45	['N2-Malonyl-D-tryptophan']
207.04994	0.002408805	0.018348179	0.009366546	0.008951516						0

215.01769	4.79E-10	0.02181524	0.009308708	0.00911371	C6H10O7	[M+Na-2H]-	194.042655	215.0173236	1.7	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']
273.01036	1.82E-11	0.079492172	0.019645468	0.01100845						0
461.18576	1.74E-05	0.071125351	0.015758176	0.011052202	C23H37O5P	[M+K-2H]-	424.237863	461.1864696	-1.54	['3beta-Hydroxy-16-phosphonopregn-5-en-20-one monoethyl ester']
379.04991	1.51E-10	0.056543304	0.014110242	0.0126704						0
475.13098	1.96E-11	0.039472899	0.014184307	0.012822007	C28H24O5	[M+Cl]-	440.162375	475.1317766	-1.68	['Marchantin A']
395.04485	3.32E-08	0.058882192	0.016239291	0.012903764	C11H23N2O7PS	[M+K-2H]-	358.096363	395.0449696	-0.3	['Pantetheine 4-phosphate']
476.13452	1.67E-11	0.039962607	0.014899046	0.013327292						0
395.02387	3.93E-08	0.06816137	0.01799405	0.013863973						0
445.0844	1.69E-11	0.070504677	0.018624773	0.015133199						0
475.20139	6.81E-05	0.079681231	0.020394274	0.017069455	C17H34N4O10	[M+Na-2H]-	454.227496	475.2021646	-1.63	['Ribostamycin', 'Xylostasin']
149.00871	4.32E-11	0.051111537	0.021029003	0.017442906						0
149.00929	2.19E-10	0.069968361	0.021218781	0.018218955	C2H2O4	[M+Hac-H]-	89.99531	149.0091636	0.85	['Oxalate']
149.00929	2.19E-10	0.069968361	0.021218781	0.018218955	C4H6O6	[M-H]-	150.01644	149.0091636	0.85	['(R,R)-Tartaric acid', '(S,S)-Tartaric acid', 'meso-Tartaric acid']
150.01266	1.58E-10	0.071815728	0.023493804	0.018979126						0
455.04508	2.86E-10	0.092563172	0.02180407	0.019623426						0
235.04602	5.01E-11	0.117763125	0.02964588	0.022862654	C12H10N2O	[M+(37Cl)]-	198.079313	235.0457646	1.09	['4-(2-Pyrazinylethenyl)phenol', '4-Hydroxyazobenzene', 'N-Nitrosodiphenylamine', 'para-Nitrosodiphenylamine']
235.04602	5.01E-11	0.117763125	0.02964588	0.022862654	C6H8O6	[M+Hac-H]-	176.03209	235.0459436	0.33	['(4S)-4,6-Dihydroxy-2,5-dioxohexanoate', '(4S,5S)-4,5-Dihydroxy-2,6-dioxohexanoate', '2-Dehydro-D-glucono-1,5-lactone', '2-Hydroxy-3-oxoadipate', '5-Dehydro-4-deoxy-D-glucuronate', 'Ascorbate', 'D-Galacturonolactone', 'D-Glucurono-6,2-lactone', 'D-Glucuronolactone', 'L-xylo-Hexulonolactone', 'Parapyruvate']
235.04602	5.01E-11	0.117763125	0.02964588	0.022862654	C7H15N3O2S2	[M-2H]-	237.060571	235.0460182	0.01	['Cartap']
159.03012	5.78E-13	0.038298153	0.024040367	0.022962486	C4H4O3	[M+Hac-H]-	100.016045	159.0298986	1.39	['Succinic anhydride']
159.03012	5.78E-13	0.038298153	0.024040367	0.022962486	C6H8O5	[M-H]-	160.037175	159.0298986	1.39	['2-Formylglutarate', '2-Oxoadipate', '3-Oxoadipate', '3D-(3,5/4)-Trihydroxycyclohexane-1,2-dione', 'D-2,3-Diketo 4-deoxy-epi-inositol']

667.30171	1.78E-05	0.094261171	0.033148825	0.023217509	C37H40N2O6	[M+Hac-H]-	608.288638	667.3024916	-1.17	['Berbamine', 'Gyrocarpine', 'Oxyacanthine', 'Pycnamine', 'Thalmine']
653.28604	1.90E-05	0.104129058	0.03367471	0.024867149	C36H38N2O6	[M+Hac-H]-	594.272988	653.2868416	-1.23	['(+)-Atherospermoline', '(+)-Bebeerine', 'Aromoline', 'Daphnandrine', 'Isochondrodendrine', 'Obamegine']
653.17932	8.30E-11	0.060729644	0.028385866	0.026915447						0
201.04086	5.64E-10	0.129743173	0.054020291	0.027090118	C6H14NO2S	[M+(37Cl)]-	164.074526	201.0409776	-0.58	['S-Methyl-L-methionine']
201.04086	5.64E-10	0.129743173	0.054020291	0.027090118	C6H6O4	[M+Hac-H]-	142.02661	201.0404636	1.97	['(S)-5-Oxo-2,5-dihydrofuran-2-acetate', '1,2,3,5-Tetrahydroxybenzene', '2,5-Dihydro-5-oxofuran-2-acetate', '2-Hydroxymuconate semialdehyde', '2-Oxo-2,3-dihydrofuran-5-acetate', 'Kojic acid', 'cis,cis-4-Hydroxymuconic semialdehyde', 'cis,cis-Muconate', 'cis,trans-Hexadienedioate']
681.31757	5.76E-05	0.109003786	0.035200065	0.027256159	C38H42N2O6	[M+Hac-H]-	622.304288	681.3181416	-0.84	['(+)-O-Methylthalicberine', '(+)-Tetrandrine', 'Cycleanine', 'Isotetrandrine', 'Obaberine', 'Rodiasine']
681.31757	5.76E-05	0.109003786	0.035200065	0.027256159	C40H46N2O8	[M-H]-	682.325418	681.3181416	-0.84	['Fetidine']
461.11554	2.08E-10	0.073571178	0.030181809	0.027857301						0
193.07211	7.91E-11	0.082547223	0.038425293	0.028959632	C5H10O4	[M+Hac-H]-	134.05791	193.0717636	1.79	['(R)-2,3-Dihydroxy-3-methylbutanoate', '1-Deoxy-D-xylulose', '2,3-Dihydroxy-3-methylbutanoate', '2-Deoxy-L-arabinose', '2-Deoxy-alpha-D-ribopyranose', 'Deoxyribose']
193.07211	7.91E-11	0.082547223	0.038425293	0.028959632	C7H14O6	[M-H]-	194.07904	193.0717636	1.79	['(-)-Quebrachitol', '1-O-Methyl-myo-inositol', '3-O-Methyl-myo-inositol', '4-O-Methyl-myo-inositol', '5-O-Methyl-myo-inositol', '6-O-Methyl-myo-inositol', 'D-Pinitol', 'Methyl beta-D-galactoside', 'O-Methyl-scyllo-inositol']
313.07796	2.02E-09	0.060072078	0.031853652	0.029339709	C7H16N8O4	[M+K-2H]-	276.129452	313.0780586	-0.31	['Trimethylenetetraurea']
205.08926	4.49E-11	0.031905477	0.027091918	0.029921495						0
603.0742	7.20E-10	0.207119834	0.043014147	0.029932791						0
647.14495	1.23E-10	0.114785036	0.038741534	0.032369544	C34H30N2O9	[M+K-2H]-	610.195133	647.1437396	1.87	['Atalanine']
265.02022	1.27E-11	0.175690012	0.048598865	0.033842732	C6H6O8	[M+Hac-H]-	206.00627	265.0201236	0.36	['3-Oxalomalate']
265.02022	1.27E-11	0.175690012	0.048598865	0.033842732	C8H12N4OS2	[M+Na-2H]-	244.045255	265.0199236	1.12	['CGP 52608']
789.51353	0.02313151	0.073035042	0.026354515	0.03519344						0
535.15252	5.69E-11	0.061989824	0.035987139	0.03519659						0
209.06711	3.13E-10	0.113343246	0.054017361	0.036216013						0
379.02002	6.77E-12	0.133384005	0.04215413	0.036508971						0
607.13747	1.81E-10	0.117756881	0.037599964	0.037555258						0

191.02007	1.45E-10	0.088398859	0.037153199	0.03914626	C4H4O5	[M+Hac-H]-	132.005875	191.0197286	1.79	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']
191.02007	1.45E-10	0.088398859	0.037153199	0.03914626	C6H8O7	[M-H]-	192.027005	191.0197286	1.79	['(1R,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(1S,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(4R,5S)-4,5,6-Trihydroxy-2,3-dioxohexanoate', '2,5-Didehydro-D-gluconate', '2-Dehydro-3-deoxy-D-glucarate', '5-Dehydro-4-deoxy-D-glucarate', 'Carboxymethyloxysuccinate', 'Citrate', 'Isocitrate']
439.08654	9.28E-11	0.087740147	0.042411723	0.039616762						0
195.08779	3.03E-10	0.071849065	0.041338294	0.040918021						0
197.08232	2.73E-10	0.078577557	0.044864742	0.041770362	C10H14O4	[M-H]-	198.08921	197.0819336	1.96	['1,2-Dihydroxymint lactone', '1-(3,4-Dimethoxyphenyl)ethane-1,2-diol', 'Paeonilactone A', 'cis-2,3-Dihydroxy-2,3-dihydro-p-cumate']
197.08232	2.73E-10	0.078577557	0.044864742	0.041770362	C7H16NO3	[M+Cl]-	162.113019	197.0824206	-0.51	['Carnitine']
197.08232	2.73E-10	0.078577557	0.044864742	0.041770362	C8H10O2	[M+Hac-H]-	138.06808	197.0819336	1.96	['1-(4-Hydroxyphenyl)ethanol', '2-Methyl-6-oxohepta-2,4-dienal', '3-Ethylcatechol', '3-Methoxybenzyl alcohol', '4-Hydroxyphenylethanol', 'Styrene cis-glycol']
197.08232	2.73E-10	0.078577557	0.044864742	0.041770362	C8H18NO2	[M+K-2H]-	160.133754	197.0823606	-0.21	['Methacholine']
587.0794	4.81E-10	0.181507725	0.047689598	0.042061405						0
395.01498	3.08E-09	0.177130432	0.055127267	0.042391402						0
489.21712	0.000205573	0.138175441	0.049835569	0.042679958	C18H36N4O10	[M+Na-2H]-	468.243146	489.2178146	-1.42	['Gentamicin A']
489.21712	0.000205573	0.138175441	0.049835569	0.042679958	C24H34N4O5S	[M-H]-	490.224993	489.2177166	-1.22	['Glimepiride']
401.13047	7.52E-12	0.103102444	0.053257771	0.043887464	C12H22O11	[M+Hac-H]-	342.116215	401.1300686	1	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']
402.13402	1.55E-11	0.103932714	0.054329495	0.04490222						0

329.10925	2.53E-05	0.131275838	0.038938896	0.04534704						0
459.20664	0.000119174	0.103195333	0.048958951	0.046068796	C23H37O5P	[M+Cl]-	424.237863	459.2072646	-1.36	['3beta-Hydroxy-16-phosphonopregn-5-en-20-one monoethyl ester']
513.10296	8.65E-09	0.039976458	0.04428579	0.047288625	C22H22N6O5S2	[M-H]-	514.109313	513.1020366	1.8	['Cefpirome']
597.35007	1.53E-08	0.12314928	0.053009628	0.048117539	C40H50O2	[M+Cl]-	562.38108	597.3504816	-0.69	['Rhodoxanthin']
312.06563	2.86E-09	0.09035123	0.049783729	0.04839714	C10H11N3O3S	[M+Hac-H]-	253.052114	312.0659676	-1.08	['Sulfamethoxazole']
312.06563	2.86E-09	0.09035123	0.049783729	0.04839714	C12H15N3O5S	[M-H]-	313.073244	312.0659676	-1.08	['Albendazole-beta-hydroxysulphone', 'Albendazole-gamma-hydroxysulphone']
625.38174	7.78E-08	0.110120554	0.049408334	0.048464379						0
375.31211	4.32E-10	0.097987968	0.046438035	0.048923044	C19H40O3	[M+Hac-H]-	316.297745	375.3115986	1.36	['1-O-Hexadecyl-sn-glycerol']
663.13944	3.62E-09	0.176182896	0.107312562	0.049098936						0
151.06118	2.07E-11	0.102217169	0.057190991	0.049153532	C3H8O3	[M+Hac-H]-	92.047345	151.0611986	-0.12	['Glycerol']
151.06118	2.07E-11	0.102217169	0.057190991	0.049153532	C5H12O5	[M-H]-	152.068475	151.0611986	-0.12	['D-Apiitol', 'L-Arabitol', 'Ribitol', 'Xylitol']
393.04451	7.03E-09	0.130718025	0.054776525	0.049267042	C15H16O11	[M+Na-2H]-	372.069265	393.0439336	1.47	['2-O-Caffeoylglucarate']
637.18449	9.44E-11	0.095505722	0.051681209	0.049780565						0
181.07209	1.69E-11	0.093147239	0.057911699	0.049998257	C4H10O4	[M+Hac-H]-	122.05791	181.0717636	1.8	['D-Threitol', 'Erythritol']
181.07209	1.69E-11	0.093147239	0.057911699	0.049998257	C6H14O6	[M-H]-	182.07904	181.0717636	1.8	['D-Iditol', 'D-Sorbitol', 'Galactitol', 'Mannitol']
181.07209	1.69E-11	0.093147239	0.057911699	0.049998257	C7H14NO3	[M+Na-2H]-	160.097369	181.0720376	0.29	['3-Dehydrocarnitine']
421.04797	4.10E-09	0.242283728	0.072903508	0.050578967	C15H18N2O8S	[M+Cl]-	386.07839	421.0477916	0.42	['(7R)-7-(4-Carboxybutanamido)cephalosporanate']
311.06233	1.93E-09	0.10507567	0.052817971	0.050843259						0
376.31547	2.28E-10	0.091829837	0.047481077	0.051567417						0
445.19259	5.60E-09	0.086377245	0.05266785	0.052716877						0
587.09651	1.94E-10	0.191425056	0.061405247	0.052764334	C32H22O10	[M+Na-2H]-	566.1213	587.0959686	0.92	['Ginkgetin']
152.06466	8.23E-11	0.102989816	0.059588393	0.053174979						0
211.08275	3.75E-10	0.097456614	0.063438294	0.054985309	C5H12O5	[M+Hac-H]-	152.068475	211.0823286	2	['D-Apiitol', 'L-Arabitol', 'Ribitol', 'Xylitol']
211.08275	3.75E-10	0.097456614	0.063438294	0.054985309	C7H16O7	[M-H]-	212.089605	211.0823286	2	['Perseitol', 'Volemitol']
269.08792	1.04E-11	0.08182843	0.065848621	0.056184409	C13H16N2O2	[M+(37Cl)]-	232.121178	269.0876296	1.08	['Aminoglutethimide', 'Melatonin']
269.08792	1.04E-11	0.08182843	0.065848621	0.056184409	C7H14O7	[M+Hac-H]-	210.073955	269.0878086	0.41	['Sedoheptulose', 'alpha-D-Mannoheptulopyranose', 'beta-D-Sedoheptulopyranose']
333.05959	5.77E-11	0.10261159	0.06062334	0.056873314	C7H15O9P	[M+Hac-H]-	274.045373	333.0592266	1.09	['1-Deoxy-D-altro-heptulose 7-phosphate']
333.05959	5.77E-11	0.10261159	0.06062334	0.056873314	C9H19O11P	[M-H]-	334.066503	333.0592266	1.09	['2-(alpha-D-Galactosyl)-sn-glycerol 3-phosphate', '2-(beta-D-Glucosyl)-sn-glycerol 3-phosphate', "alpha-D-Galactosyl-(1,1')-sn-glycerol 3-phosphate", 'sn-glycero-3-Phospho-1-inositol']
251.0774	1.31E-12	0.195313221	0.064052991	0.056885716	C13H14N2O	[M+(37Cl)]-	214.110613	251.0770646	1.34	['Harmaline']

251.0774	1.31E-12	0.195313221	0.064052991	0.056885716	C7H12O6	[M+Hac-H]-	192.06339	251.0772436	0.62	['2D-5-O-Methyl-2,3,5/4,6-pentahydroxycyclohexanone', 'Quinate', 'Valiolone']
216.00597	1.02E-10	0.07809873	0.054972799	0.057404172						0
309.08308	2.74E-10	0.150225212	0.0673631	0.058162226						0
473.22211	1.17E-06	0.115053539	0.062169826	0.05956796						0
373.06294	4.46E-11	0.206818671	0.075142631	0.060415739						0
195.05139	4.28E-12	0.056538268	0.051339729	0.061388003	C4H8O5	[M+Hac-H]-	136.037175	195.0510286	1.85	['Threonate']
195.05139	4.28E-12	0.056538268	0.051339729	0.061388003	C6H12O7	[M-H]-	196.058305	195.0510286	1.85	['2-Carboxy-D-arabinitol', 'D-Altronate', 'D-Gluconic acid', 'D-Mannonate']
215.00519	2.79E-11	0.082883135	0.06591649	0.063075443	C3H8O3S2	[M+Hac-H]-	155.991489	215.0053426	-0.71	['2-(Methylthio)ethanesulfonate']
799.23813	4.49E-11	0.150298171	0.07226101	0.067589117						0
401.09429	2.92E-09	0.124978605	0.065598927	0.068086103						0
403.10993	5.70E-07	0.116447098	0.067949052	0.070151245						0
549.16798	3.75E-10	0.241225096	0.087366254	0.071603034						0
347.28066	1.33E-06	0.150881041	0.06610394	0.071689294						0
550.17148	1.02E-09	0.239304354	0.087924587	0.072525585						0
207.0494	0.008654954	0.160231481	0.076488629	0.074016551						0
153.06554	3.09E-05	0.139289193	0.078667782	0.075339857						0
447.13628	3.50E-11	0.119856297	0.073511781	0.075491426	C20H30N2O5S	[M+K-2H]-	410.187545	447.1361516	0.29	['Benfuracarb']
219.05151	5.01E-10	0.182982357	0.094231059	0.076117718						0
267.07225	6.52E-12	0.140726105	0.081730699	0.079198856	C9H16O9	[M-H]-	268.079435	267.0721586	0.34	['2(alpha-D-Mannosyl)-D-glycerate']
601.13955	1.02E-09	0.181573233	0.093103371	0.081400273						0
293.10316	1.72E-09	0.16210397	0.083880985	0.082945031	C13H14O4	[M+Hac-H]-	234.08921	293.1030636	0.33	['1-Acetoxychavicol acetate', 'Coixinden B']
293.10316	1.72E-09	0.16210397	0.083880985	0.082945031	C15H18O6	[M-H]-	294.11034	293.1030636	0.33	['Tutin']
241.09297	7.14E-11	0.180398462	0.102519536	0.084006749	C12H16N2O	[M+(37Cl)]-	204.126263	241.0927146	1.06	['Bufotenine', 'Caulophylline', 'Psilocin']
241.09297	7.14E-11	0.180398462	0.102519536	0.084006749	C6H14O6	[M+Hac-H]-	182.07904	241.0928936	0.32	['D-Iditol', 'D-Sorbitol', 'Galactitol', 'Mannitol']
253.09303	2.86E-11	0.19835296	0.093804177	0.084356426	C13H16N2O	[M+(37Cl)]-	216.126263	253.0927146	1.25	['Girgensonine', 'Tetrahydroharmine']
253.09303	2.86E-11	0.19835296	0.093804177	0.084356426	C7H14O6	[M+Hac-H]-	194.07904	253.0928936	0.54	['(-)-Quebrachitol', '1-O-Methyl-myo-inositol', '3-O-Methyl-myo-inositol', '4-O-Methyl-myo-inositol', '5-O-Methyl-myo-inositol', '6-O-Methyl-myo-inositol', 'D-Pinitol', 'Methyl beta-D-galactoside', 'O-Methyl-scyllo-inositol']
253.09303	2.86E-11	0.19835296	0.093804177	0.084356426	C9H18O8	[M-H]-	254.10017	253.0928936	0.54	['2-(beta-D-Glucosyl)-sn-glycerol', '3-beta-D-Galactosyl-sn-glycerol']
313.11436	1.03E-09	0.159974651	0.095005472	0.087081929	C9H18O8	[M+Hac-H]-	254.10017	313.1140236	1.07	['2-(beta-D-Glucosyl)-sn-glycerol', '3-beta-D-Galactosyl-sn-glycerol']
471.14196	7.89E-06	0.013807634	0.078868163	0.088540887	C26H26O7	[M+Na-2H]-	450.167855	471.1425236	-1.2	["4-Benzyloxy-2-hydroxy-3',4',5',6-

										tetramethoxychalcone"]
208.05476	3.87E-10	0.15850753	0.092174244	0.091720853						0
505.2121	1.32E-05	0.293907487	0.111494174	0.092647365	C18H36N4O11	[M+Na-2H]-	484.238061	505.2127296	-1.25	['Kanamycin A', 'Kanamycin C']
727.2162	1.84E-09	0.217301758	0.100315415	0.09489121						0
177.04078	4.07E-11	0.135734242	0.097132949	0.095329537	C4H6O4	[M+Hac-H]-	118.02661	177.0404636	1.79	['Methyl oxalate', 'Methylmalonate', 'Succinate']
177.04078	4.07E-11	0.135734242	0.097132949	0.095329537	C6H10O6	[M-H]-	178.04774	177.0404636	1.79	['2,4,6/3,5-Pentahydroxycyclohexanone', '2-Dehydro-3-deoxy-D-galactonate', '2-Dehydro-3-deoxy-D-gluconate', '2-Dehydro-D-glucose', '2-Deoxy-5-keto-D-gluconic acid', '3-Dehydro-2-deoxy-D-gluconate', '3-Keto-beta-D-galactose', '5-Dehydro-2-deoxy-D-gluconate', '5-Dehydro-D-fructose', '5-Deoxy glucuronic acid', 'D-Galactono-1,4-lactone', 'D-Galactono-1,5-lactone', 'D-Glucono-1,4-lactone', 'D-Glucono-1,5-lactone', 'D-galacto-Hexodialdose', 'Gulono-1,4-lactone', 'Hexose-1,5-lactone', 'L-Galactono-1,4-lactone', 'L-Gulono-1,4-lactone', 'myo-Inosose-5']
196.01025	2.38E-07	0.255151118	0.116332763	0.096308774						0
207.04887	0.192271166	0.203922085	0.100018597	0.096945125						0
565.16309	1.81E-09	0.23452454	0.112702062	0.097771087	C32H32O7	[M+K-2H]-	528.214805	565.1634116	-0.57	['Karwinskione']
661.11705	3.02E-07	0.388075599	0.110035477	0.098485985	C27H30O17	[M+Cl]-	626.148305	661.1177066	-0.99	['Quercetin 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside']
325.07798	8.74E-10	0.291153964	0.120265491	0.099923288						0
175.06142	4.40E-09	0.179155739	0.106633986	0.100043262	C5H8O3	[M+Hac-H]-	116.047345	175.0611986	1.26	['2-Oxopentanoic acid', '3-Methyl-2-oxobutanoic acid', '3-Oxopentanoic acid', '5-Oxopentanoate']
175.06142	4.40E-09	0.179155739	0.106633986	0.100043262	C7H12O5	[M-H]-	176.068475	175.0611986	1.26	['(2R,3S)-3-Isopropylmalate', '(R)-2-(n-Propyl)-malate', '2-Propylmalate', '3-Propylmalate', 'alpha-Isopropylmalate']
311.16633	0.000567328	0.274957849	0.124997908	0.100235903						0
176.06487	3.09E-09	0.169800627	0.104689951	0.10072411						0
255.23041	1.73E-05	0.200331389	0.108090514	0.10202572						0
533.13687	1.18E-09	0.168253996	0.109991227	0.103627661						0
445.12074	1.69E-11	0.166852097	0.101146585	0.104227501	C20H28N2O5S	[M+K-2H]-	408.171895	445.1205016	0.54	['Tamsulosin']
445.12074	1.69E-11	0.166852097	0.101146585	0.104227501	C23H24N2O4S	[M+Na-2H]-	424.14568	445.1203486	0.88	['Eprosartan']
207.0529	1.48E-09	0.215060469	0.10778754	0.104289025						0
207.05244	3.49E-09	0.3459751	0.107787547	0.104289032						0
326.08134	9.59E-10	0.302100646	0.106775979	0.105423338	C11H13N3O3S	[M+Hac-H]-	267.067764	326.0816176	-0.85	['Sulfisoxazole']

563.18385	7.53E-11	0.150184015	0.107552214	0.107471812	C18H32O16	[M+Hac-H]-	504.16904	563.1828936	1.7	['1F-beta-D-Fructosylsucrose', '6-alpha-Maltosylglucose', '6F-alpha-D-Galactosylsucrose', 'Cellotriose', 'D-Gal alpha 1->6D-Gal alpha 1->6D-Glucose', 'Galactomannan', 'Isomaltotriose', 'Maltotriose', 'Melezitose', 'Panose', 'Raffinose', 'Umbelliferose', 'beta-D-Fructofuranosyl O-beta-D-glucopyranosyl-(1-6)-alpha-D-glucopyranoside']
563.18385	7.53E-11	0.150184015	0.107552214	0.107471812	C32H32O7	[M+Cl]-	528.214805	563.1842066	-0.63	['Karwinskione']
555.10601	1.72E-09	0.212908622	0.117994652	0.109793106						0
463.3284	1.19E-07	0.208102375	0.1062898	0.109934737						0
549.15949	2.24E-09	0.218145255	0.119322984	0.111711353	C24H32O13	[M+Na-2H]-	528.184295	549.1589636	0.96	['Deoxyloganic acid tetraacetate']
179.05643	1.24E-12	0.165783433	0.118732854	0.113267233	C4H8O4	[M+Hac-H]-	120.04226	179.0561136	1.77	['D-Erythrose', 'D-Erythrulose', 'D-Threose']
179.05643	1.24E-12	0.165783433	0.118732854	0.113267233	C6H12O6	[M-H]-	180.06339	179.0561136	1.77	['2-Deoxy-D-gluconate', 'Aldohexose', 'D-Aldose', 'D-Allose', 'D-Altrose', 'D-Fructose', 'D-Fuconate', 'D-Galactose', 'D-Glucose', 'D-Gulose', 'D-Hamamelose', 'D-Hexose', 'D-Idose', 'D-Mannose', 'D-Psicose', 'D-Sorbose', 'D-Tagatose', 'D-Talose', 'Fructose(pyranose)', 'Ketose', 'L-Fructose', 'L-Fuconate', 'L-Galactose', 'L-Gulose', 'L-Rhamnonate', 'L-Sorbose', 'Sorbose', 'alpha-D-Galactose', 'alpha-D-Glucose', 'alpha-D-Mannose', 'alpha-L-Sorbopyranose', 'beta-D-Fructose', 'beta-D-Galactose', 'beta-D-Glucose', 'beta-D-Hamamelopyranose', 'beta-D-Mannose', 'muco-Inositol', 'myo-Inositol', 'scyllo-Inositol']
399.07867	2.54E-08	0.234706605	0.11306597	0.117026159	C14H17N2O4PS	[M+Hac-H]-	340.064668	399.0785216	0.37	['Pyridafenthion']
464.15452	9.68E-07	0.342749728	0.14025758	0.117727181						0
437.09158	3.99E-07	0.623432324	0.142851868	0.118572821	C23H18N2O5	[M+Cl]-	402.121573	437.0909746	1.39	['Saphenamycin']
725.23663	1.69E-11	0.166322045	0.129258675	0.119182786	C24H42O21	[M+Hac-H]-	666.221865	725.2357186	1.26	['1,3-alpha-D-Mannosyl-1,2-alpha-D-mannosyl-1,2-alpha-D-mannosyl-D-mannose', 'Cellotetraose', 'Glycogen', 'Isolychnose', 'Lychnose', 'Maltotetraose', 'Stachyose', 'alpha-D-Galactosyl-(1-6)-alpha-D-galactosyl-(1-6)-beta-D-fructosyl-(2-1)-alpha-D-glucoside']
389.13058	5.54E-09	0.282563766	0.127553855	0.119592558	C17H26N2O4S	[M+Cl]-	354.16133	389.1307316	-0.39	['Sultopride']
679.20741	9.30E-06	0.092614305	0.117734912	0.119795402						0
566.16667	2.96E-09	0.231748516	0.131435186	0.12113899						0
445.18213	0.000373126	0.204927049	0.173077102	0.121550319	C22H32O7	[M+(37Cl)]-	408.214805	445.1812566	1.96	['Cascarillin', 'Nigakilactone F']

606.19782	2.37E-11	0.169412792	0.129141592	0.122838168						0
571.10137	1.09E-08	0.302679776	0.133642529	0.123057278	C29H26O10	[M+K-2H]-	534.1526	571.1012066	0.29	['Cercosporin']
555.0979	5.16E-09	0.284701706	0.136014702	0.123373931						0
473.07922	1.88E-09	0.711138401	0.16425272	0.123785942						0
387.11491	2.72E-10	0.270685307	0.133857817	0.124387842	C17H24N2O4S	[M+Cl]-	352.14568	387.1150816	-0.44	['Mercaptoacetyl-Phe-Leu']
295.06743	1.89E-10	0.250105408	0.149402149	0.125557774						0
587.11108	1.12E-08	0.106166699	0.130162595	0.125920409	C24H26N2O13	[M+(37Cl)]-	550.143493	587.1099446	1.93	['Betanin', 'Gomphrenin-I', 'Isobetanin']
388.11829	2.52E-10	0.271781525	0.134266105	0.12701935	C19H21N5O2	[M+K-2H]-	351.169525	388.1181316	0.41	['Pirenzepine']
388.11829	2.52E-10	0.271781525	0.134266105	0.12701935	C23H19NO5	[M-H]-	389.126324	388.1190476	-1.95	['(+/-)-6-Acetonyldihydrosanguinarine']
461.13077	1.40E-11	0.219489486	0.132722132	0.128969413						0
462.27163	0.031506332	0.195423403	0.075487839	0.129270103						0
415.07357	9.42E-07	0.185519912	0.123389127	0.129315866	C17H20N2O6S	[M+Cl]-	380.10421	415.0736116	-0.1	['Methicillin']
445.1793	0.000225892	0.141290623	0.145254425	0.130650729	C25H30O5	[M+Cl]-	410.209325	445.1787266	1.29	['Vismione D']
207.04832	2.01E-07	0.299470054	0.124961556	0.132826007						0
617.1219	5.59E-09	0.174768161	0.1192817	0.134550179	C34H28O9	[M+K-2H]-	580.173335	617.1219416	-0.07	['Mulberrofuran C']
445.1873	2.06E-08	0.164840874	0.138513395	0.135978849	C22H26O6	[M+Hac-H]-	386.17294	445.1867936	1.14	['(+)-Eudesmin', 'Burseran']
445.1873	2.06E-08	0.164840874	0.138513395	0.135978849	C24H30O8	[M-H]-	446.19407	445.1867936	1.14	['Estrone glucuronide', 'Yangambin']
321.02321	2.43E-08	0.255480124	0.130266484	0.13726328	C14H14NO4PS	[M-2H]-	323.038119	321.0235662	-1.11	['EPN']
321.02321	2.43E-08	0.255480124	0.130266484	0.13726328	C6H15O5PS2	[M+Hac-H]-	262.009857	321.0237106	-1.56	['Demeton-S-methylsulphon']
529.07349	4.17E-09	0.228140774	0.137345263	0.140801083						0
453.06601	1.12E-08	0.484691323	0.151698817	0.141761157	C13H19N4O12P	[M-H]-	454.073714	453.0664376	-0.94	['1-(5-Phosphoribosyl)-5-amino-4-(N-succinocarboxamide)-imidazole']
453.06601	1.12E-08	0.484691323	0.151698817	0.141761157	C15H20N4O8S	[M+(37Cl)]-	416.100188	453.0666396	-1.39	['O-Carbamoyl-deacetylcephalosporin C']
483.0765	5.34E-07	0.197216796	0.128115735	0.142011575	C16H22N4O9S	[M+(37Cl)]-	446.110753	483.0772046	-1.46	['Cephamycin C']
268.11197	1.14E-10	0.191525674	0.125557732	0.142207832						0
207.05344	0.003781863	0.298613004	0.14661007	0.142667309						0
459.13629	7.24E-11	0.218579431	0.154048299	0.14421659						0
192.05982	1.20E-09	0.313369854	0.168718416	0.14526497						0
605.19426	1.93E-13	0.18394206	0.142639057	0.147809402						0
460.13967	3.05E-10	0.201778688	0.142789618	0.148145063	C17H29NO11	[M+(37Cl)]-	423.174064	460.1405156	-1.84	['Neolinustatin']
223.04345	2.73E-07	0.315771397	0.154850674	0.148914673						0
519.22784	0.000578357	0.49327588	0.181741693	0.149017224						0
453.08642	2.75E-05	0.547482484	0.187459452	0.149309733						0
202.07254	2.81E-10	0.288709663	0.173937785	0.149354257	C9H13N2O2	[M+Na-2H]-	181.097703	202.0723716	0.83	['Pyridostigmine']
186.11252	0.008378706	0.112593668	0.042966029	0.152555408	C7H16N4O2	[M-2H]-	188.127326	186.1127732	-1.36	['Homoarginine', 'Ngamma-Monomethyl-L-arginine']
533.17315	3.12E-10	0.349179011	0.174261044	0.153522697						0
209.05572	3.66E-09	0.281249754	0.164311259	0.154027393						0

520.23132	0.000857341	0.510975489	0.187072962	0.1551474	C27H38O10	[M-2H]-	522.2465	520.2319472	-1.21	['Trilobolide']
489.11074	2.22E-08	0.309698628	0.161157528	0.15693472	C20H26N2O8S	[M+Cl]-	454.14099	489.1103916	0.71	['Sirodesmin H']
489.11074	2.22E-08	0.309698628	0.161157528	0.15693472	C28H22O6	[M+Cl]-	454.14164	489.1110416	-0.62	['Gnetin A', 'epsilon-Viniferin']
475.16744	1.01E-09	0.313605543	0.177772706	0.156937526						0
321.08301	3.92E-07	0.289071576	0.160027408	0.158636224	C16H16N2O3	[M+(37Cl)]-	284.116093	321.0825446	1.45	['Metominostrobin']
321.08301	3.92E-07	0.289071576	0.160027408	0.158636224	C17H20N2S	[M+K-2H]-	284.13472	321.0833266	-0.99	['Promazine', 'Promethazine']
341.06958	8.39E-08	0.285603355	0.146178739	0.162200026	C19H16N2O2	[M+K-2H]-	304.121178	341.0697846	-0.6	['Ceceline']
446.27671	0.015210175	0.222029583	0.094452282	0.162659041						0
396.34887	0.001730809	0.31099978	0.159031123	0.164364921						0
460.25591	0.066961158	0.190450987	0.091570441	0.165205051						0
371.12001	1.69E-11	0.230845377	0.163166294	0.165395505	C11H20O10	[M+Hac-H]-	312.10565	371.1195036	1.36	['6-O-(beta-D-Xylopyranosyl)-beta-D-glucopyranose', '6-O-beta-D-Xylopyranosyl-D-glucose', 'Arabino-galactose', 'Vicianose']
478.26654	0.047619382	0.171373535	0.089577751	0.165620973						0
323.09871	1.27E-10	0.262748958	0.161847721	0.167090986	C12H20O10	[M-H]-	324.10565	323.0983736	1.04	["Bis-D-fructose 2',1:2,1-dianhydride", "D-Fructofuranose 1,2':2,3-dianhydride"]
323.09871	1.27E-10	0.262748958	0.161847721	0.167090986	C16H18N2O3	[M+(37Cl)]-	286.131743	323.0981946	1.6	['Cromakalim', 'Difenoxuron', 'Levcromakalim', 'Pilosine']
323.09871	1.27E-10	0.262748958	0.161847721	0.167090986	C8H24N4O3P2	[M+(37Cl)]-	286.132367	323.0988186	-0.34	['Schradan']
267.10862	1.47E-10	0.212058175	0.161099801	0.167849609	C14H18N2O	[M+(37Cl)]-	230.141913	267.1083646	0.96	['Camoensine']
477.14687	2.35E-06	0.360863868	0.158614948	0.169118014						0
339.19716	0.000336891	0.461390107	0.213496187	0.169133172	C22H28O3	[M-H]-	340.203845	339.1965686	1.74	['17-Hydroxy-3-oxo-17alpha-pregna-1,4-diene-21-carboxylic acid, gamma-lactone', 'Norethindrone acetate']
305.02828	5.26E-08	0.402039626	0.170630851	0.169977117	C6H15O4PS2	[M+Hac-H]-	246.014942	305.0287956	-1.69	['Oxydemeton-methyl']
305.02828	5.26E-08	0.402039626	0.170630851	0.169977117	C9H16O9	[M+K-2H]-	268.079435	305.0280416	0.78	['2(alpha-D-Mannosyl)-D-glycerate']
417.12559	2.62E-06	0.294769827	0.205368663	0.170276529	C18H26N2O5S	[M+Cl]-	382.156245	417.1256466	-0.14	['Furathiocarb']
262.09333	1.66E-09	0.320489828	0.192801808	0.171392231	C14H15N3	[M+(37Cl)]-	225.126597	262.0930486	1.07	['Cyprodinil', 'ortho-Aminoazotoluene', 'para-(Dimethylamino)azobenzene']
262.09333	1.66E-09	0.320489828	0.192801808	0.171392231	C8H13NO5	[M+Hac-H]-	203.079374	262.0932276	0.39	['N2-Acetyl-L-aminoadipate']
240.08066	1.70E-10	0.275261645	0.187774532	0.172443871						0
341.07285	2.51E-09	0.304861725	0.16694804	0.175350997						0
651.1639	1.35E-10	0.278147953	0.176136077	0.175943429						0
695.31327	9.08E-09	0.267078563	0.201753162	0.176307268						0
534.24688	0.000493546	0.536092585	0.211369194	0.17717667						0

239.07731	1.12E-10	0.306502922	0.198685718	0.178200051	C6H12O6	[M+Hac-H]-	180.06339	239.0772436	0.28	['2-Deoxy-D-gluconate', 'Aldohexose', 'D-Aldose', 'D-Allose', 'D-Altrose', 'D-Fructose', 'D-Fuconate', 'D-Galactose', 'D-Glucose', 'D-Gulose', 'D-Hamamelose', 'D-Hexose', 'D-Idose', 'D-Mannose', 'D-Psicose', 'D-Sorbose', 'D-Tagatose', 'D-Talose', 'Fructose(pyranose)', 'Ketose', 'L-Fructose', 'L-Fuconate', 'L-Galactose', 'L-Gulose', 'L-Rhamnonate', 'L-Sorbose', 'Sorbose', 'alpha-D-Galactose', 'alpha-D-Glucose', 'alpha-D-Mannose', 'alpha-L-Sorbopyranose', 'beta-D-Fructose', 'beta-D-Galactose', 'beta-D-Glucose', 'beta-D-Hamamelopyranose', 'beta-D-Mannose', 'muco-Inositol', 'myo-Inositol', 'scyllo-Inositol']
309.11947	7.91E-11	0.328766764	0.192061619	0.181556467	C16H20N2O2	[M+(37Cl)]-	272.152478	309.1189296	1.75	['4-(3-Methylbut-2-enyl)-L-tryptophan', 'Dimethylallyltryptophan']
533.24336	0.001538379	0.559270459	0.211596767	0.181799398	C26H42NO7S	[M+Na-2H]-	512.268201	533.2428696	0.92	['Sulfoglycolithocholate']
200.12961	0.001322894	0.177780396	0.096442619	0.182861947	C8H15NO	[M+Hac-H]-	141.115364	200.1292176	1.96	['(-)-Hygrine', 'Hygrine', 'Pelletierine', 'Physoperuvine', 'Trachelanthamidine', 'Tropine']
314.27041	7.43E-05	0.279156614	0.16416566	0.183215598						0
326.27041	0.000110993	0.348192373	0.202101311	0.18347147						0
535.11623	4.36E-05	0.400722188	0.186811389	0.18368769						0
327.09361	6.65E-10	0.262299198	0.178058044	0.185606325	C9H16O9	[M+Hac-H]-	268.079435	327.0932886	0.98	['2(alpha-D-Mannosyl)-D-glycerate']
591.14277	8.22E-10	0.272142168	0.161281848	0.186492966						0
249.06173	2.31E-11	0.401460938	0.192445669	0.186967812	C13H12N2O	[M+(37Cl)]-	212.094963	249.0614146	1.27	['Harmine', 'Pyocyanine']
249.06173	2.31E-11	0.401460938	0.192445669	0.186967812	C7H10O6	[M+Hac-H]-	190.04774	249.0615936	0.55	['2,4-Dihydroxyhept-2-enedioate', '3-Dehydroquinate', '4-Hydroxy-2-oxo-heptanedioate', '4-Hydroxy-4-methyl-2-oxoadipate']
649.14817	2.04E-08	0.294668822	0.19068029	0.187763668						0
342.07628	1.20E-08	0.311498272	0.177002835	0.187884503	C17H15N5O	[M+K-2H]-	305.12766	342.0762666	0.04	['Zaleplon']
457.0843	9.14E-10	0.674923098	0.238430542	0.187890051						0
519.12124	3.32E-09	0.464728687	0.208792792	0.191370604						0
534.17673	6.23E-11	0.402073673	0.211157844	0.191510455	C20H35NO13	[M+(37Cl)]-	497.210844	534.1772956	-1.06	['Validamycin A']
237.06168	9.39E-09	0.317005072	0.202711873	0.192354019	C12H12N2O	[M+(37Cl)]-	200.094963	237.0614146	1.12	['4,4-Diaminodiphenyl ether', 'Harmalol']

237.06168	9.39E-09	0.317005072	0.202711873	0.192354019	C6H10O6	[M+Hac-H]-	178.04774	237.0615936	0.36	['2,4,6/3,5-Pentahydroxycyclohexanone', '2-Dehydro-3-deoxy-D-galactonate', '2-Dehydro-3-deoxy-D-gluconate', '2-Dehydro-D-glucose', '2-Deoxy-5-keto-D-gluconic acid', '3-Dehydro-2-deoxy-D-gluconate', '3-Keto-beta-D-galactose', '5-Dehydro-2-deoxy-D-gluconate', '5-Dehydro-D-fructose', '5-Deoxy glucuronic acid', 'D-Galactono-1,4-lactone', 'D-Galactono-1,5-lactone', 'D-Glucono-1,4-lactone', 'D-Glucono-1,5-lactone', 'D-galacto-Hexodialdose', 'Gulono-1,4-lactone', 'Hexose-1,5-lactone', 'L-Galactono-1,4-lactone', 'L-Gulono-1,4-lactone', 'myo-Inosose-5']
237.06168	9.39E-09	0.317005072	0.202711873	0.192354019	C8H14O8	[M-H]-	238.06887	237.0615936	0.36	['3-Deoxy-D-manno-octulosonate']
205.07218	8.38E-10	0.473539503	0.23023341	0.19424863						0
203.05654	9.62E-09	0.336299332	0.2319242	0.194425103						0
327.2657	0.000214538	0.363880153	0.210790591	0.195390142						0
312.25477	0.000689718	0.31385083	0.205744252	0.198699	C18H35NO3	[M-H]-	313.261694	312.2544176	1.13	['(+)-Prosopinine']
223.04398	4.36E-10	0.388627349	0.223620043	0.199294136						0
469.06081	2.01E-07	0.455248885	0.229579778	0.200615483	C15H20N4O9S	[M+(37Cl)]-	432.095103	469.0615546	-1.59	['7a-Hydroxy-O-carbamoyl-deacetylcephalosporin C']
186.11385	0.008951729	0.070804779	0.051082966	0.201681397	C7H13NO	[M+Hac-H]-	127.099714	186.1135676	1.52	['N-Cyclohexylformamide']
186.11385	0.008951729	0.070804779	0.051082966	0.201681397	C9H17NO3	[M-H]-	187.120844	186.1135676	1.52	['(E)-2-Butenyl-4-methyl-threonine', '8-Amino-7-oxononanoate', 'N2-Acetyl-L-lysine']
520.16102	4.02E-09	0.404443637	0.210901388	0.202235327						0
592.1459	3.08E-10	0.256310781	0.181289639	0.203193972						0
205.03583	1.55E-11	0.168867642	0.192812829	0.203933116						0
458.12395	4.53E-10	0.474368176	0.236143756	0.204091187						0
457.12049	4.80E-10	0.47451844	0.235765773	0.204470695	C20H24N2O8	[M+(37Cl)]-	420.153268	457.1197196	1.69	['2-N,6-N-Bis(2,3-dihydroxy-N-benzoyl)-L-serine']
328.2861	7.79E-05	0.30487013	0.194071981	0.206398976	C17H35NO	[M+Hac-H]-	269.271864	328.2857176	1.16	['Capsi-amide']
461.15191	3.65E-09	0.611495276	0.24530262	0.207706102						0
529.08229	3.26E-08	0.402992496	0.205526621	0.208902923						0
326.23405	0.034159797	0.274475177	0.140394139	0.209044445						0
547.25904	0.002668841	0.616855884	0.266308142	0.210165583						0
513.08727	1.34E-08	0.622846283	0.225858875	0.212667645	C13H19N4O12P	[M+Hac-H]-	454.073714	513.0875676	-0.58	['1-(5-Phosphoribosyl)-5-amino-4-(N-succinocarboxamide)-imidazole']
519.15753	1.86E-09	0.400057042	0.221445581	0.212870217						0
205.03342	1.19E-05	0.256621674	0.175401365	0.214065347						0

328.24968	0.003757131	0.288993959	0.162515853	0.214733185						0
371.26386	0.029536825	0.296039416	0.18499692	0.214873888						0
191.05638	3.89E-09	0.440378898	0.231357274	0.217954356	C5H8O4	[M+Hac-H]-	132.04226	191.0561136	1.39	['(4S)-4,5-Dihydroxypentan-2,3-dione', '(S)-2-Acetolactate', '2-(Hydroxymethyl)-4-oxobutanoate', '2-Acetolactate', '3-Hydroxy-3-methyl-2-oxobutanoic acid', '4-Hydroxy-2-oxopentanoate', 'Deoxyribonolactone', 'Glutarate']
191.05638	3.89E-09	0.440378898	0.231357274	0.217954356	C7H12O6	[M-H]-	192.06339	191.0561136	1.39	['2D-5-O-Methyl-2,3,5/4,6-pentahydroxycyclohexanone', 'Quinate', 'Valiolone']
445.15717	1.04E-09	0.462481132	0.18751109	0.219387826	C20H30N2O5S	[M+Cl]-	410.187545	445.1569466	0.5	['Benfuracarb']
439.07749	2.29E-07	0.368242052	0.236323157	0.223250409						0
368.24479	0.025239854	0.259072143	0.164009026	0.227008966						0
399.25058	1.90E-05	0.275264297	0.198171653	0.233209577						0
311.09872	3.39E-10	0.292177966	0.22243845	0.233210718	C11H20O10	[M-H]-	312.10565	311.0983736	1.11	['6-O-(beta-D-Xylopyranosyl)-beta-D-glucopyranose', '6-O-beta-D-Xylopyranosyl-D-glucose', 'Arabino-galactose', 'Vicianose']
311.09872	3.39E-10	0.292177966	0.22243845	0.233210718	C7H16N8O4	[M+Cl]-	276.129452	311.0988536	-0.43	['Trimethylenetetraurea']
745.4872	0.080534177	0.290001891	0.162389862	0.234256507						0
607.46046	0.008345374	0.634567228	0.300117447	0.236728052						0
677.11147	0.000179672	0.792150361	0.292971073	0.240517172						0
374.2829	0.079392584	0.253073942	0.174797196	0.242924056	C24H40O3	[M-2H]-	376.297745	374.2831922	-0.78	['Isolithocholate', 'Lithocholic acid']
592.44168	0.016502644	0.683661941	0.334514602	0.243911949						0
666.18265	1.91E-09	0.860676671	0.302690849	0.245031815						0
370.26045	0.04281981	0.328019651	0.185778953	0.24707317						0
665.21513	4.31E-12	0.320724378	0.246276003	0.247584441	C24H42O21	[M-H]-	666.221865	665.2145886	0.81	['1,3-alpha-D-Mannosyl-1,2-alpha-D-mannosyl-1,2-alpha-D-mannosyl-D-mannose', 'Cellotetraose', 'Glycogen', 'Isolychnose', 'Lychnose', 'Maltotetraose', 'Stachyose', 'alpha-D-Galactosyl-(1-6)-alpha-D-galactosyl-(1-6)-beta-D-fructosyl-(2-1)-alpha-D-glucoside']
665.21513	4.31E-12	0.320724378	0.246276003	0.247584441	C37H40O9	[M+K-2H]-	628.267235	665.2158416	-1.07	['Resiniferatoxin']
606.45728	0.010683619	0.691024884	0.314762186	0.249805293						0
374.29173	0.029900715	0.272333208	0.178090486	0.250036235						0
535.22271	0.002583654	0.717689014	0.287057789	0.251143706						0
196.04663	0.000239887	0.455234815	0.290284274	0.252479219						0
262.1661	0.000462728	0.198524749	0.1202221	0.25311304						0
376.23465	0.131159736	0.242863813	0.259294368	0.254325834						0

207.05113	7.87E-08	0.488098892	0.260571104	0.254781529	C5H8O5	[M+Hac-H]-	148.037175	207.0510286	0.49	['(R)-2-Hydroxyglutarate', '(R)-2-Methylmalate', '(S)-2-Hydroxyglutarate', '(S)-2-Methylmalate', '2-Dehydro-3-deoxy-D-xylonate', '2-Dehydro-3-deoxy-L-arabinonate', '2-Hydroxyglutarate', 'Citramalate', 'D-Arabinono-1,4-lactone', 'D-Xylono-1,4-lactone', 'D-Xylonolactone', 'D-erythro-3-Methylmalate', 'D-threo-3-Methylmalate', 'L-Arabinono-1,4-lactone', 'L-Arabinono-1,5-lactone', 'L-Xylono-1,4-lactone', 'L-threo-3-Methylmalate']
729.49239	0.112175319	0.31973611	0.181039442	0.25562958						0
338.27038	0.000732058	0.468767068	0.322829018	0.255660135						0
342.22892	0.037461259	0.280391536	0.23189892	0.256535327						0
339.27379	0.000707686	0.465498683	0.320302293	0.256781331						0
343.12493	3.08E-10	0.251148028	0.26238229	0.256857184	C12H24O11	[M-H]-	344.131865	343.1245886	0.99	['Clusianose', 'Melibiitol']
343.12493	3.08E-10	0.251148028	0.26238229	0.256857184	C20H22N2S	[M+Na-2H]-	322.15037	343.1250386	-0.32	['Mequitazine']
593.44485	0.015265747	0.683420968	0.326270796	0.257102773						0
373.27942	0.108954381	0.30535211	0.170783103	0.259336649						0
385.13573	2.14E-10	0.404606006	0.291269684	0.261309595	C12H22O10	[M+Hac-H]-	326.1213	385.1351536	1.5	['2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose', 'Robinobiose', 'Rutinose']
385.13573	2.14E-10	0.404606006	0.291269684	0.261309595	C13H18N4O6	[M+Hac-H]-	326.122636	385.1364896	-1.97	['6,7-Dimethyl-8-(D-ribityl)lumazine']
385.13573	2.14E-10	0.404606006	0.291269684	0.261309595	C18H24N2O5	[M+(37Cl)]-	348.168523	385.1349746	1.96	['Enalaprilate', 'Funebrine']
372.27583	0.110921536	0.305366805	0.171357939	0.261374344	C18H35NO3	[M+Hac-H]-	313.261694	372.2755476	0.76	['(+)-Prosopinine']
537.50905	5.38E-05	0.446944979	0.271351303	0.262680345						0
536.50573	7.79E-05	0.453643844	0.266184825	0.264754937						0
611.08809	4.34E-07	0.39696814	0.273821408	0.267529968						0
517.14186	2.86E-11	0.297913281	0.25092289	0.2679909						0
593.22502	0.000290318	0.512659721	0.340211347	0.268167806	C27H34O11	[M+Hac-H]-	534.210115	593.2239686	1.77	['Arctiin', 'Forsythin', 'Undulatone']
340.29026	0.000181274	0.427631098	0.284727863	0.268759818						0
397.10331	0.057478696	0.294004452	0.263787352	0.271432353						0
453.07452	5.25E-09	0.263772386	0.221025449	0.273684489						0
340.28574	0.001470604	0.461237037	0.303866076	0.277258659	C18H35NO	[M+Hac-H]-	281.271864	340.2857176	0.07	['Dodemorph']
319.06741	8.27E-07	0.685500708	0.328302434	0.278263529	C16H14N2O3	[M+(37Cl)]-	282.100443	319.0668946	1.62	['Saphenic acid methyl ester']
372.27008	0.090837552	0.340441328	0.224313989	0.278907501						0
585.06374	2.11E-05	0.624977637	0.326402137	0.281510574						0
795.18942	5.64E-08	0.181464031	0.213763525	0.282102217						0
719.13114	1.25E-06	0.668746972	0.373576178	0.282411198						0
655.49166	0.122839501	0.422297759	0.215248161	0.282915857						0
549.23833	0.006858522	0.718708207	0.33820218	0.283129628	C24H34N4O5S	[M+Hac-H]-	490.224993	549.2388466	-0.94	['Glimepiride']

341.28932	0.001424711	0.455198361	0.311466972	0.284213915						0
390.28668	0.06278445	0.336148971	0.202848538	0.284665452						0
665.17925	1.92E-09	0.860100752	0.337441788	0.286286868	C36H36O10	[M+K-2H]-	628.23085	665.1794566	-0.31	['Gnidicin']
613.12675	2.38E-07	0.324113114	0.250199108	0.287446183						0
596.52701	0.042087911	0.421717046	0.191250568	0.288346152						0
223.04482	0.000625661	0.468731761	0.273959257	0.290003339						0
402.2503	0.072347946	0.319274782	0.193786662	0.290478787						0
294.24394	0.085324266	0.321995254	0.200933294	0.291257498						0
277.05661	5.23E-07	0.446974503	0.251592264	0.29261993	C11H14NO6	[M+Na-2H]-	256.082114	277.0567826	-0.62	['Nicotinate D-ribonucleoside']
449.07093	3.87E-06	0.641107767	0.315286305	0.295585034						0
308.22349	0.099221168	0.331524548	0.184975969	0.296188273						0
201.02593	2.90E-05	0.160919725	0.22686788	0.297286404	C8H10N2S	[M+Cl]-	166.05647	201.0258716	0.29	['Ethionamide']
356.28104	0.000165059	0.378841059	0.239975243	0.29990542	C18H35NO2	[M+Hac-H]-	297.266779	356.2806326	1.14	['3-Ketosphingosine', 'Cassine', 'Spiroxamine']
400.23465	0.061844571	0.33455239	0.211460318	0.302126171						0
747.50281	0.08382317	0.326503115	0.199084378	0.30251208						0
342.29269	0.007001517	0.491951594	0.332808422	0.302766258	C24H40O	[M-2H]-	344.307915	342.2933622	-1.96	['3beta-Cyclopentyl-5alpha-androstan-17beta-ol']
404.26595	0.116718132	0.298607347	0.185146331	0.30331513						0
386.25532	0.105091148	0.317317296	0.190393201	0.304900788						0
405.26934	0.112175319	0.303331971	0.187669928	0.305210561						0
449.2512	0.048279776	0.247415853	0.182563255	0.308199017						0
388.27101	0.036778574	0.325987744	0.192983024	0.308227434						0
731.50787	0.130750816	0.338412092	0.200210619	0.309417746						0
654.44203	0.025508357	0.199970008	0.075090903	0.309523089						0
603.12106	9.80E-08	0.507680158	0.30841514	0.312038815						0
623.16875	3.49E-07	0.293221457	0.329812654	0.312293143						0
387.25875	0.103639754	0.302180658	0.196870744	0.31284468						0
483.13667	9.97E-07	0.379563259	0.336171663	0.31612154						0
192.01533	2.46E-05	0.214341697	0.258681428	0.317899184						0
469.05194	9.61E-05	0.580311814	0.358804957	0.319369477						0
659.1304	7.26E-08	0.356374739	0.337477476	0.321129093						0
517.17826	1.03E-07	0.42809667	0.304474581	0.322034878						0
689.14354	0.025239854	0.219643787	0.311084776	0.322597432	C38H28N4O7	[M+K-2H]-	652.195801	689.1444076	-1.26	['Esmeraldin B']
563.14753	3.65E-07	0.403601693	0.298971329	0.323864797						0
722.17273	4.83E-07	0.501359068	0.361543518	0.326105966						0
716.5163	0.159459598	0.397651455	0.229000523	0.328226736						0
389.27442	0.06238205	0.338290702	0.205438545	0.330686006						0

293.05153	1.25E-05	0.619793384	0.348980131	0.33157477						0
379.08482	2.45E-05	0.163180452	0.291867384	0.332335762						0
667.52784	0.112175319	0.36815064	0.224088088	0.333204461						0
383.08366	5.00E-08	0.594078982	0.351638091	0.334841921	C17H18N2O6	[M+(37Cl)]-	346.116488	383.0829396	1.88	['Miraxanthin-V', 'Nifedipine']
715.51285	0.178263027	0.399881615	0.231331861	0.336557482						0
342.30169	0.015975666	0.516010743	0.304611547	0.338875443	C18H37NO	[M+Hac-H]-	283.287514	342.3013676	0.94	['Octadecanamide']
344.2446	0.146595516	0.412110227	0.239068542	0.339603337						0
683.52303	0.126278304	0.451300246	0.248368854	0.339904583						0
721.16973	3.10E-07	0.518393891	0.374858917	0.341540582						0
681.17457	2.41E-09	0.937027422	0.401791737	0.341719475						0
671.48654	0.159013601	0.318223823	0.190111233	0.341949698						0
340.28284	0.00476544	0.556416444	0.375218904	0.343968587						0
645.13925	1.37E-07	0.76416854	0.347532268	0.345802448						0
265.05659	3.64E-09	0.557516972	0.429634993	0.346323288	C7H10O7	[M+Hac-H]-	206.042655	265.0565086	0.31	['(2S,3R)-3-Hydroxybutane-1,2,3-tricarboxylate', '(R)-2-Hydroxybutane-1,2,4-tricarboxylate', '2-Methylcitrate', 'Homoisocitrate']
265.05659	3.64E-09	0.557516972	0.429634993	0.346323288	C9H16N4S2	[M+Na-2H]-	244.08164	265.0563086	1.06	['Metiamide']
568.49558	0.061897456	0.471451261	0.22065709	0.347678881	C32H63NO3	[M+Hac-H]-	509.480794	568.4946476	1.64	['N-(Tetradecanoyl)-sphing-4-enine']
410.23195	0.066961158	0.394160977	0.232638437	0.349995551	C22H36O7	[M-2H]-	412.246105	410.2315522	0.97	['Grayanotoxin I']
266.05993	8.51E-09	0.580757008	0.440647657	0.352105468						0
649.25463	0.002187499	1.427248885	0.437110412	0.352820204	C35H41NO11	[M-2H]-	651.267964	649.2534112	1.88	['Rifamycin Z']
386.10271	4.43E-07	0.520292693	0.321133082	0.354590369						0
221.06716	1.32E-08	0.441418363	0.346229548	0.358032977						0
354.26539	0.013313179	0.431104104	0.25557659	0.359387426						0
685.53844	0.101197722	0.30036119	0.252785041	0.360617255						0
383.12004	1.05E-09	0.58858046	0.393053547	0.362659434	C12H20O10	[M+Hac-H]-	324.10565	383.1195036	1.4	["Bis-D-fructose 2',1:2,1-dianhydride", "D-Fructofuranose 1,2':2,3-dianhydride"]
383.12004	1.05E-09	0.58858046	0.393053547	0.362659434	C14H24O12	[M-H]-	384.12678	383.1195036	1.4	['Acetyl-maltose']
385.09929	6.15E-07	0.51311762	0.32559028	0.362905929						0
608.17741	9.92E-08	0.486489934	0.393941772	0.364094192						0
563.25412	0.020065624	0.760877883	0.413590484	0.368060518	C24H42O7P2	[M+Hac-H]-	504.240581	563.2544346	-0.56	['SR 12813']
607.17368	7.78E-08	0.493540507	0.405805855	0.373710772						0
411.0787	9.93E-05	0.695377644	0.421788433	0.373955206	C12H16O12	[M+Hac-H]-	352.06418	411.0780336	1.62	['4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-galacturonate', '4-(4-Deoxy-beta-D-gluc-4-enuronosyl)-D-galacturonate']
340.28119	0.115034403	0.633208601	0.410290731	0.377517034						0
147.06642	3.18E-06	0.387771775	0.248598999	0.37914359	C4H8O2	[M+Hac-H]-	88.05243	147.0662836	0.93	['(R)-Acetoin', '1,4-Dioxane', '2-Methylpropanoate', 'Acetoin', 'Butanoic

										acid', 'Ethyl acetate']
147.06642	3.18E-06	0.387771775	0.248598999	0.37914359	C6H12O4	[M-H]-	148.07356	147.0662836	0.93	['(R)-2,3-Dihydroxy-3-methylpentanoate', '(R)-Mevalonate', '(R)-Pantoate', '(S)-Mevalonate', '2,3-Dihydroxy-3-methylpentanoate', '3,6-Dideoxy-L-galactose', 'Abequose']
397.06315	0.000337831	0.829262075	0.475667461	0.38758541						0
337.07798	8.42E-08	0.470652896	0.370366553	0.390855437	C12H18O11	[M-H]-	338.084915	337.0776386	1.01	['L-Ascorbic acid-2-glucoside']
337.07798	8.42E-08	0.470652896	0.370366553	0.390855437	C16H16N2O4	[M+(37Cl)]-	300.111008	337.0774596	1.54	['5-Nitro-2-(3-phenylpropylamino)benzoic acid', 'Desmedipham', 'Phenmedipham']
593.19438	4.21E-08	0.628146927	0.415285455	0.392866043	C34H36O7	[M+K-2H]-	556.246105	593.1947116	-0.56	['Ingenol 3,20-dibenzoate']
216.12462	0.02226835	0.353058249	0.230686487	0.405223416						0
323.13513	2.16E-08	0.519594712	0.444451543	0.405911814						0
453.05712	0.000435195	1.029632534	0.480135913	0.411180946						0
543.40238	0.020936268	0.526139937	0.106312266	0.41304159						0
369.10439	4.21E-08	0.640834543	0.449129609	0.416953223						0
473.1155	1.38E-07	0.793922124	0.452791755	0.423861783						0
263.04096	2.63E-09	0.266692662	0.355207168	0.424084341	C6H15N2O6P	[M+Na-2H]-	242.066776	263.0414446	-1.84	['2-Deoxystreptamine 4-phosphate', '2-Deoxystreptamine phospahte', '5-Phosphonooxy-L-lysine', 'Phosphoallohydroxy-L-lysine']
263.04096	2.63E-09	0.266692662	0.355207168	0.424084341	C7H8O7	[M+Hac-H]-	204.027005	263.0408586	0.39	['Oxaloglutarate']
793.12193	6.34E-05	1.66740441	0.587537817	0.427418685						0
223.04816	3.29E-06	0.606056869	0.459515598	0.434321557						0
217.00355	0.000126961	0.414715257	0.44307043	0.435164111						0
225.09813	4.66E-05	0.520444187	0.388220931	0.442264656	C12H16N2	[M+(37Cl)]-	188.131348	225.0977996	1.47	['N,N-Dimethyltryptamine']
225.09813	4.66E-05	0.520444187	0.388220931	0.442264656	C9H18NO4	[M+Na-2H]-	204.123584	225.0982526	-0.54	['O-Acetylcarnitine']
765.23213	2.21E-09	0.464917189	0.47998572	0.445445771						0
753.1961	5.64E-08	0.466908781	0.426603582	0.44661319						0
223.08285	0.001384765	0.782212868	0.605114934	0.450900564						0
474.11889	0.000149693	0.855332926	0.489559446	0.456153986	C16H21N3O8S	[M+Hac-H]-	415.104939	474.1187926	0.21	['Cephalosporin C']
441.08941	7.91E-07	1.226247482	0.594275294	0.457915809						0
763.21671	1.21E-09	0.332391416	0.442908973	0.460309032						0
756.21428	1.52E-05	0.706603512	0.551205648	0.463408923						0
467.08156	1.40E-05	0.727219545	0.473758643	0.465517689	C16H22N4O8S	[M+(37Cl)]-	430.115838	467.0822896	-1.56	['N-Ethylmaleimide-S-glutathione']
261.06167	1.27E-07	0.769003848	0.542475576	0.466668699						0
629.12214	4.06E-06	0.454560254	0.458737119	0.469792378						0
295.10382	7.46E-07	0.719090615	0.548842867	0.49512866	C15H20N2S	[M+Cl]-	260.13472	295.1041216	-1.02	['Methaphenilene']

621.18947	3.92E-07	0.67799198	0.473821327	0.505752417						0
443.10506	4.44E-09	0.627801169	0.495788942	0.508422104						0
635.1689	7.16E-06	0.687636708	0.489560184	0.508663593						0
517.10557	3.76E-06	0.762354481	0.472362585	0.514123447	C30H24O6	[M+K-2H]-	480.15729	517.1058966	-0.63	['Blestriarene B']
279.07226	1.81E-06	1.070940162	0.645805286	0.518782685	C8H12O7	[M+Hac-H]-	220.058305	279.0721586	0.36	['(R)-(Homo)2-citrate', '1-Hydroxypentane-1,2,5-tricarboxylate']
659.1082	0.000566944	0.43285009	0.343851858	0.525494407						0
323.0623	1.72E-06	1.389760074	0.557169017	0.52609037						0
797.18706	2.87E-06	1.260493091	0.515535254	0.534503616						0
441.1257	9.48E-07	0.85763559	0.570577307	0.535202872						0
203.05547	6.25E-05	1.037688591	0.655793226	0.545170182						0
421.03959	3.57E-06	1.50033872	0.615525964	0.545874144						0
681.21013	2.38E-07	0.544704155	0.514389729	0.546986756						0
737.23702	5.19E-08	0.420286896	0.510873371	0.553775483						0
437.03459	4.34E-06	1.376614817	0.6686147	0.558929367						0
553.10326	9.96E-05	1.50458501	0.548720854	0.561607836						0
448.10329	2.35E-06	0.99900636	0.533069535	0.561740986						0
324.06569	1.81E-06	1.448767016	0.600324694	0.563978536						0
677.14332	3.87E-05	0.502981331	0.493098646	0.569545294						0
415.1055	2.49E-05	0.635153336	0.569207387	0.575888796						0
620.17735	0.001463923	0.740493144	0.596512377	0.576870458						0
725.20068	0.007483464	0.699870205	0.583861396	0.590973868						0
263.07562	0.046270723	0.965795821	0.521306924	0.591749063						0
409.13581	0.015605426	0.9036873	0.758824874	0.59452714	C21H28N2S2	[M+(37Cl)]-	372.169392	409.1358436	-0.08	['Buthiobate']
205.07115	0.00332185	1.332650983	0.728602215	0.604974925						0
196.00922	0.049745326	1.927514658	0.579182402	0.60748005						0
609.15306	0.000315518	0.97204515	0.606533789	0.608330383						0
755.21144	1.56E-05	0.80105343	0.667269441	0.62262907						0
739.1809	0.023755073	0.850774363	0.811741574	0.629284667						0
239.11372	0.11284827	0.719741008	0.532207854	0.642332251						0
381.06805	1.42E-05	1.570344041	0.799964689	0.647729096						0
595.17367	3.76E-06	0.509146699	0.56211032	0.651192117						0
667.19495	1.81E-05	0.885712895	0.621937823	0.655030135						0
761.18387	0.00011843	5.80095297	0.606159658	0.657457903						0
603.15248	0.000148349	0.323164509	0.543898948	0.659606284						0
335.09874	0.000176028	1.097953882	0.648527404	0.662115714	C11H16O8	[M+Hac-H]-	276.08452	335.0983736	1.09	['Ranunculin']
335.09874	0.000176028	1.097953882	0.648527404	0.662115714	C18H22N2S	[M+K-2H]-	298.15037	335.0989766	-0.71	['Trimeprazine']
224.04976	0.000337259	0.883859809	0.697390506	0.663684741						0

797.25898	0.001648919	0.685629917	0.682672522	0.666900272						0
669.12974	5.88E-06	0.390577547	0.590177469	0.668828247						0
447.09987	1.47E-06	1.100633256	0.670682033	0.671610217						0
769.22739	0.000106507	0.590067729	0.651161197	0.673800216						0
604.18204	9.44E-08	0.672404622	0.606174615	0.675522725						0
723.18532	0.000585119	0.990117272	0.753125954	0.679897896						0
521.31701	0.192271166	1.009149881	0.382618619	0.684283547						0
611.14565	0.122568765	0.795893006	0.671857234	0.68593956	C20H32N6O12S2	[M-H]-	612.151968	611.1446916	1.57	['Glutathione disulfide']
603.17858	1.75E-08	0.657431803	0.610382644	0.686044341						0
619.17385	2.53E-05	0.85685503	0.676149635	0.686080998						0
415.10254	0.173785416	0.777313423	0.679511682	0.686634774	C17H22N4O6	[M+K-2H]-	378.153936	415.1025426	-0.01	['Reduced riboflavin']
353.07296	0.033162358	0.676096578	0.699801106	0.695529805						0
645.11689	0.008234153	0.689438468	0.759088206	0.697369728						0
307.06744	5.89E-07	1.58527508	0.773777462	0.697748695	C12H16NO7	[M+Na-2H]-	286.092679	307.0673476	0.3	['N-Glucosylnicotinate']
307.06744	5.89E-07	1.58527508	0.773777462	0.697748695	C15H14N2O3	[M+(37Cl)]-	270.100443	307.0668946	1.78	['Dihydroxycarbazepine']
337.11439	0.028774901	1.005798509	0.768387984	0.698141256	C11H18O8	[M+Hac-H]-	278.10017	337.1140236	1.09	['Tuliposide A']
337.11439	0.028774901	1.005798509	0.768387984	0.698141256	C17H20N2O3	[M+(37Cl)]-	300.147393	337.1138446	1.62	['Bifenazate']
416.11324	3.24E-06	1.46429757	0.759630265	0.701545715	C14H19N3O6S	[M+Hac-H]-	357.099459	416.1133126	-0.17	['Deacetoxycephalosporin C']
536.30854	0.298093796	0.98981838	0.396715089	0.704042002						0
681.20122	0.003315422	0.702817821	0.663703938	0.705762961						0
520.31359	0.258643198	1.060350567	0.450105061	0.706070624						0
534.29288	0.474744409	0.888687248	0.432272308	0.712280451						0
535.16781	0.002194322	0.723735552	0.612763222	0.713035546						0
417.11386	1.42E-05	1.506073583	0.714965698	0.717698206	C19H26O6S	[M+Cl]-	382.145012	417.1144136	-1.33	['2-Methoxyestradiol-17beta 3-sulfate']
561.13179	0.000959703	0.922915123	0.702725666	0.725480664						0
532.12448	2.08E-07	1.364713024	0.785317718	0.725578981						0
749.16578	0.173785416	0.656669837	0.705102644	0.725646739						0
264.08068	0.001418363	0.76685749	0.732362523	0.726738148						0
263.0773	0.000978128	0.771773208	0.733257005	0.730188366	C14H14N2O	[M+(37Cl)]-	226.110613	263.0770646	0.89	['Metyrapone']
263.0773	0.000978128	0.771773208	0.733257005	0.730188366	C8H12O6	[M+Hac-H]-	204.06339	263.0772436	0.21	['3-o-Ethyl-L-ascorbic acid']
495.0678	0.004998252	0.922213548	0.712826928	0.734862387						0
587.12624	1.17E-05	1.317081623	0.949364012	0.737292796						0
587.12465	1.17E-05	1.317081632	0.949364127	0.737292842						0
785.2231	1.56E-06	0.447973789	0.620333219	0.739474227						0
415.10971	4.35E-06	1.521172422	0.809203	0.749376432						0
194.031	0.366499125	0.636623235	0.682991132	0.7500321						0
735.14935	7.61E-05	0.41750339	0.66874656	0.758049626						0
353.10934	0.001188747	1.176728823	0.896009101	0.761228663	C11H18O9	[M+Hac-H]-	294.095085	353.1089386	1.14	['Tuliposide B']

353.10934	0.001188747	1.176728823	0.896009101	0.761228663	C20H21NO3S	[M-2H]-	355.124216	353.1096632	-0.92	['AL-321']
798.22553	0.031506332	0.649375224	0.790201899	0.768871518						0
501.14681	8.04E-05	1.076107957	0.76465077	0.769569281	C18H30O16	[M-H]-	502.15339	501.1461136	1.39	['alpha-L-Rhamnopyranosyl-(1->2)-beta-D-galactopyranosyl-(1->2)-beta-D-glucuronopyranoside']
738.16786	0.001283603	1.214348369	0.913766399	0.771229415						0
339.09363	0.000373126	0.689254358	0.682814926	0.774338572	C12H20O11	[M-H]-	340.100565	339.0932886	1.01	['3-Ketolactose', '3-Ketosucrose', 'Cellobiono-1,5-lactone']
516.12961	0.000121116	1.076511667	0.817920602	0.778044156						0
270.04677	0.510547104	1.295584696	1.047352758	0.780537073						0
601.12698	0.01665336	0.841783843	0.768556529	0.783020621						0
263.07453	0.018379776	0.869237119	0.494653499	0.78349077						0
555.12126	0.042973061	0.673048529	0.835566976	0.784780468						0
223.04614	0.00761868	1.061816871	0.844927251	0.789898118	C11H10N2O	[M+(37Cl)]-	186.079313	223.0457646	1.68	['Credazine', 'Deoxyvasicinone']
223.04614	0.00761868	1.061816871	0.844927251	0.789898118	C5H8O6	[M+Hac-H]-	164.03209	223.0459436	0.88	['2-Dehydro-D-xylonate']
650.18786	7.92E-06	1.530812868	1.018816498	0.797115026						0
400.11839	7.35E-05	1.257188965	0.849379633	0.7979741						0
615.14245	0.010558303	0.5976506	0.742246757	0.802056706	C34H28O9	[M+Cl]-	580.173335	615.1427366	-0.47	['Mulberrofuran C']
737.16479	0.001226956	1.197409544	0.89312352	0.802353488						0
511.06321	0.005638508	0.672795123	0.619668217	0.814860008						0
399.11491	3.87E-05	1.295489367	0.875274963	0.823759844	C12H20O11	[M+Hac-H]-	340.100565	399.1144186	1.23	['3-Ketolactose', '3-Ketosucrose', 'Cellobiono-1,5-lactone']
399.11491	3.87E-05	1.295489367	0.875274963	0.823759844	C19H26N2O3S	[M+K-2H]-	362.166415	399.1150216	-0.28	['DU 122290']
552.30357	0.410104488	1.005134787	0.44864007	0.830553577	C24H39N5O6	[M+Hac-H]-	493.290035	552.3038886	-0.58	['Syringolin A']
392.24887	0.119537469	0.337696952	0.335318905	0.837867788						0
791.17651	0.058195886	1.035675441	0.810797837	0.858985585						0
266.05186	0.552331795	1.468229663	1.085869627	0.866086659						0
579.17902	0.00203753	0.749662709	0.759786205	0.872615098						0
395.06023	0.015669573	0.624034348	0.714021724	0.878351889	C14H19N2O7P	[M+(37Cl)]-	358.092991	395.0594426	1.99	['N1-(5-Phospho-alpha-D-ribosyl)-5,6-dimethylbenzimidazole']
446.31308	0.586533994	0.740834163	0.446103317	0.884270278						0
648.20852	0.133460873	0.745617619	0.96693484	0.912243504						0
323.05951	0.000776221	2.05833405	0.778885123	0.913872573	C12H16NO7	[M+(37Cl)]-	286.092679	323.0591306	1.17	['N-Glucosylnicotinate']
323.05951	0.000776221	2.05833405	0.778885123	0.913872573	C13H12O4S	[M+Hac-H]-	264.045632	323.0594856	0.08	['(1R,2R)-3-[(1,2-Dihydro-2-hydroxy-1-naphthalenyl)thio]-2-oxopropanoic acid']
649.18434	7.89E-07	1.395167772	0.975879432	0.923512938						0
297.08309	0.063869114	0.647522665	0.801672782	0.927433066	C14H16N2O3	[M+(37Cl)]-	260.116093	297.0825446	1.84	['Maculosin']
297.08309	0.063869114	0.647522665	0.801672782	0.927433066	C15H20N2S	[M+K-2H]-	260.13472	297.0833266	-0.8	['Methaphenilene']
297.08309	0.063869114	0.647522665	0.801672782	0.927433066	C8H14O8	[M+Hac-H]-	238.06887	297.0827236	1.23	['3-Deoxy-D-manno-octulosonate']

439.074	3.21E-07	1.814348935	0.991542963	0.936693669						0
673.14802	0.000493546	0.538566307	0.828961924	0.948778612						0
740.21959	5.42E-09	1.70001067	1.128641628	0.949919581						0
739.21642	1.10E-08	1.663162005	1.125989247	0.967573025						0
171.03023	0.125458505	0.913765106	1.04588089	0.967965841	C5H4O3	[M+Hac-H]-	112.016045	171.0298986	1.94	['2-Furoate']
171.03023	0.125458505	0.913765106	1.04588089	0.967965841	C7H8O5	[M-H]-	172.037175	171.0298986	1.94	['2-Hydroxy-5-methyl-cis,cis-muconate', '2-Hydroxyhepta-2,4-dienedioate', '2-Oxo-5-methyl-cis-muconate', '2-Oxohept-3-enedioate', '3-Dehydroshikimate', '5-Dehydroshikimate', '5-Methylmaleylacetate', 'cis,cis-2,4-Dihydroxy-5-methyl-6-oxo-2,4-hexadienoate']
797.22293	0.911166782	1.025392454	1.05201031	0.983366587						0
593.15816	1.61E-05	1.470505754	1.006516628	0.98838187						0
210.02597	0.953174626	0.932018475	1.111555566	0.99297092						0
254.05189	0.100068085	2.190182284	1.28681286	0.997011189						0
657.1297	0.032076287	1.218997155	0.898026356	0.9996675						0
343.26057	0.859868907	1.228799917	0.934579331	1.002416067						0
589.12728	4.74E-10	2.13036596	1.108469937	1.002433735						0
343.11365	0.919124952	0.940848474	0.983482444	1.003037953	C17H22O5	[M+(37Cl)]-	306.146725	343.1131766	1.38	['Arnicolide A', 'Confertiflorin', 'Eupaformonin', 'Gaillardin', 'Isotenulin', 'Ligulatin B', 'Lipiferolide', 'Matricin', 'Ovatifolin', 'Pyrethrosin', 'Tenulin', 'Viscidulin B', 'Xanthinin', 'Xanthumin']
735.22122	0.000233714	0.873232319	1.102811441	1.030580114						0
341.10906	0.746287328	1.079290714	1.029591337	1.037100222	C10H18O9	[M+Hac-H]-	282.095085	341.1089386	0.36	['Xylobiose']
341.10906	0.746287328	1.079290714	1.029591337	1.037100222	C12H22O11	[M-H]-	342.116215	341.1089386	0.36	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']

342.11264	0.778762398	1.076680357	1.036802489	1.041225971						0
791.24751	0.027723709	0.752559405	1.100706092	1.047192099						0
391.24553	0.133394441	0.348440606	0.225847465	1.051067099						0
594.16172	5.73E-06	1.549653263	1.039818321	1.056045489						0
547.15248	0.099451368	0.938816606	0.966977974	1.070307171						0
459.11523	9.29E-06	0.719190034	0.954481093	1.077279656	C23H24N2O4S	[M+Cl]-	424.14568	459.1150816	0.32	['Eprosartan']
472.10334	0.675156518	1.073832871	1.082554719	1.081027113						0
733.16951	0.00138755	0.761442	1.041301726	1.085687875						0
751.18034	0.373256035	1.126545059	1.051012233	1.090092552						0
647.20483	0.017136506	0.774473458	1.049448566	1.09518145						0
379.05597	0.454594974	1.034859375	0.921585912	1.104858229						0
325.11147	0.351328837	1.463622615	1.393348125	1.112518638						0
240.03621	0.384159557	1.683118603	1.325376632	1.118962372						0
341.10419	0.877249114	0.868876765	1.115434007	1.13324231	C11H20N4O6	[M+(37Cl)]-	304.138286	341.1047376	-1.61	['Nopaline']
575.18428	6.67E-06	1.399240828	1.231556207	1.154952989						0
569.08575	0.027215533	1.62955519	1.087042613	1.165263975						0
585.1088	1.25E-06	0.673609485	0.916524951	1.174179921						0
266.09632	0.002522976	0.81709569	1.111277421	1.183842394						0
265.09297	0.00178862	0.870955187	1.090116333	1.186481978						0
661.14781	0.001036454	0.886358364	1.010067757	1.19078204						0
443.14135	2.58E-05	1.44975123	1.250524596	1.208725752	C14H24O12	[M+Hac-H]-	384.12678	443.1406336	1.62	['Acetyl-maltose']
443.14135	2.58E-05	1.44975123	1.250524596	1.208725752	C20H28N2O5S	[M+Cl]-	408.171895	443.1412966	0.12	['Tamsulosin']
578.16707	2.56E-08	1.95360876	1.294105186	1.210737111	C23H34N3O10P	[M+Cl]-	543.198185	578.1675866	-0.89	['Phosphoramidon']
351.09367	0.000278057	1.706430659	1.339758346	1.218910871						0
413.13055	0.000122308	1.612868884	1.424759567	1.226149128						0
437.07952	0.045932976	1.174866234	1.123262758	1.236836684						0
545.17322	0.000417514	1.260379478	1.276442226	1.236877854						0
531.12113	1.34E-10	2.4822952	1.387706536	1.269609079						0
723.22149	1.58E-07	1.778411749	1.436651019	1.272880447						0
651.20007	3.64E-07	1.731467193	1.364605937	1.290951429						0
743.15	0.057682136	1.567118972	1.455660058	1.308078684						0
427.10995	5.74E-05	1.726635114	1.35560492	1.309623645						0
325.11435	0.017136506	1.382520197	1.312772488	1.32299601	C12H22O10	[M-H]-	326.1213	325.1140236	1	['2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose', 'Robinobiose', 'Rutinose']
325.11435	0.017136506	1.382520197	1.312772488	1.32299601	C16H20N2O3	[M+(37Cl)]-	288.147393	325.1138446	1.55	['Methyl 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-p-toluate', 'Methyl 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-m-toluate']
479.1022	0.552473972	1.342508104	1.165269974	1.32687929	C21H27N3O4S3	[M-2H]-	481.116373	479.1018202	0.79	['Yersiniabactin']

577.16337	1.07E-09	2.185459224	1.44759664	1.327018447						0
749.23724	0.000566944	1.090265788	1.371592036	1.327712374						0
591.17887	1.00E-05	1.495515193	1.378824324	1.341123565						0
367.08876	2.58E-05	1.470951889	1.467728053	1.347338415	C18H22N2O2S	[M+K-2H]-	330.1402	367.0888066	-0.13	['Pyributicarb']
413.16699	0.157948056	1.020665607	1.2217286	1.351044384	C20H28N2O5	[M+(37Cl)]-	376.199823	413.1662746	1.73	['Enalapril', 'Remifentanil']
729.21117	3.31E-09	0.440619582	1.115682129	1.368964619						0
763.18065	0.000404022	1.1065813	1.35657262	1.381554439						0
498.11896	4.93E-05	1.3677969	1.356928384	1.390292052						0
727.19535	1.00E-08	0.49466379	1.056670674	1.390407943	C40H36O11	[M+Cl]-	692.225765	727.1951666	0.25	['Kuwanone G']
719.22612	0.00135294	1.012156169	1.42514717	1.398736328						0
340.06064	5.58E-07	2.597695685	1.501329299	1.408152781						0
556.1611	5.67E-09	0.594517688	1.219793772	1.41705226						0
562.17165	7.31E-05	1.76854529	1.554011068	1.420756575						0
326.11776	0.002444671	1.473961698	1.430913858	1.426326981						0
753.1603	0.003038714	1.621694851	1.654156527	1.427740239						0
339.05719	6.42E-07	2.647515795	1.543462462	1.441392964	C14H17N2O4PS	[M-H]-	340.064668	339.0573916	-0.59	['Pyridafenthion']
339.13003	8.87E-06	2.399543535	1.652842563	1.456481976	C13H24O10	[M-H]-	340.13695	339.1296736	1.05	['Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside']
592.18228	2.86E-06	1.646207354	1.497139216	1.45735462						0
745.16996	2.21E-05	1.671139033	1.479648775	1.458716778	C40H36O12	[M+K-2H]-	708.22068	745.1692866	0.9	['Sanggenon C', 'Sanggenon D']
692.16232	0.000432863	0.96868551	1.293997589	1.461079615						0
522.14029	0.082796135	1.282255768	1.404445281	1.464846228						0
416.14972	0.010975374	0.984858925	1.371299846	1.466142011						0
327.1089	0.022373545	1.142356399	1.340390355	1.467594706	C15H20O8	[M-H]-	328.11582	327.1085436	1.09	['Anisatin', 'Paeonoside']
471.09993	0.001704966	1.339620185	1.358290999	1.483595418						0
569.0983	0.024112234	1.446486839	1.160147862	1.497422295						0
453.16212	0.003331989	1.154941462	1.544039641	1.523560684						0
652.20336	0.000180909	1.932361742	1.591398334	1.543007525						0
415.14624	0.000107725	1.013775528	1.463242332	1.56821849						0
797.329	0.23731873	1.735278591	1.914264303	1.569051918						0
738.20374	1.12E-05	1.145796361	1.420546748	1.573020213						0
487.16755	4.14E-08	2.39723522	1.779336289	1.615983931						0
473.15173	2.43E-08	1.906222289	1.604532608	1.623113851	C16H22N4O9	[M+Hac-H]-	414.138681	473.1525346	-1.7	['Clavamycin A']
474.15522	6.10E-08	1.917931414	1.621992638	1.634029321						0
721.20561	3.02E-07	1.150321382	1.561423304	1.635708865						0
676.16725	3.63E-07	1.104281235	1.599061085	1.654278032						0
515.12622	7.21E-08	2.256321696	1.779001586	1.658552192	C30H24O6	[M+Cl]-	480.15729	515.1266916	-0.92	['Blestriarene B']
793.17225	0.003415801	1.586051873	1.368559758	1.659065855						0

691.15887	3.23E-06	1.111946803	1.492771012	1.661452154						0
799.20248	0.000214066	1.285821209	1.511843188	1.664838117						0
443.12029	2.28E-05	1.201363151	1.421584387	1.676282129						0
617.19455	0.001657699	1.454433128	1.672971091	1.676585868						0
191.05518	0.441774569	1.216983651	0.501812835	1.684064394	C6H10N4O2	[M+Na-2H]-	170.080376	191.0550446	0.71	['N-Isopropylammelide']
558.14013	0.004100429	1.43950663	1.600823654	1.692602873						0
675.16376	6.20E-08	1.151204669	1.643969294	1.694839654						0
599.14731	1.28E-06	1.22780813	1.55583541	1.696676339						0
497.11547	1.91E-06	1.693579873	1.677395286	1.702387394	C22H24N2O9	[M+(37Cl)]-	460.148183	497.1146346	1.68	['Oxytetracycline']
553.09072	2.34E-07	2.093897434	1.632471585	1.708030135						0
467.10524	4.92E-06	1.291361513	1.625852577	1.708189399						0
569.06841	0.000561386	3.674563864	1.867743595	1.709129937						0
722.20899	1.81E-07	1.184054929	1.643549458	1.724539715						0
685.18435	1.95E-08	1.003370536	1.540442833	1.737141115						0
547.11616	7.44E-11	4.052528009	2.120818313	1.74575381						0
677.21569	9.38E-07	1.893343721	1.845321218	1.749142299						0
571.14347	3.83E-07	0.568681018	1.43938307	1.78118855	C17H35N4O13P	[M+K-2H]-	534.193829	571.1424356	1.81	["5'-Phosphoribostamycin"]
483.09993	8.32E-05	1.565930442	1.621499609	1.782336229						0
397.13553	1.19E-07	1.947547695	1.895291108	1.783600722	C19H26N2O3S	[M+Cl]-	362.166415	397.1358166	-0.72	['DU 122290']
737.20067	1.52E-05	1.330521894	1.671595929	1.800839499						0
678.21901	0.011608632	1.808478173	1.723195471	1.814991524						0
590.16658	2.73E-07	2.064263078	1.844895156	1.842119631	C26H37NO8S2	[M+Cl]-	555.196063	590.1654646	1.89	['Tiapamil']
689.17952	3.00E-07	1.326684896	1.822824157	1.865609667						0
481.12065	1.82E-07	1.938000271	1.942806952	1.866265626	C22H24N2O8	[M+(37Cl)]-	444.153268	481.1197196	1.93	['Doxycycline', 'Tetracycline']
481.12065	1.82E-07	1.938000271	1.942806952	1.866265626	C31H24O3	[M+K-2H]-	444.172545	481.1211516	-1.04	['Difenacoum']
249.09813	0.000213196	1.054540496	1.297373993	1.882258278	C14H16N2	[M+(37Cl)]-	212.131348	249.0977996	1.33	['3,3-Dimethylbenzidine']
249.09813	0.000213196	1.054540496	1.297373993	1.882258278	C8H14O5	[M+Hac-H]-	190.084125	249.0979786	0.61	['(R)-3-((R)-3-Hydroxybutanoyloxy)butanoate']
657.18961	4.26E-07	1.440205612	1.921320786	1.911565465						0
499.13113	4.69E-06	2.055712533	2.027425321	1.914222024						0
471.17249	3.19E-07	2.678904741	2.134031934	1.930069234						0
657.15279	1.16E-05	0.987101537	1.705125569	1.94834811						0
633.15337	8.17E-07	0.925384434	1.650071598	1.948568748						0
485.15195	7.61E-10	1.603369316	1.907840331	1.949273132						0
455.06862	1.35E-10	5.102849973	2.385196443	1.951052285						0
573.13188	4.71E-09	2.971710326	2.120191373	1.955393378						0
766.19904	8.45E-06	1.019826193	1.576595597	1.964293643						0

561.16808	2.89E-08	2.571945638	2.196411911	1.98843462	C18H30O16	[M+Hac-H]-	502.15339	561.1672436	1.49	['alpha-L-Rhamnopyranosyl-(1->2)-beta-D-galactopyranosyl-(1->2)-beta-D-glucuronopyranoside']
471.12649	0.002122029	1.595998681	1.951038742	1.993593865	C22H26O10	[M+Na-2H]-	450.1526	471.1272686	-1.65	['Auriculoside']
795.24244	8.96E-09	1.839605213	1.904284845	2.007468249						0
576.15138	2.35E-06	2.069670473	1.837611792	2.076123726						0
509.11598	3.70E-05	1.767738939	2.205251288	2.085232023						0
723.29187	0.019321607	4.762474697	2.590454708	2.087084261						0
622.15676	0.000576191	2.543152387	2.467181306	2.091936677						0
765.19588	2.94E-07	1.089579886	1.747611498	2.098614299						0
747.22168	2.47E-08	1.418387701	2.079814691	2.102885447						0
646.15677	5.14E-09	1.195098356	1.869143777	2.141054771						0
753.23241	3.46E-09	1.745783539	2.017352342	2.163992339						0
679.15947	4.41E-07	2.291658105	2.071577909	2.176689427						0
521.1369	7.04E-07	1.6105832	1.878827097	2.178095638	C25H28N2O7S	[M+Na-2H]-	500.161725	521.1363936	0.97	['(S)-N-[3-(3,4-Methylenedioxyphenyl)-2-(acetylthio)methyl-1-oxoprolyl]-(S)-alanine benzyl ester']
796.24576	0.000587972	1.906271875	1.860585211	2.195978889						0
317.06485	0.151031231	2.066861541	2.409266644	2.198738946	C10H18O9	[M+Cl]-	282.095085	317.0644866	1.15	['Xylobiose']
317.06485	0.151031231	2.066861541	2.409266644	2.198738946	C14H16O7	[M+Na-2H]-	296.089605	317.0642736	1.82	['de-Hypoxanthine futalosine']
317.06485	0.151031231	2.066861541	2.409266644	2.198738946	C8H19O3PS2	[M+Hac-H]-	258.051327	317.0651806	-1.04	['Demeton-O', 'Demeton-S']
735.18471	3.58E-08	1.61483484	1.900372878	2.204261187						0
737.30741	0.067320882	4.251774625	2.748931828	2.208736539						0
473.1407	0.003451522	0.849237672	1.782863812	2.20977229						0
789.19594	1.91E-07	1.377441852	2.031487811	2.21417963						0
396.12351	2.26E-06	3.040133982	1.964941629	2.219815292						0
399.15136	0.000249961	1.266750564	1.937066895	2.223604444	C13H24O10	[M+Hac-H]-	340.13695	399.1508036	1.39	['Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside']
559.11603	2.75E-05	1.882420629	2.085285745	2.233710531						0
451.11033	0.000243002	1.374515411	2.2107158	2.250427411	C22H26N2O4S	[M+K-2H]-	414.16133	451.1099366	0.87	['Diltiazem']
379.12512	7.59E-06	2.21408217	2.133370911	2.254289256	C19H22N2O4	[M+(37Cl)]-	342.157958	379.1244096	1.87	['Phenisopham']
499.09477	0.000344769	1.977897593	2.010523258	2.262174553						0
645.15287	3.78E-09	1.35444009	2.002630766	2.266078084						0
607.28034	0.059543669	3.887132	2.608288023	2.285746954	C35H39N5O5	[M-2H]-	609.29512	607.2805672	-0.37	['Ergocristine']
607.28034	0.059543669	3.887132	2.608288023	2.285746954	C37H40N2O6	[M-H]-	608.288638	607.2813616	-1.68	['Berbamine', 'Gyrocarpine', 'Oxyacanthine', 'Pycnamine', 'Thalmine']
513.11034	2.16E-09	2.39469831	2.24790236	2.319696374						0
593.26473	0.076528266	3.774954646	2.711357178	2.336083775	C36H38N2O6	[M-H]-	594.272988	593.2657116	-1.65	['(+)-Atherospermoline', '(+)-Bebeerine', 'Aromoline', 'Daphnandrine', 'Isochondrodendrine', 'Obamegine']

575.1479	2.07E-06	2.309996761	2.067221105	2.338468411						0
754.23508	0.000283793	1.86910588	2.304446828	2.34289936						0
469.15691	4.75E-10	1.969059025	2.398758857	2.352981405						0
505.1347	5.60E-07	1.884235347	2.228746618	2.357714557	C22H22O10	[M+Hac-H]-	446.1213	505.1351536	-0.9	['Biochanin A-beta-D-glucoside', 'Dihydrogranaticin', 'Glycitin', 'Obtusifolin 2-glucoside', 'Physcion 8-glucoside', 'Trifolirhizin']
473.14595	0.000209106	2.182828932	2.245634248	2.412200176	C22H22O8	[M+Hac-H]-	414.13147	473.1453236	1.32	['Picropodophyllin', 'Podophyllotoxin']
441.16206	0.000323735	2.256964611	2.321373203	2.420042198						0
379.06528	3.29E-06	2.241822327	2.10823736	2.431835114	C12H22O11	[M+K-2H]-	342.116215	379.0648216	1.21	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']
425.13075	4.87E-05	2.33522705	2.457835254	2.43314838						0
660.17223	8.05E-09	1.519331442	2.588973733	2.474051479						0
395.12008	4.30E-10	3.486362231	2.612580737	2.474393882	C19H24N2O3S	[M+Cl]-	360.150765	395.1201666	-0.22	['LY395153']
545.13686	6.01E-10	1.710424751	2.061547017	2.488508621						0
736.18838	1.33E-07	1.823511365	2.072580622	2.492186492						0
514.11395	3.70E-10	2.632729928	2.461372407	2.541736813	C24H23N5O6	[M+K-2H]-	477.164835	514.1134416	0.99	['CB3717']
467.14145	1.90E-05	3.233017686	3.048211671	2.568600283						0
412.1184	1.27E-09	3.840928808	2.677916986	2.592684057						0
793.22687	3.37E-10	1.837388508	2.295659194	2.595720801						0
687.20027	1.72E-09	1.139776852	2.133661546	2.59575888	C32H38N6O7S	[M+K-2H]-	650.252271	687.2008776	-0.88	['BQ 518']
715.19533	6.27E-11	1.141351715	2.322803275	2.618126919						0
795.20651	3.64E-09	2.233212048	2.622929941	2.656763345						0
621.29624	0.036778574	4.489798235	2.807770628	2.664134247	C38H42N2O6	[M-H]-	622.304288	621.2970116	-1.24	['(+)-O-Methylthalicberine', '(+)-Tetrandrine', 'Cycleanine', 'Isotetrandrine', 'Obaberine', 'Rodiasine']
546.14022	1.43E-06	1.874507461	2.116413172	2.685226728						0
409.09949	5.11E-07	3.014227093	2.883945702	2.704049498	C12H25N2O10P	[M+Na-2H]-	388.124686	409.0993546	0.33	['Fructoselysine 6-phosphate']

747.18525	5.18E-09	1.465588842	2.554647319	2.706021125						0
489.13645	1.38E-10	3.025073191	2.539911647	2.716173298						0
395.08378	4.96E-08	1.529613064	2.375563256	2.71691426						0
341.14564	0.00042444	1.052538558	1.681749258	2.738476232						0
631.17405	4.79E-10	2.356962113	2.610526813	2.743448503						0
724.22447	2.59E-12	3.816078314	3.144001756	2.750426433						0
560.15603	9.44E-09	2.30112041	2.637427444	2.80452897						0
621.15322	1.75E-08	3.445730059	2.903824426	2.812619648						0
589.19943	1.10E-06	1.713064548	2.584505227	2.855861904						0
506.14526	2.85E-10	2.484954515	2.642729932	2.866426713						0
673.18434	4.81E-10	2.123418644	2.883815157	2.867385118						0
411.11493	1.40E-09	4.196233778	2.947600094	2.867751546						0
532.16084	1.70E-10	2.277724669	2.685335674	2.885867923						0
685.12461	5.67E-10	1.649456344	2.468970733	2.89264675						0
761.16625	2.68E-05	2.584288052	2.76280654	2.901296589						0
505.14172	2.20E-10	2.586391237	2.684769417	2.908809903						0
661.22013	4.35E-07	2.605328534	2.989101633	2.922644584						0
529.17826	8.05E-09	2.790159579	2.976503144	2.94327752						0
796.20988	6.99E-09	2.320716048	2.855457929	2.954039555						0
491.15054	6.27E-07	3.816693141	2.711050452	3.004661885						0
659.16872	5.05E-11	1.981138182	3.047365676	3.028470572						0
457.15692	3.68E-10	3.044761742	2.968636405	3.037934982						0
615.17892	9.13E-11	2.506580327	3.019867201	3.08388978						0
531.1573	1.47E-10	2.446465597	2.868559787	3.089806587						0
557.17319	2.19E-10	2.857270634	3.101207764	3.101747719						0
717.17436	1.06E-08	2.029264627	2.923261058	3.109210633						0
718.17774	0.000831748	2.333676794	3.204320325	3.120844195						0
743.16668	3.29E-05	2.394438559	2.556545418	3.169699849						0
671.16884	1.70E-10	1.685062573	2.915321133	3.182141128						0
511.07158	8.38E-10	2.770077293	2.787761769	3.196311449						0
775.18054	7.44E-11	1.25121271	2.740632765	3.259161324						0
196.04562	0.09578271	6.089523306	3.674827543	3.333372299						0
489.13978	1.45E-10	4.325777326	3.245706741	3.34824049	C20H22N8O5	[M+Cl]-	454.171317	489.1407186	-1.92	['Methotrexate']
489.13978	1.45E-10	4.325777326	3.245706741	3.34824049	C22H22O9	[M+Hac-H]-	430.126385	489.1402386	-0.94	['Ononin']
768.21456	5.57E-09	2.668568924	3.526609864	3.350596702						0
643.17396	3.43E-11	2.137005211	3.547285347	3.370998864						0
689.21601	3.47E-07	1.868677943	2.739696299	3.379817282	C38H38O10	[M+Cl]-	654.2465	689.2159016	0.16	['Mezerein']
377.10957	2.06E-06	3.418397389	3.180538363	3.449513949	C20H24N2OS	[M+K-2H]-	340.160935	377.1095416	0.08	['Lucanthone', 'Propiomazine']

544.1611	1.39E-09	2.5844992	3.481247384	3.467860049						0
759.16176	8.26E-12	1.062664438	2.681836965	3.471567284						0
439.11006	5.65E-12	3.445480744	3.556550101	3.509476586						0
719.18951	1.15E-10	1.671626023	2.764331886	3.512068512						0
748.18865	4.27E-06	2.382512062	4.387903773	3.573934551						0
767.21162	2.37E-10	2.891256848	3.717247581	3.604218828						0
355.05219	9.62E-09	4.551262282	3.735514557	3.648020463						0
383.10862	0.000159989	4.079802118	3.358448301	3.67710006	C19H22O6	[M+(37Cl)]-	346.14164	383.1080916	1.38	['Alectrol', 'Antheridic acid', 'Cynaropicrin', 'Gibberellin A29-catabolite', 'Gibberellin A3', 'Gibberellin A34-catabolite', 'Gibberellin A6', 'Hallactone A', 'Molephantin', 'Ponalactone A', 'Saupirin', 'Strigol']
600.17963	0.000118916	3.298177268	3.459973412	3.678559556						0
489.14658	2.21E-10	4.663141357	3.591516716	3.681681787						0
487.12436	6.44E-12	2.910435489	3.318125875	3.688932956	C22H20O9	[M+Hac-H]-	428.110735	487.1245886	-0.47	['epsilon-Rhodomycinone']
487.12436	6.44E-12	2.910435489	3.318125875	3.688932956	C24H24O11	[M-H]-	488.131865	487.1245886	-0.47	['Phrymarolin I']
517.15717	1.81E-07	1.811057232	2.768895692	3.694152036						0
365.1095	3.70E-10	3.80775443	4.053270793	3.698354567	C18H22N2O2S	[M+Cl]-	330.1402	365.1096016	-0.28	['Pyributicarb']
365.1095	3.70E-10	3.80775443	4.053270793	3.698354567	C19H24N2OS	[M+K-2H]-	328.160935	365.1095416	-0.11	['Methotrimeprazine']
687.16372	1.55E-11	1.916101313	3.39363725	3.710978783						0
691.1952	1.41E-10	2.39679074	3.356274031	3.719176266	C38H38O10	[M+K-2H]-	654.2465	691.1951066	0.14	['Mezerein']
490.15017	2.19E-10	4.767629511	3.686731148	3.755967363						0
751.21638	2.86E-11	3.155217176	4.020853515	3.761711386						0
586.17195	1.38E-07	2.592526018	3.625954552	3.794897181						0
789.23242	4.30E-12	2.05914133	3.517800342	3.797732497						0
486.11898	1.57E-10	4.828384836	4.030195739	3.801812804						0
663.16388	2.26E-12	4.066635353	3.804725005	3.820365181						0
585.13212	1.35E-10	1.384118149	2.984409006	3.858598157						0
664.16731	5.16E-12	4.328232559	3.889301964	3.873349896						0
627.1407	2.81E-05	5.14700632	3.911303231	3.881421601						0
676.20358	3.68E-10	2.063054146	3.618453109	3.940564043						0
453.09037	9.15E-07	2.031509707	3.623289144	3.953558304						0
485.11547	7.91E-11	5.109475395	4.244580656	3.986840378						0
794.23062	1.12E-10	2.560875205	3.503580197	4.006155964						0
693.21074	1.28E-09	4.826607976	4.174919451	4.015324693						0
759.18494	8.17E-11	1.422439356	3.167745588	4.019921413						0
645.18924	6.80E-13	0.961201366	3.157588204	4.054415418						0
606.16153	2.66E-10	5.150983331	4.004153973	4.074932673						0
393.10448	2.38E-09	5.040189041	4.817078678	4.077418647						0

554.14536	5.93E-09	0.821209529	2.815783405	4.092856167						0
471.13603	2.24E-12	1.937360094	3.607159607	4.113393453						0
537.32888	0.000493546	4.023136094	3.561425247	4.139472814	C30H48N2O4	[M+(37Cl)]-	500.361408	537.3278596	1.9	['Vicenistatin']
719.1539	2.57E-10	1.713619251	3.68176235	4.142581965						0
472.1396	7.26E-13	1.954909628	3.643482755	4.177190147						0
693.17449	3.69E-11	2.807454258	3.479730089	4.258179627						0
488.13465	5.71E-11	2.654114556	3.680824629	4.374446636	C23H25N5O5	[M+K-2H]-	451.18557	488.1341766	0.97	['Doxazosin']
523.10792	6.94E-08	3.810701924	4.11745457	4.375291952						0
688.16705	3.08E-09	1.762696976	3.901104196	4.401586667						0
694.17777	3.81E-08	2.685321918	3.643761875	4.408753083	C34H33NO15	[M-H]-	695.185024	694.1777476	0.03	['Dexylosylbenanomicin A']
539.16278	1.69E-11	2.391329383	4.040883111	4.477816913	C26H34N2O4S2	[M+(37Cl)]-	502.196002	539.1624536	0.61	['Myxothiazol Z']
487.13115	1.67E-11	2.798772214	3.836834226	4.588931619						0
727.37731	0.00017282	2.328257951	4.114939944	4.596725382						0
675.20007	5.30E-11	2.525854239	4.28543379	4.608239213						0
643.13574	8.02E-06	4.52183037	4.413814459	4.639277788						0
527.16265	7.91E-11	3.334364273	4.757275201	4.660225056						0
720.19337	4.99E-10	1.651115881	3.598275344	4.662642827						0
743.18958	1.94E-10	1.330544192	3.602324443	4.663686752	C40H36O12	[M+Cl]-	708.22068	743.1900816	-0.67	['Sanggenon C', 'Sanggenon D']
529.14176	4.97E-13	2.453160123	3.698118959	4.665763578						0
717.21085	2.84E-10	1.994811971	4.39998019	4.668347503						0
749.20086	1.67E-11	2.44720464	4.266731952	4.674140066						0
397.10526	2.38E-07	4.314901928	4.720191358	4.70415438	C19H22O7	[M+Cl]-	362.136555	397.1059566	-1.75	['Gibberellin A8-catabolite', 'Nagilactone C', 'Vernolide']
397.10526	2.38E-07	4.314901928	4.720191358	4.70415438	C20H24O6	[M+K-2H]-	360.15729	397.1058966	-1.6	['Dibenzo-18-crown-6', 'Euponin', 'Fastigilin C', 'Lariciresinol', 'Molephantinin', 'Multigilin', 'Triptolide']
502.11387	3.81E-06	4.006320702	4.520358566	4.724317912						0
514.15036	6.67E-12	3.101219268	3.983628503	4.726185651						0
613.16325	2.57E-08	2.289900793	4.292989227	4.731788282						0
565.36025	0.000406045	4.480787461	4.22304107	4.741376546						0
453.12573	1.33E-10	5.757709379	5.371211588	4.747587113						0
646.19321	4.29E-13	1.037904235	3.65176193	4.768280488						0
399.10706	2.06E-06	5.090507696	4.285070575	4.786059523						0
694.21402	2.74E-10	5.85191063	5.095542751	4.829639723						0
779.2121	1.33E-05	2.17461212	3.921464007	4.89337338						0
733.20578	8.69E-12	2.270805552	4.553617086	4.909655716						0
513.14684	7.10E-14	3.310480507	4.160019218	4.915287422						0
413.0869	1.48E-07	3.265293234	4.706068149	4.944208024	C17H20N4O6	[M+K-2H]-	376.138286	413.0868926	0.02	['Riboflavin']
589.16306	8.46E-11	5.445025692	4.957310126	5.017031836						0

659.20512	6.18E-11	2.389409434	4.619794724	5.032548668						0
573.16785	1.16E-07	4.339183629	4.782558888	5.057011303						0
557.13686	2.05E-11	3.730786299	4.847002005	5.079249156						0
731.19006	9.35E-12	2.251535881	4.66490343	5.136309148						0
605.15788	1.47E-10	6.241849846	5.082148194	5.153301963						0
701.17948	3.72E-08	2.290197733	4.59315865	5.18581835	C42H32O9	[M+Na-2H]-	680.204635	701.1793036	0.25	['Canaliculatol', 'Copalliferol B']
571.11639	1.70E-10	4.731136154	5.293297377	5.255212703	C21H26N8O6S2	[M+Na-2H]-	550.141676	571.1163446	0.08	['Cefclidin']
345.08312	3.12E-08	2.360171021	4.649231416	5.288149231	C18H16N2O3	[M+(37Cl)]-	308.116093	345.0825446	1.67	['Citrus Red No.2']
759.2216	3.15E-11	1.798810421	4.559938641	5.332079012						0
661.18403	1.67E-11	1.612875242	4.237786577	5.341303733						0
381.09822	5.64E-06	6.149024326	5.835607973	5.341414123	C12H24O11	[M+(37Cl)]-	344.131865	381.0983166	-0.25	['Clusianose', 'Melibiitol']
381.09822	5.64E-06	6.149024326	5.835607973	5.341414123	C19H14O5	[M+Hac-H]-	322.084125	381.0979786	0.63	['11-Deoxylandomycinone', 'Decarboxytetracenomycin F1', 'Tetrangomycin']
381.09822	5.64E-06	6.149024326	5.835607973	5.341414123	C21H18O7	[M-H]-	382.105255	381.0979786	0.63	['12-Deoxyaklanonic acid', 'Austrobailignan 1']
530.14524	6.80E-14	2.828210418	4.207140139	5.348573076						0
618.16178	1.07E-12	3.671466761	4.853278989	5.350494582						0
647.1686	2.12E-11	5.743247251	5.536474969	5.360319657						0
617.15819	1.66E-12	3.638403671	4.977546041	5.377949175						0
679.19472	2.06E-10	3.525742234	5.015495206	5.476751731						0
752.21963	4.32E-11	4.510922069	5.998024793	5.490722673						0
511.13154	2.87E-12	4.68716125	4.808392506	5.519845922						0
515.16246	1.09E-11	2.539111307	4.726693924	5.539315506						0
516.16594	1.02E-11	2.553197267	4.733402147	5.595365477						0
587.18306	1.50E-11	3.472716447	4.974032729	5.683575364						0
527.1263	7.92E-11	6.316120548	5.51824541	5.732339742						0
720.15742	1.45E-10	2.291393695	5.098757958	5.807161968						0
456.1084	2.89E-12	5.923451133	5.916196631	5.82801174						0
328.08893	0.011037115	4.509415588	5.841314214	5.835602371						0
695.19009	1.26E-10	2.874788141	4.989185067	5.86197269						0
755.40875	4.77E-05	2.730065205	4.870748274	5.881440332	C37H56N8O9	[M-H]-	756.417027	755.4097506	-1.32	['Nostocyclopeptide A1']
680.19838	1.49E-10	3.751405815	5.399771219	5.926927215						0
703.19511	1.12E-11	1.564937359	4.320594688	6.043981212						0
469.12049	4.29E-13	7.662945467	7.089481604	6.06900641						0
696.19332	4.45E-11	2.840880343	5.086165884	6.110786651						0
446.0997	3.53E-07	1.636396885	5.837012437	6.156311658						0
602.16659	4.99E-13	4.976848889	5.93675965	6.182487467						0
583.15292	5.60E-09	3.29784217	6.031533634	6.379546719						0

397.09234	4.62E-10	6.185792347	6.160626416	6.3900215	C19H14O6	[M+Hac-H]-	338.07904	397.0928936	-1.39	['8-O-Methylsterigmatocystin', 'Rabelomycin']
397.09234	4.62E-10	6.185792347	6.160626416	6.3900215	C19H17N3O7	[M-2H]-	399.106652	397.0920992	0.61	['Nocardicin E', 'Nocardicin F']
397.09234	4.62E-10	6.185792347	6.160626416	6.3900215	C21H18O8	[M-H]-	398.10017	397.0928936	-1.39	['Auramycinone', 'Dihydro-NAME', 'Nogalavinone']
541.14197	7.26E-13	4.019850483	6.254775031	6.523959072						0
794.1944	1.67E-11	1.333453701	5.027521396	6.546063813						0
531.14928	6.80E-13	4.820450536	5.742780238	6.556680162						0
662.18789	1.47E-11	1.879992222	5.132835076	6.560013106						0
470.12398	1.15E-11	8.186232737	7.574028134	6.574617087						0
501.11042	5.05E-11	6.952788685	6.354813894	6.603745333						0
546.10383	1.82E-11	4.046323324	5.880156886	6.606658837						0
543.15762	9.30E-13	4.856716006	6.64790088	6.627444047						0
543.12127	3.34E-10	6.579225407	6.593936079	6.738426122						0
585.16841	1.68E-12	4.845749712	6.453400107	6.757625761						0
648.17241	2.68E-11	7.325608775	6.945043704	6.768653526						0
793.19075	1.17E-11	1.498040829	5.402887803	7.034997915						0
779.17569	1.53E-05	5.908123284	7.632136046	7.107758118						0
455.10491	2.43E-13	7.033367087	7.276403284	7.123615427						0
528.12973	4.49E-11	8.346128362	7.019242918	7.172258695						0
599.18407	3.41E-09	5.549457022	7.215140775	7.395128143	C28H38N2O8S	[M+K-2H]-	562.23489	599.1834966	0.96	['TMC 126']
537.14704	4.70E-14	1.863116594	5.66984365	7.595079287						0
507.11318	0.001717682	4.878033816	7.686915993	7.682674913						0
413.08921	8.22E-08	4.97158766	7.196870679	7.705675037	C14H18N6O7S	[M-H]-	414.095771	413.0884946	1.73	['Pyrazosulfuron-ethyl']
750.20418	7.81E-10	3.102416835	7.066703625	7.764498072						0
343.06747	3.51E-08	3.925149487	7.19797476	7.935675357	C14H16O10	[M-H]-	344.07435	343.0670736	1.16	['Theogallin']
570.14018	1.24E-09	2.141984972	5.660455602	7.938923684						0
495.13628	1.40E-08	5.799401034	7.294473683	7.972850947						0
525.14702	2.00E-12	5.486476207	7.595411095	7.975444857						0
495.07638	3.65E-11	10.08800853	7.851615168	8.006697883						0
677.17936	5.16E-12	3.308652367	6.442091375	8.299973545						0
629.15839	1.19E-12	3.844999016	7.271293606	8.306780078						0
415.0986	9.72E-10	6.734471344	8.153393803	8.363372353	C19H22O8	[M+(37Cl)]-	378.13147	415.0979216	1.63	['Hydroxyvernolide']
415.0986	9.72E-10	6.734471344	8.153393803	8.363372353	C19H24O6S	[M+Cl]-	380.129362	415.0987636	-0.39	['2-Methoxyestrone 3-sulfate']
497.15195	2.75E-12	5.294737203	7.005104167	8.392643735						0
437.13083	1.38E-09	7.542201299	8.697908411	8.416934744						0
779.20064	3.01E-11	2.06644701	6.785560799	8.803377161						0
663.19964	1.45E-10	5.726016774	8.341556022	8.907918858	C36H36O10	[M+Cl]-	628.23085	663.2002516	-0.92	['Gnidicin']

479.08155	3.48E-11	10.14688996	8.737774404	8.984282499						0
553.1185	1.09E-10	3.387685263	7.478865509	9.294921505						0
344.08383	0.023783086	6.109645326	9.797794241	9.430234125						0
559.15252	2.09E-12	7.734747016	8.920349818	9.441713357						0
746.20935	1.06E-13	1.337498395	7.636541121	9.65844685						0
791.21131	3.31E-12	4.231635902	8.841848106	9.768733626						0
745.20572	1.51E-13	2.132874421	7.938524152	9.796124872						0
567.15767	1.91E-07	4.812891169	8.936862843	10.33728904						0
503.12613	6.27E-11	7.31499257	9.20656377	10.35513103						0
382.10777	1.46E-09	9.457479385	10.19610114	10.39993222	C14H20N6O5S	[M-2H]-	384.121591	382.1070382	1.92	['S-Adenosyl-L-homocysteine']
775.15728	3.71E-10	1.992654063	6.721157393	10.4980891						0
678.1828	3.31E-12	4.263292996	8.523987458	11.06139991						0
398.10264	2.73E-10	11.37416403	11.10563554	11.23351374						0
704.16233	4.28E-12	4.666570414	9.601626103	11.36385756						0
414.09761	1.38E-10	8.369751407	10.9452172	11.52017027	C16H21N3O8S	[M-H]-	415.104939	414.0976626	-0.13	['Cephalosporin C']
380.09219	3.32E-09	4.891612984	10.12447648	11.52081408						0
413.094	1.49E-10	8.398399564	10.96287418	11.56203685						0
397.0989	4.99E-10	11.79525442	11.61466435	11.72303025	C12H18O11	[M+Hac-H]-	338.084915	397.0987686	0.33	['L-Ascorbic acid-2-glucoside']
397.0989	4.99E-10	11.79525442	11.61466435	11.72303025	C18H20N2O6	[M+(37Cl)]-	360.132138	397.0985896	0.78	['3-Methoxytyramine-betaxanthin', 'Nitrendipine']
397.0989	4.99E-10	11.79525442	11.61466435	11.72303025	C19H24N2O3S	[M+K-2H]-	360.150765	397.0993716	-1.19	['LY395153']
792.21485	5.77E-11	4.248025456	10.74688758	11.83059543						0
399.10352	8.16E-11	12.60420092	12.18091629	12.24257854	C19H22O7	[M+(37Cl)]-	362.136555	399.1030066	1.29	['Gibberellin A8-catabolite', 'Nagilactone C', 'Vernolide']
573.15736	9.50E-14	4.59255658	10.26025809	12.30024596	C30H29N3O7S	[M-2H]-	575.172624	573.1580712	-1.24	['4,5-Dihydro-4-hydroxy-5-S-glutathionyl-benzo[a]pyrene', '7,8-Dihydro-7-hydroxy-8-S-glutathionyl-benzo[a]pyrene']
381.10423	2.07E-09	11.07994873	11.81653602	12.33280422						0
734.20924	4.70E-12	4.642351692	11.42484538	12.34357052						0
569.11344	9.50E-14	5.563398333	9.986484331	12.34709474						0
430.09258	1.22E-09	6.112166455	10.91138786	12.54872348						0
553.14194	1.06E-13	2.315831307	9.549998813	12.92588482						0
619.13753	7.26E-13	4.10143325	9.94844802	13.06346676						0
601.16308	9.50E-14	10.47704161	12.67352134	13.36008347						0
664.20352	8.60E-11	8.821724098	13.45644948	14.4357901						0
569.17332	8.69E-12	8.400578141	13.28365651	14.79146622						0
776.21997	2.14E-13	5.19953184	14.16045389	15.90449665						0
439.14641	0	5.94810494	12.51984178	16.31109327						0
455.14121	6.30E-14	6.94786087	14.31089701	16.67183593	C21H26N2O7	[M+(37Cl)]-	418.174003	455.1404546	1.66	['Nimodipine']

456.14476	1.51E-13	7.286628259	15.24144452	17.74454803						0
429.08921	7.64E-11	9.180547371	16.57856969	18.90899972	C12H18O13	[M+Hac-H]-	370.074745	429.0885986	1.42	['1,2-beta-D-Glucuronosyl-D-glucuronate', 'Digalacturonate']
703.15874	9.11E-13	8.020614878	16.38850632	19.00504855						0
705.17443	3.73E-08	7.95055842	18.07650811	19.2106298						0
379.0887	2.44E-09	8.356312636	16.6953025	19.23470062						0
569.13689	4.99E-13	4.909548844	15.19201731	20.14635314						0
530.10891	9.50E-14	16.86506367	19.70278726	20.63243104						0
555.15756	1.51E-13	8.055690325	18.03518958	20.96759429						0
620.14098	1.16E-12	5.665917232	15.87263805	21.00711113	C28H29N2O12	[M+Cl]-	585.172053	620.1414546	-0.77	['SN38 glucuronide carboxylate form']
761.20045	1.97E-13	4.768902641	19.84072456	24.81735701						0
603.142	1.93E-13	9.191864188	20.96769137	25.54446228						0
545.10051	1.62E-13	15.8675972	24.22854326	26.5812912						0
529.10542	1.51E-13	22.10648039	25.40386507	26.69687751						0
604.14574	9.50E-14	10.3551639	23.7997447	29.07942892						0
775.21666	8.40E-14	10.89801389	28.49036441	31.50678289						0
778.19927	5.65E-13	5.595429408	25.57649614	33.1719229						0
762.20414	2.14E-13	6.271769002	29.65539184	37.34547994						0
777.1954	2.43E-12	7.386652415	32.14790835	41.26623729						0
587.13865	9.63E-08	8.13682682	36.44604042	42.1136558	C23H34O15	[M+K-2H]-	550.189775	587.1383816	0.46	['Genipin 1-beta-gentiobioside']
587.14674	1.97E-13	18.1069711	36.44604146	42.11365732						0
571.15223	6.30E-14	18.12710165	41.73818736	49.28429459						0
588.15103	6.30E-14	22.70332001	45.03445326	52.08031408						0
572.15598	6.30E-14	21.58985272	49.29691508	58.00088032						0

Table 3 Extraction of the aged laboratory sherds

	10-Apr	11-Apr-12					12-Apr-12			
Sample Identification	weight (mg)	volume of solution 1 (ul)	volume of ChCl3 (ul)	volume of water II (ul)	volume of polar layer removed (ul)	volume of non-polar removed (ul)	amount of 4M KOH (ul)	2N HCl (ul)	volume of ethyl acetate (ul)	Estimated volume of depolymerised sample removed (ul)
Water_glass_1A	104	560	400	200	400	200	150	400	450	350
Water_glass_1B	104	560	400	200	400	200	150	400	450	350
Water_glass_2A	101	560	400	200	400	200	150	400	450	350
Water_glass_2B	103	560	400	200	400	200	150	400	450	300
Water_glass_3A	100	560	400	200	400	<200	150	400	450	xxx
Water_glass_3B	102	560	400	200	400	200	150	400	450	350
Water_glass_4A	105	560	400	200	400	200	150	400	450	350
Water_glass_4B	101	560	400	200	400	200	150	400	450	350
Water_sand_1A	102	560	400	200	400	200	150	400	450	350
Water_sand_1B	101	560	400	200	400	200	150	400	450	350
Water_sand_2A	101	560	400	200	400	200	150	400	450	350
Water_sand_2B	103	560	400	200	400	200	150	400	450	350
Water_sand_3A	100	560	400	200	400	200	150	400	450	200
Water_sand_3B	103	560	400	200	400	200	150	400	450	200
Water_sand_4A	101	560	400	200	400	200	150	400	450	400
Water_sand_4B	103	560	400	200	400	200	150	400	450	400
Water_peat_1A	102	560	400	200	400	200	150	400	450	400
Water_peat_1B	104	560	400	200	400	200	150	400	450	400
Water_peat_2A	104	560	400	200	400	200	150	400	450	400
Water_peat_2B	104	560	400	200	400	200	150	400	450	400
Water_peat_3A	104	560	400	200	400	200	150	400	450	400
Water_peat_3B	103	560	400	200	400	xxx	150	400	450	400
Water_peat_4A	103	560	400	200	400	<200	150	400	450	400
Water_peat_4B	104	560	400	200	400	200	150	400	450	400
Red_glass_1A	104	560	400	200	400	xxx	150	400	450	400
Red_glass_1B	100	560	400	200	400	xxx	150	400	450	400
Red_glass_2A	101	560	400	200	400	<200	150	400	450	400
Red_glass_2B	105	560	400	200	400	200	150	400	450	400
Red_glass_3A	102	560	400	200	400	xxx	150	400	450	400
Red_glass_3B	104	560	400	200	400	200	150	400	450	200
Red_glass_4A	101	560	400	200	400	200	150	400	450	400
Red_glass_4B	104	560	400	200	400	200	150	400	450	400

Red_sand_1A	101	560	400	200	400	200	150	400	450	400
Red_sand_1B	102	560	400	200	400	200	150	400	450	400
Red_sand_2A	101	560	400	200	400	<200	150	400	450	400
Red_sand_2B	103	560	400	200	400	<200	150	400	450	400
Red_sand_3A	104	560	400	200	400	200	150	400	450	400
Red_sand_3B	100	560	400	200	400	200	150	400	450	400
Red_sand_4A	100	560	400	200	400	200	150	400	450	400
Red_sand_4B	103	560	400	200	400	<200	150	400	450	400
Red_peat_1A	101	560	400	200	400	200	150	400	450	400
Red_peat_1B	101	560	400	200	400	<200	150	400	450	400
Red_peat_2A	101	560	400	200	400	200	150	400	450	400
Red_peat_2B	105	560	400	200	400	200	150	400	450	400
Red_peat_3A	105	560	400	200	400	200	150	400	450	400
Red_peat_3B	102	560	400	200	400	200	150	400	450	400
Red_peat_4A	1004	560	400	200	400	200	150	400	450	400
Red_peat_4B	100	560	400	200	400	200	150	400	450	400
White_glass_1A	101	560	400	200	400	200	150	400	450	400
White_glass_1B	104	560	400	200	400	200	150	400	450	400
White_glass_2A	103	560	400	200	400	200	150	400	450	400
White_glass_2B	103	560	400	200	400	200	150	400	450	400
White_glass_3A	103	560	400	200	400	200	150	400	450	400
White_glass_3B	100	560	400	200	400	200	150	400	450	400
White_glass_4A	102	560	400	200	400	200	150	400	450	400
White_glass_4B	101	560	400	200	400	200	150	400	450	400
White_sand_1A	101	560	400	200	400	200	150	400	450	400
White_sand_1B	100	560	400	200	400	200	150	400	450	400
White_sand_2A	100	560	400	200	400	200	150	400	450	400
White_sand_2B	102	560	400	200	400	200	150	400	450	400
White_sand_3A	100	560	400	200	400	200	150	400	450	400
White_sand_3B	102	560	400	200	400	200	150	400	450	400
White_sand_4A	104	560	400	200	400	200	150	400	450	400
White_sand_4B	101	560	400	200	400	200	150	400	450	400
White_peat_1A	100	560	400	200	400	200	150	400	450	400
White_peat_1B	104	560	400	200	400	200	150	400	450	400
White_peat_2A	104	560	400	200	400	200	150	400	450	400
White_peat_2B	103	560	400	200	400	200	150	400	450	400
White_peat_3A	102	560	400	200	400	200	150	400	450	400
White_peat_3B	100	560	400	200	400	200	150	400	450	400

White_peat_4A	100	560	400	200	400	200	150	400	450	400
White_peat_4B	102	560	400	200	400	200	150	400	450	400

Table 4 A list of the ions, and the value at PC2 gathered from the PCA model of polar aliquot laboratory aged sherds, red vs. white. The top loadings represent the most characteristic ions for each samples, white wine and red wine.

RED			**WHITE**	
m/z	PC2		m/z	PC2
527.03951	-0.194197		215.00466	0.223288
560.98543	-0.1637913		133.01425	0.1833406
129.03594	-0.1581585		653.21532	0.1547995
271.10313	-0.1449812		483.13312	0.1534142
247.01336	-0.1370775		463.14577	0.1357549
405.00264	-0.1315703		459.13557	0.1308625
517.00433	-0.1313375		377.08555	0.1290473
463.00853	-0.1220809		387.11429	0.1130887
667.02731	-0.1188552		402.13333	0.1070036
171.01486	-0.1156026		401.12986	0.1058682
189.05752	-0.1124271		217.00433	0.1020022
605.02044	-0.1067045		483.12071	0.0987837
129.03645	-0.1029562		217.02974	0.0973852
501.02638	-0.1018672		263.04085	0.0954415
545.0116	-0.1005002		215.03271	0.0934456
329.01647	-0.0993011		397.24447	0.092283
171.02743	-0.0982178		127.01675	0.0853919
384.27546	-0.0948836		143.035	0.0846085
343.03704	-0.0936314		341.10872	0.0843529
348.98245	-0.092944		321.08015	0.0832489
490.99788	-0.0917557		425.27584	0.0827095
128.0401	-0.0912604		138.04892	0.080884
687.02433	-0.0879469		137.04553	0.0783973

297.11895	-0.0871712		217.00158	0.0757216
567.05197	-0.0856982		439.08597	0.0749727
161.06248	-0.0825848		420.23502	0.0744596
162.06596	-0.0821917		143.99149	0.0741534
415.1456	-0.0758327		129.01381	0.0730377
160.0662	-0.0752306		478.98659	0.0726884
269.08748	-0.0717381		329.2081	0.0699409
344.03805	-0.071203		130.08737	0.069071
329.23314	-0.0705731		217.00475	0.0662399
337.07742	-0.0702788		203.02304	0.064961
348.98584	-0.0637976		165.97897	0.0640402
211.0822	-0.0635616		145.05066	0.0635167
346.01155	-0.0614021		135.0663	0.0633933
345.03464	-0.0596478		559.11953	0.0631855
199.03766	-0.0590428		125.0432	0.0629459
411.00161	-0.0544935		315.22888	0.0621023
399.08197	-0.0540084		238.99371	0.06153
488.02344	-0.0537299		463.32769	0.0612237
425.04009	-0.0483255		191.07316	0.0606247
1254.7698	-0.047535		199.02233	0.059333
487.04645	-0.0451493		202.02655	0.0583972
1238.7748	-0.044271		202.02622	0.0583972
343.12436	-0.0436145		201.02539	0.0571529
441.01793	-0.0434836		202.02885	0.0567729
485.04887	-0.0431113		343.26006	0.0566648
443.19233	-0.041562		239.07699	0.0563589
412.00214	-0.0411227		173.0626	0.0550716

Table 5 The top 50 loadings from the wine polar aliquots and wine KOH aliquots (red and white wine inclusive).

POLAR			KOH	
m/z	PC1		m/z	PC1
137.05	-0.125		149.01	-0.102
133.05	-0.111		175.06	-0.094
205.09	-0.108		133.01	-0.085
134.05	-0.102		321.01	-0.082
147.07	-0.099		336.98	-0.076
269.09	-0.098		147.03	-0.076
204.09	-0.096		133.05	-0.071
173.06	-0.095		153.02	-0.07
193.04	-0.092		191.02	-0.07
271.1	-0.091		147.07	-0.069
313.11	-0.089		133.05	-0.066
135.03	-0.089		150.01	-0.063
147.03	-0.088		508.98	-0.056
215	-0.086		176.06	-0.055
167.06	-0.086		133.01	-0.055
195.05	-0.085		133.01	-0.054
133.01	-0.084		175.06	-0.054
241.09	-0.083		74.505	-0.054
206.1	-0.081		493.01	-0.053
149.05	-0.078		163.04	-0.051
206.09	-0.077		486.99	-0.051
219.07	-0.077		131	-0.05

POLAR			KOH	
m/z	PC1		m/z	PC1
197.07	-0.076		220.01	-0.047
315.13	-0.074		211.03	-0.046
138.05	-0.074		530.96	-0.046
181.07	-0.072		206.97	-0.046
117.02	-0.071		144.03	-0.045
129.04	-0.07		181.01	-0.045
148.07	-0.07		477.07	-0.044
135.05	-0.068		193	-0.044
255.11	-0.067		307.01	-0.044
165.04	-0.066		195.03	-0.044
253.09	-0.065		144.05	-0.043
475.17	-0.063		134.05	-0.042
211.08	-0.062		187.04	-0.042
461.13	-0.06		322.01	-0.042
161.06	-0.06		197.05	-0.042
217.02	-0.059		320.99	-0.042
311.1	-0.057		117.02	-0.041
146.05	-0.056		342.99	-0.04
211.01	-0.056		329.09	-0.04
179.06	-0.056		189.02	-0.04
160.07	-0.055		151.01	-0.038
212.03	-0.055		209.01	-0.038

199.02	-0.077		215.07	-0.05	209.07	-0.055		196.99	-0.038
343.12	-0.076		179.04	-0.049	397.07	-0.054		199.17	-0.038
214.99	-0.076		524.95	-0.047	189.06	-0.054		103	-0.037

Table 6 A list of the ions, corresponding intensity, and the value at PC2 gathered from the PCA model Figure 12b of the alkaline fusion laboratory aged sherds, red vs. white. The top loadings represent the most characteristic ions for each samples, white wine and red wine.

Top 200 negative loadings for white wine				Top 200 positive loadings for red wine		
M/Z	INTENSITY	PC2		M/Z	INTENSITY	PC2
133.0147	71903.8	-0.10587		338.97	1150248	0.145774
133.0137	82074.4	-0.09579		197.0454	6107928	0.132806
477.0109	199390.7	-0.09325		477.0673	531727.4	0.118234
305.0125	462880.7	-0.08926		198.0491	414474.2	0.11277
473.2122	11932.8	-0.08823		478.0708	119597.8	0.089112
267.0357	117614.7	-0.08745		204.9911	121569	0.087793
505.3357	174178.9	-0.08511		381.0171	107147.9	0.087318
470.994	139276.9	-0.08471		187.0421	313918.4	0.086387
492.9849	176803.1	-0.08138		327.0871	337434.4	0.08605
320.9863	859571.1	-0.07991		169.0143	182359	0.085592
664.9836	98735.9	-0.07882		199.0168	1125706	0.085588
476.99	115577.7	-0.07541		253.0717	211403.7	0.083339
133.0142	16494795	-0.07426		339.9733	80539.8	0.082664
493.0057	447212.5	-0.07259		201.0139	345164.1	0.082125
320.9774	81105.8	-0.07116		337.0198	206399.5	0.080551
461.0161	69129.2	-0.06989		220.0015	67679.4	0.080186

326.9943	94775.9	-0.06855		167.035	350068.8	0.078243
215.0174	167385.1	-0.06821		611.2497	75170.1	0.074704
508.9798	460347.1	-0.06695		125.0011	127918.5	0.074097
135.0186	145992.9	-0.06628		117.0189	80536.2	0.073741
648.9887	56978.3	-0.06564		293.0301	176702.6	0.073049
508.9594	65995.5	-0.06432		119.0502	379780.7	0.071845
133.0155	3609.1	-0.06416		188.9962	94910.2	0.070899
486.9889	341052	-0.06402		187.0977	399486.6	0.068436
321.9897	35761.6	-0.0633		329.0664	505228.1	0.067755
137.0609	181053	-0.06311		328.0905	31023.9	0.066742
514.967	51574.7	-0.06299		89.02438	57476.2	0.065648
275.0215	47151.7	-0.06239		363.2385	30639.7	0.065598
649.0095	47489.1	-0.06144		205.0144	98961.8	0.065051
680.9782	135886.4	-0.06075		247.0247	32656	0.064841
680.958	59923	-0.06063		295.2641	33168.3	0.064624
506.3391	52447.9	-0.0588		202.9989	86451.7	0.064011
309.0825	51537.9	-0.05846		407.0046	151572.9	0.062924
664.9633	49303.8	-0.05725		183.03	115160.7	0.062921
665.0043	83147.6	-0.057		186.0457	58457.4	0.06265
134.0183	45599.9	-0.05688		138.0323	42004.6	0.062579
658.9953	39855.5	-0.05674		331.0456	644847.9	0.062488
658.9887	66661.7	-0.05612		199.05	25216.1	0.06229
199.1703	2247787	-0.0542		211.025	262104.9	0.062031
342.9682	85536.3	-0.05419		129.0365	111334.9	0.061633
321.0072	4254193	-0.05349		374.3009	7910.7	0.06163
530.941	55674.2	-0.0534		200.0202	106458.6	0.060818
658.9676	32114	-0.05323		331.082	44186.4	0.060172
200.1738	253765.3	-0.0528		444.9607	43176.9	0.058668
393.3008	74922.2	-0.05225		163.04	1344736	0.057309

411.2359	51327.8	-0.05211		355.3215	27417.7	0.05694
454.9991	37614.9	-0.05155		190.9932	38303.2	0.05692
514.9879	75940.4	-0.05124		319.0821	27502.2	0.056117
492.9642	29224.2	-0.05048		345.228	332434.9	0.056019
322.9845	21379.3	-0.05041		126.9981	40944.7	0.055918
322.0106	344746	-0.05028		203.0119	28667.7	0.055095
509.9833	51762.1	-0.05027		385.0177	35125.4	0.054984
173.0264	102621.2	-0.05014		206.9882	19023.9	0.054152
478.0141	36201	-0.04971		398.9549	29105.4	0.053882
530.9618	190465.1	-0.04963		164.0435	114668.3	0.05311
494.0093	50165.4	-0.04951		507.1146	22029.8	0.05264
524.9537	223538.8	-0.04917		374.2991	127362.3	0.051869
483.4419	93626.7	-0.04917		329.2331	2201009	0.05175
476.9815	26358.7	-0.04786		330.2364	369463	0.051703
479.0099	63125.6	-0.04732		209.0628	220540.3	0.051293
508.9509	24105.2	-0.04647		128.0401	40673.2	0.051188
494.9835	40093.7	-0.04646		411.2517	21538.8	0.050913
130.9987	298220	-0.0462		311.1134	27918.2	0.050614
483.167	109726.7	-0.04574		333.0613	79861.1	0.050355
197.0821	183841.5	-0.04553		217.0122	11987.2	0.050111
508.972	74290	-0.04538		515.0235	22779.5	0.050109
299.0254	538505.8	-0.04538		413.3078	29674.6	0.050048
521.3308	34191.2	-0.04524		383.3529	35462.9	0.050029
613.3745	37591.6	-0.04467		153.0194	1799687	0.049715
259.1914	96167.2	-0.04463		154.0228	94899.7	0.0493
427.231	70474.6	-0.04437		481.3171	33057.7	0.04915
497.1826	104167.3	-0.0443		188.1011	27975.9	0.048762
255.2309	959173.6	-0.0441		191.0351	94746.1	0.047728
493.9881	21235	-0.04386		373.2958	625185.2	0.047393

510.9787	28825.8	-0.04367		327.2175	420766	0.047387
492.9768	31281.2	-0.04354		120.0536	26293.9	0.047363
336.9706	30259.9	-0.04351		202.0174	11448.4	0.047058
674.9618	43466.9	-0.04344		197.0466	31070.9	0.04655
648.968	16588.9	-0.04283		199.1715	9108.8	0.046404
674.9663	5725.8	-0.04256		612.2532	21074.2	0.046073
665.9869	19618.6	-0.04254		395.0983	15226.9	0.045887
199.1686	16430.2	-0.04247		148.0697	92661.8	0.045811
511.1985	51475.3	-0.04238		199.0968	8431.8	0.0458
487.9924	38597	-0.04204		152.0116	18312.8	0.045501
473.4	114888.6	-0.04194		138.0278	323556.6	0.045222
478.9885	21955.3	-0.04181		319.0457	46926.5	0.045004
455.4107	36661.2	-0.04154		161.0628	84723.9	0.044973
460.9951	16945.5	-0.04148		319.0302	58867.5	0.044962
323.0115	77952.7	-0.04137		499.0492	19536.7	0.044909
337.3109	170746.6	-0.04113		163.0389	25534.2	0.044849
524.9454	80054.9	-0.04044		137.0244	5126490	0.044817
539.3414	22925.7	-0.04035		223.025	78464.2	0.044485
516.9657	30108.4	-0.04033		173.0012	20127.5	0.044462
585.3432	23731.6	-0.04003		328.2208	31153.9	0.044406
526.9524	23553.3	-0.0398		535.1095	12683.5	0.044313
475.2008	42903.9	-0.03962		138.0273	3458.9	0.04428
471.0029	11777.3	-0.03913		221.0093	15493.1	0.044261
471.9975	10844.7	-0.03824		329.0876	214837.2	0.044233
666.9818	20078.8	-0.03787		331.2043	37486.3	0.044118
481.1877	13688.6	-0.03779		208.0665	37983.7	0.043901
199.1692	49615.9	-0.03772		147.0663	1883832	0.043791
307.0113	300145.2	-0.03759		343.2123	237771	0.043294
595.5419	136565.3	-0.03745		369.2645	24987.2	0.04322

571.1994	53079.4	-0.03733		387.1659	42851.7	0.042923
477.9933	10798	-0.03705		181.0143	180546.4	0.042825
681.982	18580.2	-0.03654		395.2775	14448	0.04117
530.9325	14962	-0.03596		199.0249	70983.8	0.041154
467.1722	10946.9	-0.03593		345.2643	159427.1	0.041043
555.2044	7387.8	-0.03565		641.4635	19354.6	0.040428
165.9792	25547.4	-0.03556		483.3327	29554.3	0.040348
463.015	17536.3	-0.03544		205.161	3472.7	0.040255
525.9572	19640.1	-0.0352		449.109	8430.7	0.039971
530.9536	37396.2	-0.03504		195.0291	7881.1	0.039855
659.9987	7092.6	-0.03475		493.0988	8212.7	0.039855
133.013	8655.3	-0.03457		197.0093	78631	0.039801
650.9867	8322.2	-0.03454		125.0244	45838.1	0.039212
439.2677	8179.1	-0.03454		213.0406	51947.8	0.039157
172.03	12744	-0.03444		581.2395	10222.3	0.038623
484.4453	23223.6	-0.03438		447.3117	31808.3	0.038492
492.9557	10313.1	-0.03428		190.952	82211	0.038238
512.4766	110661.2	-0.03408		179.0351	313212.1	0.038172
536.949	8323.3	-0.03393		147.0452	39486.2	0.038048
471.0239	17836.2	-0.03383		207.0301	116377.9	0.038018
283.2594	62435.3	-0.03381		466.9427	12645.4	0.037371
482.998	8126.3	-0.03369		131.0714	494901.4	0.037072
474.4033	31372.3	-0.03319		181.0507	70728.7	0.036934
511.4732	324923	-0.03309		255.0509	130108.2	0.036911
190.973	18146.5	-0.03292		119.0344	30980.5	0.036588
469.1513	19024.1	-0.03281		469.0776	6929.4	0.036352
396.3118	49650.3	-0.03258		76.50966	17451	0.036175
596.5453	44626.6	-0.03231		428.9865	8256.9	0.036136
514.9461	16892.5	-0.03228		333.1108	121686.8	0.036116

718.9345	20686.2	-0.03224		209.0093	101160.1	0.035661
484.1703	14999.7	-0.03166		271.0822	17185.8	0.035316
498.1859	14839.1	-0.0316		482.9166	7630.1	0.035099
729.6408	17820.8	-0.03154		195.03	323515.6	0.035098
532.9394	15306.1	-0.03149		243.1237	49227.2	0.034765
337.9845	133588.5	-0.03136		395.2747	144163.1	0.034657
462.0191	6040.5	-0.03131		211.0614	76892.5	0.034576
220.1457	897647.3	-0.03108		445.2959	17422.6	0.034392
546.9273	41210.8	-0.03104		383.2438	19751.5	0.034229
666.9608	6494	-0.03095		151.0402	191046.4	0.034143
546.9358	95855.4	-0.03087		293.2121	413741.2	0.033659
514.9588	7747	-0.03084		519.2731	6385	0.03349
336.9812	2099819	-0.03043		213.1134	160215.6	0.03341
531.9653	17235.9	-0.0303		217.1083	324345.5	0.033214
338.9793	89595.1	-0.02989		260.8975	30841.4	0.033117
529.4626	244608.1	-0.02982		313.0927	260684.2	0.033098
489.2165	13518.7	-0.02963		139.0287	14041.7	0.032826
757.6717	23179.7	-0.02932		148.0333	40194.2	0.032436
461.1851	25286.8	-0.0293		427.234	25770.2	0.03238
679.3176	79936.9	-0.0293		191.0198	860206.9	0.0317
484.9968	6046.4	-0.02927		146.9831	4200.4	0.031593
533.4065	52927.3	-0.02924		357.1965	229198.5	0.031495
342.9892	223427.3	-0.02916		123.0452	9926.4	0.030545
336.9723	119011.1	-0.02828		147.03	1233057	0.030543
666.0079	8297	-0.02784		327.1082	26625.9	0.030443
193.1348	209198.8	-0.02781		294.2154	26600.1	0.03032
132.002	5160.5	-0.02765		639.4479	7313.5	0.030171
530.4661	82362.7	-0.0276		743.1623	10860.9	0.030088
536.9699	14082.7	-0.02751		383.2074	22294.2	0.030068

582.3496	8426.6	-0.02741		325.2018	54031.8	0.029803
552.9441	37987.2	-0.02731		73.53314	17336.7	0.029782
265.1478	173713.2	-0.02731		205.009	1850.8	0.029757
396.9935	11606.8	-0.02723		665.1516	9882.7	0.029719
650.0128	3838.6	-0.02701		132.9464	291371.9	0.029676
527.1724	31993.2	-0.02648		413.2543	10933	0.029663
671.1398	7420.8	-0.02628		317.0665	22657.4	0.029496
198.0409	23883	-0.02625		555.3539	5563.9	0.029231
476.9691	11858.9	-0.02621		132.0748	18603.9	0.029085
561.4378	90041.5	-0.02615		329.1653	162442.6	0.029007
597.5575	37226.8	-0.02609		270.978	14639.2	0.028862
552.923	7037.5	-0.0259		151.0038	55326.4	0.028731
513.1567	39052.7	-0.02571		341.1969	25697.2	0.028413
670.9713	11941.6	-0.02557		403.1657	16295.2	0.028097
499.1409	34451.8	-0.02532		655.4436	9303.3	0.027996
227.2016	306456.6	-0.02512		121.0295	1673497	0.027766
221.1491	66900.8	-0.025		505.0622	4661	0.027596
614.3779	8730	-0.02486		333.22	11255	0.027191
474.2214	599522.3	-0.02478		412.8931	21428.7	0.027183
473.2178	2145201	-0.02476		173.082	29434.1	0.026982
669.1818	11162.9	-0.02471		122.0329	135513.6	0.02698
498.9719	7622.3	-0.02468		198.0481	15130.8	0.026768
669.173	5045.3	-0.02459		209.0457	173610.6	0.026647
395.1925	63212.7	-0.02436		142.9809	5252.3	0.026597
157.1235	33239.7	-0.02425		201.1134	112884.4	0.026515
485.2827	531808.4	-0.02425		401.2543	15403.3	0.026027
465.1449	27083.3	-0.0241		635.1772	31613.4	0.025988
651.008	7697.4	-0.02366		395.2439	20226.9	0.025653
221.1549	157998.6	-0.02345		135.0452	49124.8	0.025589

655.166	10449.8	-0.02332		192.9492	23599.5	0.025412
473.2825	30373.5	-0.02316		192.0232	33143.7	0.025314
685.1553	5685.1	-0.0228		525.1035	8640.3	0.024654
133.0501	74438.4	-0.02277		127.0037	16504.5	0.024618
572.2027	4288.4	-0.02266		152.0354	14025.8	0.024386
397.2566	24519.9	-0.02259		339.3266	192104.1	0.023777
727.2669	64700.5	-0.02209		371.2802	26932.3	0.023713
304.9915	88300.3	-0.02205		401.0877	125876.4	0.023196
403.3248	110704.7	-0.02184		277.2172	333304	0.02306
455.2417	9024.5	-0.0218		144.0456	256363.6	0.022653

Table 7 A list of the ions, corresponding intensity, and the value at PC1 gathered from figure 3b of water vs. wine, KOH aliquot. The table is sorted on the top 150 values of PC1 from the smallest to the largest value.

M/Z	INTENSITY	PC1	Empirical formula (parent)	Empirical formula (peak)	Ion form	Theoretical mass (neutral) (Da)	Theoretical m/z (Da)	Mass error (ppm)	KEGG_COMPOUND
149.00912	12839920.3	-0.102401503	C2H2O4	C2H2O4	[M+Hac-H]-	89.99531	149.0091636	-0.29	['Oxalate']
149.00912	12839920.3	-0.102401503	C4H6O6	C4H6O6	[M-H]-	150.01644	149.0091636	-0.29	['(R,R)-Tartaric acid', '(S,S)-Tartaric acid', 'meso-Tartaric acid']
175.06112	4971425.5	-0.094286393	C5H8O3	C5H8O3	[M+Hac-H]-	116.047345	175.0611986	-0.45	['2-Oxopentanoic acid', '3-Methyl-2-oxobutanoic acid', '3-Oxopentanoic acid', '5-Oxopentanoate']
175.06112	4971425.5	-0.094286393	C7H12O5	C7H12O5	[M-H]-	176.068475	175.0611986	-0.45	['(2R,3S)-3-Isopropylmalate', '(R)-2-(n-Propyl)-malate', '2-Propylmalate', '3-Propylmalate', 'alpha-Isopropylmalate']
133.01418	16494794.9	-0.085369204	C2H2O3	C2H2O3	[M+Hac-H]-	74.000395	133.0142486	-0.52	['Glyoxylate']
133.01418	16494794.9	-0.085369204	C4H6O5	C4H6O5	[M-H]-	134.021525	133.0142486	-0.52	['(R)-Malate', '(S)-Malate', '3-Dehydro-L-threonate', 'Malate']
321.00724	4254192.7	-0.082271453		0					0
336.98117	2099819.3	-0.075821148		0					0
147.02996	1233057	-0.075751737	C3H4O3	C3H4O3	[M+Hac-H]-	88.016045	147.0298986	0.42	['3-Hydroxypropenoate', '3-Oxopropanoate', 'Pyruvate']
147.02996	1233057	-0.075751737	C5H8O5	C5H8O5	[M-H]-	148.037175	147.0298986	0.42	['(R)-2-Hydroxyglutarate', '(R)-2-Methylmalate', '(S)-2-Hydroxyglutarate', '(S)-2-Methylmalate', '2-Dehydro-3-deoxy-D-xylonate', '2-Dehydro-3-deoxy-L-arabinonate', '2-Hydroxyglutarate', 'Citramalate', 'D-Arabinono-1,4-lactone', 'D-Xylono-1,4-lactone', 'D-Xylonolactone', 'D-erythro-3-Methylmalate', 'D-threo-3-Methylmalate', 'L-Arabinono-1,4-lactone', 'L-Arabinono-1,5-lactone', 'L-Xylono-1,4-lactone', 'L-threo-3-Methylmalate']

133.05012	74438.4	-0.070841319		0					0
153.01939	1799686.6	-0.070222273	C7H6O4	C7H6O4	[M-H]-	154.02661	153.0193336	0.37	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']
191.01982	860206.9	-0.070063079	C4H4O5	C4H4O5	[M+Hac-H]-	132.005875	191.0197286	0.48	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']
191.01982	860206.9	-0.070063079	C6H8O7	C6H8O7	[M-H]-	192.027005	191.0197286	0.48	['(1R,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(1S,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(4R,5S)-4,5,6-Trihydroxy-2,3-dioxohexanoate', '2,5-Didehydro-D-gluconate', '2-Dehydro-3-deoxy-D-glucarate', '5-Dehydro-4-deoxy-D-glucarate', 'Carboxymethyloxysuccinate', 'Citrate', 'Isocitrate']
147.06634	1883832.2	-0.068889369	C4H8O2	C4H8O2	[M+Hac-H]-	88.05243	147.0662836	0.38	['(R)-Acetoin', '1,4-Dioxane', '2-Methylpropanoate', 'Acetoin', 'Butanoic acid', 'Ethyl acetate']
147.06634	1883832.2	-0.068889369	C6H12O4	C6H12O4	[M-H]-	148.07356	147.0662836	0.38	['(R)-2,3-Dihydroxy-3-methylpentanoate', '(R)-Mevalonate', '(R)-Pantoate', '(S)-Mevalonate', '2,3-Dihydroxy-3-methylpentanoate', '3,6-Dideoxy-L-galactose', 'Abequose']
133.05061	3301580.2	-0.06607991	C3H6O2	C3H6O2	[M+Hac-H]-	74.03678	133.0506336	-0.18	['(R)-Lactaldehyde', '(S)-Lactaldehyde', '3-Hydroxypropanal', 'Glycidol', 'Hydroxyacetone', 'Lactaldehyde', 'Methyl acetate', 'Propanoate']
133.05061	3301580.2	-0.06607991	C5H10O4	C5H10O4	[M-H]-	134.05791	133.0506336	-0.18	['(R)-2,3-Dihydroxy-3-methylbutanoate', '1-Deoxy-D-xylulose', '2,3-Dihydroxy-3-methylbutanoate', '2-Deoxy-L-arabinose', '2-Deoxy-alpha-D-ribopyranose', 'Deoxyribose']
150.01262	395150	-0.062934511		0					0
508.97983	460347.1	-0.055860612		0					0
176.06465	321586.9	-0.05546201		0					0
133.01365	82074.4	-0.055236115		0					0

133.0147	71903.8	-0.054447121		0					0
175.06207	7760.3	-0.053993069		0					0
74.50455	214325.8	-0.053743837		0					0
493.00574	447212.5	-0.052869566		0					0
163.04002	1344736.2	-0.050834612	C9H8O3	C9H8O3	[M-H]-	164.047345	163.0400686	-0.3	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']
486.98886	341052	-0.050511794		0					0
130.99865	298220	-0.049814628	C4H4O5	C4H4O5	[M-H]-	132.005875	130.9985986	0.39	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']
215.07339	235640.8	-0.049587507		0					0
179.03508	313212.1	-0.048992668	C9H8O4	C9H8O4	[M-H]-	180.04226	179.0349836	0.54	['2-Hydroxy-3-(4-hydroxyphenyl)propenoate', '3-(4-Hydroxyphenyl)pyruvate', 'Aspirin', 'Caffeate', 'trans-2,3-Dihydroxycinnamate']
524.95372	223538.8	-0.047072946		0					0
220.00756	258793.6	-0.046855575		0					0
211.02497	262104.9	-0.046164544	C9H8O6	C9H8O6	[M-H]-	212.03209	211.0248136	0.74	['2-Hydroxy-6-ketononatrienedioate', '3-(2-Carboxyethenyl)-cis,cis-muconate', '5-Carboxyvanillic acid']
530.96179	190465.1	-0.045647873		0					0
206.96793	191453.9	-0.045614948	C3H7O7P	C3H7O7P	[M+Na-2H]-	185.992943	206.9676116	1.54	['2-Phospho-D-glycerate', '3-Phospho-D-glycerate', '3-Phospho-DL-glycerate']
144.03033	141336.5	-0.045401167	C5H7NO4	C5H7NO4	[M-H]-	145.037509	144.0302326	0.68	['2-Oxoglutaramate', '4-Oxoglutaramate']

181.01434	180546.4	-0.04498824	C8H6O5	C8H6O5	[M-H]-	182.021525	181.0142486	0.5	['2-Hydroxyisophthalic acid', '3,5-Dihydroxyphenylglyoxylate', '4-Hydroxyphthalate', 'Stipitatate']
477.06728	531727.4	-0.044173816		0					0
192.99662	136176.3	-0.0439438		0					0
307.01125	300145.2	-0.043572018		0					0
195.03004	323515.6	-0.043538515	C9H8O5	C9H8O5	[M-H]-	196.037175	195.0298986	0.73	['3-(3,4-Dihydroxyphenyl)pyruvate']
144.04555	256363.6	-0.043125123	C9H7NO	C9H7NO	[M-H]-	145.052764	144.0454876	0.43	['1(2H)-Isoquinolinone', '3-Methyleneoxindole', '8-Hydroxyquinoline', 'Indole-3-carboxaldehyde', 'Quinolin-2-ol', 'Quinolin-4-ol']
134.05405	160816.9	-0.042315225		0					0
187.04205	313918.4	-0.042302435		0					0
322.01063	344746	-0.042106671	C10H13O10P	C10H13O10P	[M-2H]-	324.024638	322.0100852	1.69	['5-O-(1-Carboxyvinyl)-3-phosphoshikimate']
322.01063	344746	-0.042106671	C7H14NO9P	C7H14NO9P	[M+Cl]-	287.040622	322.0100236	1.88	['AminoDAHP']
322.01063	344746	-0.042106671	C8H18NO4PS2	C8H18NO4PS2	[M+Cl]-	287.041491	322.0108926	-0.82	['Vamidothion']
322.01063	344746	-0.042106671	C9H20NO3PS2	C9H20NO3PS2	[M+K-2H]-	285.062226	322.0108326	-0.63	['Prothoate']
197.04542	6107928.3	-0.041884423	C7H6O3	C7H6O3	[M+Hac-H]-	138.031695	197.0455486	-0.65	['2-Hydroxy-5-methylquinone', '3,4-Dihydroxybenzaldehyde', '3-Hydroxybenzoate', '4-Hydroxybenzoate', 'Gentisate aldehyde', 'Salicylate', 'Sesamol']
197.04542	6107928.3	-0.041884423	C9H10O5	C9H10O5	[M-H]-	198.052825	197.0455486	-0.65	['3-(3,4-Dihydroxyphenyl)lactate', '3-Methoxy-4-hydroxymandelate', 'Syringic acid']
320.98631	859571.1	-0.041633171		0					0
117.0193	2144058.9	-0.041306416	C2H2O2	C2H2O2	[M+Hac-H]-	58.00548	117.0193336	-0.29	['Glyoxal']

117.0193	2144058.9	-0.041306416	C4H6O4	C4H6O4	[M-H]-	118.02661	117.0193336	-0.29	['Methyl oxalate', 'Methylmalonate', 'Succinate']
342.98921	223427.3	-0.040309349		0					0
329.08755	214837.2	-0.040272106	C18H16N2O2	C18H16N2O2	[M+(37Cl)]-	292.121178	329.0876296	-0.24	['INF271']
189.02132	99540	-0.040084789		0					0
151.01354	79540.4	-0.038475372		0					0
209.00931	101160.1	-0.038331243		0					0
196.99156	94211.2	-0.037744932		0					0
199.17149	9108.8	-0.037652533		0					0
103.00368	88796.1	-0.037181229	C3H4O4	C3H4O4	[M-H]-	104.01096	103.0036836	-0.03	['2-Hydroxy-3-oxopropanoate', 'Hydroxypyruvate', 'Malonate']
103.00368	88796.1	-0.037181229	CO2	CO2	[M+Hac-H]-	43.98983	103.0036836	-0.03	['CO2']
169.01433	182359	-0.036673686	C7H6O5	C7H6O5	[M-H]-	170.021525	169.0142486	0.48	['Gallate']
154.0228	94899.7	-0.036359313		0					0
197.00934	78631	-0.035967678	C8H6O6	C8H6O6	[M-H]-	198.01644	197.0091636	0.9	['3,4-Dihydroxyphthalate', '4,5-Dihydroxyphthalate']
148.06974	92661.8	-0.035767219		0					0
546.93579	95855.4	-0.035692706		0					0
524.9454	80054.9	-0.035499922		0					0
200.09299	198539.2	-0.035489295	C7H11NO2	C7H11NO2	[M+Hac-H]-	141.078979	200.0928326	0.79	['Arecaidine', 'Ethosuximide', 'Guvacoline', 'L-Hypoglycin']
305.01245	462880.7	-0.035406426		0					0
103	133588.5	-0.035353525		0					0

207.03006	116377.9	-0.035199659	C10H8O5	C10H8O5	[M-H]-	208.037175	207.0298986	0.78	['Fraxetin']
199.01683	1125705.9	-0.03496134	C10H10O2	C10H10O2	[M+K-2H]-	162.06808	199.0166866	0.72	['(1S,2S)-1,2-Dihydronaphthalene-1,2-diol', '1,2-Dihydronaphthalene-1,2-diol', '4-Hydroxycinnamoylmethane', 'Isosafrole', 'Methyl cinnamate', 'Safrole', 'cis-1,2-Dihydronaphthalene-1,2-diol', 'p-Methoxycinnamaldehyde', 'trans-2-Phenylcyclopropanecarboxylic acid']
199.01683	1125705.9	-0.03496134	C9H8O3	C9H8O3	[M+Cl]-	164.047345	199.0167466	0.42	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']
299.02541	538505.8	-0.034655317	C12H10N2O5	C12H10N2O5	[M+(37Cl)]-	262.058973	299.0254246	-0.05	['Cinoxacin']
135.01857	145992.9	-0.034305511		0					0
164.04351	114668.3	-0.033715967		0					0
183.03001	115160.7	-0.033479499	C6H4O3	C6H4O3	[M+Hac-H]-	124.016045	183.0298986	0.61	['2-Hydroxy-1,4-benzoquinone']
183.03001	115160.7	-0.033479499	C8H8O5	C8H8O5	[M-H]-	184.037175	183.0298986	0.61	['3,4-Dihydroxymandelate', '3-O-Methylgallate']
373.29583	625185.2	-0.03328096		0					0
223.02498	78464.2	-0.033183711		0					0
199.02492	70983.8	-0.033044207	C6H4O4	C6H4O4	[M+Hac-H]-	140.01096	199.0248136	0.53	['cis-4-Carboxymethylenebut-2-en-4-olide', 'trans-4-Carboxymethylenebut-2-en-4-olide']
199.02492	70983.8	-0.033044207	C8H8O6	C8H8O6	[M-H]-	200.03209	199.0248136	0.53	['(3S,4R)-3,4-Dihydroxycyclohexa-1,5-diene-1,4-dicarboxylate', '2-Hydroxy-5-carboxymethylmuconate semialdehyde', '3-Carboxymethylmuconate', '4-Fumarylacetoacetate', '4-Maleylacetoacetate', 'Phthalate 3,4-cis-dihydrodiol', 'cis-4,5-Dihydroxycyclohexa-1(6),2-diene-1,2-dicarboxylate']
319.02824	54064.5	-0.03293396	C12H15N2O3PS	C12H15N2O3PS	[M+Na-2H]-	298.054103	319.0287716	-1.67	['Phoxim', 'Quinalphos']

149.00817	15207.7	-0.03286484	C3H4N4O2	C3H4N4O2	[M+Na-2H]-	128.033426	149.0080946	0.51	['Ammelide']
119.05024	379780.7	-0.032784001	C8H8O	C8H8O	[M-H]-	120.057515	119.0502386	0.01	['2-Methylbenzaldehyde', '3-Methylbenzaldehyde', '4-Hydroxystyrene', 'Acetophenone', 'Phenylacetaldehyde', 'Styrene oxide', 'p-Tolualdehyde']
680.97824	135886.4	-0.032728389		0					0
205.01439	98961.8	-0.032722519	C10H6O5	C10H6O5	[M-H]-	206.021525	205.0142486	0.69	['Flaviolin']
173.02638	102621.2	-0.032478461		0					0
198.04905	414474.2	-0.032454592		0					0
201.01393	345164.1	-0.032280828	C9H8O3	C9H8O3	[M+(37Cl)]-	164.047345	201.0137966	0.66	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']
189.07696	334513.5	-0.031522479	C6H10O3	C6H10O3	[M+Hac-H]-	130.062995	189.0768486	0.59	['(3R)-3-Methyl-2-oxopentanoic acid', '(R)-Pantolactone', '(S)-3-Methyl-2-oxopentanoic acid', '1-Oxa-2-oxo-3-hydroxycycloheptane', '2-Hydroxyethyl methacrylate', '2-Oxohexanoic acid', '3-Methyl-2-oxopentanoate', '3-Oxohexanoic acid', '4-Methyl-2-oxopentanoate', '5-Oxohexanoic acid', '6-Hydroxyhexan-6-olide', 'Adipate semialdehyde', 'Ethyl 3-oxobutanoate', 'beta-Ketoisocaproate']
189.07696	334513.5	-0.031522479	C8H14O5	C8H14O5	[M-H]-	190.084125	189.0768486	0.59	['(R)-3-((R)-3-Hydroxybutanoyloxy)butanoate']
329.06638	505228.1	-0.031465144	C15H10O5	C15H10O5	[M+Hac-H]-	270.052825	329.0666786	-0.91	['2-Hydroxydaidzein', "3',4',7-Trihydroxyisoflavone", '3,6,4-Trihydroxyflavone', "4',6,7-Trihydroxyisoflavone", '5-Deoxykaempferol', 'Aloe-emodin', 'Apigenin', 'Baicalein', 'Emodin', 'Galangin', 'Genistein', 'Islandicin', 'Lucidin', 'Morindone', 'Norobtusifolin', 'Norwogonin', 'Purpurin 1-methyl ether', 'Sulphuretin']

329.06638	505228.1	-0.031465144	C17H14O7	C17H14O7	[M-H]-	330.073955	329.0666786	-0.91	['(+)-Bisdechlorogeodin', '(-)-Bisdechlorogeodin', "3',4',5-Trihydroxy-3,7-dimethoxyflavone", 'Aflatoxin G2', 'Aurantio-obtusin', 'Cirsiliol', 'Hildecarpin', 'Tricin']
325.07742	78652.1	-0.031399294		0					0
378.99191	64958.4	-0.031343078		0					0
205.03551	30085.2	-0.03117134	C5H6O5	C5H6O5	[M+Hac-H]-	146.021525	205.0353786	0.64	['2-Oxoglutarate', '5-Hydroxy-2,4-dioxopentanoate', 'Dehydro-D-arabinono-1,4-lactone', 'Methyloxaloacetate', 'Oxaloacetate 4-methyl ester']
205.03551	30085.2	-0.03117134	C7H10O7	C7H10O7	[M-H]-	206.042655	205.0353786	0.64	['(2S,3R)-3-Hydroxybutane-1,2,3-tricarboxylate', '(R)-2-Hydroxybutane-1,2,4-tricarboxylate', '2-Methylcitrate', 'Homoisocitrate']
492.98493	176803.1	-0.031145628		0					0
202.99892	86451.7	-0.031129631		0					0
358.9631	134325.5	-0.031096467		0					0
508.97201	74290	-0.030919315		0					0
407.00459	151572.9	-0.030868335		0					0
161.04562	67109.1	-0.030741974	C4H6O3	C4H6O3	[M+Hac-H]-	102.031695	161.0455486	0.44	['(S)-Methylmalonate semialdehyde', '2-Methyl-3-oxopropanoate', '2-Oxobutanoate', 'Acetoacetate', 'Succinate semialdehyde']
161.04562	67109.1	-0.030741974	C6H10O5	C6H10O5	[M-H]-	162.052825	161.0455486	0.44	['(2R,3S)-2,3-Dimethylmalate', '(R)-2-Ethylmalate', '(R)-3,3-Dimethylmalate', '(S)-2-(Hydroxymethyl)glutarate', '1,5-Anhydro-D-fructose', '2-Dehydro-3-deoxy-D-fuconate', '2-Dehydro-3-deoxy-L-fuconate', '2-Dehydro-3-deoxy-L-rhamnonate', '2-Deoxy-scyllo-inosose', '2-Hydroxyadipate', '3,6-Anhydrogalactose', '3,6-Anhydroglucose', '3-Ethylmalate', '3-Hydroxy-3-methylglutarate', 'D-Fucono-1,4-lactone', 'Diethyl pyrocarbonate', 'L-Fucono-1,5-lactone', 'L-Rhamnono-

									1,4-lactone', 'Lichenin']
145.01434	43325.5	-0.030439508	C5H6O5	C5H6O5	[M-H]-	146.021525	145.0142486	0.63	['2-Oxoglutarate', '5-Hydroxy-2,4-dioxopentanoate', 'Dehydro-D-arabinono-1,4-lactone', 'Methyloxaloacetate', 'Oxaloacetate 4-methyl ester']
514.98789	75940.4	-0.03026179		0					0
118.0227	102759.2	-0.03018279		0					0
477.01091	199390.7	-0.030080016		0					0
181.05073	70728.7	-0.029838377	C7H6O2	C7H6O2	[M+Hac-H]-	122.03678	181.0506336	0.53	['3-Hydroxybenzaldehyde', '4-Hydroxybenzaldehyde', 'Benzoate', 'Salicylaldehyde', 'Tropolone']
181.05073	70728.7	-0.029838377	C9H10O4	C9H10O4	[M-H]-	182.05791	181.0506336	0.53	['(R)-3-(4-Hydroxyphenyl)lactate", "2',6-Dihydroxy-4-methoxyacetophenone", '3,4-Dihydroxyphenylpropanoate', '3-(2,3-Dihydroxyphenyl)propanoate', '3-(4-Hydroxyphenyl)lactate', '3-Methoxy-4-hydroxyphenylglycolaldehyde', 'Homovanillate', 'cis-3-(3-Carboxyethenyl)-3,5-cyclohexadiene-1,2-diol']
277.2172	333304	-0.029509292	C18H30O2	C18H30O2	[M-H]-	278.22458	277.2173036	-0.37	['(6Z,9Z,12Z)-Octadecatrienoic acid', '(9Z,11E,13E)-Octadecatrienoic acid', '(9Z,12Z,15Z)-Octadecatrienoic acid', '5beta-Estrane-3alpha,17beta-diol', 'Crepenynate', 'Punicic acid']
154.01471	48661.1	-0.029363054	C6H5NO4	C6H5NO4	[M-H]-	155.021859	154.0145826	0.83	['2,6-Dihydroxynicotinate', '4-Nitrocatechol']
478.07077	119597.8	-0.029229517		0					0

163.03888	25534.2	-0.029194754		0					0
327.08714	337434.4	-0.029188092	C14H18N4O3	C14H18N4O3	[M+K-2H]-	290.137891	327.0864976	1.96	['Benomyl', 'Trimethoprim']
327.08714	337434.4	-0.029188092	C16H12O4	C16H12O4	[M+Hac-H]-	268.07356	327.0874136	-0.84	['1-[6-Hydroxy-2-(4-hydroxyphenyl)-1-benzofuran-3-yl]ethanone', '6-Hydroxy-2-methoxyflavone', 'Dalbergin', 'Formononetin', 'Isoformononetin', 'Tectochrysin']
327.08714	337434.4	-0.029188092	C18H16O6	C18H16O6	[M-H]-	328.09469	327.0874136	-0.84	['2-(4-Hydroxyphenyl)-5,6,7-trimethoxy-4H-1-benzopyran-4-one', '6-Hydroxy-2-(4-methoxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one', "7-Hydroxy-2',4',5-trimethoxyisoflavone", '9-Demethylmunduserone', 'Betagarin', 'Ophiopogonanone A']
193.05078	194456.2	-0.029083865	C10H10O4	C10H10O4	[M-H]-	194.05791	193.0506336	0.76	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']
129.01937	657155	-0.028954418	C3H2O2	C3H2O2	[M+Hac-H]-	70.00548	129.0193336	0.28	['Propynoate']
129.01937	657155	-0.028954418	C5H6O4	C5H6O4	[M-H]-	130.02661	129.0193336	0.28	['(E)-Glutaconate', '2,5-Dioxopentanoate', '2-Methylmaleate', '4,5-Dioxopentanoate', 'Acetylpyruvate', 'Itaconate', 'Mesaconate']
209.06283	220540.3	-0.028917582	C6H12N4O2	C6H12N4O2	[M+(37Cl)]-	172.096026	209.0624776	1.69	['L-Capreomycidine']
255.05092	130108.2	-0.028903558	C9H8O5	C9H8O5	[M+Hac-H]-	196.037175	255.0510286	-0.43	['3-(3,4-Dihydroxyphenyl)pyruvate']
470.99402	139276.9	-0.028876043		0					0
148.03333	40194.2	-0.028582145		0					0
313.0563	28914.6	-0.02852408		0					0
193.0144	24727.2	-0.028402811		0					0
208.96501	25035.8	-0.028231207		0					0

337.0198	206399.5	-0.028186546	C10H12N4O5S	C10H12N4O5S	[M+(37Cl)]-	300.052843	337.0192946	1.5	['Tazobactam']
337.0198	206399.5	-0.028186546	C10H12N4O7	C10H12N4O7	[M+K-2H]-	300.070601	337.0192076	1.76	['Urate-3-ribonucleoside']
214.07704	13444.7	-0.028051426		0					0
157.0507	47407.2	-0.02795424	C7H10O4	C7H10O4	[M-H]-	158.05791	157.0506336	0.42	['(1S,4S)-4-Hydroxy-3-oxocyclohexane-1-carboxylate', '2-Isopropylmaleate', '5-D-(5/6)-5-C-(Hydroxymethyl)-2,6-dihydroxycyclohex-2-en-1-one', 'Dimethyl citraconate']
497.18261	104167.3	-0.027923037		0					0
188.99615	94910.2	-0.027731671	C7H6O4	C7H6O4	[M+Cl]-	154.02661	188.9960116	0.73	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']
188.99615	94910.2	-0.027731671	C8H8O3	C8H8O3	[M+K-2H]-	152.047345	188.9959516	1.05	['(R)-Mandelate', '(S)-Mandelate', "2',4-Dihydroxyacetophenone", '2-(Hydroxymethyl)benzoic acid', '2-Hydroxyphenylacetate', "3',4-Dihydroxyacetophenone", '3,4-Dihydroxyphenylacetaldehyde', '3-Hydroxyphenylacetate', '3-Methoxytropolone', '3-Methylsalicylate', '4-Hydroxy-3-methoxy-benzaldehyde', '4-Hydroxymethylsalicylaldehyde', '4-Hydroxyphenacyl alcohol', '4-Hydroxyphenyl acetate', '4-Hydroxyphenylacetate', '4-Methoxybenzoate', '4-Methylsalicylate', '6-Methylsalicylate', 'Menisdaurilide', 'Methyl salicylate', 'Phenoxyacetate', 'Resorcinol monoacetate']
151.00376	55326.4	-0.027711898		0					0
192.02322	33143.7	-0.027567359		0					0
213.04063	51947.8	-0.027101305	C7H6O4	C7H6O4	[M+Hac-H]-	154.02661	213.0404636	0.78	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']
213.04063	51947.8	-0.027101305	C9H10O6	C9H10O6	[M-H]-	214.04774	213.0404636	0.78	['2-Hydroxy-6-oxonona-2,4-diene-1,9-dioate']

200.02024	106458.6	-0.026854654	C5H3NO4	C5H3NO4	[M+Hac-H]-	141.006209	200.0200626	0.89	['5-Nitrofurfural']
206.04604	36618	-0.026829802	C10H9NO4	C10H9NO4	[M-H]-	207.053159	206.0458826	0.76	['1-Nitro-5,6-dihydroxy-dihydronaphthalene', '2-Formaminobenzoylacetate', '3-Amino-4,7-dihydroxy-8-methylcoumarin', '4-(2-Aminophenyl)-2,4-dioxobutanoate']
206.04604	36618	-0.026829802	C8H5NO2	C8H5NO2	[M+Hac-H]-	147.032029	206.0458826	0.76	['Indole-5,6-quinone', 'Isatin']
215.01744	167385.1	-0.026803166	C12H8O2S	C12H8O2S	[M-H]-	216.024502	215.0172256	1	['1,2-Dihydroxydibenzothiophene']
215.01744	167385.1	-0.026803166	C6H10O7	C6H10O7	[M+Na-2H]-	194.042655	215.0173236	0.54	['2-Dehydro-D-galactonate', '2-Keto-D-gluconic acid', '3-Dehydro-L-gulonate', '5-Dehydro-D-gluconate', 'D-Fructuronate', 'D-Galacturonate', 'D-Glucuronate', 'D-Glucuronic acid', 'D-Mannuronate', 'D-Tagaturonate', 'Galacturonic acid', 'L-Guluronic acid', 'L-Iduronic acid', 'beta-D-Glucopyranuronic acid']
571.19938	53079.4	-0.026680312		0					0
546.92729	41210.8	-0.026633111		0					0
293.03014	176702.6	-0.026481388	C11H14NO6	C11H14NO6	[M+K-2H]-	256.082114	293.0307206	-1.98	['Nicotinate D-ribonucleoside']
293.03014	176702.6	-0.026481388	C13H10O8	C13H10O8	[M-H]-	294.03757	293.0302936	-0.52	['Tricrozarin A']
253.07166	211403.7	-0.026398465	C10H10O4	C10H10O4	[M+Hac-H]-	194.05791	253.0717636	-0.41	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']
479.00988	63125.6	-0.026325852		0					0
338.97931	89595.1	-0.026238656		0					0
191.03512	94746.1	-0.026221889	C10H8O4	C10H8O4	[M-H]-	192.04226	191.0349836	0.71	['1,3,6,8-Naphthalenetetrol', '2-Hydroxychromene-2-carboxylate', '3,4-Dehydro-6-hydroxymellein', 'Acamelin', 'Anemonin', 'Isoscopoletin', 'Methylenedioxycinnamic acid', 'Naphthazarin',

									'Scopoletin', 'trans-O-Hydroxybenzylidenepyruvate']
204.06676	32734.7	-0.026175765	C11H11NO3	C11H11NO3	[M-H]-	205.073894	204.0666176	0.7	['5-Methoxyindoleacetate', 'Gentianamine', 'Indolelactate', 'Swietenidin B']
204.06676	32734.7	-0.026175765	C9H7NO	C9H7NO	[M+Hac-H]-	145.052764	204.0666176	0.7	['1(2H)-Isoquinolinone', '3-Methyleneoxindole', '8-Hydroxyquinoline', 'Indole-3-carboxaldehyde', 'Quinolin-2-ol', 'Quinolin-4-ol']
141.01942	49453.5	-0.025952037	C6H6O4	C6H6O4	[M-H]-	142.02661	141.0193336	0.61	['(S)-5-Oxo-2,5-dihydrofuran-2-acetate', '1,2,3,5-Tetrahydroxybenzene', '2,5-Dihydro-5-oxofuran-2-acetate', '2-Hydroxymuconate semialdehyde', '2-Oxo-2,3-dihydrofuran-5-acetate', 'Kojic acid', 'cis,cis-4-Hydroxymuconic semialdehyde', 'cis,cis-Muconate', 'cis,trans-Hexadienedioate']
167.03504	350068.8	-0.025797423	C6H4O2	C6H4O2	[M+Hac-H]-	108.02113	167.0349836	0.34	['o-Benzoquinone', 'p-Benzoquinone']
167.03504	350068.8	-0.025797423	C8H8O4	C8H8O4	[M-H]-	168.04226	167.0349836	0.34	['(R)-4-Hydroxymandelate', '(S)-4-Hydroxymandelate', '1,2-Dihydrophthalic acid', '2,6-Dihydroxyphenylacetate', '2,6-Dimethoxybenzoquinone', '2-Hydroxy-6-oxoocta-2,4,7-trienoate', '3,4-Dihydroxymandelaldehyde', '3,4-Dihydroxyphenylacetate', '4-Hydroxymandelate', '4-Hydroxymethylsalicylate', '4-Hydroxypheoxyacetate', 'Homogentisate', 'Orsellinate', 'Vanillate']
509.98334	51762.1	-0.025703446		0					0
205.08907	78697.8	-0.025682607		0					0
455.26252	64305.3	-0.025562019		0					0
304.99145	88300.3	-0.025556559		0					0
165.01859	1381.8	-0.025271306		0					0

89.02438	57476.2	-0.024994887	C3H6O3	C3H6O3	[M-H]-	90.031695	89.0244186	-0.43	['(R)-Lactate', '(S)-Lactate', '3-Hydroxypropanoate', 'D-Glyceraldehyde', 'Glyceraldehyde', 'Glycerone', 'L-Glyceraldehyde', 'Lactate']
89.02438	57476.2	-0.024994887	CH2O	CH2O	[M+Hac-H]-	30.010565	89.0244186	-0.43	['Formaldehyde']
319.03016	58867.5	-0.024993576	C15H10N2O4	C15H10N2O4	[M+(37Cl)]-	282.064058	319.0305096	-1.1	['N-(p-Nitrobenzyl)phthalimide']
494.00931	50165.4	-0.024876577		0					0
664.98363	98735.9	-0.024656939		0					0
476.99003	115577.7	-0.024618005		0					0
513.15673	39052.7	-0.024602819	C21H32O12	C21H32O12	[M+(37Cl)]-	476.18938	513.1558316	1.75	['Kanokoside A']
335.00195	44001.3	-0.024371536		0					0
499.14089	34451.8	-0.024270569		0					0
381.01709	107147.9	-0.024114182		0					0
186.04569	58457.4	-0.024106908		0					0
658.99532	39855.5	-0.023732286		0					0
550.99066	19262	-0.023583887		0					0
658.98866	66661.7	-0.023415301		0					0
530.95356	37396.2	-0.023403802		0					0
487.99237	38597	-0.023236294		0					0
403.26996	79648	-0.02323353		0					0
202.07224	7061.7	-0.023170765	C6H9NO3	C6H9NO3	[M+Hac-H]-	143.058244	202.0720976	0.7	['2-Hydroxymethylclavam']
202.07224	7061.7	-0.023170765	C8H13NO5	C8H13NO5	[M-H]-	203.079374	202.0720976	0.7	['N2-Acetyl-L-aminoadipate']

202.07224	7061.7	-0.023170765	C9H13N2O2	C9H13N2O2	[M+Na-2H]-	181.097703	202.0723716	-0.65	['Pyridostigmine']
220.00153	67679.4	-0.023119295	C7H7NO5	C7H7NO5	[M+Cl]-	185.032424	220.0018256	-1.34	['2-Amino-3-carboxymuconate semialdehyde']
220.00153	67679.4	-0.023119295	C8H9NO2S	C8H9NO2S	[M+(37Cl)]-	183.035401	220.0018526	-1.47	['p-Hydroxyphenylacetothiohydroximate']
220.00153	67679.4	-0.023119295	C8H9NO4	C8H9NO4	[M+K-2H]-	183.053159	220.0017656	-1.07	['3,5-Dihydroxy-phenylglycine', '3-Carboxy-4-methoxy-N-methyl-2-pyridone', '3-Hydroxy-4-hydroxymethyl-2-methylpyridine-5-carboxylate', '4-Pyridoxate', '5-Methoxy-3-hydroxyanthranilate']

(a)

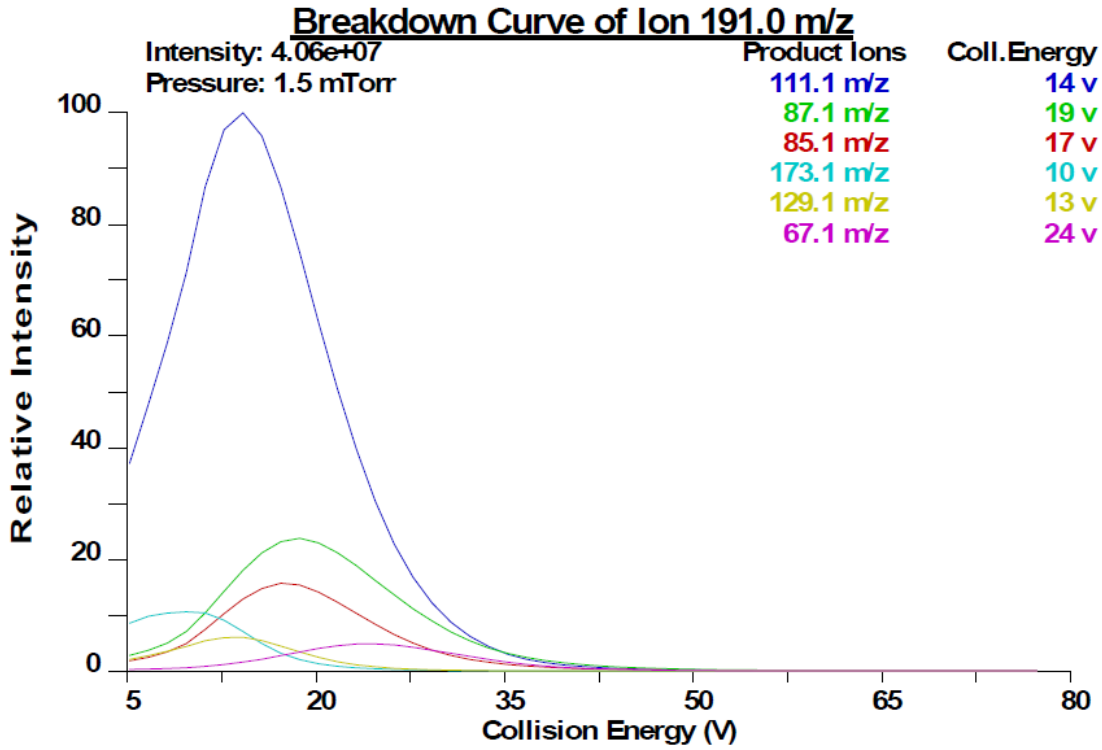

Figure 1a A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 191, citric acid.

(b)

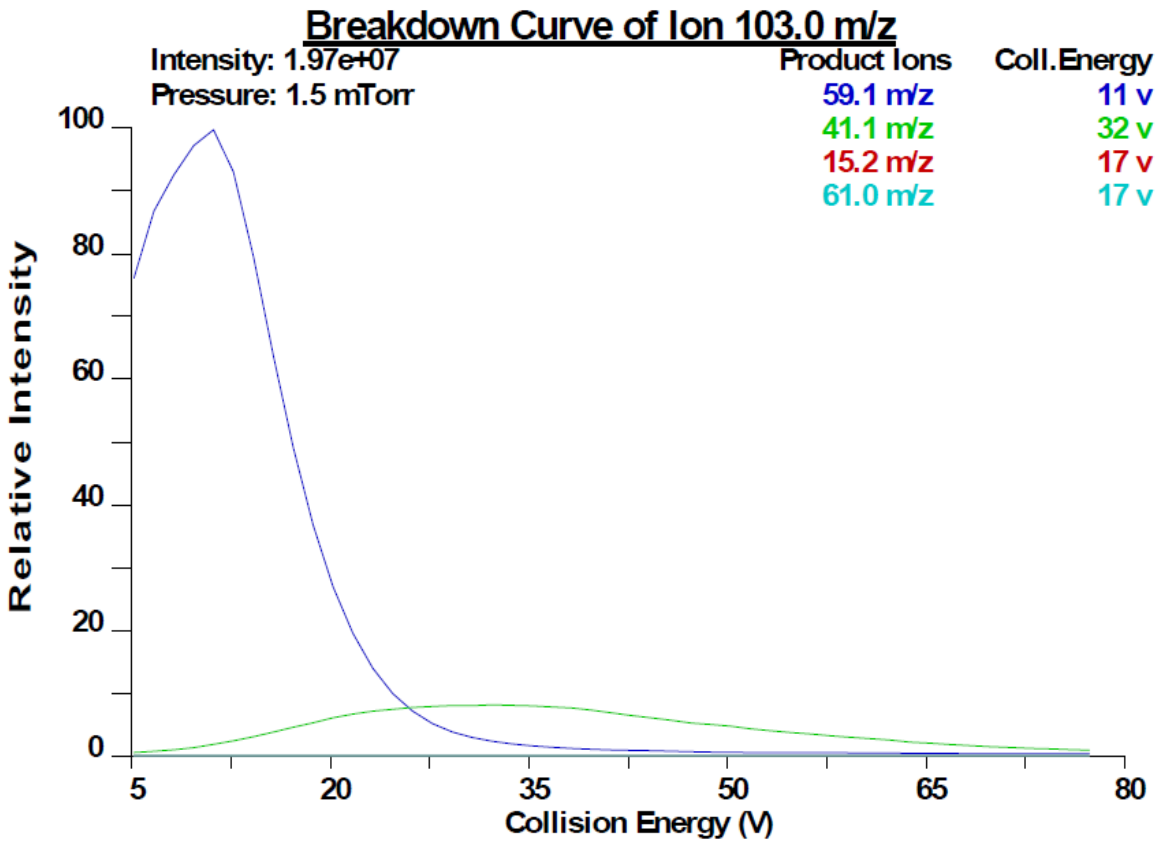

Figure 2b A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 103, malonic acid.

(c)

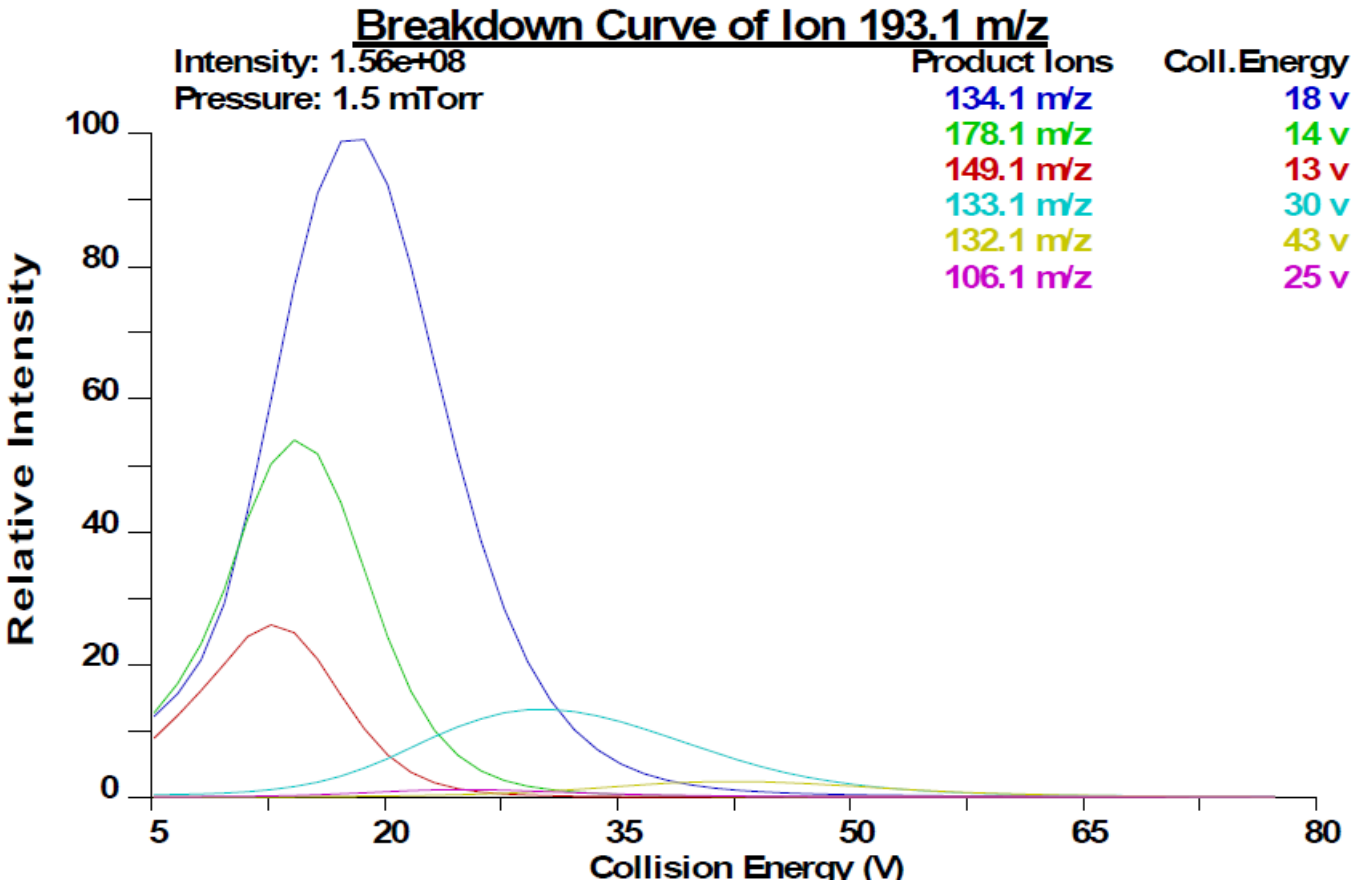

Figure 3c A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 193, ferulic acid.

(d)

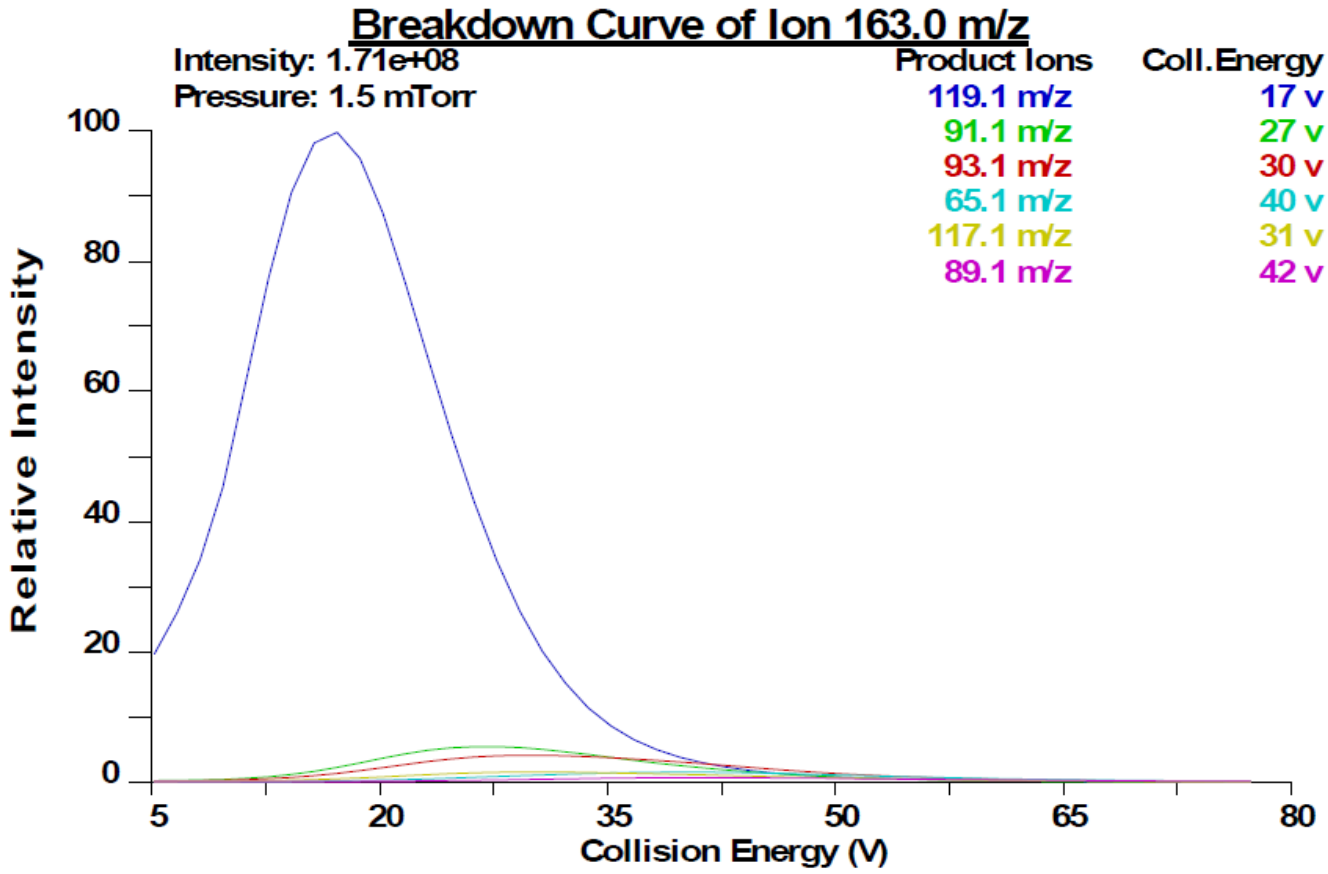

Figure 4d A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 163, m-coumaric acid.

(e)

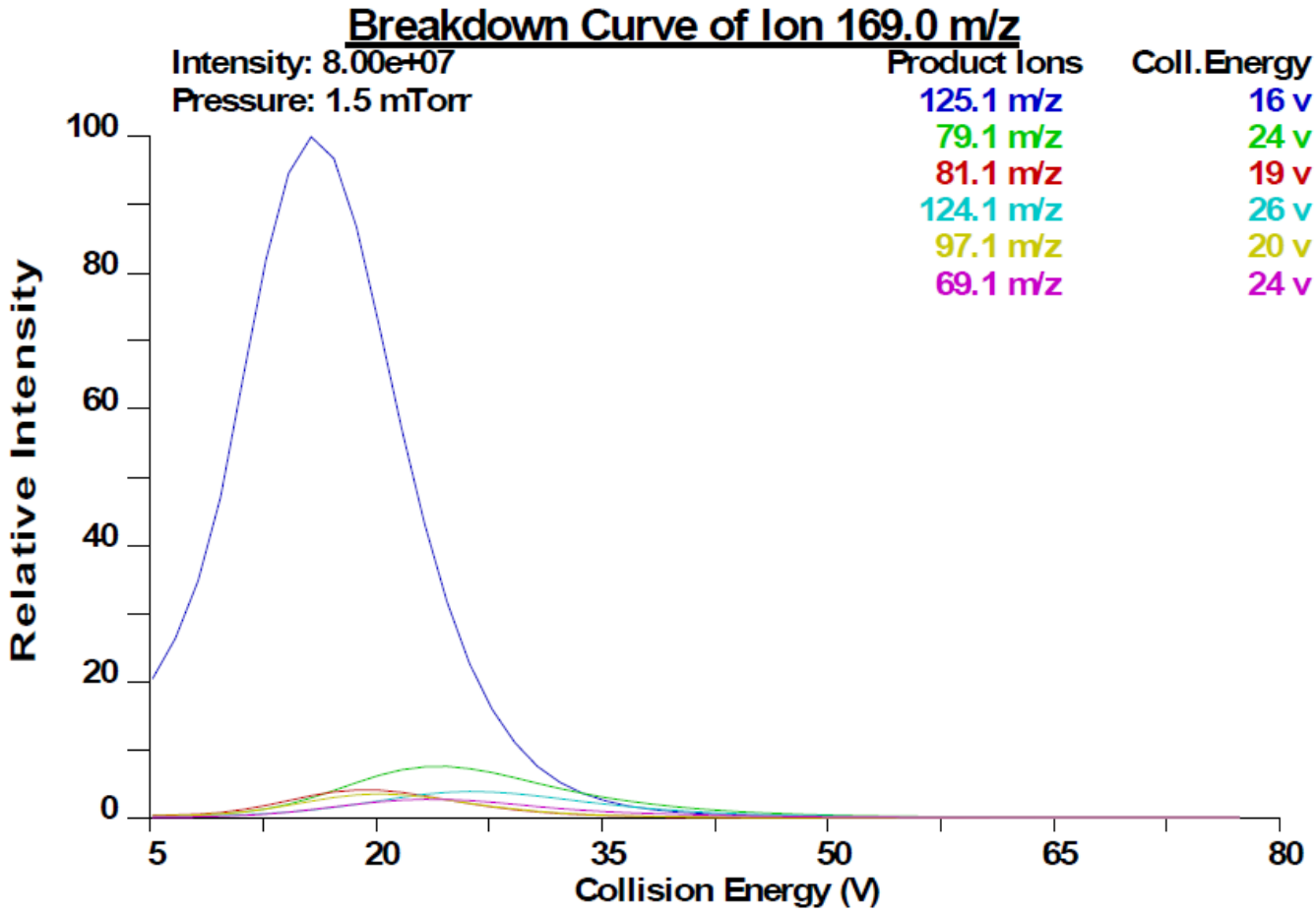

Figure 5e A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 169, gallic acid.

(f)

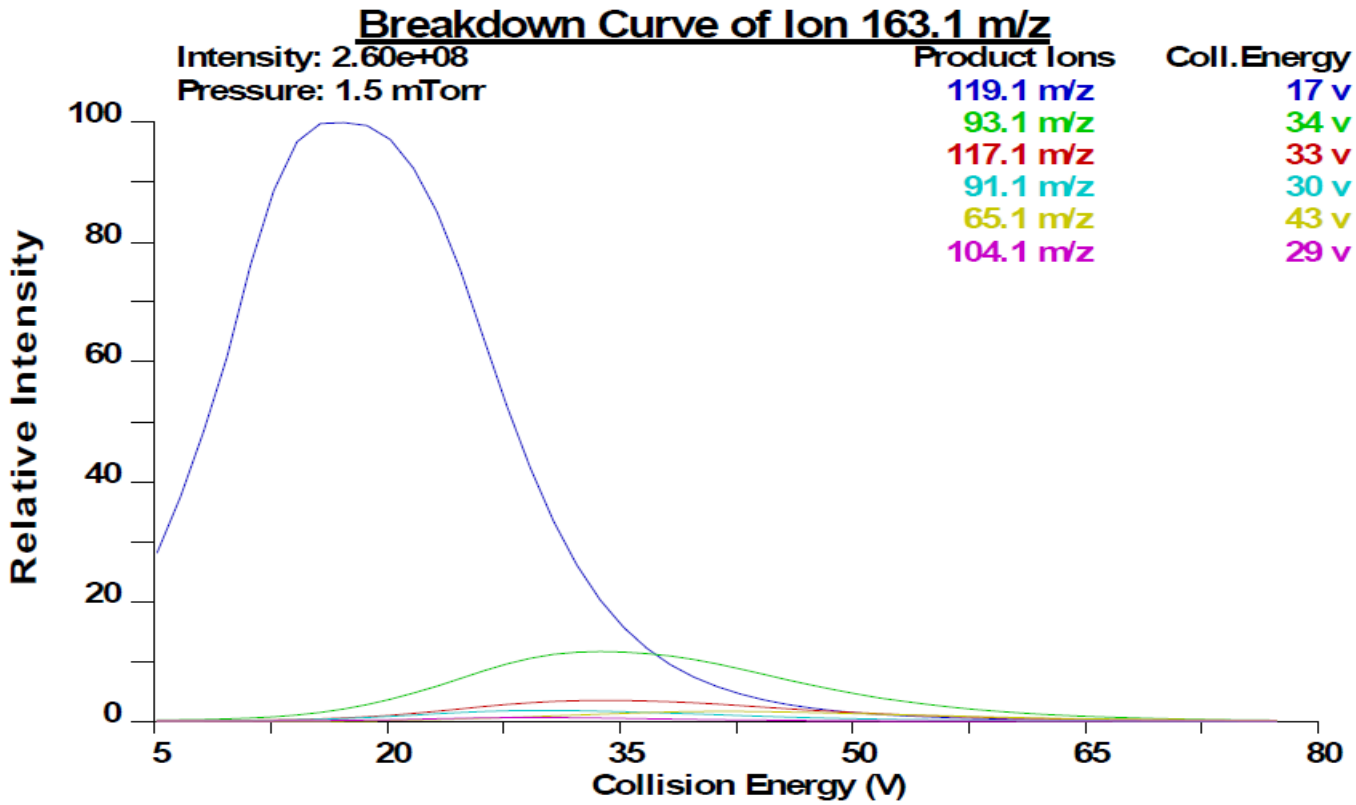

Figure 6f A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 163, p-coumaric acid.

(g)

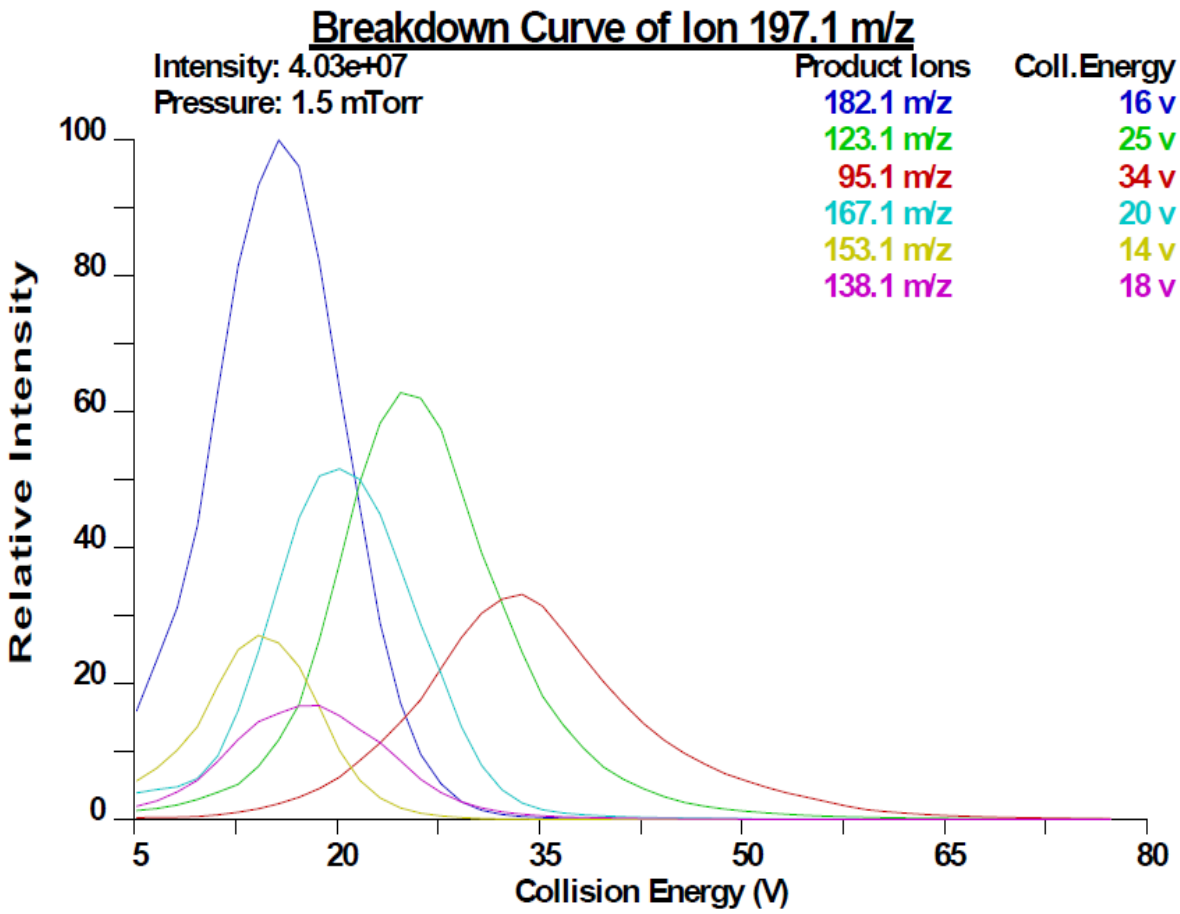

Figure 7g A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 197, syringic acid.

(h)

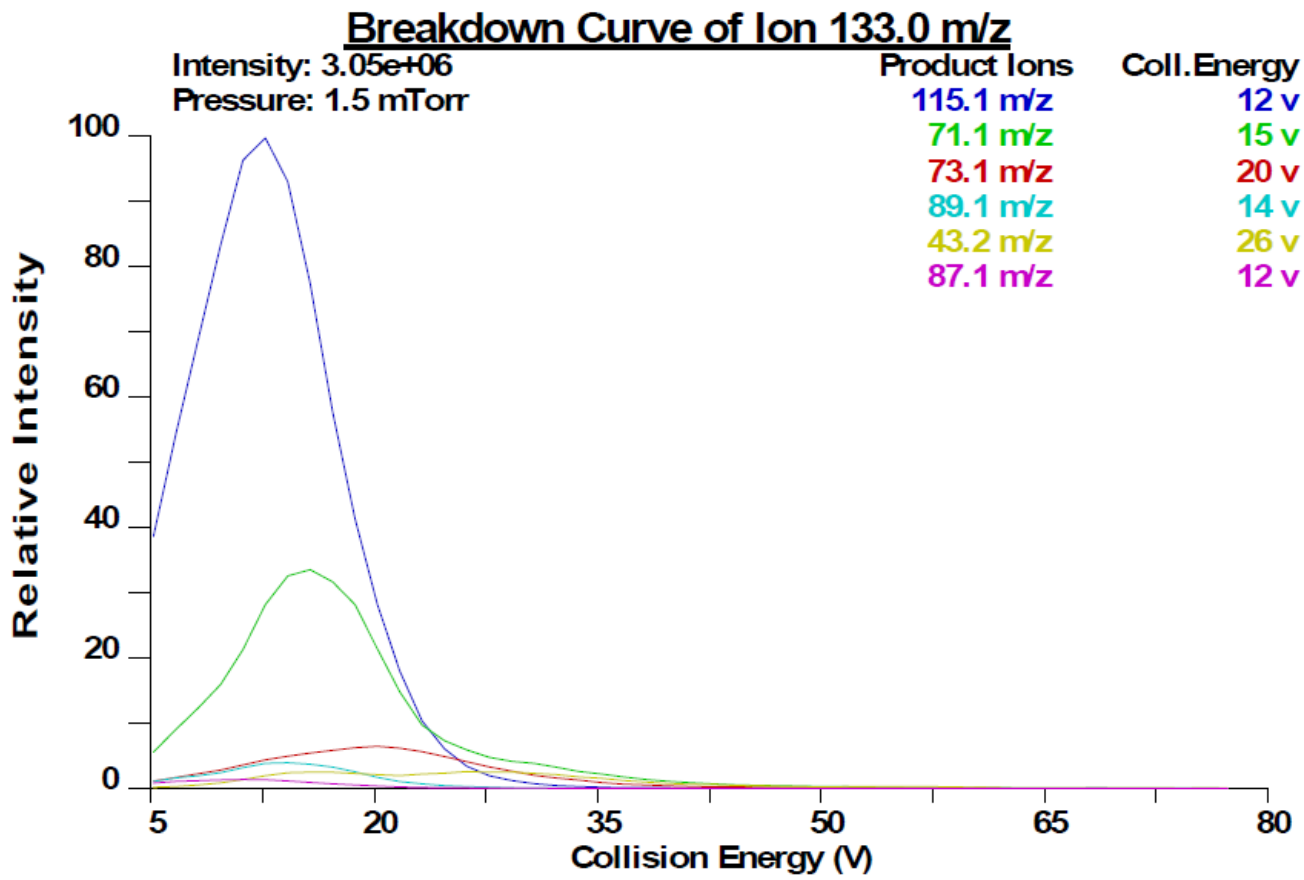

Figure 8h A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 133, malic acid.

(i)

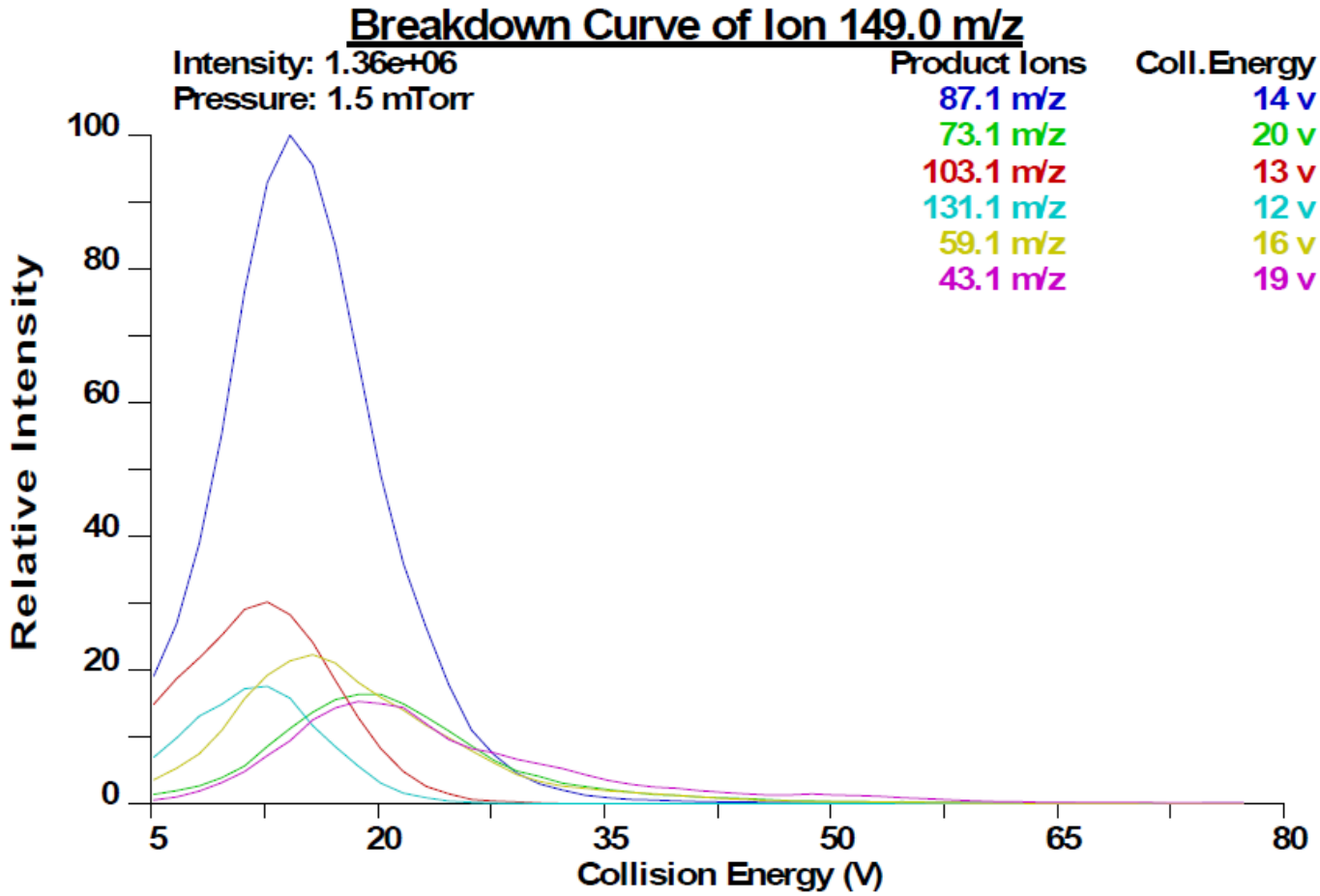

Figure 9i A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 149, tartaric acid.

(j)

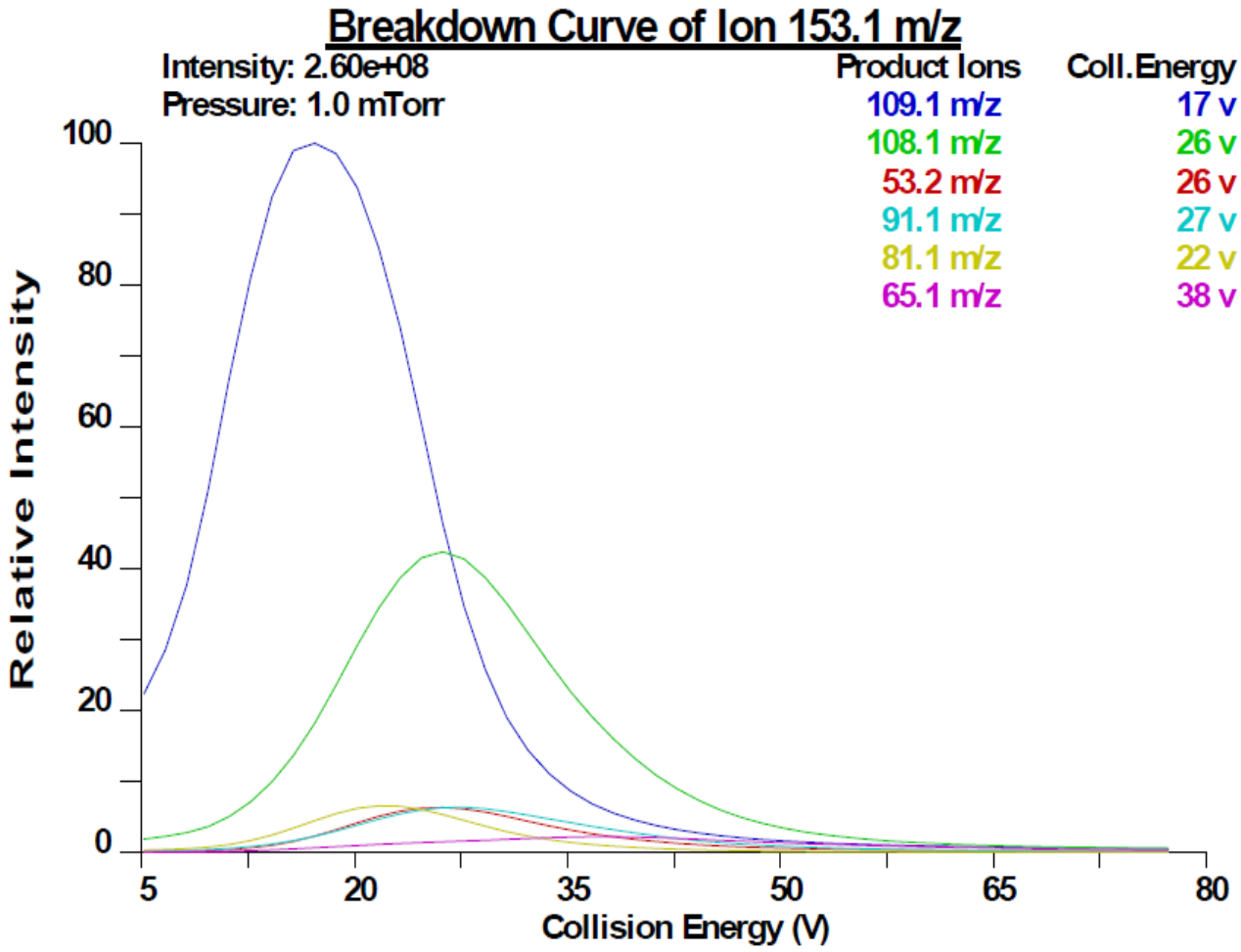

Figure 10j A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 153, 2,3-DHB acid.

(k)

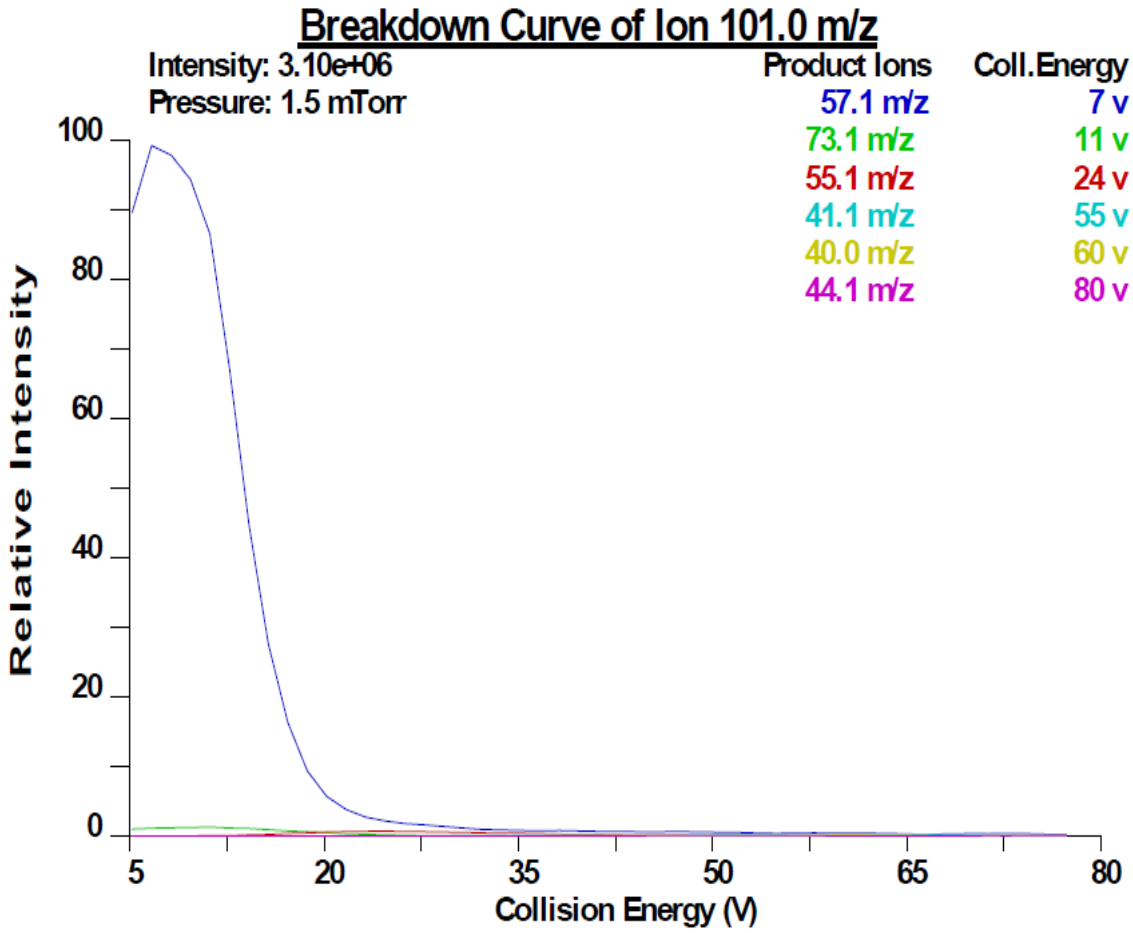

Figure 11k A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 101, ketobutyric acid.

(I)

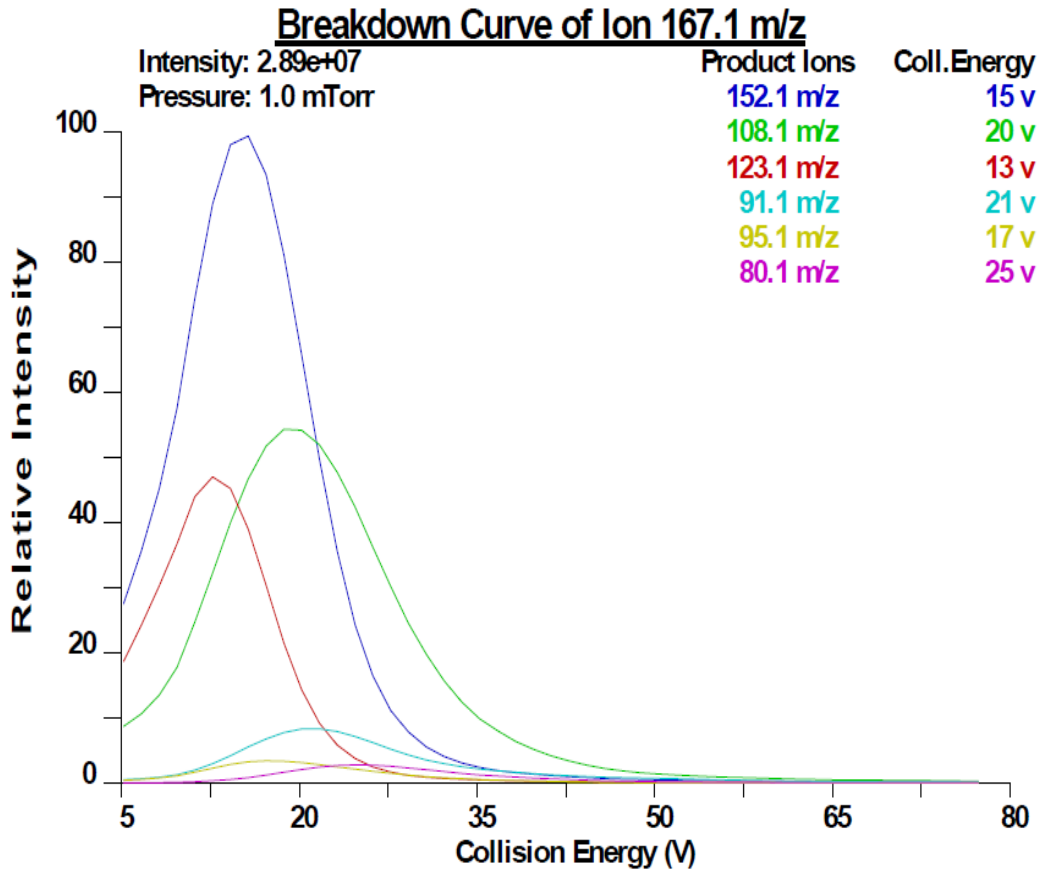

Figure 12l A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 167, vanillic acid.

(m)

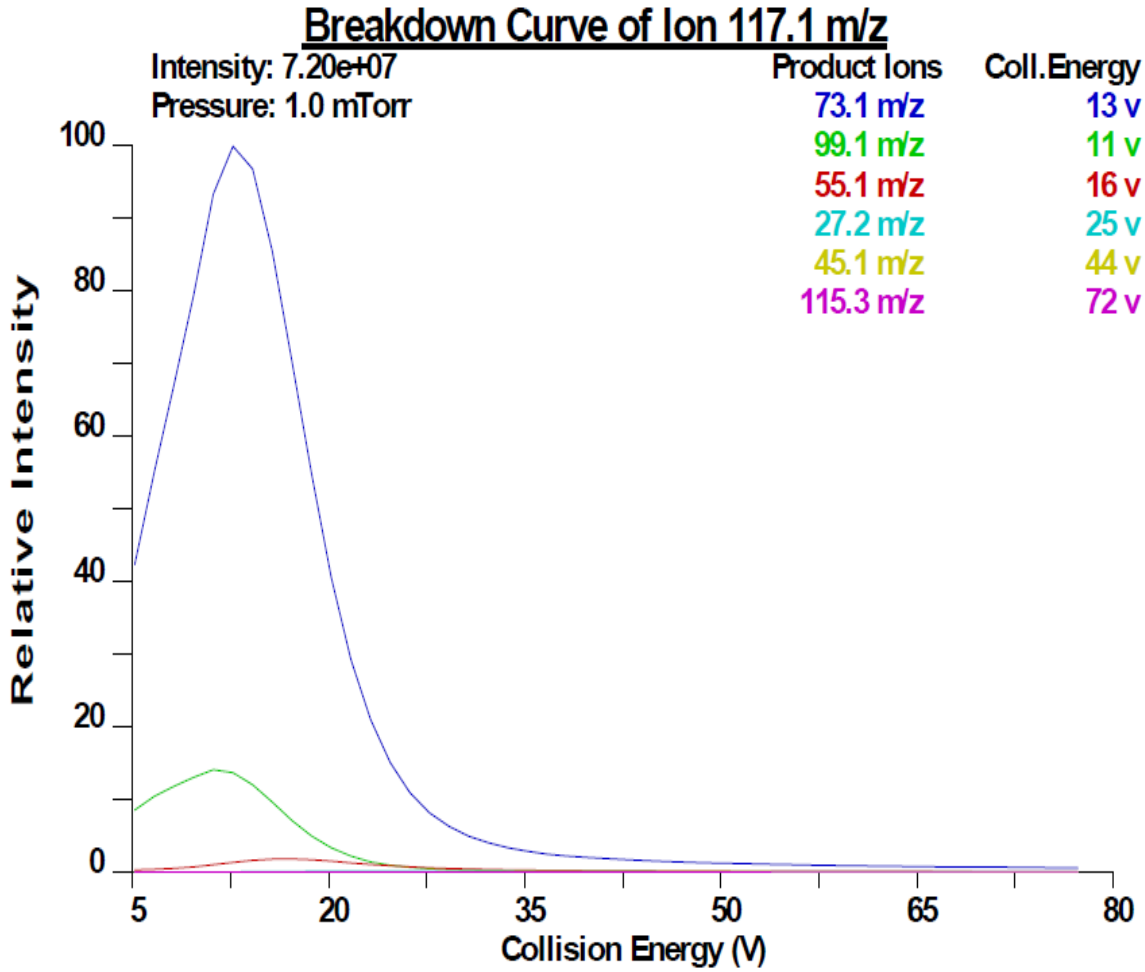

Figure 13m A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 117, succinic acid.

(n)

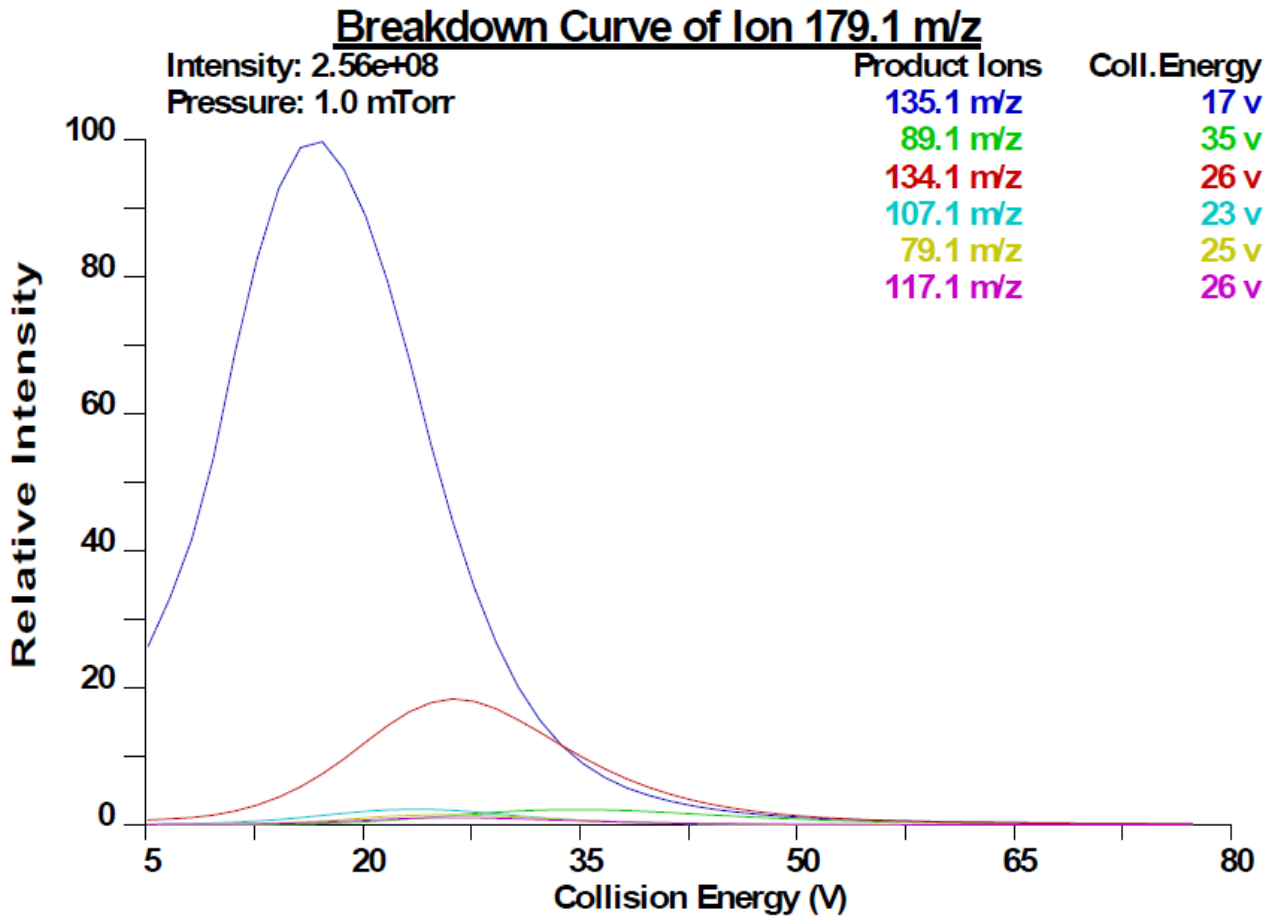

Figure 14n A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 179, caffeic acid.

(o)

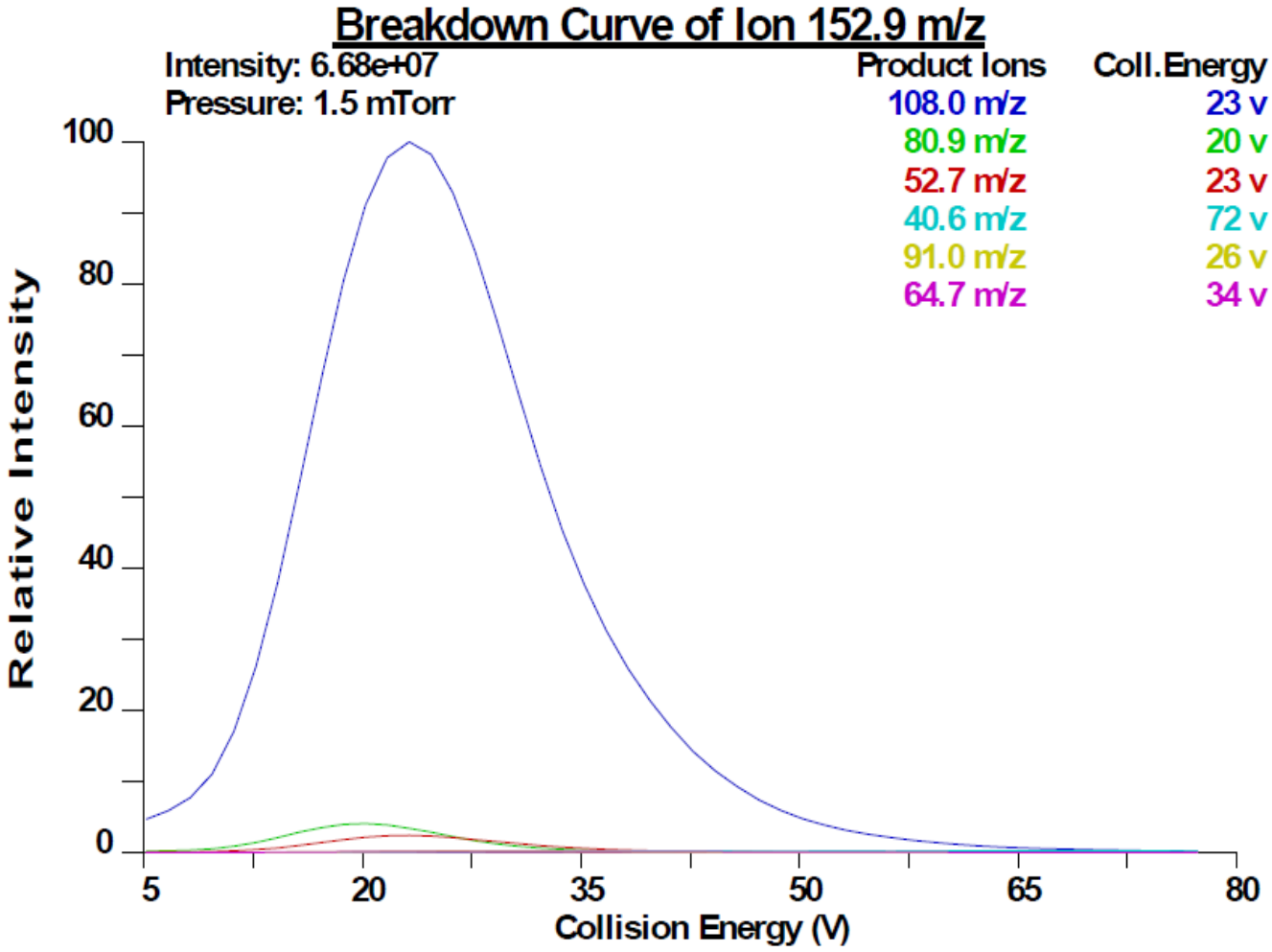

Figure 15o A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 153, 2,5 DHB (gentisic) acid.

(p)

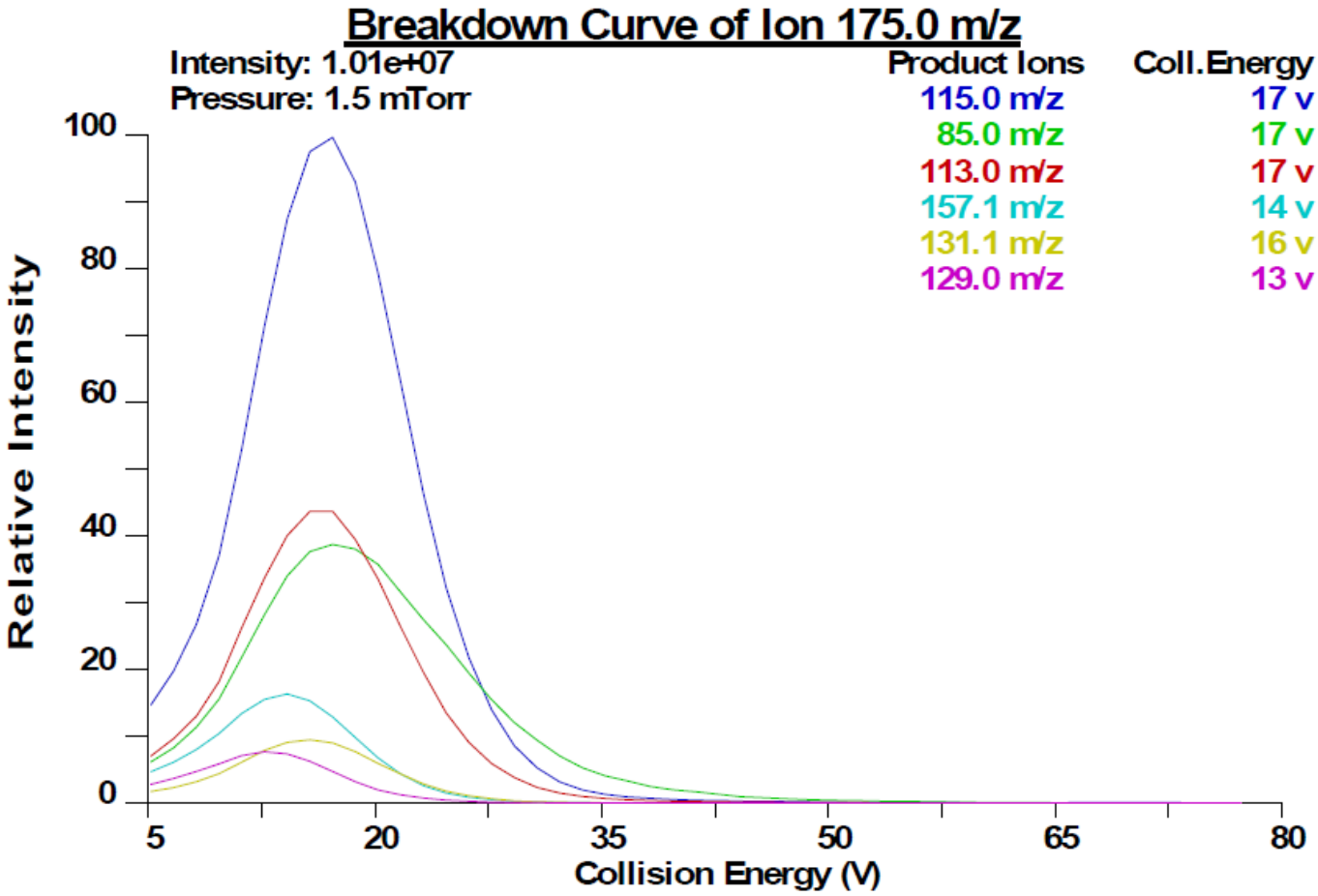

Figure 16p A graph of relative intensity vs optimum collision energy for the formation of the product ions from m/z 175, isopropyl malic acid.

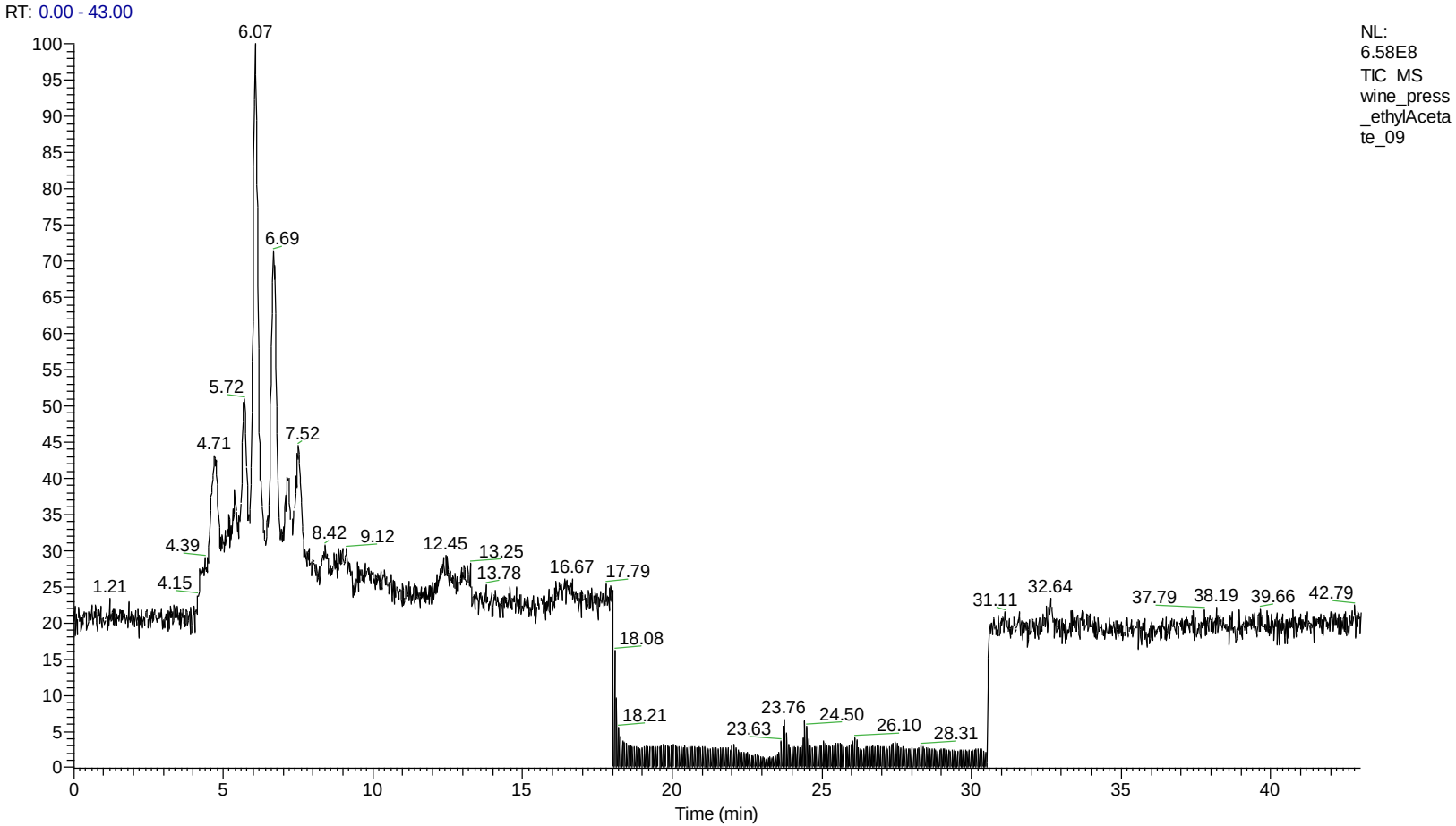

Figure 2 The total ion chromatogram from the Sardinian wine press from the ethyl acetate liquid/liquid extraction.

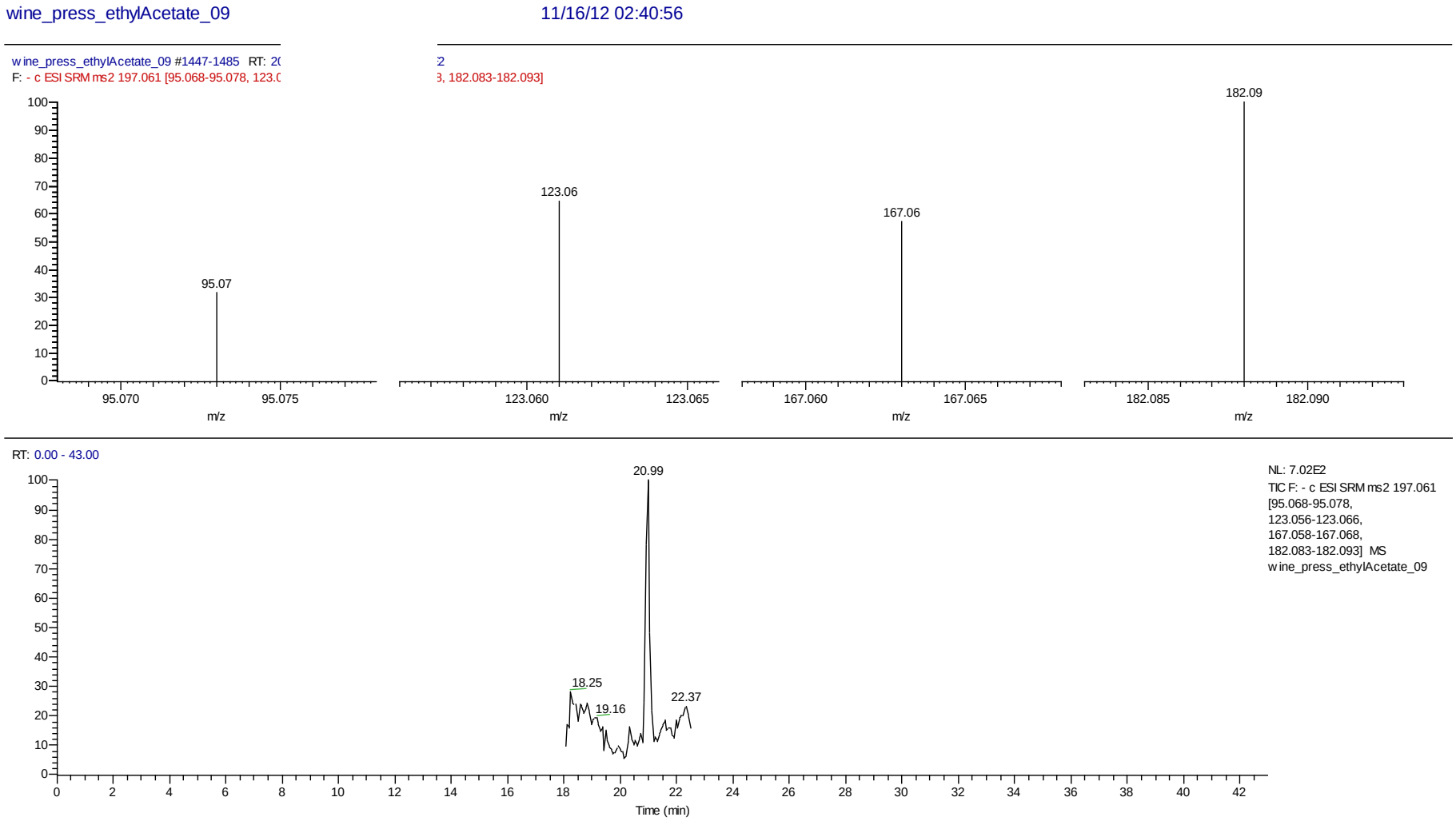

Figure 3a The identification of syringic acid from the Sardinian wine press based upon a) four product ions from *m/z* 197 and b) retention time (20.99).

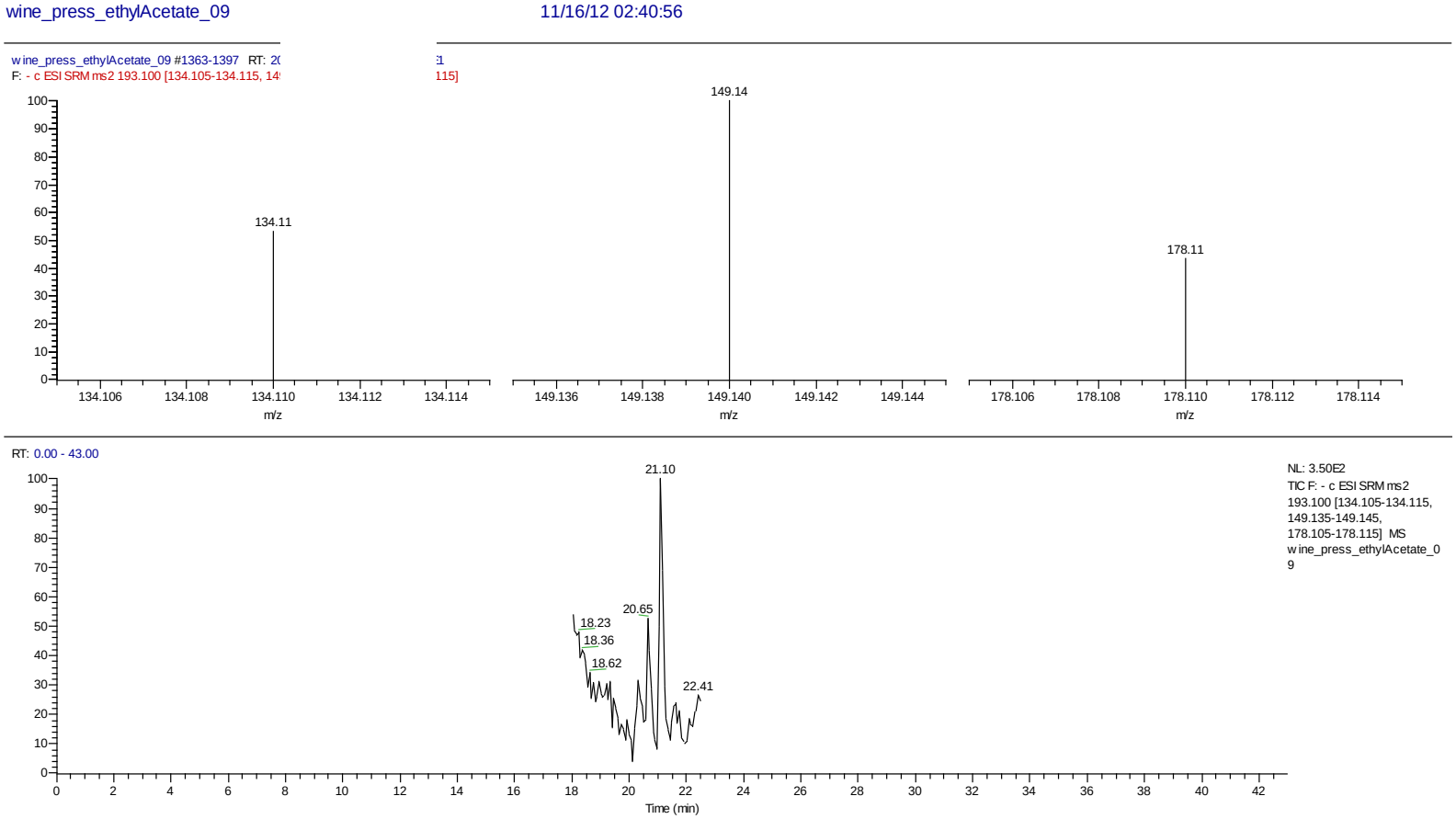

Figure 3b The identification of ferulic acid from the Sardinian wine press based upon a) three product ions from *m/z* 193 and b) retention time (20.32).

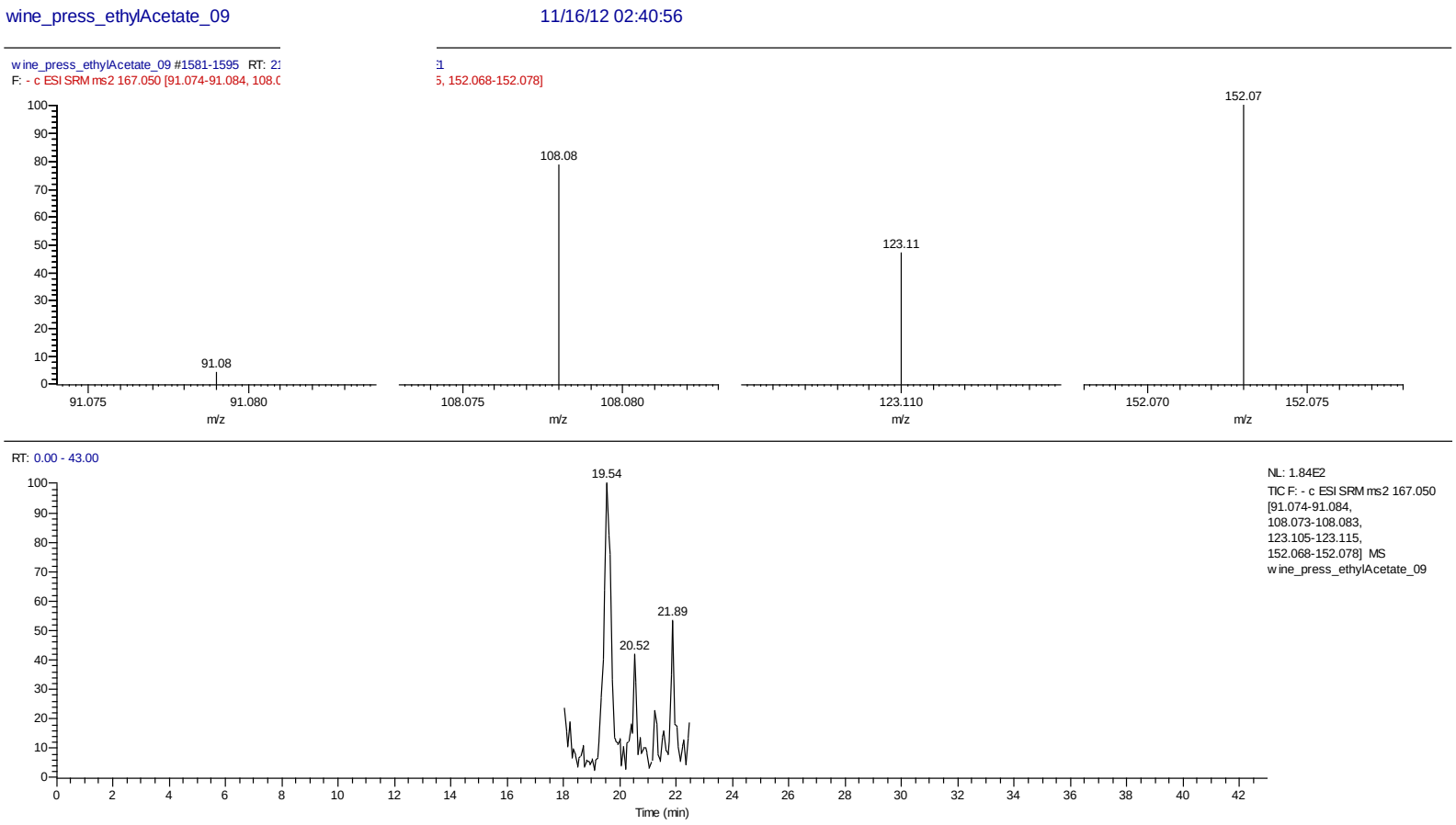

Figure 3c The identification of vanillic acid from the Sardinian wine press based upon a) four product ions from *m/z* 167 and b) retention time (21.89).

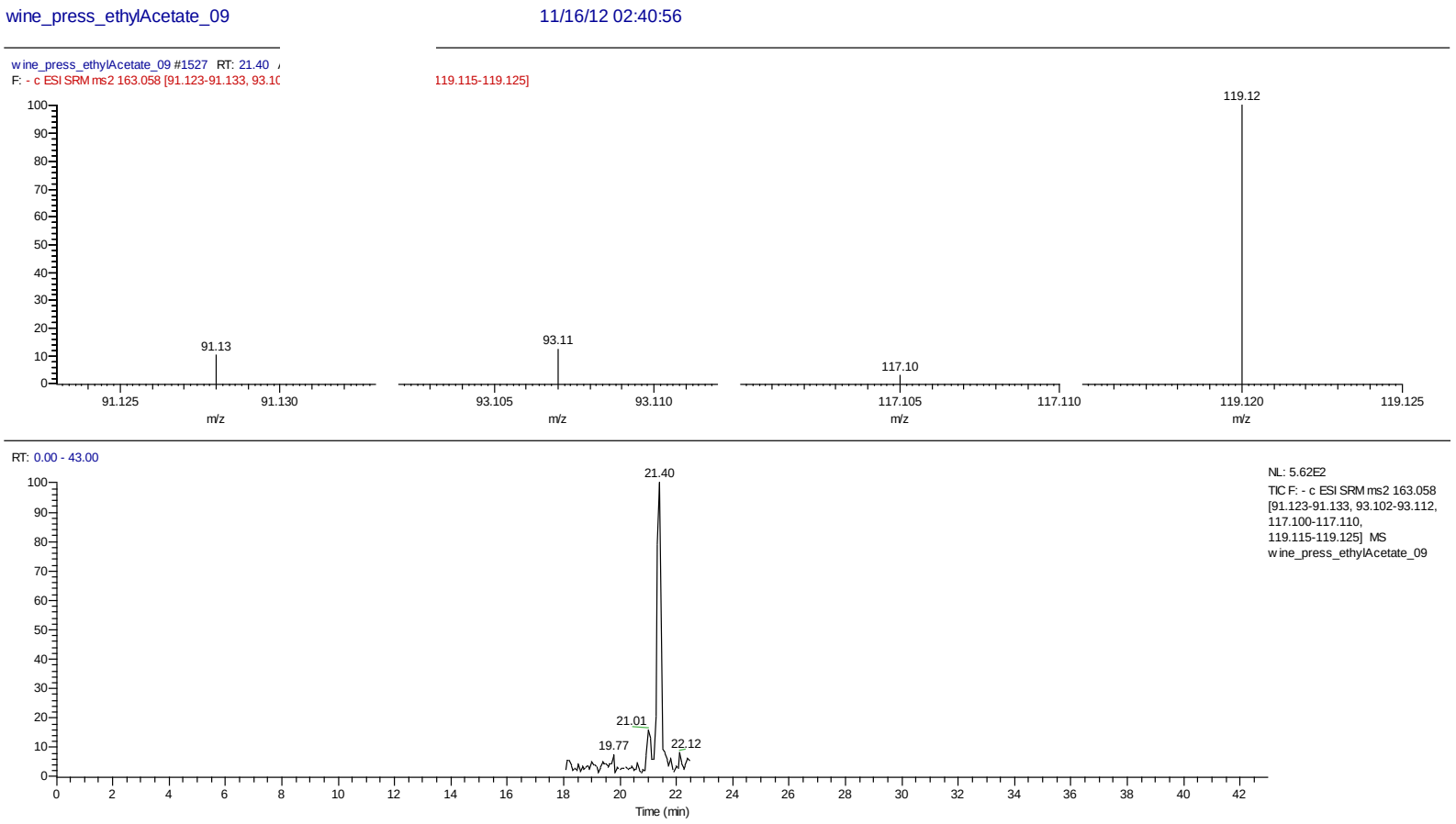

Figure 3d The identification of p-coumaric acid from the Sardinian wine press based upon a) four product ions from *m/z* 163 and b) retention time (21.40).

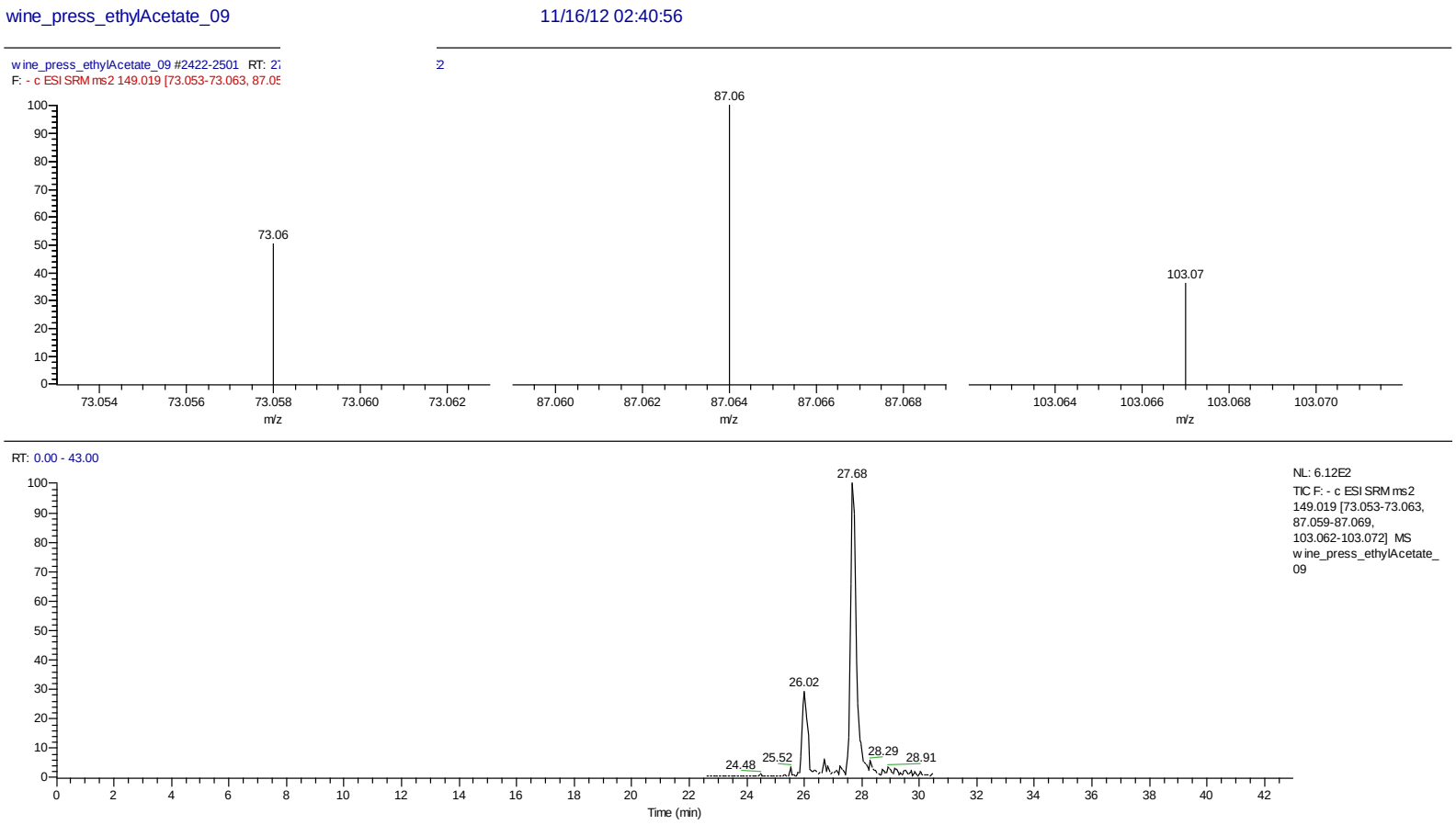

Figure 3e The identification of tartaric acid from the Sardinian wine press based upon a) three product ions from *m/z* 149 and b) retention time (27.68).

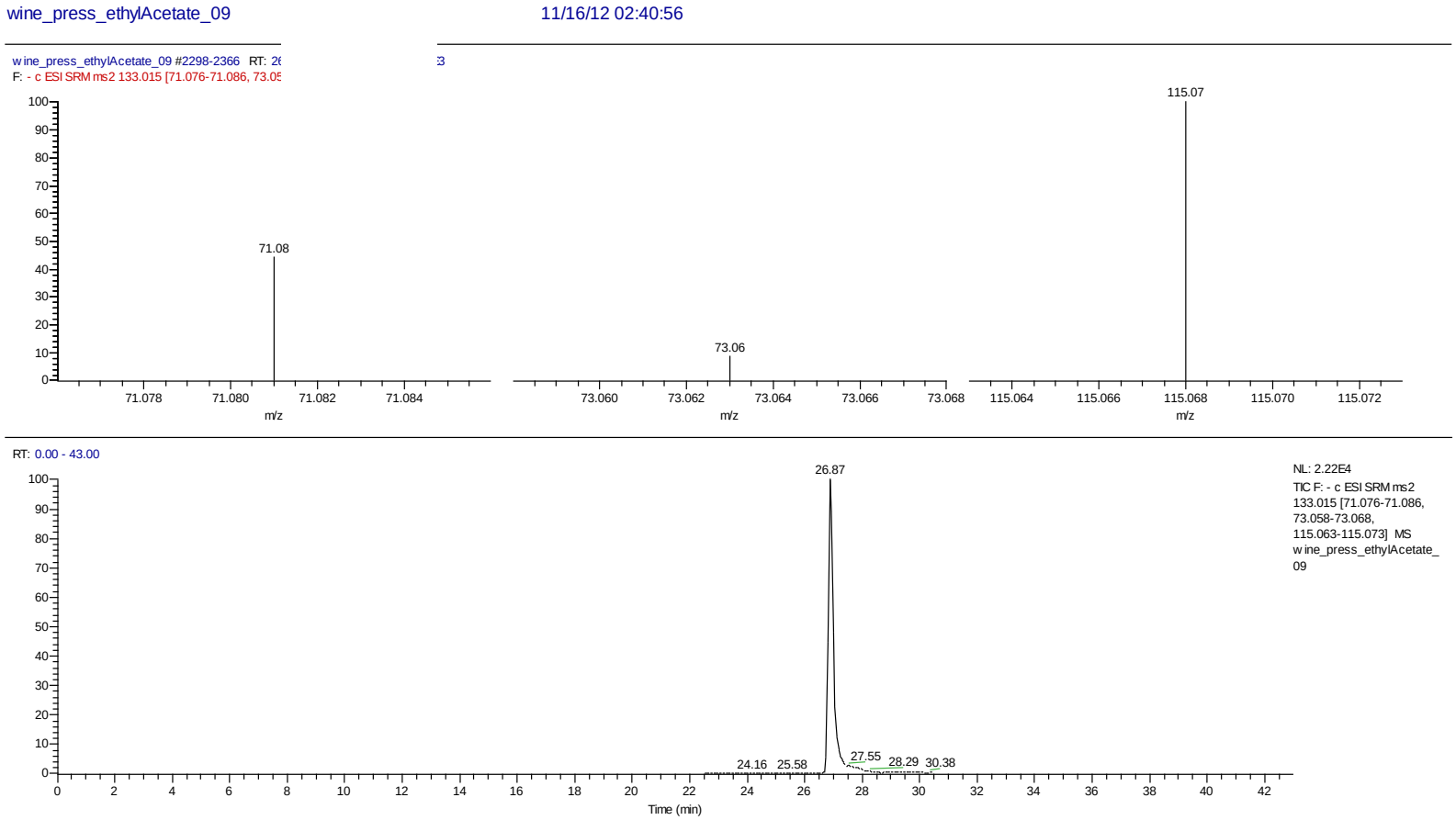

Figure 3f The identification of malic acid from the Sardinian wine press based upon a) three product ions from *m/z* 133 and b) retention time (26.87).

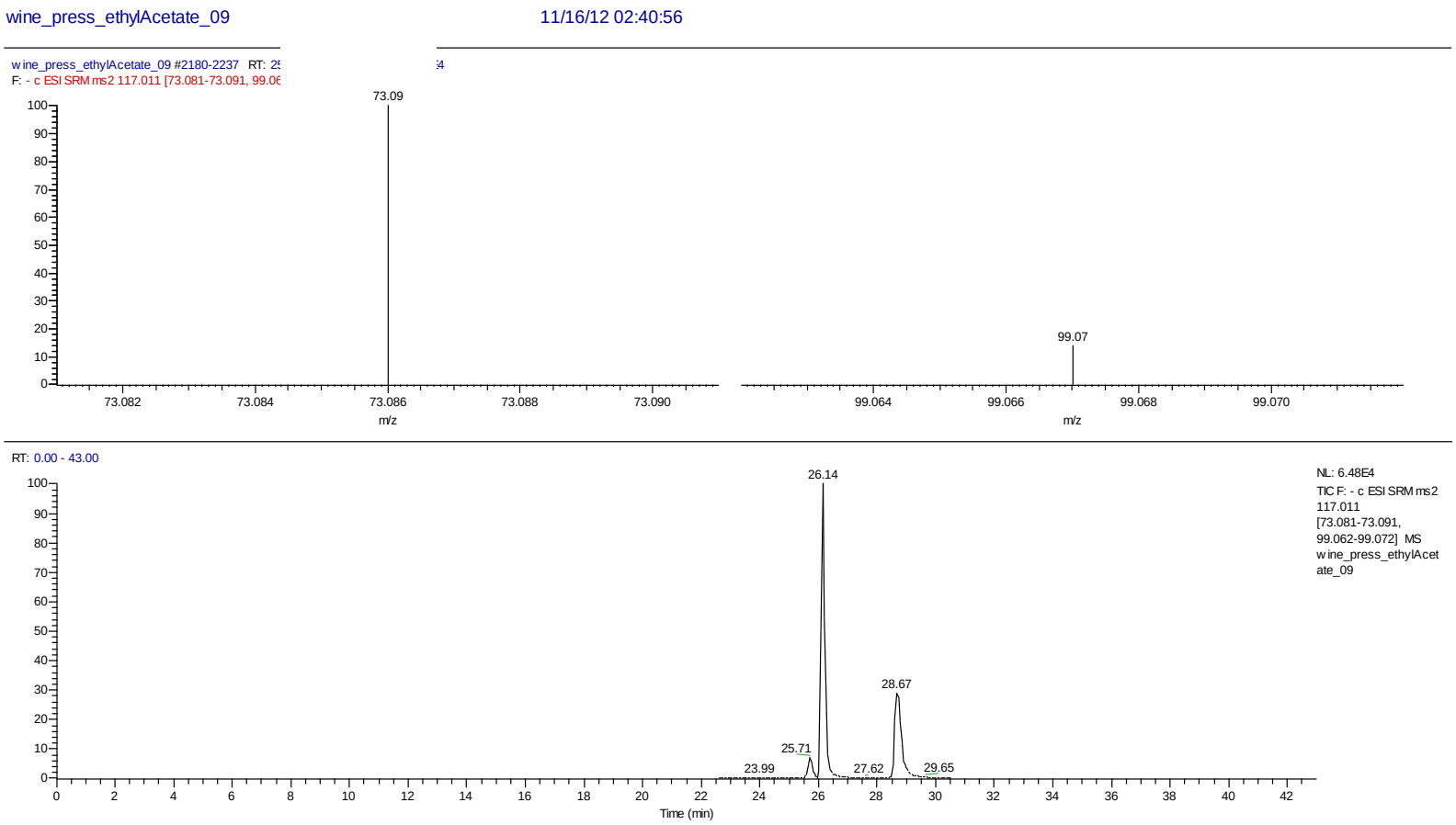

Figure 3g The identification of succinic acid from the Sardinian wine press based upon a) two product ions from *m/z* 117 and b) retention time (26.14).

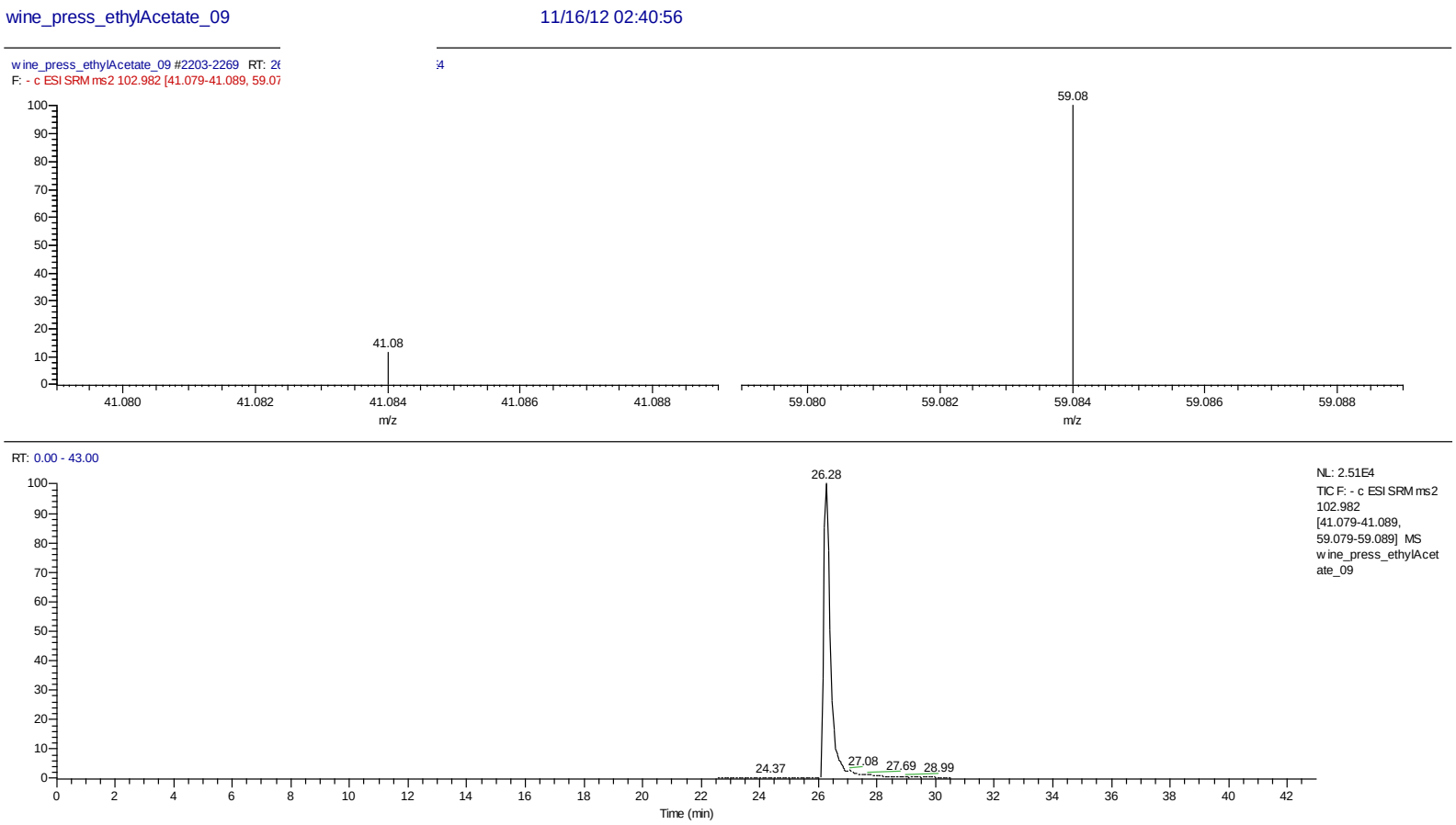

Figure 3h The identification of malonic acid from the Sardinian wine press based upon a) two product ions from *m/z* 103 and b) retention time (26.28).

Table 1 Sample preparation for the three extraction procedures described in 4.2.1 and 4.2.2.

	291012						301012			
Sample Identification	**weight (g)**	**volume of solution 1 (ml)**	**volume of $CHCl_3$ (ml)**	**volume of water II (ml)**	**volume of polar layer removed (ul)**	**volume of non-polar removed (ul)**	**amount of 1M KOH (ul)**	**2N HCl (ml)**	**volume of ethyl acetate (ml)**	**volume of depolymerised sample removed (ul)**
1. TM.003.3/ prepatory layer	0.994	5.6	4	2	NA	NA	600	2.5	3.0	NA
2. TM.003.3/ prepatory layer	0.994	5.6	4	2	NA	NA	600	2.5	3.0	NA
3. TM.003.3/ wine press A	0.991	5.6	4	2	NA	NA	600	2.5	3.0	NA
4. TM.003.3/ wine press A	0.996	5.6	4	2	NA	NA	600	2.5	3.0	NA
5. extraction blank: 1 hour	NA	5.6	4	2	NA	NA	600	2.5	3.0	NA
6. extraction blank: 24 hours	NA	5.6	4	2	NA	NA	600	2.5	3.0	NA
	91112								**141112**	
1. TM.003.3/ wine press	0.95	5.6	4	2	NA	NA	600	2.5	1	NA
2. TM.003.3/ wine press	0.95	5.6	4	2	NA	NA	600	2.5	1	NA
3. TM.003.3/ prep layer	0.96	5.6	4	2	NA	NA	600	2.5	1	NA
4. extraction blank	NA	5.6	4	2	NA	NA	600	2.5	1	NA
	181112								**211112**	

1. TM.003.3/ prepatory layer	0.974	5.6	4	2	NA	NA	600	2.5	3.0	NA
2. TM.003.3/ prepatory layer	0.986	5.6	4	2	NA	NA	600	2.5	3.0	NA
3. TM.003.3/ wine press A	0.981	5.6	4	2	NA	NA	600	2.5	3.0	NA
4. extraction blank	NA	5.6	4	2	NA	NA	600	2.5	3.0	NA

Table 2 The results of the targeted analysis for 16 acids on samples of Sardinian wine press, the prepatory layer of the wine press, and the extraction blanks. The results were from the following extraction procedures: (1) an extended KOH preparation (2) C18 SPE flow through collect, water eluate, methanol eluate, and ethyl acetate eluate, (3) ethyl acetate liquid liquid extraction, and (4) re-preparation of ethyl acetate extraction method. SPE spiked sample spiked with syringic and p-coumaric acids.

chemical name	RT	KOH EXTRACTION BLANK one hour	KOH prep layer, 1 hour	KOH prep layer, 1 hour, rerun	KOH EXTRACTION BLANK 24 hours	KOH prep layer,24 hours	KOH prep layer, 24 hours, rerun	KOH wine press, 24 hours
ketobutyric acid	19.66	N	N	N	N	N	N	N
ferulic acid	20.55	N	N	N	N	N	N	N
gentisic acid	20.94	N	N	N	N	N	N	N
syringic acid	21.11	N	N	(20.93)	N	20.93	20.93	N
m-hydroxycinnamic acid	21.13	N	N	N	N	N	N	N
p-coumaric acid	21.43	N	21.34	21.34	N	21.34	21.34	21.34
2,3-DHB	21.52	N	N	N	N	N	N	N
vanillic acid	21.92	N	21.83	21.83	N	21.83	21.84	N
caffeic acid	24.1	N	N	N	N	N	N	N
ascorbic acid	25.67	N	N	N	N	N	N	N
succinic acid	26.09	N	26.21	26.21	N	N	N	N
malonic acid	26.23	N	26.34	26.35	N	N	N	N
malic acid	26.79	N	N	N	N	N	N	N
tartaric acid	27.62	N	N	N	N	N	N	N

citric acid	28.05	N	N	N	N	N	N	N
gallic acid	28.32	N	N	N	N	N	N	N
chemical name	RT	**SPE extraction_blank Water elution**	**SPE Spiked wine press Water elution**	**SPE extraction_blank Methanol elution**	**SPE Spiked wine press Methanol elution**			
ketobutyric acid	19.66	N	N	N	N			
ferulic acid	20.55	N	(20.57)	N	N			
gentisic acid	20.94	N	N	N	N			
syringic acid	21.11	N	21.31	N	21.04			
m-hydroxycinnamic acid	21.13	N	N (21.25)	N	N			
p-coumaric acid	21.43	N	21.65	N	21.45			
2,3-DHB	21.52	N	N	N	N			
vanillic acid	21.92	N	N (21.29)	N	N (21.03)			
caffeic acid	24.1	N	N	N	N			
ascorbic acid	25.67	N	N	N	N			
succinic acid	26.09	N	(26.56)	26.44	26.31			
malonic acid	26.23	N	N	(26.69)	N			
malic acid	26.79	N	N	N	N			
tartaric acid	27.62	N	N	N	N			
citric acid	28.05	N	N	N	N			
gallic acid	28.32	N	N	N	N			

chemical name	RT	SPE wine press Water elution	SPE prep layer Water elution	SPE wine press Methanol elution	SPE prep layer Methanol elution	161112_blank 90/10		
ketobutyric acid	19.66	N	N	N	N	N		
ferulic acid	20.55	N	N	20.32	21.10	N		
gentisic acid	20.94	N	N	N	N	N		
syringic acid	21.11	N	N	20.99	20.99	N		
m-hydroxycinnamic acid	21.13	N	N	N	N	N		
p-coumaric acid	21.43	N	N	21.4	21.4	N		
2,3-DHB	21.52	N	N	N	N	N		
vanillic acid	21.92	N	N (19.74)	21.89	21.89	N		
caffeic acid	24.1	N	N	N	N	N		
ascorbic acid	25.67	N	N	N	N	N		
succinic acid	26.09	26.51	26.26	26.26	26.2	26.27		
malonic acid	26.23	N (26.70)	N (26.52)	26.4	26.4	26.46, possible carryover		
malic acid	26.79	N	N	N	27.12	N		
tartaric acid	27.62	N	N	N	N	N		
citric acid	28.05	N	N	N	N	N		
gallic acid	28.32	N	N	N	N	N		
chemical name	RT	SPE flow through extraction_blank	SPE flow through Wine press	161112_blank_90/10	SPE flow through Spiked_wine_press			
ketobutyric acid	19.66	N	N	N	N			
ferulic acid	20.55	N	N	N	N			
gentisic acid	20.94	N	N	N	N			
syringic acid	21.11	N	N	N	21.19			

m-hydroxycinnamic acid	21.13	N	N	N	N (21.18)			
p-coumaric acid	21.43	N	N	N	21.49			
2,3-DHB	21.52	N	N	N	N			
vanillic acid	21.92	N	N	N	N (21.17)			
caffeic acid	24.1	N	N	N	N			
ascorbic acid	25.67	N	N	N	N			
succinic acid	26.09	26.27	26.27	26.27	26.26			
malonic acid	26.23	N (26.34)	N (26.53)	N (26.58)	26.52			
malic acid	26.79	N	N	N	N			
tartaric acid	27.62	N	N	N	N			
citric acid	28.05	N	N	N	N			
gallic acid	28.32	N	N	N	N			
chemical name	RT	**151112_90/10_blank**	**Ethyl_acetate_elution_ext_blank**	**Ethyl_acetate_elution_press**				
ketobutyric acid	19.66	N	N	N				
ferulic acid	20.55	N	N	N				
gentisic acid	20.94	N	N	N				
syringic acid	21.11	N	N	N				
m-hydroxycinnamic acid	21.13	N	N	N (21.38)				
p-coumaric acid	21.43	N	N	21.4				
2,3-DHB	21.52	N	N	N				
vanillic acid	21.92	N	N	N				
caffeic acid	24.1	N	N	N				
ascorbic acid	25.67	N	N	N				

succinic acid	26.09	26.26	26.33	26.27				
malonic acid	26.23	(26.46)	26.64	(26.46)				
malic acid	26.79	N	(27.49)	(27.61)				
tartaric acid	27.62	N	N	N				
citric acid	28.05	N	N	N				
gallic acid	28.32	N	N	N				
chemical name	RT	**Ethyl_acetate elution spiked_press**	**Ethyl_acetate elution_prep**	**Ethyl_acetate_extraction extraction_blank**	**Ethyl_acetate extraction wine press**	**Ethyl_acetate extraction spiked_press**	**Ethyl_acetate extraction prep layer**	
ketobutyric acid	19.66	N	N	N	N	N	N	
ferulic acid	20.55	N	N	N	20.65	N	(20.65)	
gentisic acid	20.94	N	N	N	N	N	N	
syringic acid	21.11	20.99	N	N	20.99	(20.99)	20.99	
m-hydroxycinnamic acid	21.13	N (21.39)	N (21.39)	N	N	N	N	
p-coumaric acid	21.43	N	N	N	21.4	21.4	21.4	
2,3-DHB	21.52	N	N	N	N	N	N	
vanillic acid	21.92	N (20.98)	N	N	21.89	(21.89)	21.89	
caffeic acid	24.1	N	N	N	N	N	N	
ascorbic acid	25.67	N	N	N	N	N	N	
succinic acid	26.09	26.26	26.27	26.26	26.26	26.27	26.14	
malonic acid	26.23	26.64	26.58	(26.52), possible carryover	(26.52)	(26.52)	26.28	
malic acid	26.79	N	N	N	N	N	26.87	
tartaric acid	27.62	N	N	N	N	N	27.68	
citric acid	28.05	N	N	N	N	N	N	
gallic acid	28.32	N	N	N	N	N	N	

chemical name	RT	211112_90/10_blank	211112_ethyl_acetate_extraction_prep_sample3					
ketobutyric acid	19.66	N	N					
ferulic acid	20.55	N	20.51					
gentisic acid	20.94	N	N					
syringic acid	21.11	N	20.66					
m-hydroxycinnamic acid	21.13	N	N					
p-coumaric acid	21.43	N	21.19					
2,3-DHB	21.52	N	N					
vanillic acid	21.92	N	21.82					
caffeic acid	24.1	N	N					
ascorbic acid	25.67	N	N					
succinic acid	26.09	N	26.32					
malonic acid	26.23	N	N					
malic acid	26.79	N (27.84, possible ghosting effect)	(26.92)					
tartaric acid	27.62	N	N					
citric acid	28.05	N	N					
gallic acid	28.32	N	N					

Chapter 5

Table 1 Sample identification for the Sardinian wine study analyzed 20130221

	20130205	20130206					20130207		20130208	
Sample Identification	**weight (g)**	**volume of solution 1 (ml)**	**volume of CHCl3 (ml)**	**volume of water II (ml)**	**volume of polar layer removed (ul)**	**volume of non-polar removed (ul)**	**amount of 1M KOH (ul)**	**2N HCl (ml)**	**volume of ethyl acetate (ml)**	**volume of depolymerised sample removed (ul)**
TM.003.3/ wine press A	0.981	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ wine press A	0.982	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ wine press A	0.983	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ wine press A	1.003	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ wine press A	1.002	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.027.3/ wine press B	0.989	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.027.3/ wine press B	1.013	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.027.3/ wine press B	1.003	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.027.3/ wine press B	0.983	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.027.3/ wine press B	0.985	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ prepatory layer	0.998	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ prepatory layer	1	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ prepatory layer	1.008	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ prepatory layer	0.988	5.6	4	2	NA	NA	600	2.5	3.0	NA
TM.003.3/ prepatory layer	0.996	5.6	4	2	NA	NA	600	2.5	3.0	NA

1.TM.044.1.3/ neck	0.985	5.6	4	2	NA	NA	600	2.5	3.0	NA
SAR.TM.67/ neck	0.988	5.6	4	2	NA	NA	600	2.5	3.0	NA
Sample Identification	**weight (g)**	**volume of solution 1 (ml)**	**volume of ChCl3 (ml)**	**volume of water II (ml)**	**volume of polar layer removed (ul)**	**volume of non-polar removed (ul)**	**amount of 1M KOH (ul)**	**2N HCl (ml)**	**volume of ethyl acetate (ml)**	**volume of depolymerised sample removed (ul)**
1.TM.028.1.33/ neck	0.996	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM..060.1.15/ neck	1.002	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.042.1.7/ neck	0.968	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.033.1.14/ side wall	0.989	5.6	4	2	NA	NA	600	2.5	3.0	NA
SAR.TM.4/side wall	1	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.034.1.20/ side wall	0.987	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.033.1.15/ side wall	0.985	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.060.1.45/ side wall	1.01	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM..060.1.56/ base	0.986	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.035.1.1/ base	1.004	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.036.1.2/ base	0.991	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.028.1.31/ base	1.004	5.6	4	2	NA	NA	600	2.5	3.0	NA
1.TM.033.1.20/ base	1.006	5.6	4	2	NA	NA	600	2.5	3.0	NA
extraction blank	NA	5.6	4	2	NA	NA	600	2.5	3.0	NA

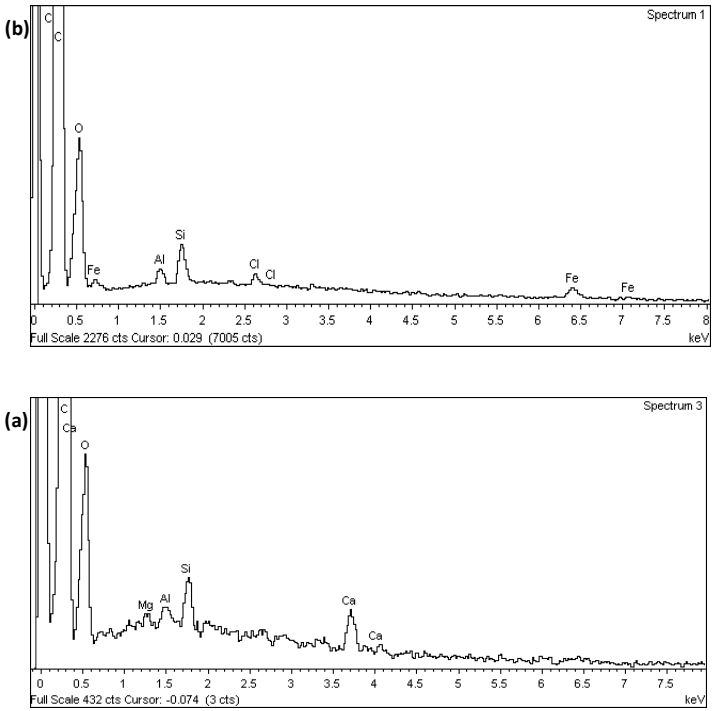

Figure 1 Elemental results of white powder remaining after extraction (neck, side wall). The common elements in both spectra are aluminum and silicon, presumably leached out of the clay matrix during the alkaline fusion.

Table 2 Sample identification for the Sardinian wine study analyzed 20130607

	20130601	20130602					20130603		20130604	
Sample Identification	**weight (g)**	**volume of solution 1 (ml)**	**volume of CHCl3 (ml)**	**volume of water II (ml)**	**volume of polar layer removed (ul)**	**volume of non-polar removed (ul)**	**amount of 1M KOH (ul)**	**2N HCl (ml)**	**volume of ethyl acetate (ml)**	**volume of depolymerised sample removed (ul)**
TM.003.3/ wine press A	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ wine press A	1.02	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ wine press A	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ wine press A	1.02	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ wine press A	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/ wine press B	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/ wine press B	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/ wine press B	1.03	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/ wine press B	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/ wine press B	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ prepatory layer	1.02	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ prepatory layer	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ prepatory layer	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ prepatory layer	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
TM.003.3/ prepatory layer	1.01	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.044.1.3/ neck	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
SAR.TM.67/ neck	1.03	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.028.1.33/ neck	1.01	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM..060.1.15/ neck	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.042.1.7/ neck	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.033.1.14/ side wall	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
SAR.TM.4/side wall	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.034.1.20/ side wall	0.97	5.6	4	2	NA	NA	600	2.5	3	NA

1.TM.033.1.15/ side wall	1.03	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.060.1.45/ side wall	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM..060.1.56/ base	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
Sample Identification	**weight (mg)**	**volume of solution 1 (ml)**	**volume of ChCl3 (ml)**	**volume of water II (ml)**	**volume of polar layer removed (ul)**	**volume of non-polar removed (ul)**	**amount of 1M KOH (ul)**	**2N HCl (ml)**	**volume of ethyl acetate (ml)**	**volume of depolymerised sample removed (ul)**
1.TM.035.1.1/ base	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.036.1.2/ base	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.028.1.31/ base	0.98	5.6	4	2	NA	NA	600	2.5	3	NA
1.TM.033.1.20/ base	0.99	5.6	4	2	NA	NA	600	2.5	3	NA
extraction blank	NA	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/SPE spike	0.97	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/SPE spike	1	5.6	4	2	NA	NA	600	2.5	3	NA
TM.027.3/SPE spike	1.03	5.6	4	2	NA	NA	600	2.5	3	NA

Table 3 Logged ratios of identified organic acids for laboratory aged sherds and for archaeological samples.

	103:117	103:133	117:133	103:149	117:149	133:149	103:163	117:163	133:163	149:163	103:167	163:167	103:175	117:175	133:175
WHTG2B	-1.32318	-2.42251	-2.25186	-0.78193	-0.57027	0.35329	-0.92868	0.54125	0.75291	1.67647	1.14247	2.77580	0.80303	2.24180	2.38177
WHS4A	-1.21750	-2.43764	-1.92448	-0.45863	-0.40660	0.50679	-0.70698	0.75886	0.81089	1.72429	1.02918	2.94443	0.91372	2.24932	2.50525
WHS1A	-1.63107	-2.49965	-1.74284	-0.89993	-0.57133	0.32398	-0.11177	0.73114	1.05974	1.95504	1.18234	2.82363	0.79298	2.05093	2.13513
WHG4B	-1.21446	-2.28964	-2.11150	-0.47690	-0.41227	0.55404	-0.89704	0.73757	0.80219	1.76850	1.07058	2.84368	0.78989	2.14575	2.45693
WHS4B	-2.07219	-2.85631	-1.41691	-0.84783	-0.80196	-0.00571	0.65527	1.22436	1.27022	2.06648	1.37953	2.85060	0.75173	2.16366	2.33174
WHG2A	-1.25654	-2.32411	-1.98211	-0.76019	-0.39783	0.45086	-0.72557	0.49635	0.85870	1.70740	1.00803	2.77497	0.69734	2.07560	2.39861
WHS3B	-1.25140	-2.64846	-2.15343	-0.69456	-0.64269	0.35294	-0.90203	0.55684	0.60871	1.60434	0.97454	3.00141	0.99874	2.37160	2.77904
WHS2A	-1.25866	-2.55381	-2.14036	-0.83669	-0.58720	0.35377	-0.88170	0.42197	0.67146	1.61242	1.01378	2.90758	0.98883	2.30894	2.57662
WHG3A	-1.18554	-2.33391	-2.12742	-0.63032	-0.43523	0.42915	-0.94188	0.55522	0.75031	1.61470	1.06533	2.76306	0.97789	2.21369	2.30416
WHG1A	-1.34660	-2.40739	-2.28874	-0.86790	-0.53184	0.18789	-0.94214	0.47870	0.81476	1.53449	1.05938	2.59528	0.83539	2.12016	2.37747
WHS3A	-1.19076	-2.26796	-1.92298	-0.37063	-0.48240	0.62885	-0.73223	0.82013	0.70836	1.81961	1.13053	2.89681	0.86389	2.20773	2.61733
WHTG3B	-1.05903	-2.62644	-1.97712	-0.65185	-0.37422	0.52062	-0.91809	0.40718	0.68481	1.57966	0.89085	3.14706	1.10501	2.45826	2.59990
WHTS2B	-1.93945	-2.40572	-0.82731	-0.76425	-1.05996	0.17009	1.11214	1.17519	0.87948	2.10954	1.00436	2.57582	1.32899	1.47064	1.50318
RG3A	-1.47106	-1.50041	-2.50770	-1.48006	-1.54159	-0.97274	-1.03664	-0.00901	-0.07053	0.49831	1.01143	0.52767	-0.63431	1.04078	-0.64000
RS3B	-1.45267	-1.38201	-2.03877	-1.85403	-1.75648	-1.20192	-0.58611	-0.40137	-0.30382	0.25074	0.90832	0.18008	-0.41522	0.83766	-1.07503
RS2B	-1.57420	-1.69206	-2.43994	-2.10307	-1.87293	-1.31789	-0.86574	-0.52887	-0.29873	0.25631	0.81086	0.37417	-0.57817	0.92872	-0.84941
RG4A	-1.28959	-1.46066	-2.25733	-1.57074	-1.41886	-0.86802	-0.96774	-0.28114	-0.12927	0.42157	1.07172	0.59264	-0.26615	1.24280	-0.54547
RG1A	-1.84215	-1.64200	-2.06484	-2.02399	-1.90313	-1.32289	-0.22269	-0.18183	-0.06097	0.51926	1.05150	0.31911	-0.57759	0.85134	-0.84976
RS1A	-1.70278	-1.54046	-1.79581	-1.50232	-1.62963	-1.06385	-0.09303	0.20045	0.07315	0.63893	1.30077	0.47661	-0.23818	1.13845	-0.83577
RS4A	-1.43297	-1.47870	-1.88438	-1.52619	-1.51338	-0.88655	-0.45141	-0.09322	-0.08042	0.54642	1.06407	0.59215	-0.17098	1.10980	-0.75453
RS2A	-1.39828	-1.53215	-2.48595	-1.39064	-1.34250	-0.83468	-1.08767	0.00764	0.05578	0.56361	1.23974	0.69747	-0.47507	1.37360	-0.30989
RS4B	-1.54929	-1.58637	-2.16048	-1.69141	-1.63200	-1.06702	-0.61119	-0.14211	-0.08271	0.48228	0.92434	0.51936	-0.40120	0.96142	-0.77586
RS3A	-1.45338	-1.48450	-2.05726	-1.66818	-1.65280	-1.05218	-0.60388	-0.21481	-0.19943	0.40119	1.00450	0.43232	-0.30367	1.03562	-0.86101
white 2, 1:2	-0.93645	-1.58864	-0.65219	-1.53749	-0.60104	0.05114	-0.41395	0.52249	1.17468	1.12354	1.92366	2.33761	-0.69503	0.24142	0.89361
white 3, 1:2	-1.26799	-1.97835	-0.71036	-1.81857	-0.55058	0.15978	-0.79386	0.47413	1.18449	1.02472	1.64109	2.43495	-0.94435	0.32364	1.03400
red 1, 1:10	-0.44752	-0.63316	-0.18565	-0.46627	-0.01875	0.16689	-0.77047	-0.32296	-0.13731	-0.30420	0.91984	1.69032	-0.13682	0.31069	0.49634
red 2, 1:10	-0.45113	-0.58501	-0.13388	-0.44493	0.00620	0.14009	-0.74217	-0.29104	-0.15716	-0.29725	0.89049	1.63266	-0.20173	0.24940	0.38328
red 3, 1:10	-0.38493	-0.67971	-0.29478	-0.64963	-0.26470	0.03009	-0.82972	-0.44479	-0.15001	-0.18009	0.78170	1.61142	-0.22874	0.15619	0.45097
WP1_TM.003	0.20524	0.34535	0.14011	2.41034	2.20510	2.06499	2.10367	1.89842	1.75832	-0.30667	1.96526	-0.13840	1.11066	0.90542	0.76531
WP2_TM.003	0.21128	0.58272	0.37144	2.64771	2.43643	2.06499	2.19994	1.98866	1.61722	-0.44777	2.08413	-0.11582	1.34804	1.13675	0.76531
WP3_TM.003	0.53635	-0.09829	-0.63464	1.96671	1.43036	2.06499	1.96099	1.42464	2.05927	-0.00572	2.40490	0.44391	0.66703	0.13068	0.76531
WP4_TM.003	-0.02324	-0.35437	-0.33114	1.53371	1.55695	1.88808	1.64265	1.66589	1.99702	0.10894	1.74291	0.10026	-0.05554	-0.03231	0.29883
WP5_TM.003	0.27437	-0.27182	-0.54619	2.10147	1.82710	2.37328	1.67692	1.40255	1.94874	-0.42455	1.87813	0.20121	0.60894	0.33457	0.88076
WP6_TM.027	0.00449	0.58947	0.58498	2.65446	2.64997	2.06499	1.53012	1.52563	0.94065	-1.12434	1.68002	0.14990	1.35478	1.35029	0.76531
WP7_TM.027	0.83737	-0.09829	-0.93566	1.96671	1.12933	2.06499	1.96099	1.12362	2.05927	-0.00572	1.76507	-0.19592	0.66703	-0.17035	0.76531
WP8_TM.027	-0.10275	0.25600	0.35876	2.62203	2.72478	2.36602	1.56575	1.66850	1.30974	-1.05628	1.58388	0.01813	1.32235	1.42510	1.06634
WP9_TM.027	0.06190	-0.09428	-0.15618	2.56353	2.50163	2.65781	1.60147	1.53957	1.69575	-0.96206	1.62760	0.02613	0.96282	0.90092	1.05710

WP10_TM.027	0.07212	0.50670	0.43458	2.57169	2.49957	2.06499	1.96563	1.89351	1.45893	-0.60606	2.18393	0.21831	1.27201	1.19990	0.76531
prep11	-0.22809	0.16860	0.39669	2.57461	2.80270	2.40602	1.83901	2.06710	1.67042	-0.73560	1.56695	-0.27206	1.27494	1.50303	1.10634
prep12	0.25771	0.46674	0.20904	2.53174	2.27403	2.06499	2.52602	2.26831	2.05927	-0.00572	2.66890	0.14288	1.23206	0.97435	0.76531
prep13	-0.05231	-0.09829	-0.04598	1.96671	2.01902	2.06499	1.96099	2.01330	2.05927	-0.00572	2.40490	0.44391	0.66703	0.71934	0.76531
prep14	-0.27526	0.55435	0.82961	2.61935	2.89461	2.06499	1.95793	2.23319	1.40358	-0.66141	1.86210	-0.09583	1.31967	1.59493	0.76531
prep15	-0.17240	0.53753	0.70994	2.60253	2.77493	2.06499	1.59415	1.76655	1.05662	-1.00837	1.65225	0.05810	1.30285	1.47525	0.76531

	149:175	**163:175**	**103:193**	**133:193**	**149:193**	**163:193**	**175:193**	**103:197**	**117:197**	**133:197**	**149:197**	**167:197**	**175:197**	**193:197**
WHTG2B	1.46993	2.60515	2.07115	0.21166	1.13522	0.60122	0.92356	-1.04921	0.38956	0.52953	-1.97277	1.43877	1.57874	0.13997
WHS4A	1.46585	2.43127	1.73617	0.05203	0.96543	0.27032	0.91340	-1.11731	0.21829	0.47422	-2.03071	1.33560	1.59153	0.25593
WHS1A	0.84291	2.06682	1.29411	0.32860	1.22390	0.45120	0.89531	-1.13534	0.12260	0.20680	-2.03064	1.25794	1.34214	0.08420
WHG4B	1.63461	2.66555	1.96762	0.06463	1.03094	0.33301	0.96631	-1.08747	0.26839	0.57957	-2.05378	1.35586	1.66704	0.31118
WHS4B	0.56909	1.41120	0.72426	0.04586	0.84212	0.15517	0.79625	-1.30262	0.10931	0.27739	-2.09888	1.41194	1.58001	0.16808
WHG2A	1.22192	2.43297	1.73360	0.36236	1.21105	0.51168	0.84870	-1.22893	0.14932	0.47234	-2.07763	1.37825	1.70127	0.32302
WHS3B	1.45887	2.50638	1.87657	0.05187	1.04750	0.41770	0.99563	-1.00703	0.36583	0.77327	-2.00266	1.37286	1.78030	0.40744
WHS2A	1.30367	2.49413	1.89549	0.24949	1.19046	0.59182	0.94097	-0.97779	0.34233	0.61001	-1.91875	1.32011	1.58779	0.26768
WHG3A	1.49710	2.55657	2.00720	0.19509	1.05947	0.51010	0.86438	-0.92079	0.31501	0.40548	-1.78517	1.23580	1.32627	0.09047
WHG1A	1.42083	2.47663	2.00151	0.33606	1.05579	0.58068	0.71973	-1.04016	0.24462	0.50192	-1.75989	1.28478	1.54208	0.25730
WHS3A	1.55235	2.55184	1.86276	-0.11177	0.99948	0.31040	1.11125	-0.92167	0.42217	0.83177	-2.03292	1.34384	1.75343	0.40959
WHTG3B	1.32527	2.49774	1.80894	0.27763	1.17247	0.48367	0.89484	-1.14720	0.20604	0.34768	-2.04205	1.35325	1.49488	0.14164
WHTS2B	0.06305	0.99740	-0.10778	-0.29571	0.93434	-0.17083	1.23005	-0.01678	0.12488	0.15742	-1.24683	0.14166	0.17419	0.03254
RG3A	1.02763	1.53496	2.04807	-0.06153	0.50732	1.02044	0.56885	-0.59313	1.08196	-0.59882	-1.16198	1.67509	-0.00569	-1.68078
RS3B	0.18474	0.83685	1.49443	0.09755	0.65211	1.30969	0.55456	-0.04075	1.21214	-0.70055	-0.59531	1.25289	-0.65980	-1.91269
RS2B	0.33687	1.12205	1.67661	0.23014	0.78518	1.33974	0.55504	-0.39729	1.10960	-0.66853	-0.95234	1.50689	-0.27124	-1.77813
RG4A	0.68660	1.38931	2.03947	0.15188	0.70272	1.35287	0.55084	-0.30796	1.20099	-0.58727	-0.85880	1.50895	-0.27932	-1.78826
RG1A	0.04085	0.74195	1.27419	0.12086	0.70110	1.23333	0.58023	-0.31647	1.11247	-0.58863	-0.89670	1.42893	-0.27216	-1.70110
RS1A	0.29348	0.73196	1.39381	-0.12730	0.43848	1.10032	0.56578	-0.14901	1.22763	-0.74660	-0.71479	1.37663	-0.59759	-1.97422
RS4A	0.35818	0.99783	1.51547	0.01281	0.63965	1.15729	0.62684	-0.13630	1.14448	-0.71985	-0.76314	1.28078	-0.58355	-1.86433
RS2A	1.09531	1.65128	2.32741	0.04813	0.55596	1.23209	0.50783	-0.66472	1.18396	-0.49953	-1.17254	1.84867	0.16518	-1.68349
RS4B	0.46907	1.09346	1.53552	0.05940	0.62439	1.06645	0.56499	-0.35557	1.00705	-0.73022	-0.92056	1.36262	-0.37465	-1.73727
RS3A	0.38907	1.00507	1.60838	0.01538	0.61600	1.21931	0.60062	-0.13537	1.20393	-0.69270	-0.73599	1.33930	-0.55733	-1.89663
white 2, 1:2	0.84246	-0.28108	0.29953	1.88817	1.83702	0.71348	0.99456	1.55121	2.48765	3.13984	3.08870	-0.37245	2.24624	1.25167
white 3, 1:2	0.87422	-0.15049	-0.14071	1.83764	1.67787	0.65315	0.80365	1.21655	2.48454	3.19490	3.03513	-0.42454	2.16090	1.35726
red 1, 1:10	0.32945	0.63365	1.02837	1.66154	1.49465	1.79885	1.16520	-0.60076	-0.15324	0.03240	-0.13449	-1.52061	-0.46394	-1.62914
red 2, 1:10	0.24320	0.54044	1.05886	1.64387	1.50378	1.80103	1.26059	-0.53419	-0.08306	0.05082	-0.08927	-1.42468	-0.33246	-1.59305
red 3, 1:10	0.42088	0.60098	0.96423	1.64395	1.61386	1.79396	1.19298	-0.63851	-0.25358	0.04121	0.01112	-1.42021	-0.40976	-1.60274
WP1_TM.003	-1.29968	-0.99300	1.86533	1.51998	-0.54502	-0.23834	0.75466	1.65662	1.45138	1.31127	-0.75372	-0.44704	0.54596	-0.20870
WP2_TM.003	-1.29968	-0.85191	1.98585	1.40313	-0.66187	-0.21410	0.63781	1.78621	1.57493	1.20349	-0.86150	-0.41373	0.43818	-0.19964
WP3_TM.003	-1.29968	-1.29396	2.36694	2.46523	0.40023	0.40595	1.69991	2.82590	2.28955	2.92419	0.85920	0.86491	2.15887	0.45896
WP4_TM.003	-1.58925	-1.69819	1.67181	2.02618	0.13810	0.02916	1.72735	1.78461	1.80784	2.13898	0.25090	0.14196	1.84015	0.11280

WP5_TM.003	-1.49252	-1.06798	1.57334	1.84516	-0.52812	-0.10358	0.96440	1.71560	1.44123	1.98742	-0.38586	0.03868	1.10666	0.14226
WP6_TM.027	-1.29968	-0.17534	2.27958	1.69011	-0.37488	0.74946	0.92480	1.97799	1.97350	1.38852	-0.67647	0.44787	0.62321	-0.30159
WP7_TM.027	-1.29968	-1.29396	2.06610	2.16438	0.09939	0.10511	1.39907	2.15560	1.31823	2.25389	0.18890	0.19461	1.48857	0.08951
WP8_TM.027	-1.29968	-0.24340	2.27484	2.01884	-0.34719	0.70909	0.95249	1.90048	2.00324	1.64448	-0.72154	0.33474	0.57814	-0.37436
WP9_TM.027	-1.60071	-0.63865	2.06304	2.15732	-0.50049	0.46157	1.10022	1.79533	1.73343	1.88961	-0.76820	0.19386	0.83251	-0.26771
WP10_TM.027	-1.29968	-0.69361	2.26052	1.75383	-0.31117	0.29490	0.98851	2.29198	2.21987	1.78528	-0.27971	0.32635	1.01997	0.03146
prep11	-1.29968	-0.56408	2.10483	1.93624	-0.46978	0.26582	0.82990	1.64090	1.86899	1.47230	-0.93371	-0.19811	0.36597	-0.46393
prep12	-1.29968	-1.29396	2.93197	2.46523	0.40023	0.40595	1.69991	3.29896	3.04125	2.83221	0.76722	0.77294	2.06690	0.36699
prep13	-1.29968	-1.29396	2.36694	2.46523	0.40023	0.40595	1.69991	3.12587	3.17818	3.22415	1.15916	1.16488	2.45884	0.75893
prep14	-1.29968	-0.63826	2.07328	1.51893	-0.54607	0.11535	0.75361	1.86610	2.14136	1.31174	-0.75325	-0.09183	0.54643	-0.20718
prep15	-1.29968	-0.29130	2.21260	1.67506	-0.38993	0.61845	0.90975	1.76551	1.93792	1.22798	-0.83701	0.17136	0.46267	-0.44709

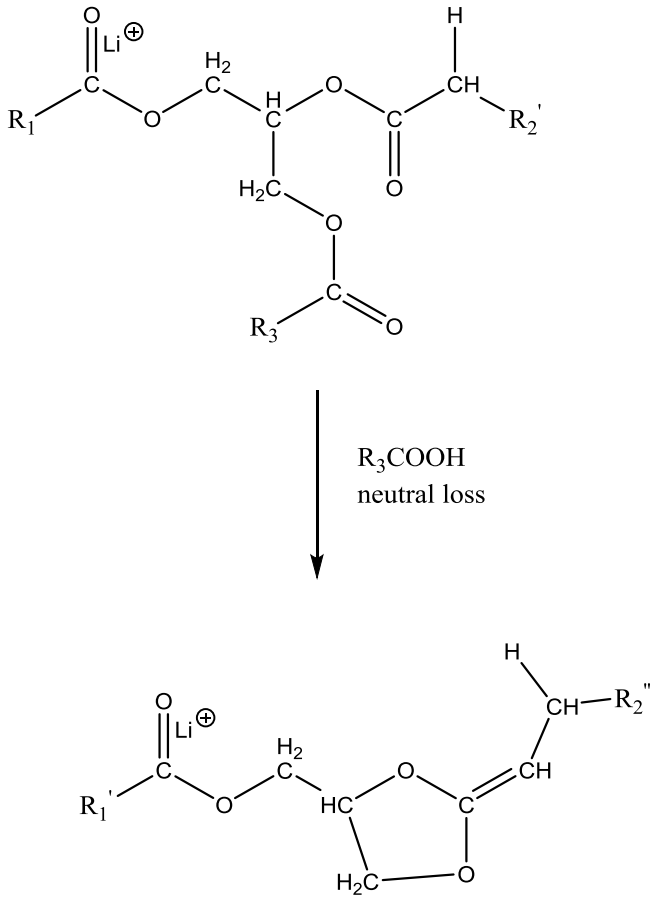

Figure 1 Proposed fragmentation scheme of lithiated TAGs, MS^1 to MS^2, reproduced from Hsu and Turk, 2010.

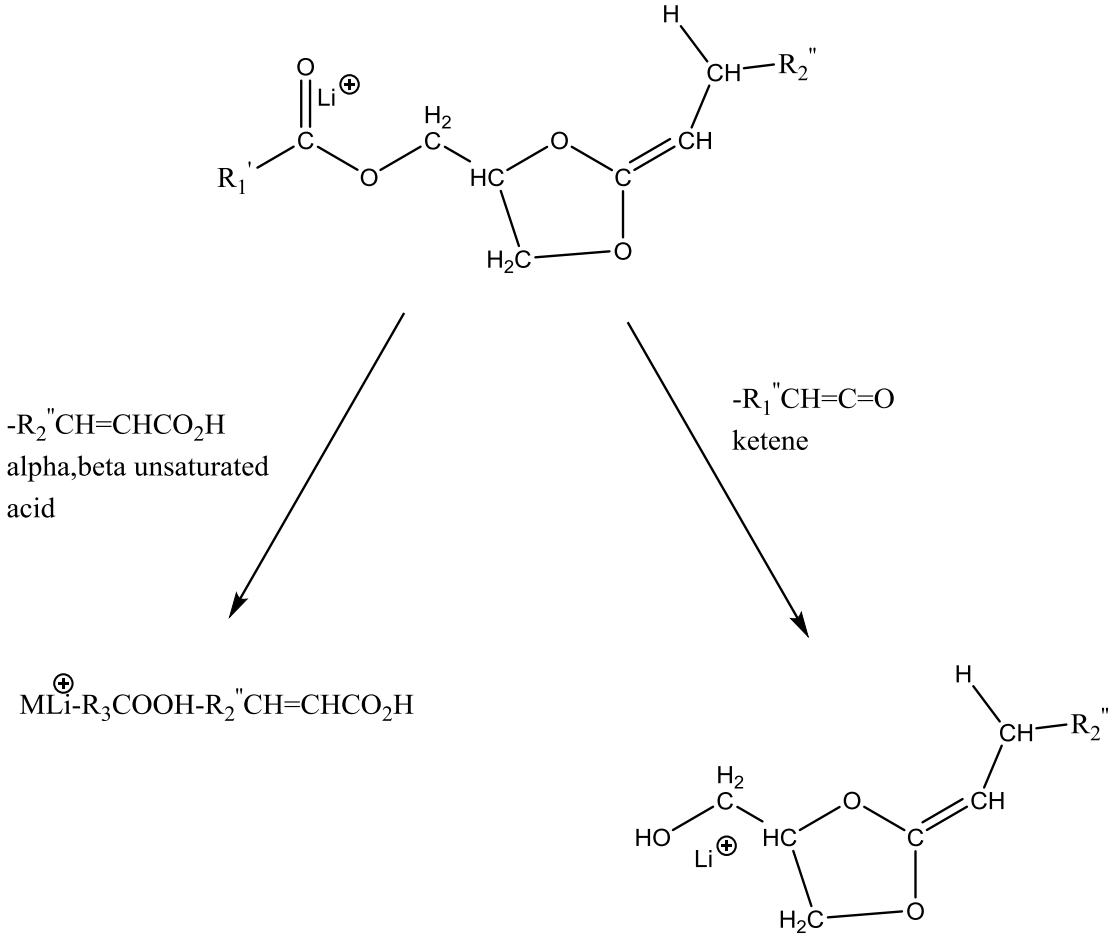

Figure 2 Proposed fragmentation scheme of lithiated TAGs, MS^2 to MS^3, reproduced from Hsu and Turk, 2010.

Mussels with lentils

1. one pint of mussels
2. 350g lentils
3. spices (peppercorn, cumin seeds, coriander seeds, dried mint, rue, pennyroyal)
4. 1 onion
5. 15ml vinegar
6. 15ml honey
7. 15ml defructum
8. 5ml anchovy essence and olive oil

Lamb stew

1. 900g lamb
2. 1 onion
3. 1 teaspoon coriander seeds
4. pinch of pepper, lovage, and cumin
5. 5ml anchovy essence
6. 15ml olive oil
7. 15ml wine
8. 1 tablespoon cornflour

Truffles

1. 12 large truffles
2. salt
3. 15ml olive oil
4. 15ml wine
5. 15ml caroenum
6. 15ml honey
7. pinch of pepper
8. 2 teaspoons of cornflour

Figure 3 Three recipes reproduced from (Renfrew, 1985: 35,40,41.

Table 1 Results of the TAG analysis of laboratory aged sherd doped with goats' cheese aged three months

acyl carbon number	types of TAGs	fatty acid constituents													
C24	3	4/12/18	4/14/16	4/10/20											
C26	2	4/12/20	4/16/16												
C28	5	10/10/18	10/12/16	10/8/20	6/16/16	6/14/18									
C30	10	10/12/18	10/16/14	10/10/20	14/12/14	14/6/20	14/16/10	16/08/16	16/10/14	16/4/20	16/06/18				
C32	13	12/14/16	16/15/11	16/18/8	16/9/17	16/16/10	16/7/19	16/8/18	16/6/20	10/12/20	10/13/19	10/18/14	10/15/17	14/14/14	
C34	9	14/14/16	14/10/20	12/16/16	14/18/12	16/8/20	16/10/18	16/9/19	10/15/19	10/17/17					
C38	3	18/10/18	14/16/16	14/14/18											

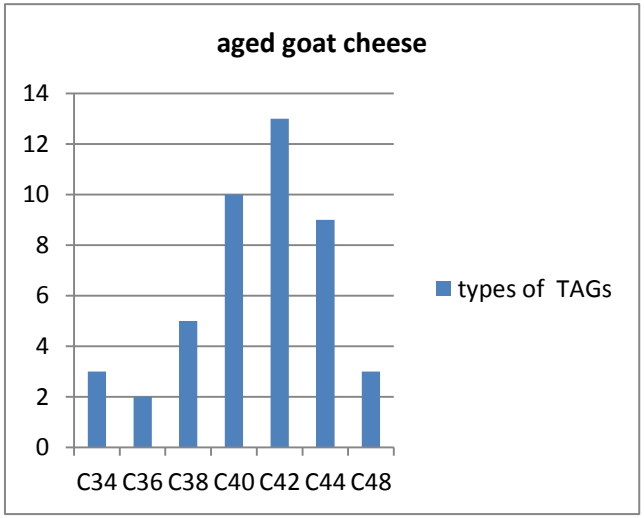

Table 2 Results of the TAG analysis of laboratory aged sherd doped with cows milk aged three months

Cow 3A Acyl carbon number	types of TAGs	fatty acid constituents	
C38:1	1	4.18.16:1	
C40	1	16.8.16	
C40:1	2	4.18.18:1	6.18:1.16
C42	1	16.10.16	
C44	2	12:14:18	12:16:16
C46			
C48			
C50			
C50:1	2	16:16:18:1	14:18:18:1

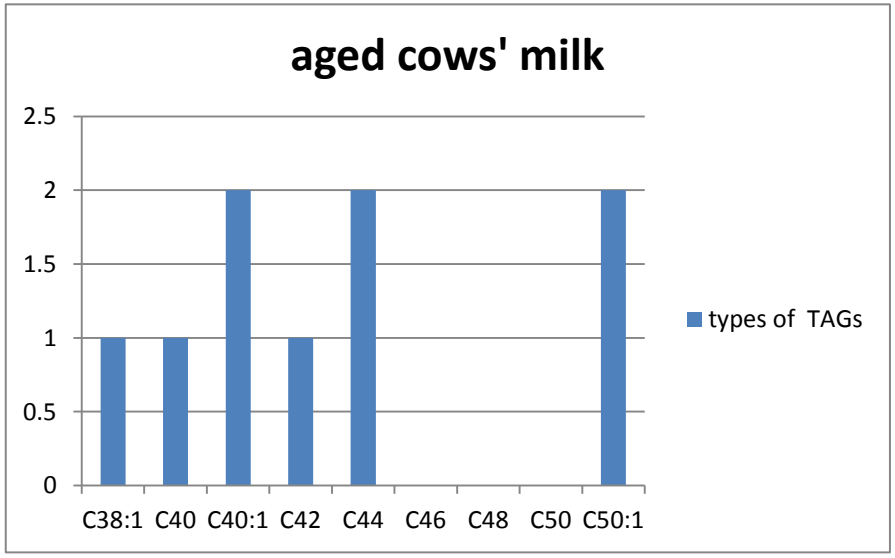

Table 3 Results of the TAG analysis of sherd doped with sheep cheese aged three months

sheep 3A acyl carbon number	types of TAGs	fatty acid constituents										
C34	8	4.10.20	4.15.15	4.12.18	4.14.16	14.10.10	14.6.14	16.8.10	16.4.14			
C34:1	1	4.12.18:1										
C36	7	4.12.20	4.14.18	4.15.17	4.16.16	6.16.14	16.8.12	16.10.10				
C36:1	1	4.14.18:1										
C38	9	4.16.18	4.15.19	4.14.20	6.16.16	6.15.17	6.12.20	6.18.14	8.14.16	16.12.10		
C38:1	4	4.14.20:1	4.18.16:1	4.18:1.16	16.6.16:1							
C40	10	18.4.18	18.6.16	18.8.14	10.10.20	10.12.18	10.14.16	16.4.20	16.6.18	16.8.16	16.5.19	
C40:1	11	6.16.18:1	6.14.20:1	6.18.16:1	6.15.19:1	4.17:1.19	4.18:1.18	4.17.19:1	4.16.20:1	4.16:1.20	18:1.8.14	18:1.10.12
C42												
C42:1												
C44												
C44:1	2	10.18:1.16	10.16.18:1									

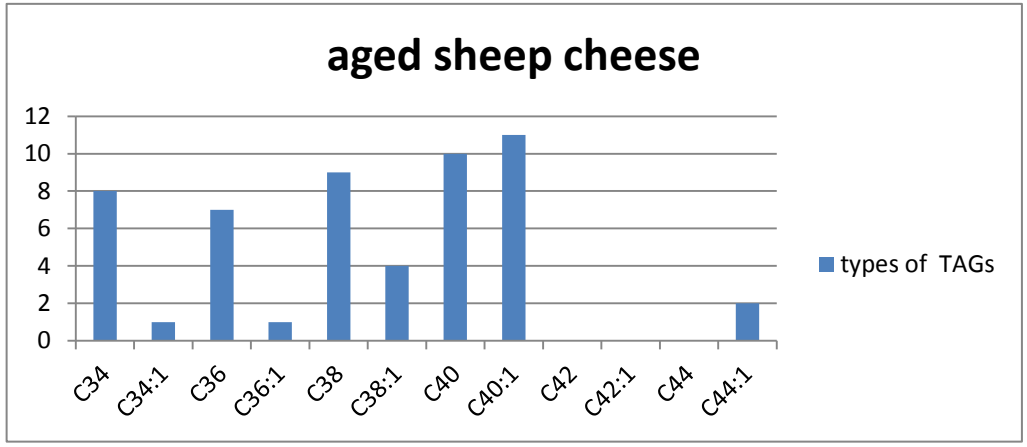

Table 4 The top 200 loadings based upon the PCA scores plot from Chapter 6, Figure 6.4. The table is sorted on PC1 values staring from lowest to highest. The three yellow masses are phenolic acids common to the wine biomarker list.

M/Z	INTENSITY	PC1	Empirical formula (parent)	Empirical formula (peak)	Ion form	Theoretical mass (neutral) (Da)	Theoretical mass (Da)	Mass error (ppm)	KEGG_Putative identificaiton
153.01922	5394464	-0.0567179	C7H6O4	C7H6O4	[M-H]-	154.02661	153.0193336	-0.74	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']
187.04179	6531863.5	-0.0502019		0					0
179.03482	14153511	-0.0490905	C9H8O4	C9H8O4	[M-H]-	180.04226	179.0349836	-0.91	['2-Hydroxy-3-(4-hydroxyphenyl)propenoate', '3-(4-Hydroxyphenyl)pyruvate', 'Aspirin', 'Caffeate', 'trans-2,3-Dihydroxycinnamate']
277.21703	15907124	-0.0485573	C18H30O2	C18H30O2	[M-H]-	278.22458	277.2173036	-0.99	['(6Z,9Z,12Z)-Octadecatrienoic acid', '(9Z,11E,13E)-Octadecatrienoic acid', '(9Z,12Z,15Z)-Octadecatrienoic acid', '5beta-Estrane-3alpha,17beta-diol', 'Crepenynate', 'Punicic acid']
197.04548	6917421.6	-0.0477345	C7H14NO3	C7H14NO3	[M+K-2H]-	160.097369	197.0459756	-2.52	['3-Dehydrocarnitine']
197.04548	6917421.6	-0.0477345	C7H6O3	C7H6O3	[M+Hac-H]-	138.031695	197.0455486	-0.35	['2-Hydroxy-5-methylquinone', '3,4-Dihydroxybenzaldehyde', '3-Hydroxybenzoate', '4-Hydroxybenzoate', 'Gentisate aldehyde', 'Salicylate', 'Sesamol']
197.04548	6917421.6	-0.0477345	C9H10O5	C9H10O5	[M-H]-	198.052825	197.0455486	-0.35	['3-(3,4-Dihydroxyphenyl)lactate', '3-Methoxy-4-hydroxymandelate', 'Syringic acid']
129.03634	1362343.4	-0.047612		0					0
161.06253	1633131.8	-0.0469184		0					0
154.02263	351008.9	-0.0464517		0					0
137.02428	6583738.3	-0.0460582	C7H6O3	C7H6O3	[M-H]-	138.031695	137.0244186	-1.01	['2-Hydroxy-5-methylquinone', '3,4-Dihydroxybenzaldehyde', '3-Hydroxybenzoate', '4-Hydroxybenzoate', 'Gentisate aldehyde', 'Salicylate', 'Sesamol']
209.0627	814900.4	-0.0453093	C6H12N4O2	C6H12N4O2	[M+(37Cl)]-	172.096026	209.0624776	1.06	['L-Capreomycidine']

163.04002	489449.1	-0.0451876	C9H8O3	C9H8O3	[M-H]-	164.047345	163.0400686	-0.3	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']
221.04557	724963.2	-0.045185	C11H10O5	C11H10O5	[M-H]-	222.052825	221.0455486	0.1	['2-Hydroxy-4-hydroxymethylbenzalpyruvate', '2-Hydroxy-7-hydroxymethylchromene-2-carboxylate', '2-Succinylbenzoate', '6-(2-Methoxyvinyl)benzo[1,3]dioxole-5-carboxylic acid', 'Fraxidin', 'Isofraxidin', 'Leptodactylone', 'Sphagnum acid']
221.04557	724963.2	-0.045185	C8H13N2O2P	C8H13N2O2P	[M+Na-2H]-	200.071466	221.0461346	-2.55	['Diamidafos']
221.04557	724963.2	-0.045185	C9H6O3	C9H6O3	[M+Hac-H]-	162.031695	221.0455486	0.1	['Umbelliferone']
203.035	608807.9	-0.0443507	C11H8O4	C11H8O4	[M-H]-	204.04226	203.0349836	0.08	['1,2-Dihydroxy-8-carboxynaphthalene', '1,4-Dihydroxy-2-naphthoate', '1,4-Dihydroxy-6-naphthoate', 'Droserone', 'Spirodilactone']
277.2152	488837.7	-0.0432642	C16H32O2	C16H32O2	[M+Na-2H]-	256.24023	277.2148986	1.09	['Hexadecanoic acid']
195.0299	332449.2	-0.0429359	C9H8O5	C9H8O5	[M-H]-	196.037175	195.0298986	0.01	['3-(3,4-Dihydroxyphenyl)pyruvate']
141.01925	330838.9	-0.0429189	C6H6O4	C6H6O4	[M-H]-	142.02661	141.0193336	-0.59	['(S)-5-Oxo-2,5-dihydrofuran-2-acetate', '1,2,3,5-Tetrahydroxybenzene', '2,5-Dihydro-5-oxofuran-2-acetate', '2-Hydroxymuconate semialdehyde', '2-Oxo-2,3-dihydrofuran-5-acetate', 'Kojic acid', 'cis,cis-4-Hydroxymuconic semialdehyde', 'cis,cis-Muconate', 'cis,trans-Hexadienedioate']
377.08762	2423971.5	-0.0424825	C16H14O7	C16H14O7	[M+Hac-H]-	318.073955	377.0878086	-0.5	['Demethylsulochrin', 'Orsellinate depside']
259.06281	197681.4	-0.0421662		0					0
329.06644	1159814	-0.0421168	C15H10O5	C15H10O5	[M+Hac-H]-	270.052825	329.0666786	-0.73	['2-Hydroxydaidzein', "3',4',7-Trihydroxyisoflavone", '3,6,4-Trihydroxyflavone', "4',6,7-Trihydroxyisoflavone", '5-Deoxykaempferol', 'Aloe-emodin', 'Apigenin', 'Baicalein', 'Emodin', 'Galangin', 'Genistein', 'Islandicin', 'Lucidin', 'Morindone', 'Norobtusifolin', 'Norwogonin', 'Purpurin 1-methyl ether', 'Sulphuretin']
329.06644	1159814	-0.0421168	C17H14O7	C17H14O7	[M-H]-	330.073955	329.0666786	-0.73	['(+)-Bisdechlorogeodin', '(-)-Bisdechlorogeodin', "3',4',5-Trihydroxy-3,7-dimethoxyflavone", 'Aflatoxin G2', 'Aurantio-obtusin', 'Cirsiliol', 'Hildecarpin', 'Tricin']

207.02993	190672.7	-0.0419134	C10H8O5	C10H8O5	[M-H]-	208.037175	207.0298986	0.15	['Fraxetin']
139.00362	289490.2	-0.0418592	C6H4O4	C6H4O4	[M-H]-	140.01096	139.0036836	-0.46	['cis-4-Carboxymethylenebut-2-en-4-olide', 'trans-4-Carboxymethylenebut-2-en-4-olide']
138.0277	478185.6	-0.0415011		0					0
331.08208	1462635.1	-0.0413294	C15H12O5	C15H12O5	[M+Hac-H]-	272.068475	331.0823286	-0.75	['(6aS,11aS)-3,6a,9-Trihydroxypterocarpan', '2,7,4-Trihydroxyisoflavanone', '2-Hydroxydihydrodaidzein', '6,7,4-Trihydroxyflavanone', 'Butein', 'Butin', 'Dihydrogenistein', 'Garbanzol', 'Licodione', 'Naringenin', 'Naringenin chalcone', 'Pinobanksin', 'Rubrofusarin', 'Toralactone', 'p-Coumaroyltriacetic acid lactone']
331.08208	1462635.1	-0.0413294	C17H16O7	C17H16O7	[M-H]-	332.089605	331.0823286	-0.75	['Sulochrin']
128.03998	439849	-0.0411237		0					0
279.2327	9181564.9	-0.0411056	C18H32O2	C18H32O2	[M-H]-	280.24023	279.2329536	-0.91	['9-cis,11-trans-Octadecadienoate', 'Chaulmoogric acid', 'Linoleate', 'Malvalic acid', 'Stearolic acid']
191.05607	659197.6	-0.0408467	C5H8O4	C5H8O4	[M+Hac-H]-	132.04226	191.0561136	-0.23	['(4S)-4,5-Dihydroxypentan-2,3-dione', '(S)-2-Acetolactate', '2-(Hydroxymethyl)-4-oxobutanoate', '2-Acetolactate', '3-Hydroxy-3-methyl-2-oxobutanoic acid', '4-Hydroxy-2-oxopentanoate', 'Deoxyribonolactone', 'Glutarate']
191.05607	659197.6	-0.0408467	C7H12O6	C7H12O6	[M-H]-	192.06339	191.0561136	-0.23	['2D-5-O-Methyl-2,3,5/4,6-pentahydroxycyclohexanone', 'Quinate', 'Valiolone']
76.5096	121821.6	-0.0403706		0					0
278.22044	3167042.7	-0.0398457		0					0
187.00554	318658.4	-0.0397204	C6H6N4S	C6H6N4S	[M+Na-2H]-	166.031318	187.0059866	-2.39	['6-Methylmercaptopurine']
395.09817	1920252.2	-0.0396321	C16H16O8	C16H16O8	[M+Hac-H]-	336.08452	395.0983736	-0.52	['5-O-Caffeoylshikimic acid', 'Altersolanol A']
139.04	300639.2	-0.0395079	C7H8O3	C7H8O3	[M-H]-	140.047345	139.0400686	-0.49	['2,3,5-Trihydroxytoluene', '2,4,5-Trihydroxytoluene', '2,4,6-Trihydroxytoluene', '4-Hydroxymethylcatechol', 'Gentisyl alcohol']

199.01676	249908.8	-0.0393805	C10H10O2	C10H10O2	[M+K-2H]-	162.06808	199.0166866	0.37	['(1S,2S)-1,2-Dihydronaphthalene-1,2-diol', '1,2-Dihydronaphthalene-1,2-diol', '4-Hydroxycinnamoylmethane', 'Isosafrole', 'Methyl cinnamate', 'Safrole', 'cis-1,2-Dihydronaphthalene-1,2-diol', 'p-Methoxycinnamaldehyde', 'trans-2-Phenylcyclopropanecarboxylic acid']
199.01676	249908.8	-0.0393805	C9H8O3	C9H8O3	[M+Cl]-	164.047345	199.0167466	0.07	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']
201.03801	953816.9	-0.0389213	C6H12O6	C6H12O6	[M+Na-2H]-	180.06339	201.0380586	-0.24	['2-Deoxy-D-gluconate', 'Aldohexose', 'D-Aldose', 'D-Allose', 'D-Altrose', 'D-Fructose', 'D-Fuconate', 'D-Galactose', 'D-Glucose', 'D-Gulose', 'D-Hamamelose', 'D-Hexose', 'D-Idose', 'D-Mannose', 'D-Psicose', 'D-Sorbose', 'D-Tagatose', 'D-Talose', 'Fructose(pyranose)', 'Ketose', 'L-Fructose', 'L-Fuconate', 'L-Galactose', 'L-Gulose', 'L-Rhamnonate', 'L-Sorbose', 'Sorbose', 'alpha-D-Galactose', 'alpha-D-Glucose', 'alpha-D-Mannose', 'alpha-L-Sorbopyranose', 'beta-D-Fructose', 'beta-D-Galactose', 'beta-D-Glucose', 'beta-D-Hamamelopyranose', 'beta-D-Mannose', 'muco-Inositol', 'myo-Inositol', 'scyllo-Inositol']
199.02483	240163.1	-0.0389118	C6H4O4	C6H4O4	[M+Hac-H]-	140.01096	199.0248136	0.08	['cis-4-Carboxymethylenebut-2-en-4-olide', 'trans-4-Carboxymethylenebut-2-en-4-olide']
199.02483	240163.1	-0.0389118	C8H8O6	C8H8O6	[M-H]-	200.03209	199.0248136	0.08	['(3S,4R)-3,4-Dihydroxycyclohexa-1,5-diene-1,4-dicarboxylate', '2-Hydroxy-5-carboxymethylmuconate semialdehyde', '3-Carboxymethylmuconate', '4-Fumarylacetoacetate', '4-Maleylacetoacetate', 'Phthalate 3,4-cis-dihydrodiol', 'cis-4,5-Dihydroxycyclohexa-1(6),2-diene-1,2-dicarboxylate']
181.05062	234605.6	-0.0387545	C7H6O2	C7H6O2	[M+Hac-H]-	122.03678	181.0506336	-0.08	['3-Hydroxybenzaldehyde', '4-Hydroxybenzaldehyde', 'Benzoate', 'Salicylaldehyde', 'Tropolone']

181.05062	234605.6	-0.0387545	C9H10O4	C9H10O4	[M-H]-	182.05791	181.0506336	-0.08	['(R)-3-(4-Hydroxyphenyl)lactate', "2',6-Dihydroxy-4-methoxyacetophenone", '3,4-Dihydroxyphenylpropanoate', '3-(2,3-Dihydroxyphenyl)propanoate', '3-(4-Hydroxyphenyl)lactate', '3-Methoxy-4-hydroxyphenylglycolaldehyde', 'Homovanillate', 'cis-3-(3-Carboxyethenyl)-3,5-cyclohexadiene-1,2-diol']
317.06649	2037180	-0.0387343	C11H14N4O5	C11H14N4O5	[M+Cl]-	282.096421	317.0658226	2.1	['8-Oxocoformycin']
317.06649	2037180	-0.0387343	C14H10O5	C14H10O5	[M+Hac-H]-	258.052825	317.0666786	-0.59	['3-Carbethoxypsoralen', 'Alternariol', 'Gentisin', 'Isogentisin', 'Kigelinone', 'Mesuaxanthone A', 'Norlichexanthone', 'Norrubrofusarin']
317.06649	2037180	-0.0387343	C16H14O7	C16H14O7	[M-H]-	318.073955	317.0666786	-0.59	['Demethylsulochrin', 'Orsellinate depside']
317.06649	2037180	-0.0387343	C17H14NO4	C17H14NO4	[M+Na-2H]-	296.092284	317.0669526	-1.46	['2,3,9,10-Tetrahydroxyberberine']
223.06123	486261.6	-0.0387005	C11H12O5	C11H12O5	[M-H]-	224.068475	223.0611986	0.14	['Sinapate']
223.06123	486261.6	-0.0387005	C9H8O3	C9H8O3	[M+Hac-H]-	164.047345	223.0611986	0.14	['2-Hydroxy-3-phenylpropenoate', '3-Coumaric acid', '4-Coumarate', 'Benzoyl acetate', 'Caffeic aldehyde', 'Phenylpyruvate', 'cis-2-Hydroxycinnamate', 'cis-p-Coumarate', 'trans-2-Hydroxycinnamate']
333.09773	1300773.5	-0.0385117	C15H14O5	C15H14O5	[M+Hac-H]-	274.084125	333.0979786	-0.75	['(-)-Epiafzelechin', '5-Deoxyleucopelargonidin', 'Afzelechin', 'Apiforol', 'Fisetinidol', 'H37', 'Luteoliflavan', 'Methysticin', 'Phloretin', 'Ptaerochromenol', 'alpha-Pyrufuran', 'beta-Pyrufuran']
333.09773	1300773.5	-0.0385117	C17H18O7	C17H18O7	[M-H]-	334.105255	333.0979786	-0.75	['Byakangelicin']
211.0248	910183.8	-0.0384871	C9H8O6	C9H8O6	[M-H]-	212.03209	211.0248136	-0.06	['2-Hydroxy-6-ketononatrienedioate', '3-(2-Carboxyethenyl)-cis,cis-muconate', '5-Carboxyvanillic acid']
197.00918	236432	-0.0384558	C8H6O6	C8H6O6	[M-H]-	198.01644	197.0091636	0.08	['3,4-Dihydroxyphthalate', '4,5-Dihydroxyphthalate']
205.01426	83451.9	-0.0383958	C10H6O5	C10H6O5	[M-H]-	206.021525	205.0142486	0.06	['Flaviolin']
280.23607	1802041.1	-0.0383548		0					0
191.03497	122503.1	-0.0382761	C10H8O4	C10H8O4	[M-H]-	192.04226	191.0349836	-0.07	['1,3,6,8-Naphthalenetetrol', '2-Hydroxychromene-2-carboxylate', '3,4-Dehydro-6-hydroxymellein', 'Acamelin', 'Anemonin', 'Isoscopoletin', 'Methylenedioxycinnamic acid', 'Naphthazarin', 'Scopoletin', 'trans-O-'

									Hydroxybenzylidenepyruvate']
198.04892	487943.8	-0.0380821		0					0
219.02991	74589.7	-0.0378621	C11H8O5	C11H8O5	[M-H]-	220.037175	219.0298986	0.05	['(3E)-4-(2-Carboxyphenyl)-2-oxobut-3-enoate', '(3Z)-4-(2-Carboxyphenyl)-2-oxobut-3-enoate', '3-[6-(Carboxymethylene)cyclohexa-2,4-dien-1-ylidene]-2-oxopropanate', 'Purpurogallin']
133.01416	728773.7	-0.0378254	C2H2O3	C2H2O3	[M+Hac-H]-	74.000395	133.0142486	-0.67	['Glyoxylate']
133.01416	728773.7	-0.0378254	C4H6O5	C4H6O5	[M-H]-	134.021525	133.0142486	-0.67	['(R)-Malate', '(S)-Malate', '3-Dehydro-L-threonate', 'Malate']
193.05044	5042080.5	-0.0376497	C10H10O4	C10H10O4	[M-H]-	194.05791	193.0506336	-1	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']
193.0506	1110858.3	-0.0376497	C10H10O4	C10H10O4	[M-H]-	194.05791	193.0506336	-0.17	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']
201.05757	313794.3	-0.0376384		0					0
125.02433	164676.6	-0.0376006	C6H6O3	C6H6O3	[M-H]-	126.031695	125.0244186	-0.71	['1,2,3-Trihydroxybenzene', '5-Hydroxymethyl-2-furaldehyde', 'Benzene-1,2,4-triol', 'Maltol', 'Phloroglucinol', 'Triacetate lactone']
359.07695	6421016.9	-0.0375989	C13H16N4O6	C13H16N4O6	[M+Cl]-	324.106986	359.0763876	1.57	['Levofuraltadone']
359.07695	6421016.9	-0.0375989	C16H12O6	C16H12O6	[M+Hac-H]-	300.06339	359.0772436	-0.82	['(+)-6a-Hydroxymaackiain', '(+)-Sophorol', '(-)-Sophorol', '2-Hydroxybiochanin A', '3-Methoxyapigenin', 'Cajanin', 'Chrysoeriol', 'Diosmetin', 'Hispidulin', 'Kaempferide', 'Leptosidin', 'Pratensein', 'Questinol', 'Rhamnocitrin', 'Tectorigenin']
359.07695	6421016.9	-0.0375989	C18H16O8	C18H16O8	[M-H]-	360.08452	359.0772436	-0.82	["3',4',5-Trihydroxy-3,6,7-trimethoxyflavone", '6-Methoxyaromadendrin 3-O-acetate', 'Acerosin', 'Arcapillin', 'Chrysosplenol C', 'Irigenin', 'Oxyayanin A', 'Oxyayanin B', 'Rosmarinate', 'Thymonin']
197.04436	66226.5	-0.0375919		0					0

186.0455	1001322.2	-0.0375563		0					0
154.02205	12699.9	-0.0375245		0					0
180.03832	1177706.9	-0.0373633		0					0
259.06098	113359.9	-0.0370877	C10H14N4O2	C10H14N4O2	[M+K-2H]-	222.111676	259.0602826	2.69	['IBMX']
259.06098	113359.9	-0.0370877	C10H14N4S	C10H14N4S	[M+(37Cl)]-	222.093918	259.0603696	2.36	['6-(Pentylthio)purine']
259.06098	113359.9	-0.0370877	C12H8O3	C12H8O3	[M+Hac-H]-	200.047345	259.0611986	-0.84	['5-Methylangelicin']
259.06098	113359.9	-0.0370877	C14H12O5	C14H12O5	[M-H]-	260.068475	259.0611986	-0.84	['Khellin']
417.08261	1642861.2	-0.0370123	C14H19N4O9P	C14H19N4O9P	[M-H]-	418.088969	417.0816926	2.2	['5-Butyrylphosphoinosine']
537.10396	1109936.2	-0.0369292	C23H24N4O9	C23H24N4O9	[M+K-2H]-	500.154331	537.1029376	1.9	['Isonocardicin A', 'Nocardicin A', 'Nocardicin B']
537.10396	1109936.2	-0.0369292	C27H22O12	C27H22O12	[M-H]-	538.11113	537.1038536	0.2	['Lithospermic acid']
215.01158	6512568.1	-0.0369175	C10H10O3	C10H10O3	[M+K-2H]-	178.062995	215.0116016	-0.1	['1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene', '3-Acetyl-6-methoxybenzaldehyde', 'Coniferyl aldehyde', 'Vermelone']
215.01158	6512568.1	-0.0369175	C9H8O4	C9H8O4	[M+Cl]-	180.04226	215.0116616	-0.38	['2-Hydroxy-3-(4-hydroxyphenyl)propenoate', '3-(4-Hydroxyphenyl)pyruvate', 'Aspirin', 'Caffeate', 'trans-2,3-Dihydroxycinnamate']
181.01425	321909.5	-0.0367707	C8H6O5	C8H6O5	[M-H]-	182.021525	181.0142486	0.01	['2-Hydroxyisophthalic acid', '3,5-Dihydroxyphenylglyoxylate', '4-Hydroxyphthalate', 'Stipitatate']
395.05379	446992.2	-0.0366284	C18H16O8	C18H16O8	[M+Cl]-	360.08452	395.0539216	-0.33	["3',4',5-Trihydroxy-3,6,7-trimethoxyflavone", '6-Methoxyaromadendrin 3-O-acetate', 'Acerosin', 'Arcapillin', 'Chrysosplenol C', 'Irigenin', 'Oxyayanin A', 'Oxyayanin B', 'Rosmarinate', 'Thymonin']
395.05379	446992.2	-0.0366284	C19H18O7	C19H18O7	[M+K-2H]-	358.105255	395.0538616	-0.18	['Chryso-obtusin', 'Gardenin B']
395.05379	446992.2	-0.0366284	C22H14O6	C22H14O6	[M+Na-2H]-	374.07904	395.0537086	0.21	['Diospyrin', 'Isodiospyrin']
224.06128	81715.1	-0.0366262		0					0
395.05463	32736.4	-0.0365081	C18H16O8	C18H16O8	[M+Cl]-	360.08452	395.0539216	1.79	["3',4',5-Trihydroxy-3,6,7-trimethoxyflavone", '6-Methoxyaromadendrin 3-O-acetate', 'Acerosin', 'Arcapillin', 'Chrysosplenol C', 'Irigenin', 'Oxyayanin A', 'Oxyayanin B', 'Rosmarinate', 'Thymonin']
395.05463	32736.4	-0.0365081	C19H18O7	C19H18O7	[M+K-2H]-	358.105255	395.0538616	1.95	['Chryso-obtusin', 'Gardenin B']

395.05463	32736.4	-0.0365081	C22H14O6	C22H14O6	[M+Na-2H]-	374.07904	395.0537086	2.33	['Diospyrin', 'Isodiospyrin']
205.05065	87172.7	-0.0364532	C11H10O4	C11H10O4	[M-H]-	206.05791	205.0506336	0.08	['2-Hydroxy-3-methylbenzalpyruvate', '2-Hydroxy-8-methylchromene-2-carboxylate', 'Lathodoratin', 'Scoparone', 'cis-1,2-Dihydroxy-1,2-dihydro-8-carboxynaphthalene']
205.05065	87172.7	-0.0364532	C9H6O2	C9H6O2	[M+Hac-H]-	146.03678	205.0506336	0.08	['Coumarin']
269.08172	1631837.3	-0.036289	C14H10O2	C14H10O2	[M+Hac-H]-	210.06808	269.0819336	-0.79	['1,2-Anthracenediol', '9,10-Dihydroxyanthracene', 'Phenanthrene-3,4-diol']
269.08172	1631837.3	-0.036289	C16H14O4	C16H14O4	[M-H]-	270.08921	269.0819336	-0.79	['(+)-Medicarpin', '(-)-Medicarpin', '2-O-Methylisoliquiritigenin', 'Alloimperatorin', 'Imperatorin', 'Isoimperatorin', 'Pinostrobin', 'Stropinin', 'Vignafuran']
205.05247	558213	-0.0361867		0					0
357.06134	2441756	-0.035946	C16H10O6	C16H10O6	[M+Hac-H]-	298.04774	357.0615936	-0.71	["2',7-Dihydroxy-4',5-methylenedioxyisoflavone", 'Bowdichione', 'Irilone']
161.04546	466227.9	-0.0357169	C4H6O3	C4H6O3	[M+Hac-H]-	102.031695	161.0455486	-0.55	['(S)-Methylmalonate semialdehyde', '2-Methyl-3-oxopropanoate', '2-Oxobutanoate', 'Acetoacetate', 'Succinate semialdehyde']
161.04546	466227.9	-0.0357169	C6H10O5	C6H10O5	[M-H]-	162.052825	161.0455486	-0.55	['(2R,3S)-2,3-Dimethylmalate', '(R)-2-Ethylmalate', '(R)-3,3-Dimethylmalate', '(S)-2-(Hydroxymethyl)glutarate', '1,5-Anhydro-D-fructose', '2-Dehydro-3-deoxy-D-fuconate', '2-Dehydro-3-deoxy-L-fuconate', '2-Dehydro-3-deoxy-L-rhamnonate', '2-Deoxy-scyllo-inososе', '2-Hydroxyadipate', '3,6-Anhydrogalactose', '3,6-Anhydroglucose', '3-Ethylmalate', '3-Hydroxy-3-methylglutarate', 'D-Fucono-1,4-lactone', 'Diethyl pyrocarbonate', 'L-Fucono-1,5-lactone', 'L-Rhamnono-1,4-lactone', 'Lichenin']
335.077	558972.3	-0.0356401	C16H16O8	C16H16O8	[M-H]-	336.08452	335.0772436	-0.73	['5-O-Caffeoylshikimic acid', 'Altersolanol A']
385.09276	827361.7	-0.0356303	C18H14O6	C18H14O6	[M+Hac-H]-	326.07904	385.0928936	-0.35	['Jacareubin', 'Ophiopogonone A']
385.09276	827361.7	-0.0356303	C20H18O8	C20H18O8	[M-H]-	386.10017	385.0928936	-0.35	['Cleomiscosin A', 'Daphneticin', 'Diferulic acid', 'Glucosyloxyanthraquinone', 'Irisflorentin']
493.11397	2028202.5	-0.0355765	C24H18O8	C24H18O8	[M+Hac-H]-	434.10017	493.1140236	-0.11	['Knipholone']
493.11397	2028202.5	-0.0355765	C26H22O10	C26H22O10	[M-H]-	494.1213	493.1140236	-0.11	['5-Methoxyhydnocarpin-D', 'Salvianolic acid A']

209.00921	171169.2	-0.0355146		0					0
375.07198	2208432.8	-0.0353964	C16H12O7	C16H12O7	[M+Hac-H]-	316.058305	375.0721586	-0.48	["3',4',5,7-Tetrahydroxy-3-methoxyflavone", 'Azaleatin', 'Capillarisin', 'Isorhamnetin', 'Pedalitin', 'Pinoquercetin', 'Rhamnetin', 'Sexangularetin', 'Tamarixetin']
160.06623	321288	-0.0351747		0					0
188.99604	111766.4	-0.0350723	C7H6O4	C7H6O4	[M+Cl]-	154.02661	188.9960116	0.15	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']
188.99604	111766.4	-0.0350723	C8H8O3	C8H8O3	[M+K-2H]-	152.047345	188.9959516	0.47	['(R)-Mandelate', '(S)-Mandelate', "2',4-Dihydroxyacetophenone", '2-(Hydroxymethyl)benzoic acid', '2-Hydroxyphenylacetate', "3',4-Dihydroxyacetophenone", '3,4-Dihydroxyphenylacetaldehyde', '3-Hydroxyphenylacetate', '3-Methoxytropolone', '3-Methylsalicylate', '4-Hydroxy-3-methoxy-benzaldehyde', '4-Hydroxymethylsalicylaldehyde', '4-Hydroxyphenacyl alcohol', '4-Hydroxyphenyl acetate', '4-Hydroxyphenylacetate', '4-Methoxybenzoate', '4-Methylsalicylate', '6-Methylsalicylate', 'Menisdaurilide', 'Methyl salicylate', 'Phenoxyacetate', 'Resorcinol monoacetate']
207.05101	329111.7	-0.0350042	C5H8O5	C5H8O5	[M+Hac-H]-	148.037175	207.0510286	-0.09	['(R)-2-Hydroxyglutarate', '(R)-2-Methylmalate', '(S)-2-Hydroxyglutarate', '(S)-2-Methylmalate', '2-Dehydro-3-deoxy-D-xylonate', '2-Dehydro-3-deoxy-L-arabinonate', '2-Hydroxyglutarate', 'Citramalate', 'D-Arabinono-1,4-lactone', 'D-Xylono-1,4-lactone', 'D-Xylonolactone', 'D-erythro-3-Methylmalate', 'D-threo-3-Methylmalate', 'L-Arabinono-1,4-lactone', 'L-Arabinono-1,5-lactone', 'L-Xylono-1,4-lactone', 'L-threo-3-Methylmalate']
211.04198	246769.6	-0.0349502		0					0
391.06693	684237.4	-0.0347623	C10H13N4O7P	C10H13N4O7P	[M+Hac-H]-	332.052189	391.0660426	2.27	['2-Deoxyinosine 5-phosphate', 'Purine mononucleotide']
391.06693	684237.4	-0.0347623	C16H12O8	C16H12O8	[M+Hac-H]-	332.05322	391.0670736	-0.37	["3,3',4',5,7-Pentahydroxy-8-methoxyflavone", 'Laricitrin', 'Patuletin']
333.06137	2087294	-0.0347116	C14H10O6	C14H10O6	[M+Hac-H]-	274.04774	333.0615936	-0.67	["2,2',4,4-Tetrahydroxybenzil", 'Athyriol', 'Bellidifolin', 'Isoathyriol', 'Swertianin']

333.06137	2087294	-0.0347116	C16H14O8	C16H14O8	[M-H]-	334.06887	333.0615936	-0.67	['5,5-Dehydrodivanillate', '6-Methoxytaxifolin']
257.06642	99395.5	-0.0346071	C7H16N4O4	C7H16N4O4	[M+K-2H]-	220.117156	257.0657626	2.56	['1D-1-Guanidino-3-amino-1,3-dideoxy-scyllo-inositol']
257.06642	99395.5	-0.0346071	C9H10O5	C9H10O5	[M+Hac-H]-	198.052825	257.0666786	-1.01	['3-(3,4-Dihydroxyphenyl)lactate', '3-Methoxy-4-hydroxymandelate', 'Syringic acid']
183.02992	103910.5	-0.0342626	C6H4O3	C6H4O3	[M+Hac-H]-	124.016045	183.0298986	0.12	['2-Hydroxy-1,4-benzoquinone']
183.02992	103910.5	-0.0342626	C8H8O5	C8H8O5	[M-H]-	184.037175	183.0298986	0.12	['3,4-Dihydroxymandelate', '3-O-Methylgallate']
255.05077	103293.7	-0.0342531	C9H8O5	C9H8O5	[M+Hac-H]-	196.037175	255.0510286	-1.01	['3-(3,4-Dihydroxyphenyl)pyruvate']
119.05016	101690.3	-0.0342277	C8H8O	C8H8O	[M-H]-	120.057515	119.0502386	-0.66	['2-Methylbenzaldehyde', '3-Methylbenzaldehyde', '4-Hydroxystyrene', 'Acetophenone', 'Phenylacetaldehyde', 'Styrene oxide', 'p-Tolualdehyde']
243.05082	126889	-0.0342201	C8H8O5	C8H8O5	[M+Hac-H]-	184.037175	243.0510286	-0.86	['3,4-Dihydroxymandelate', '3-O-Methylgallate']
125.00098	990024.9	-0.0340907	C2H7O4P	C2H7O4P	[M-H]-	126.008198	125.0009216	0.47	['2-Hydroxyethylphosphonate']
125.00098	990024.9	-0.0340907	C3H6O3	C3H6O3	[M+Cl]-	90.031695	125.0010966	-0.93	['(R)-Lactate', '(S)-Lactate', '3-Hydroxypropanoate', 'D-Glyceraldehyde', 'Glyceraldehyde', 'Glycerone', 'L-Glyceraldehyde', 'Lactate']
125.00098	990024.9	-0.0340907	C4H8O2	C4H8O2	[M+K-2H]-	88.05243	125.0010366	-0.45	['(R)-Acetoin', '1,4-Dioxane', '2-Methylpropanoate', 'Acetoin', 'Butanoic acid', 'Ethyl acetate']
125.00098	990024.9	-0.0340907	H3O2P	H3O2P	[M+Hac-H]-	65.987068	125.0009216	0.47	['Phosphinate']
144.04543	334245.1	-0.0340845	C9H7NO	C9H7NO	[M-H]-	145.052764	144.0454876	-0.4	['1(2H)-Isoquinolinone', '3-Methyleneoxindole', '8-Hydroxyquinoline', 'Indole-3-carboxaldehyde', 'Quinolin-2-ol', 'Quinolin-4-ol']
179.05605	468250	-0.0340037	C4H8O4	C4H8O4	[M+Hac-H]-	120.04226	179.0561136	-0.36	['D-Erythrose', 'D-Erythrulose', 'D-Threose']

179.05605	468250	-0.0340037	C6H12O6	C6H12O6	[M-H]-	180.06339	179.0561136	-0.36	['2-Deoxy-D-gluconate', 'Aldohexose', 'D-Aldose', 'D-Allose', 'D-Altrose', 'D-Fructose', 'D-Fuconate', 'D-Galactose', 'D-Glucose', 'D-Gulose', 'D-Hamamelose', 'D-Hexose', 'D-Idose', 'D-Mannose', 'D-Psicose', 'D-Sorbose', 'D-Tagatose', 'D-Talose', 'Fructose(pyranose)', 'Ketose', 'L-Fructose', 'L-Fuconate', 'L-Galactose', 'L-Gulose', 'L-Rhamnonate', 'L-Sorbose', 'Sorbose', 'alpha-D-Galactose', 'alpha-D-Glucose', 'alpha-D-Mannose', 'alpha-L-Sorbopyranose', 'beta-D-Fructose', 'beta-D-Galactose', 'beta-D-Glucose', 'beta-D-Hamamelopyranose', 'beta-D-Mannose', 'muco-Inositol', 'myo-Inositol', 'scyllo-Inositol']
194.05398	673956.5	-0.0339748		0					0
147.04692	98274.6	-0.0339481		0					0
191.01973	295009.9	-0.0339398	C4H4O5	C4H4O5	[M+Hac-H]-	132.005875	191.0197286	0.01	['2-Hydroxyethylenedicarboxylate', 'Oxaloacetate', 'trans-2,3-Epoxysuccinate']
191.01973	295009.9	-0.0339398	C6H8O7	C6H8O7	[M-H]-	192.027005	191.0197286	0.01	['(1R,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(1S,2S)-1-Hydroxypropane-1,2,3-tricarboxylate', '(4R,5S)-4,5,6-Trihydroxy-2,3-dioxohexanoate', '2,5-Didehydro-D-gluconate', '2-Dehydro-3-deoxy-D-glucarate', '5-Dehydro-4-deoxy-D-glucarate', 'Carboxymethyloxysuccinate', 'Citrate', 'Isocitrate']
223.02489	84225.1	-0.0336847		0					0
401.12993	78988	-0.0335669	C12H22O11	C12H22O11	[M+Hac-H]-	342.116215	401.1300686	-0.35	['2-O-beta-D-Glucopyranosyl-beta-D-glucopyranose', '2-alpha-D-Glucosyl-D-glucose', 'Cellobiose', 'D-Fructosyl-D-fructofuranose', 'D-Glucosyl-D-mannose', 'Epimelibiose', 'Gentiobiose', 'Inulobiose', 'Isomaltose', 'Lactose', 'Lactulose', 'Laminaribiose', 'Levanbiose', 'Maltose', 'Mannobiose', 'Melibiose', 'Nigerose', 'Palatinose', 'Sucrose', 'alpha,alpha-Trehalose', 'alpha-Cellobiose', 'alpha-D-Aldosyl beta-D-fructoside', 'alpha-D-Galactosyl-(1->3)-1D-myo-inositol', 'alpha-D-Glucosyl-(1,3)-D-mannose', 'alpha-Maltose', 'beta-Cellobiose', 'beta-D-Fructofuranosyl-alpha-D-mannopyranoside', 'beta-Lactose', 'beta-Maltose']

133.0137	13169.7	-0.0335331		0					0
275.0559	628045.8	-0.0334329	C12H8O4	C12H8O4	[M+Hac-H]-	216.04226	275.0561136	-0.78	['Bergapten', 'Isobergapten', 'Norvisnagin', 'Sphondin', 'Xanthotoxin']
401.12412	45559.9	-0.0334004	C19H18O6	C19H18O6	[M+Hac-H]-	342.11034	401.1241936	-0.18	['3-(4-Methoxyphenyl)-5,6,7-trimethoxy-4H-1-benzopyran-4-one', "4',5,6,7-Tetramethoxyflavone", 'UWM6']
401.12412	45559.9	-0.0334004	C21H22O8	C21H22O8	[M-H]-	402.13147	401.1241936	-0.18	["(3'R,4'R)-3-Epoxyangeloyloxy-4-acetoxy-3',4-dihydroseselin", '2-(2,5-Dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one', '2-(3,5-Dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one', 'Flavanone 7-O-beta-D-glucoside', 'Nobiletin']
253.0715	789261.7	-0.033369	C10H10O4	C10H10O4	[M+Hac-H]-	194.05791	253.0717636	-1.04	['2,4,8-Trihydroxy-1-tetralone', '5-Hydroxyconiferaldehyde', '6-Hydroxymellein', 'Dimethyl phthalate', 'Ferulate', 'Isoferulic acid', 'Kakuol', 'Methyl caffeate', 'Scytalone']
210.07715	126400.7	-0.0333683	C10H13NO4	C10H13NO4	[M-H]-	211.084459	210.0771826	-0.16	['Enicoflavine', 'Methyldopa anhydrous']
210.07715	126400.7	-0.0333683	C8H9NO2	C8H9NO2	[M+Hac-H]-	151.063329	210.0771826	-0.16	['(E)-4-Hydroxyphenylacetaldehyde-oxime', '(R)-Mandelamide', '(Z)-4-Hydroxyphenylacetaldehyde-oxime', '2-Amino-3-methylbenzoate', '2-Descarboxy-cyclo-dopa', 'Acetaminophen', 'Dopamine quinone', 'L-Phenylglycine', 'N-(Acetyloxy)benzenamine', 'N-Methyl-4-aminobenzoate', 'N-Methylanthranilate']
343.08212	1300331.1	-0.0332226	C16H12O5	C16H12O5	[M+Hac-H]-	284.068475	343.0823286	-0.61	['(+)-Maackiain', '(-)-Maackiain', '2-Hydroxyformononetin', '3-Methylgalangin', 'Acacetin', 'Biochanin A', 'Calycosin', 'Cypripedin', 'Genkwanin', 'Geraldone', 'Glycitein', 'Lucidin omega-methyl ether', 'Melannin', 'Obtusifolin', 'Physcion', 'Prunetin', 'Questin', 'Texasin', 'Wogonin']
343.08212	1300331.1	-0.0332226	C18H16O7	C18H16O7	[M-H]-	344.089605	343.0823286	-0.61	['(-)-Usnic acid', "3',5-Dihydroxy-3,4',7-trimethoxyflavone", '7-Hydroxy-(S)-usnate', 'Cirsilineol', 'Eupatilin', 'Nevadensin', 'Obtusin', 'Pachypodol', 'Santin', 'Tambulin', 'Xanthomicrol']
251.07303	281953	-0.0330092		0					0
555.11465	1580125.2	-0.0330042		0					0
190.99312	37269.5	-0.0329637	C7H6O4	C7H6O4	[M+(37Cl)]-	154.02661	190.9930616	0.31	['2,3-Dihydroxybenzoate', '2,5-Dihydroxybenzoate', '3,4-Dihydroxybenzoate', 'Patulin']

209.04552	166727.3	-0.0328744	C10H10O5	C10H10O5	[M-H]-	210.052825	209.0455486	-0.14	['5-Hydroxyferulate']
209.04552	166727.3	-0.0328744	C8H6O3	C8H6O3	[M+Hac-H]-	150.031695	209.0455486	-0.14	['2-Carboxybenzaldehyde', 'Piperonal', 'alpha-Oxo-benzeneacetic acid']
217.0143	134212.3	-0.032866		0					0
403.10334	165903.3	-0.0328139	C18H16O7	C18H16O7	[M+Hac-H]-	344.089605	403.1034586	-0.29	['(-)-Usnic acid', "3',5-Dihydroxy-3,4',7-trimethoxyflavone", '7-Hydroxy-(S)-usnate', 'Cirsilineol', 'Eupatilin', 'Nevadensin', 'Obtusin', 'Pachypodol', 'Santin', 'Tambulin', 'Xanthomicrol']
213.02119	221683.1	-0.0326735	C4H8N4O4	C4H8N4O4	[M+(37Cl)]-	176.054556	213.0210076	0.86	['Allantoate']
213.02119	221683.1	-0.0326735	C8H8N4S	C8H8N4S	[M+Na-2H]-	192.046968	213.0216366	-2.1	['6-(Allylthio)purine']
208.06637	148073.6	-0.0326287		0					0
269.06646	108764.3	-0.032617	C10H10O5	C10H10O5	[M+Hac-H]-	210.052825	269.0666786	-0.81	['5-Hydroxyferulate']
269.06646	108764.3	-0.032617	C16H12N2	C16H12N2	[M+(37Cl)]-	232.100048	269.0664996	-0.15	['Cryptolepine']
327.08719	301802.1	-0.0325825	C14H18N4O3	C14H18N4O3	[M+K-2H]-	290.137891	327.0864976	2.12	['Benomyl', 'Trimethoprim']
327.08719	301802.1	-0.0325825	C16H12O4	C16H12O4	[M+Hac-H]-	268.07356	327.0874136	-0.68	['1-[6-Hydroxy-2-(4-hydroxyphenyl)-1-benzofuran-3-yl]ethanone', '6-Hydroxy-2-methoxyflavone', 'Dalbergin', 'Formononetin', 'Isoformononetin', 'Tectochrysin']
327.08719	301802.1	-0.0325825	C16H20NO4	C16H20NO4	[M+K-2H]-	290.139234	327.0878406	-1.99	['Pteleatine', 'Ribalinium']
327.08719	301802.1	-0.0325825	C18H16O6	C18H16O6	[M-H]-	328.09469	327.0874136	-0.68	['2-(4-Hydroxyphenyl)-5,6,7-trimethoxy-4H-1-benzopyran-4-one', '6-Hydroxy-2-(4-methoxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one', "7-Hydroxy-2',4',5-trimethoxyisoflavone", '9-Demethylmunduserone', 'Betagarin', 'Ophiopogonanone A']
126.99804	307852.8	-0.0324265	C3H6O3	C3H6O3	[M+(37Cl)]-	90.031695	126.9981466	-0.84	['(R)-Lactate', '(S)-Lactate', '3-Hydroxypropanoate', 'D-Glyceraldehyde', 'Glyceraldehyde', 'Glycerone', 'L-Glyceraldehyde', 'Lactate']
391.10332	87724.6	-0.0322512	C17H16O7	C17H16O7	[M+Hac-H]-	332.089605	391.1034586	-0.35	['Sulochrin']
347.07702	461859.9	-0.0321014	C15H12O6	C15H12O6	[M+Hac-H]-	288.06339	347.0772436	-0.64	['(+-)-Dalbergioidin', '2,6,7,4-Tetrahydroxyisoflavanone', '2-Hydroxy-2,3-dihydrogenistein', '2-O-Methylswertianin', '3,5-Dimethoxy-1,6-dihydroxyxanthone', 'Carthamidin', 'Dihydrokaempferol', 'Eriodictyol', 'Eriodictyol chalcone', 'Fustin',

									'Gentiacaulein', 'Micromelin', 'Okanin', 'Swerchirin']
216.01505	430701	-0.0320882		0					0
401.08768	376286.1	-0.0320434	C18H14O7	C18H14O7	[M+Hac-H]-	342.073955	401.0878086	-0.32	['Dihydromethylsterigmatocystin']
401.08768	376286.1	-0.0320434	C20H18O9	C20H18O9	[M-H]-	402.095085	401.0878086	-0.32	['1-Hydroxy-2-(beta-D-glucosyloxy)-9,10-anthraquinone', 'Frangulin B']
89.0243	226574	-0.0319944	C3H6O3	C3H6O3	[M-H]-	90.031695	89.0244186	-1.33	['(R)-Lactate', '(S)-Lactate', '3-Hydroxypropanoate', 'D-Glyceraldehyde', 'Glyceraldehyde', 'Glycerone', 'L-Glyceraldehyde', 'Lactate']
89.0243	226574	-0.0319944	CH2O	CH2O	[M+Hac-H]-	30.010565	89.0244186	-1.33	['Formaldehyde']
419.09821	132487.6	-0.0319864	C15H28O7P2	C15H28O7P2	[M+(37Cl)]-	382.131031	419.0974826	1.74	['2-cis,6-trans-Farnesyl diphosphate', 'Nerolidyl diphosphate', 'trans,trans-Farnesyl diphosphate']
419.09821	132487.6	-0.0319864	C18H16O8	C18H16O8	[M+Hac-H]-	360.08452	419.0983736	-0.39	["3',4',5-Trihydroxy-3,6,7-trimethoxyflavone", '6-Methoxyaromadendrin 3-O-acetate', 'Acerosin', 'Arcapillin', 'Chrysosplenol C', 'Irigenin', 'Oxyayanin A', 'Oxyayanin B', 'Rosmarinate', 'Thymonin']
202.94144	147951.1	-0.0318394		0					0
403.0669	759660.2	-0.0317418	C17H12O8	C17H12O8	[M+Hac-H]-	344.05322	403.0670736	-0.43	['3,4,3-Tri-O-methylellagic acid']
405.08255	190870.4	-0.0316899	C17H14O8	C17H14O8	[M+Hac-H]-	346.06887	405.0827236	-0.43	["3',4',5,6-Tetrahydroxy-3,7-dimethoxyflavone", 'Axillarin', 'Eupatolitin', 'Syringetin', 'Taxifolin 3-O-acetate']
405.08255	190870.4	-0.0316899	C19H18O10	C19H18O10	[M-H]-	406.09	405.0827236	-0.43	['Lancerin']
217.00871	1221105.3	-0.0316788	C9H8O4	C9H8O4	[M+(37Cl)]-	180.04226	217.0087116	-0.01	['2-Hydroxy-3-(4-hydroxyphenyl)propenoate', '3-(4-Hydroxyphenyl)pyruvate', 'Aspirin', 'Caffeate', 'trans-2,3-Dihydroxycinnamate']
171.02744	241846.1	-0.0315208	C5H10O5	C5H10O5	[M+Na-2H]-	150.052825	171.0274936	-0.31	['D-Apiose', 'D-Arabinose', 'D-Lyxose', 'D-Ribose', 'D-Ribulose', 'D-Xylose', 'D-Xylulose', 'L-Arabinofuranose', 'L-Arabinose', 'L-Lyxose', 'L-Ribulose', 'L-Xylose', 'L-Xylulose', 'Ribulose', 'Xylose', 'alpha-D-Lyxose', 'alpha-D-Ribulose', 'alpha-L-Arabinose', 'beta-D-Apiose', 'beta-D-Ribofuranose', 'beta-D-Ribopyranose', 'beta-D-Xylose', 'beta-L-Arabinose']

217.0525	163287.1	-0.0314781		0					0
193.01428	50836	-0.0314647		0					0
123.04507	62203	-0.0314383	C7H8O2	C7H8O2	[M-H]-	124.05243	123.0451536	-0.68	['2,3-Dihydroxytoluene', '3-Hydroxybenzyl alcohol', '4-Methylcatechol', 'Orcinol', 'Salicyl alcohol', 'o-Methoxyphenol', 'p-Hydroxybenzyl alcohol']
403.08403	102555.7	-0.0313495		0					0
313.07161	1442123.9	-0.0312855	C15H10O4	C15H10O4	[M+Hac-H]-	254.05791	313.0717636	-0.49	['1,4-Dihydroxy-2-methylanthraquinone', "4',6-Dihydroxyflavone", '7,4-Dihydroxyflavone', 'Alizarin 2-methyl ether', 'Anhydroglycinol', 'Chrysin', 'Chrysophanol', 'Daidzein', 'Digiferrugineol', 'Hispidol', 'Primetin', 'Rubiadin']
313.07161	1442123.9	-0.0312855	C17H14O6	C17H14O6	[M-H]-	314.07904	313.0717636	-0.49	['4-Methylcapillarisin', '6-Hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one', '7-Methylcapillarisin', 'Aflatoxin B2', 'Irisolidone', 'Pinobanksin 3-O-acetate', 'Pisatin', 'Ventinone A']
313.07161	1442123.9	-0.0312855	C7H14N2O6S	C7H14N2O6S	[M+Hac-H]-	254.05726	313.0711136	1.59	['5-L-Glutamyl-taurine']
285.04026	781724.3	-0.0311497	C15H10O6	C15H10O6	[M-H]-	286.04774	285.0404636	-0.71	['2-Hydroxygenistein', '6-Demethoxycapillarisin', 'Aureusidin', 'Citreorosein', 'Datiscetin', 'Fisetin', 'Isoscutellarein', 'Kaempferol', 'Luteolin', 'Maritimetin', 'Orobol', 'Scutellarein']
214.10842	336206.9	-0.0311417	C10H17NO4	C10H17NO4	[M-H]-	215.115759	214.1084826	-0.29	['2-Amino-9,10-epoxy-8-oxodecanoic acid']
214.10842	336206.9	-0.0311417	C8H13NO2	C8H13NO2	[M+Hac-H]-	155.094629	214.1084826	-0.29	['Arecoline', 'Heliotridine', 'Retronecine', 'Scopoline']
539.11975	506767.5	-0.0310363	C18H30O16	C18H30O16	[M+(37Cl)]-	502.15339	539.1198416	-0.17	['alpha-L-Rhamnopyranosyl-(1->2)-beta-D-galactopyranosyl-(1->2)-beta-D-glucuronopyranoside']
385.07351	159242.4	-0.0310198	C15H16N4O6	C15H16N4O6	[M+(37Cl)]-	348.106986	385.0734376	0.19	['Musca-aurin VII']
385.07351	159242.4	-0.0310198	C15H18N4O4S	C15H18N4O4S	[M+Cl]-	350.104878	385.0742796	-2	['Biapenem']
146.98293	114213.8	-0.030881	C2H7O4P	C2H7O4P	[M+Na-2H]-	126.008198	146.9828666	0.43	['2-Hydroxyethylphosphonate']

273.07607	59213.3	-0.0308776	C13H10O3	C13H10O3	[M+Hac-H]-	214.062995	273.0768486	-2.85	['1-Hydro-1,1a-dihydroxy-9-fluorenone', '2,2-Dihydroxybenzophenone', '2,4-Dihydroxybenzophenone', '3,4-Dihydroxy-3,4-dihydro-9-fluorenone', '4,4-Dihydroxybenzophenone', '4,4-Dimethylangelicin', '4,5-Dimethylangelicin', 'Diphenyl carbonate', 'Mycosinol', 'Phenyl salicylate']
273.07607	59213.3	-0.0308776	C15H14O5	C15H14O5	[M-H]-	274.084125	273.0768486	-2.85	['(-)-Epiafzelechin', '5-Deoxyleucopelargonidin', 'Afzelechin', 'Apiforol', 'Fisetinidol', 'H37', 'Luteoliflavan', 'Methysticin', 'Phloretin', 'Ptaerochromenol', 'alpha-Pyrufuran', 'beta-Pyrufuran']
221.06667	220150.1	-0.0307292	C12H12N2	C12H12N2	[M+(37Cl)]-	184.100048	221.0664996	0.77	['2,4-Diphenyldiamine', 'Benzidine', 'Diquat', 'Withasomnine']
221.06667	220150.1	-0.0307292	C6H10O5	C6H10O5	[M+Hac-H]-	162.052825	221.0666786	-0.04	['(2R,3S)-2,3-Dimethylmalate', '(R)-2-Ethylmalate', '(R)-3,3-Dimethylmalate', '(S)-2-(Hydroxymethyl)glutarate', '1,5-Anhydro-D-fructose', '2-Dehydro-3-deoxy-D-fuconate', '2-Dehydro-3-deoxy-L-fuconate', '2-Dehydro-3-deoxy-L-rhamnonate', '2-Deoxy-scyllo-inososе', '2-Hydroxyadipate', '3,6-Anhydrogalactose', '3,6-Anhydroglucose', '3-Ethylmalate', '3-Hydroxy-3-methylglutarate', 'D-Fucono-1,4-lactone', 'Diethyl pyrocarbonate', 'L-Fucono-1,5-lactone', 'L-Rhamnono-1,4-lactone', 'Lichenin']
221.06667	220150.1	-0.0307292	C8H14O7	C8H14O7	[M-H]-	222.073955	221.0666786	-0.04	['6-Acetyl-D-glucose']
717.14663	1099370.2	-0.0307193		0					0
147.04512	37473.7	-0.0304938	C9H8O2	C9H8O2	[M-H]-	148.05243	147.0451536	-0.23	['3-Hydroxy-1-indanone', '3-Isochromanone', '4-Hydroxycinnamyl aldehyde', 'Dihydrocoumarin', 'Pyruvophenone', 'trans-Cinnamate']
128.03522	73192	-0.030486	C5H7NO3	C5H7NO3	[M-H]-	129.042594	128.0353176	-0.76	['1-Pyrroline-4-hydroxy-2-carboxylate', '4-Oxoproline', '5-Oxo-D-proline', '5-Oxoproline', 'L-1-Pyrroline-3-hydroxy-5-carboxylate']
387.10842	197572.3	-0.0303295	C16H22N4O3S	C16H22N4O3S	[M+(37Cl)]-	350.141263	387.1077146	1.82	['Cafenstrole']

387.10842	197572.3	-0.0303295	C18H16O6	C18H16O6	[M+Hac-H]-	328.09469	387.1085436	-0.32	['2-(4-Hydroxyphenyl)-5,6,7-trimethoxy-4H-1-benzopyran-4-one', '6-Hydroxy-2-(4-methoxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one', "7-Hydroxy-2',4',5-trimethoxyisoflavone", '9-Demethylmunduserone', 'Betagarin', 'Ophiopogonanone A']
389.08766	149844.8	-0.0302431	C17H14O7	C17H14O7	[M+Hac-H]-	330.073955	389.0878086	-0.38	['(+)-Bisdechlorogeodin', '(-)-Bisdechlorogeodin', "3',4',5-Trihydroxy-3,7-dimethoxyflavone", 'Aflatoxin G2', 'Aurantio-obtusin', 'Cirsiliol', 'Hildecarpin', 'Tricin']

Table 5 Context V12-VL-53B; shallow ditch with organic material. Date of context is circa 130 A.D.

	Sample weight	Malonic acid	Succinic acid	Malci acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound' lipids
*Side of amphora V12-53B/1	1.00	ND	ND	ND	ND	ND	1788	515	ND	ND	467	ND

	Acyl carbon number	Types of TAGs	Fatty acid constituents		Acyl carbon number MANUAL ID	Types of TAGs MANUAL ID	Fatty acid constituents MANUAL ID
side of coarse, thick walled amphora VL-53B/1	C48:2	2	16:1/16/16:1	16/16/16:2	ND	ND	ND

Table 6 Context V12-VL-103B; water channel of insecure context time line, probably 3rd century

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of amphora V11-103A/2	.987	ND	ND	ND	ND	ND	ND	1404	ND	ND	182	ND
*Base of mortarium V11-103A/3	1.01	ND	ND	ND	ND	ND	ND	2555	ND	ND	925	ND

Table 7 Context V12-VL-48B, insecure context next to the B7 road, no date given, probably 3rd century

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of amphorae V12-48B/1	.983	ND	17789	ND	ND	ND	2651	1450	ND	1905	4369	oleic, palmitic, and stearic acids

Table 8 Context V12-VL-30B

	Sample weight (g)	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of amphora/storage vessel V12-30B/1	.991	ND	5437	ND	ND	ND	634	288	ND	ND	519	hexadecanoic, octadecanoic, and octadecenoic acids
*Side of amphora/storage vessel V12-30B/2	1.01	ND	30244	ND	ND	ND	11394	8929	ND	2990	6076	ND

Table 9 Context V11-VL-3B

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Base of mortarium V11-3B/5	1.01	ND	38562	ND	ND	ND	4727	7701	ND	10485	5919	low levels hexadecanoic, octadecanoic acids, as well as unidentified lipids including mass 237
*Base of storage vessel V11-3B/3	.988	1598	ND	ND	ND	ND	ND	7943	ND	ND	2793	decanedioic acid (sebacic acid), nonanedioic acid (azelaic acid), Decanoic acid (capric acid), nonanoic acid, 1,8-octanedioic acid (suberic acid), 3-hydroxy-decanedioic acid, 14-hydroxy-tetradecanoic acid (ω-hydroxy myristic acid)

Table 10 Context V11-VL-108A

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of amphora V11-108A/4	.984	ND	14108	ND	ND	ND	730	940	ND	1141	1691	ND

Table 11 Context V11-VL-124A, from the floor of a barrack;s room. 213-300 A.D.

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of amphora V11-124A/1	.983	ND	ND	ND	ND	ND	ND	1136	ND	ND	ND	ND
*Side of amphora V11-124A/2	1.01	ND	2851	ND	ND	ND	1712	1203	ND	513	1896	ND

Table 12 Context V12-VL-41B

	Sample weight (g)	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of cook pot V12-41B/3	.978	ND	6033	ND	ND	ND	3747	1477	ND	4513	3089	ND

Table 13 Context V12-VL-5B, unstratified soil separating two other contexts, 353-358 A.D.

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*cook pot V12-5B/1	1.01	ND	34523	ND	ND	ND	8265	8811	ND	9233	8112	low levels hexadecanoic, octadecanoic, and octadecenoic acids

Table 14 Context V12-VL-51B, fabric of 3rd century road, circa 213 A.D.

	Sample weight	Malonic acid	Succinic acid	Malic Acid	Tartaric acid	Gentisic acid	p-Coumaric acid	Vanillic acid	Iso-propyl malic acid	Ferulic acid	Syringic acid	'Bound Lipids'
*Side of cook pot V12-51B/5	.984	ND	22562	ND	ND	ND	9702	5292	ND	1828	1968	low levels of hexadecanoic, octadecanoicand unidentified at *m/z* 237