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INSTITUT DE POLICE SCIENTIFIQUE

THE ANALYSIS OF EXCIPIENTS IN ECSTASY TABLETS AND THEIR CONTRIBUTION IN A DRUG PROFILING CONTEXT

THÈSE DE DOCTORAT

Présentée à l'Institut de Police Scientifique (IPS) de l'Université de Lausanne

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"Education is an admirable thing, but it is well to remember from time to time that nothing that is worth knowing can be taught."

"Reisen veredelt den Geist und räumt mit allen unseren Vorurteilen auf."

Oscar Wilde
16.10.1854 - 30.11.1900
Irish dramatist & novelist

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1 INTRODUCTION

1.1 INTRODUCTION

For a long time the use of narcotics has been limited in Europe to medical practice. Drug addiction only appeared at the end of the 19th century and significantly increased since the extraction of morphine and its use during the war of 1870. Then, in the sixties of the 20th century it started to affect youth and very quickly a massive emergence of hard drugs could be observed, touching members of all social classes. (Science & Vie, n°217, 2001)

Ecstasy, which gained popularity in the seventies, designated originally a substance called MDMA, or 3,4-methylendioxymethamphetamine. Nowadays, the term is used more generally for all amphetamine type substances sold in form of tablets on the illicit market. Although, MDMA remains the most common active substance found in ecstasy tablets, a variety of other psychotropic components have made their appearance during the last decades – methamphetamine (MA), 3,4-methylendioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDEA), 4-bromo-2,5-dimethoxyphenylethylamine (2C-B), 2,5-dimethoxy-4-methylamphetamine, etc.

Contrary to the other "common narcotics", such as cocaine or heroine, ecstasy is by definition a tablet and therefore has to be treated differently. Similarly, the main substance of ecstasy is mixed with other components. The difference in the case of tablets is that these components are not defined as cutting agents, but as excipients. The term is of pharmatechnological origin and designates substances necessary to produce solid forms of drugs, such as tablets and capsules. The important point for investigation is that once excipients are added to the active substance and the tablet is pressed, its composition hardly ever changes (eventually decomposition or very slight changes in quantities – depending on the quality of the tablet). Therefore, a seized tablet presents the same composition as that of the initial production.

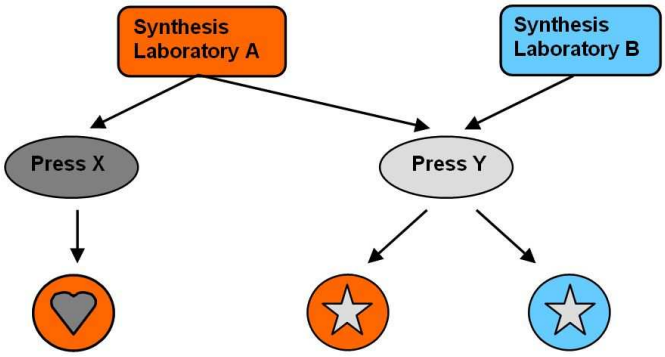


Fig 1 – Schema of illicit tablet production.

The production of ecstasy can be summarized in two steps – 1) synthesis of the active substance, and 2) addition of excipients (and eventually adulterants) followed by the compression. These two steps can be carried out by the same laboratory or by separate laboratories. Consequently, tablets of same visual appearance might not present the same composition and vice versa. This must be taken into account when trying to compare and to group ecstasy seizures.

Considering the schema in *Fig 1*, comparison of ecstasy tablets can be done at different levels :

1) by trace analysis of the active substance characterised by the way of synthesis – this was investigated for amphetamine in the (SMT, 2003), and is currently examined for MDMA in the CHAMP project (CHAMP, in progress).
2) by considering only physical characteristics, such as logo, colour, diameter, etc. Christian Zingg has investigated this problematic during his PhD project (Zingg, 2005).
3) finally, it is possible to consider the additional substances – excipients. Dyes have been examined in the PhD project of (Goldmann, 2000). Herein, carbohydrates (used as diluents and binders) and fatty acids (components of stearates which are used as lubricants) are the focus of the research.

The aim of comparison at any level is to establish eventual links (of physical or chemical nature) between different samples. By characterising the samples, it is possible to differentiate a certain number of them and furthermore to classify them into groups which in turn will allow identifying the potential source of production. This information can be used to estimate for how long a laboratory was in production, as well as to know how the illicit drug was distributed and to what extent. This form of intelligence allows a strategic vision of the illicit drugs market and is usually understood under the name of *Drug Profiling*.

1.2 AIMS – HYPOTHESES

The purpose of the presented research project is to evaluate the potential of the two types of excipients chosen – carbohydrates and fatty acids – for providing useful intelligence in the context of ecstasy profiling. This task requires the consideration of a number of hypotheses which will be detailed hereafter.

1) Similarities with pharmaceutical production

Since ecstasy is a drug in form of tablets, first information about production steps and possible ingredients has been searched in the domain of pharmaceutical technology. However, it had to be shown that this was a reliable source.

Tablets first appeared for medical use and their production is a speciality of pharmaceutical technology. It is logical to presume that producers of illicit tablets would search for basic principles of tablet production in this domain, since information is easily found. This is also corroborated by findings through seizures of illicit laboratories. A collection of these findings has been realised by Europol (*Fig 2*) and the material discovered strongly indicates similarities with pharmaceutical procedure :

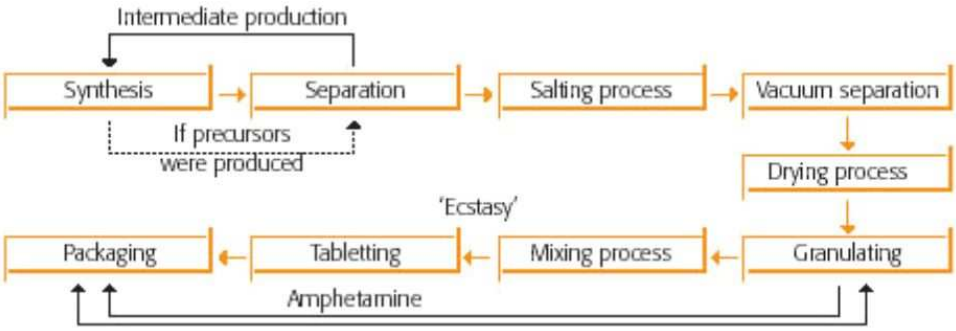


Fig 2 – Detailed procedure of ecstasy production (Europol, 2004).

However, no information is given about the use of excipients – what types of excipients are found on illicit production sites, what recipes are used, and so forth. Thanks to literature and chemical analysis of ecstasy tablets it can be demonstrated that, indeed, the term excipient – with regard to pharmaceutical applications – is more appropriate than cutting agent.

Additionally, in the following chapters it will be shown that these pharmacologically inactive substances are not only added to increase volume (such as cutting agents for powder drugs like heroin and cocaine), but that they are essential for the production of the tablets and that therefore illicit producers actually use basic pharmaceutical recipes for ecstasy production.

Nonetheless, recipes of illicit producers are much simpler than in pharmaceutical technology and a smaller number of excipients are used as well. On the other side, it does not need many substances to obtain variation between production batches. Employing a few excipients mixed in various quantities will result in a high number of possible composition combinations.

2) Variation between producers

In general, illicit producers are supposed to use substances easily available in bulk quantities, and which present characteristics allowing efficient production (e.g. applicable to direct compression). The first condition is fulfilled by most excipients because they are not only used in pharmaceutical technology, but also in other industrial sectors. Unlike for active substances, no particular control is applied to these components and prices are much lower. The criterion of direct compression excipients, e.g., does however slightly limit the number of available substances.

Presuming that a recipe for an excipient mixture resulting in tablets of good quality is not so easily modified (besides that there is no reason for it), producers will probably stick to one once they have found it. Consequently, compression batches for one producer should not considerably vary for the same recipe. When picking up the production schema again (*Fig 3*), the situation would correspond to batch 1 and 2 made on press Y with excipient amounts $x_1 \approx x_2$ and $y_1 \approx y_2$.

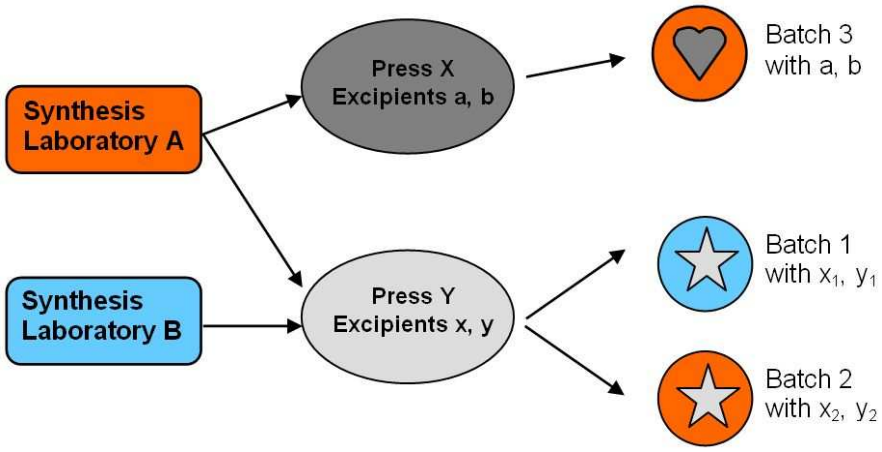


Fig 3 – Schema of illicit tablet production.

Ideally for ecstasy profiling, different illicit producers would also use different recipes and different excipients, and this would increase the variation between them to the maximum. Unfortunately, producers may know each other and/or share similar sources of information about tablet production. Additionally, a rather normal situation would be one producer asking another for his recipe as it produces good quality tablets.

However, three origins of variability between producers can be observed : first, of course, is the choice of excipients, then the used amount, and finally, the source of excipients. As a consequence, three levels of analysis have to be considered, providing specific information passing from one level to the next – qualitative analysis, quantitative analysis and trace analysis.

The optimisation of the qualitative analysis represents the basis of this research project. Relative quantification has been used for the purpose of sample grouping. However, trace analysis which would allow characterisation of the source of an excipient, is beyond the scope of this research. Nevertheless, the possibility of observing differences in cellulose and lactose standards due to different origin will be demonstrated in Chapter 7.

3) Possibility of ecstasy classification by the use of excipients

Preliminary classes have been created by Christian Zingg (2005), who has considered physical characteristics and general composition of ecstasy tablets which means determination of the active substance, its quantification and determination of eventually present adulterants (rare) and/or diluents.

By studying two types of excipients there should be sufficient information to create classes as well. It has to be verified whether the same classes than those found by Zingg can be determined, or if different classes are observed.

This approach requires specification of intra- and intervariability. Intravariability is the variation of a characteristic in one compression batch and should be low. High variation is due either to the failure of the method to give reproducible results, or to the inhomogeneity of the tablet mixture. It is also important to remember that an analysed characteristic should be variable between different batches and that this so-called intervariability should be higher than the intravariability.

The classes found through excipient analysis would express a link at the level of the blend right before compression – this is important to emphasise as these might be different from classes found through impurity analysis of the active substance, situated at the level of synthesis. Therefore, if two samples are found to belong to the same class, this can signify that they originate from the same compression batch, or from the same tablet producer having used one excipient mixture for more than one compression. In either case the origin of the tablets would be the same. Consequently, the information given by the excipients is of strategic importance as it might point towards a production site.

Furthermore, the created classes might allow identification of eventual links between police seizures. These links might be used for the allocation of several seizures to one dealer, and for the establishment of links between, until then independent, dealers through the tablets. Taking this thought even further, these links might allow the visualisation of an entire net of distribution of ecstasy tablets which does not necessarily stop at the Swiss borders.

2 THEORETICAL PART

2.1 ECSTASY GENERALITIES[1]

2.1.1 History

MDMA has been licensed in 1914 by the German company Merck, however without being commercialised. It has been developed as an appetite suppressant, but no use has been noted in the licence. It reappeared in 1953 when the American Army tested it for military use. Finally, it got "public" in 1965 thanks to Alexander Shulgin who focused his research on this type of substances. He has synthesised up to 178 other psychotropic molecules of the family of phenethylamines and has tested them himself at various dosages. In 1991, he has published a book where all his work and experiments have been described – PIHKAL – Phenethylamines I Have Known And Loved. (Shulgin and Shulgin, 1991) However, MDMA had already been before the publication of this book in the circles of psychotherapists who started to apply this substance for therapeutic use back in 1977. This type of application lasted almost a whole decade until, in 1985, the DEA (Drug Enforcement Administration) of the United States banned it as measure of urgency by emphasising a risk for public health. Thus, MDMA has been classified permanently into the *Schedule I* group (USA), only containing substances of dangerous character, creating dependency and without medical recognition. In Switzerland MDMA has been used by therapists until 1994. At this moment the substance was already very popular and often consumed at rave parties. Its production and distribution was provided by clandestine networks principally originating in Europe, although the number of seized laboratories has decreased (*Fig 6*). Until today Europe is the principal producer of amphetamine type substances, except for methamphetamine which is mainly produced in the United States and South-East Asia (*Fig 4* to *Fig 6*).

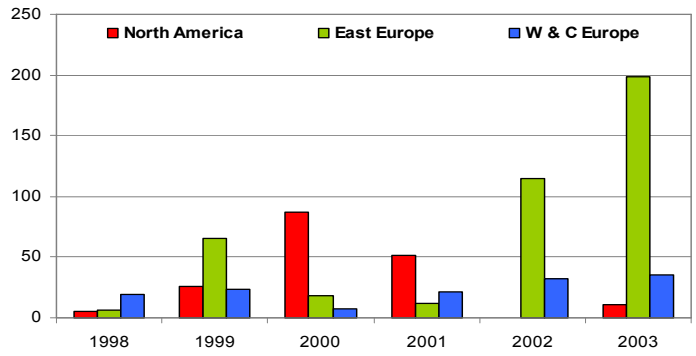


Fig 4 – Number of seized illicit laboratories producing amphetamine (ONU).

[1] (Sauer and Weilemann, 1997; Walder, 1994; Freese et al, 2002)

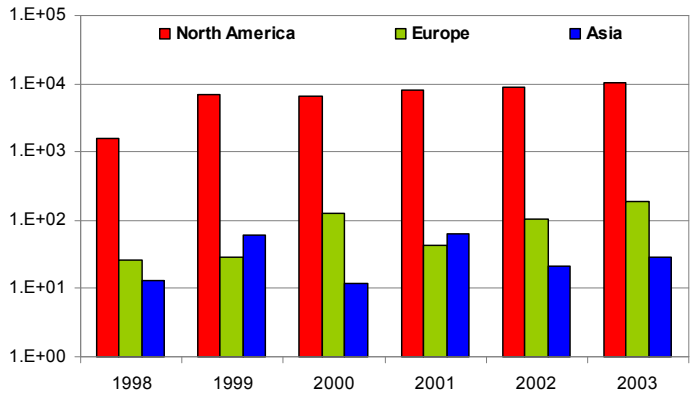


Fig 5 – Number of seized illicit laboratories producing methamphetamine (ONU).

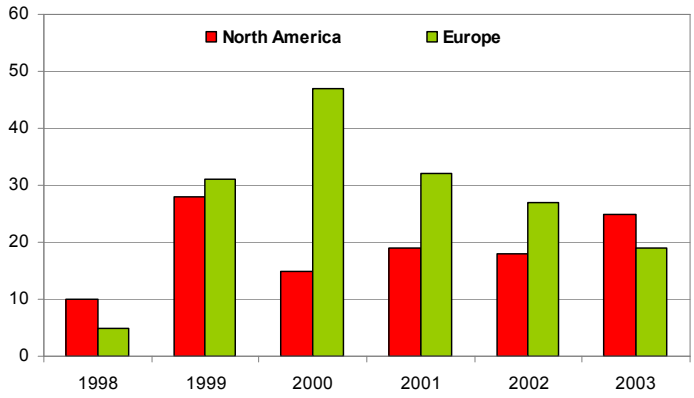


Fig 6 – Number of seized illicit laboratories producing ecstasy type substances (ONU).

2.1.2 Pharmacological aspects

The chemistry of MDMA and the other amphetamine type substances is based on the chemical structure of phenylethylamine. This common bond is represented in *Fig 7*.

It is possible to distinguish three groups among ecstasies – 1) amphetamine and methamphetamine, 2) the methylenedioxyamphetamine derivatives, and 3) the hallucinogens (2CB, DOB, etc) which are much less frequent than the first two except derivatives from serotonin. They can be differentiated by their appearance, the form of ingestion and effects. Indeed, when considering the relevant literature the three groups are in general treated individually.

Amphetamine is principally found in powder form and methamphetamine as crystals or small pills ("Thai pills"). Their effects are stimulant and can be hallucinogenic, not as much as the group of hallucinogens, but more than the methylenedioxy type derivatives. These are characterised as tablets of variable appearance and their *entactogenic* effects. This term has been proposed in 1986 to distinguish MDMA from other types of substances. It means empathogenic, euphoric, and stimulant effects, such as

increased socialibility, increased desire to touch and love, enhanced tactile sensations (it feels good to touch things) or a feeling of "oneness" with the crowd. (Internet1, 2006)

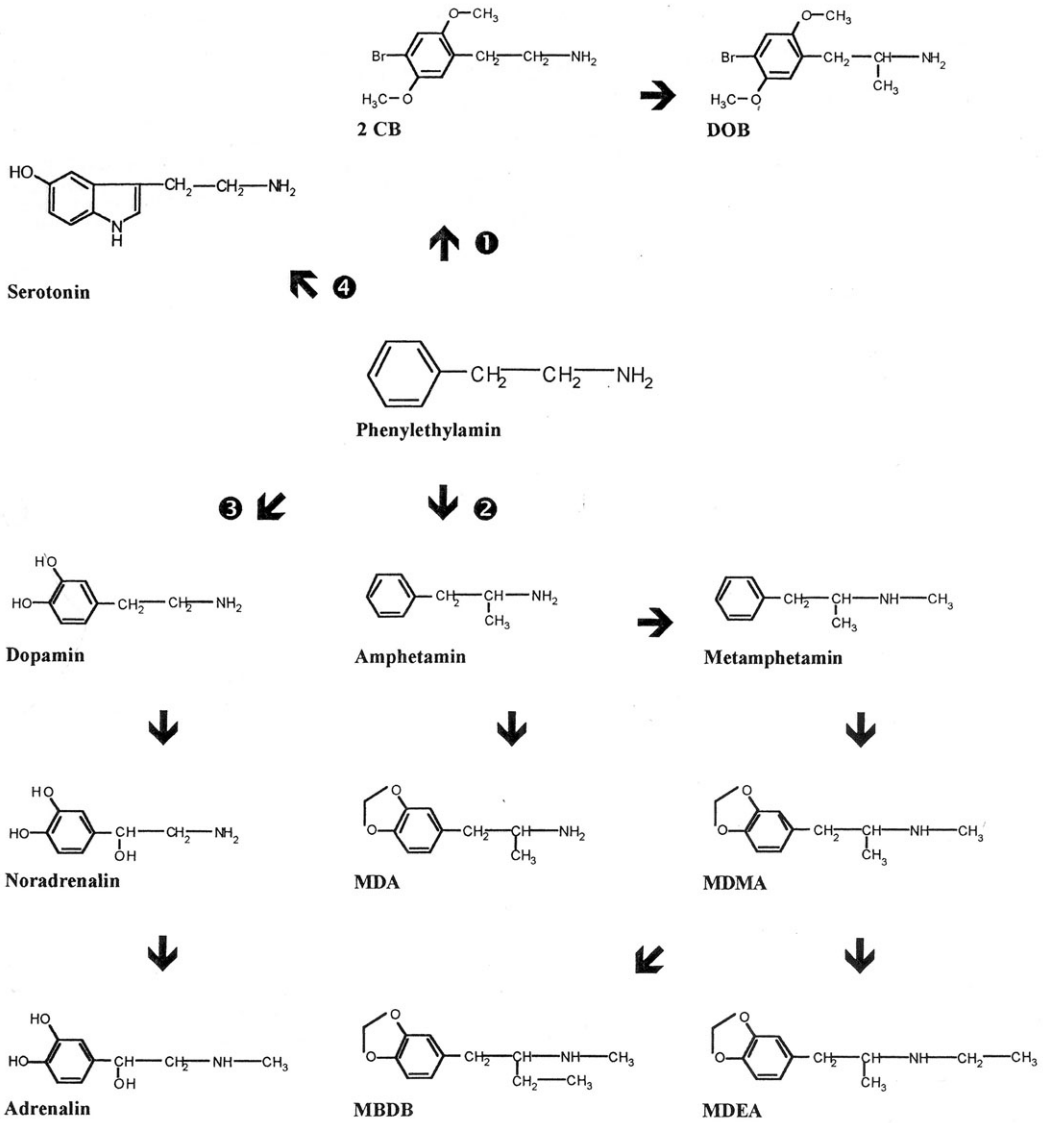


Fig 7 – Chemical structures deriving from phenylethylamine (Sauer and Weilemann, 1997), with: ① transformations into 2-CB and DOB, ② transformations into MDA, MDMA, MDEA and MBDB, ③ theoretical transformations in the group of catecholamines and ④ similarity with serotonin.

On the whole, phenylethylamine and its derivatives make intellectual tasks easier by helping to concentrate, and physical tasks as well by decreasing sleepy feelings. This explains why these substances were used by both, intellectuals and sportsmen/women. However, differences in their working mechanism, consisting principally in the use of different neurotransmitters – dopamine (amphetamine) or serotonin (MDMA) – result in preferences for either one or the other.

Common adverse side effects of MDMA ingestion include rise in blood pressure and heart rate, nystagmus (eye-wiggling), trismus (jaw tension), bruxia (teeth grinding), sweating, agitation, a post-peak

crash, muscle tension, headaches, nausea & vomiting, dry-mouth, liver problems, water retention, fatigue, confusion, mood swings, black outs, etc. While these effects have been known for years, there hasn't been much data about the prevalence of these various side effects. (Internet2, 2006)

Recently there have been increasing studies of MDMA and the neurotoxic (permanent damage of nerve terminals in the brain) effects it may cause. The main concern revolves around the long term effects of MDMA on the serotonergic system. High doses or repeated administration of MDMA cause serotonin nerve terminal degeneration and serotonin axonal degeneration in animals. Lower doses results in decreases of serotonin neural markers, such as brain tissue levels, reduced density of serotonin reuptake transporters, and reduced activity of tryptophan hydroxylase. (Internet3, 2006)

The pharmacological aspects of the discussed substances can be found in the PhD research report of Till Goldmann (2000) or the internet website – (Internet1, 2006). As for the chemical aspects, more detailed information can be found on the same website, as well as in PhD research reports of Eric Lock (SMT, 2003; Lock, 2005) and Christian Zingg (2005).

2.1.3 Illicit tablets[2]

Ecstasies are tablets and have been examined as such several times during the past decades. The observation of visual characteristics, such as size, colour, design (logo), weight and so on, can be fundamental. In the medical domain, sample description of prescription drugs and remedies is used to identify products. Lists and tables have been created to facilitate this work (Identa, 2002). Similarly, in forensic investigation the first description of items originating from the crime scene is of utmost importance as it could give evidence of what has happened.

In order to dispose of these visual and physical characteristics, as well as major chemical characteristics, a database has been created at the IPS by Christian Zingg in collaboration with the WD Zürich (Wissenschaftlicher Dienst) and contains seizures coming from two different regions in Switzerland (the Italian and French part - Ticino and Romandie – and Zürich), which are shown in *Fig 8*.

Comparison of ecstasy tablets requires knowledge of tablet production, if appropriate interpretation of observed characteristics is desired. In both, licit and illicit production, two major steps can be distinguished :

- the first step consists of the preparation of the mixture of the active substance with the excipients,
- the second of the compression of this mixture.

[2] (Marquis, 2000; Gomm and Hughes, 1991; Bauer et al, 1999; Le Hir, 1997; Kummer, 1998)

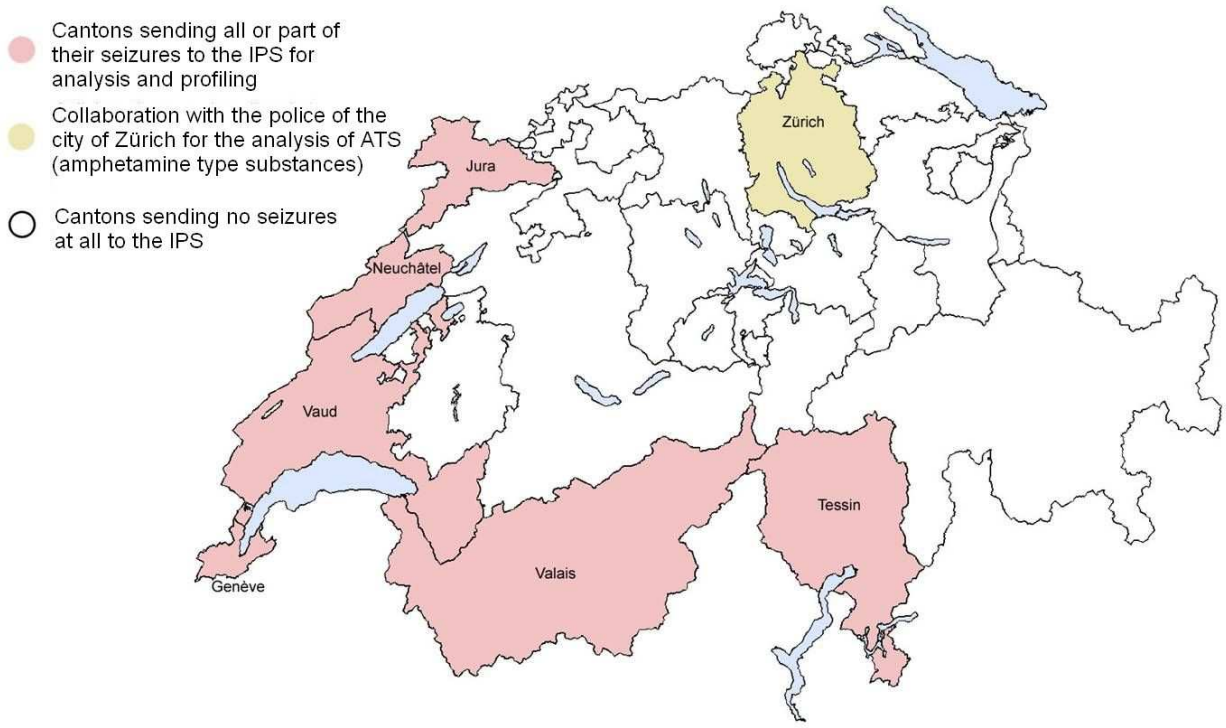


Fig 8 – Swiss map emphasising cantons sending their seizures to the IPS.

A more detailed description of the procedure with regard to illicit production is given in Appendix I. Here it will just be emphasised that the quality of the resulting tablet largely depends on the composition of the original mixture to compress. The importance of the excipients will be demonstrated in the following chapters. However, an overview of possible problems due to an inappropriate use of excipients is presented in *Table 1*.

Although this table has been taken from a book about pharmaceutical technology and it might be stricter in application than illicit producers might follow in producing tablets, the latter also encounter this kind of problems and have to find appropriate solutions. A study of excipients is an important part of this research.

Problem	Causes and solutions
Insufficient mechanical strength	○ low pressure ○ inappropriate binder or insufficient quantity ○ excessive amount of starch ○ excessive amount of lubricant ○ low humidity, overly dry mixture ○ insufficient plasticity
Capping (*Fig 9*)	○ low humidity – improved by adding a wetting agent ○ air inclusions – can be resolved by use of a particular press or by reduction of compression speed and/or pressure ○ mixture elasticity – can be resolved by adding a substance that increases deformability, such as microcrystalline cellulose ○ inhomogeneities producing differences in axial and radial strength – can be resolved by granulation of the mixture or by particle size reduction of the components
Sticking of mixture to punches and die walls (*Fig 9*)	○ insufficient amount of lubricant and "anti-sticking" substance ○ too much moisture ○ low melting point due to eutectic mixture – can be resolved by adding an adsorbing or drying agent, such as silica
Insufficient disintegration time	○ inappropriate disintegrator ○ insufficient amount or absence of disintegration accelerator ○ hydrophobic mixture, or not enough wetting agent ○ poor porosity (excessive pressure) ○ presence of soluble substances which suppresses the action of disintegration accelerators by osmotic effect
Dosage variations	○ inhomogeneous flow of mixture to compress – can be resolved by optimisation of particle size or by adding of silica

Table 1 – Possible problems during tablet manufacture (Bauer et al, 1999)

Fig 9 – Examples of *Capping* (at the left) and *Sticking* (at the right), ecstasy database IPS-WD.

2.2 EXCIPIENTS[3]

The theoretical aspects about excipients have been researched in the pharmaceutical literature, particularly in the field of galenics where preparation forms for drugs are treated. It will be shown later that the basics can be found in illicit tablet production as well.

2.2.1 Generalities

"The excipients, which can also be called auxiliary substances, are raw materials designed to enter into the composition of pharmaceutical preparations on a different basis than the active substances. They are conceived to give preparations a particular form or to be incorporated. Excipients correspond either to a chemical entity, or to a more or less complex mixture of synthetic or natural origin. Products of natural origin are used either directly, or after having been processed chemically." (Le Hir, 1997)

The excipients have three functions :

- to facilitate administration of active substances,
- to improve efficiency of the active substances,
- to ensure stability and consequently conservation until the fixed limit of use.

In pharmaceutical applications they have to meet the following requirements :

- inertia in relation to the active substance, the packing material and the organism,
- constancy in physical and chemical quality in time,
- they have to present the appropriate microbiological purity for their application.

The choice of an excipient is often a compromise between several risks. Excipients are seldom produced only for pharmaceutical use, where requirements are generally higher than in other domains. Thus, they are often chosen in the food sector. The application of an excipient can be variable and it is not rare to find excipients with several functions.

Excipients are generally defined by their physicochemical properties, and by their technological character. Only the former are described in the excipient monographs in pharmacopoeias. As for the latter, requirements have to be adapted for every production and the producers must fix the acceptance limits.

[3] (Bauer et al, 1999; Le Hir, 1997; Gurny, 1998/99; Gibson, 2001; Davies, 2001; Wasan, 2005)

2.2.2 Excipients in tablets

Tablet preparation requires particular powder/grain qualities in terms of physical and mechanical properties. The granule quality must be such as to ensure ease of powder blending and the fluidity for the precise filling of the compression chamber. Additionally granulation particles must present sufficient quality to give the tablet enough stickiness or plasticity for it to hold together and stay together as a solid dosage form.

For these reasons, most of the active substances require the presence of excipients and additionally a granulation step before compression to ensure good cohesiveness and easy disintegration.

2.2.2.1 Types of excipients

Excipients are classified by their function in tablet production :

1) *DILUENTS* – diluents have the function to fill up the volume when the amount of the active substance is insufficient to form a tablet of acceptable size. They are inert powders which are chosen from case to case depending on their accessory properties.

2) *BINDERS* – they hold particles together in the tablet that would otherwise be loose, fluffy and hard to consolidate into tablets. This is especially important for certain timed release products where the binder acts as a matrix from which the drug is gradually released. Their presence also allows reducing the compression force.

3) *LUBRICANTS* – their usage is threefold :
   - improve the fluidity of the grain,
   - decrease sticking of grain to punches and die,
   - reduce frictions between particles during compression.

   Generally, the lubricant is added to the blend right before compression, in form of a very fine powder covering particle surfaces.

4) *DISINTEGRATORS* – they allow acceleration of tablet disintegration, and hence dispersion of the active substance in water or gastric juices. They favour penetration of water into the tablet and thus allow it to breaking apart.

5) *OTHERS* – wetting agent, buffer, dyes, flavourings, absorbing and adsorbing agents.

The choice of an excipient mixture appears to be rather complex and is similar to tentative research, taking into account possible incompatibilities, desired administration mode and dosage method of the active substance. Decision about excipient ratios requires several tests and for every one, controls of

hardness, disintegration, conservation, etc. have to be realised. A basic tablet recipe in pharmacology – developed without active substance – looks like this (Bauer et al, 1999) :

I	starch (disintegrator)	10-20%
	binder[4]	1-15%
	lactose (diluent)	ad 100%
II	starch (disintegrator)	10%
	talc (lubricant)	5-8%
	Mg stearate	0.5%
	or stearic acid (lubricant)	1%

In general, tablet blends are constituted of an inner phase (I) and an outer phase (II). The inner phase with the diluent, binder and eventually a disintegrator corresponds to the actual granule. The components of the outer phase are added just before compression. A rule of thumb says that the ratio between inner and outer phase must be between 80 : 20 and 90 : 10, with regard to optimal fluidity and tap density.

2.2.3 Excipients in illicit tablets[5]

Tablets produced in clandestine laboratories can often be characterised by their unusual and crude appearance, although formulations are similar to those used in pharmaceutical production. The basic principles are the same – an active substance (amphetamine and/or derivative) is mixed with some excipients which constitute the bulk of the blend. Like in pharmacy, excipients include diluents, binders, lubricants, disintegrators and dyes. However, in illicit production a recipe does not necessarily contain every type of excipient and use different quantities as well. An example of a possible amphetamine tablet formulation is given below :

Amphetamine	5mg
Diluent	170mg
Binder	5mg
Lubricant	2mg
Disintegrator	20mg
Dye	0.03mg

The result would be a tablet of about 200mg with an amphetamine content of 2.5%. Generally, amphetamine content is not that low, but it is not unusual to observe this kind of percentages. It is important to notice the great presence of excipients, and particularly of diluent, in this formulation

[4] The most important binders in grain production and their corresponding usual concentrations (percentage in a dry grain blend) are : «starch glue» – 5-15% ; gelatine – 1-3% ; polyvinylpyrrolidone – 3-5% ; cellulose ether – 1-5%

[5] (Gomm and Hughes, 1991; Tillson and Johnson, 1974; Gomm and Humphreys, 1975)

example. Illicit tablets usually contain common and largely available diluents, which can be pharmaceutical excipients such as lactose, or simply milk powder or various forms of calcium carbonate.

2.2.3.1 Excipients observed in illicit tablets[6]

Even though excipients are regularly detected in ecstasy tablets, there is surprisingly little forensic literature concerning their use for ecstasy discrimination. Data is even almost inexistent when it comes to excipient ratios in illicit tablets. The few information found will be noted hereafter and consist more in a listing of excipients than in a description of tablet fabrication.

For a better understanding the observed substances will be classified according to pharmaceutical criteria into the four types of excipients – diluent, binder, lubricant and disintegrator. Substances noted in *italic* font were often detected in our laboratory, and those with a (*) were often cited in literature compared to the others.

DILUENTS

They are also often used for their alkaline character. Some diluents are good binders as well.

*Lactose**	*Glucose**
*Sucrose**	Maltose
Fructose	*Sorbitol**
*Mannitol**	Cellulose*
Kaolin	Methylcellulose*
Vitamin C	Talc*
Ca carbonate	Na bicarbonate*
Ca phosphate* (dibasic or tribasic)	Ca sulphate* (anhydrous and bihydrate)

BINDERS

They are often used in powder or liquid form and can be observed as combinations of two or more binders. Many only possess good cohesiveness, whereas the granulation requires also good solubility. The following binders were found in illicit tablets :

Agar	Gum	Cellulose*	Acacia
Gelatine	Methylcellulose	Dextrine	*Glucose*
Starch*	Flour	Urea	Waxes
Carbohydrates (lactose, sucrose, mannitol)		Pitch	*Stearates**

[6] (Sondermann and Kovar, 1999; King, 1997; Marquis, 2000; Gomm and Humphreys, 1975; Rhodes and Thornton, 1979; Giroud et al, 1997; Le Hir, 1997; Kibbe, 2000)

LUBRICANTS

Every tablet production requires the presence of lubricants, especially when binders are used as well. They are necessary for a good fluidity and unproblematic ejection of the tablets.

*Stearates**	Talc*	Stearic acid	Mineral oil	Vegetable oil
Colloidal silica	Starch*	Cellulose*	Boric acid	Na stearyl fumarate

DISINTEGRATORS

There are only few disintegrators of good quality compared to the other excipient types. Generally, starch is used exclusively, in form of powder or occasionally as fine grains.

Starch*, diatomaceous earth, Maizena® type products

More detailed characteristics of the most frequent excipients are described in Appendix II.

2.2.4 Synthesis

The first observation to be made is that all these substances are of very different chemical nature, which excludes immediately simultaneous analysis. There are inorganic components, carbohydrates, oils, acids, etc. However, some substances are more frequent than others – one of these is the group of carbohydrates. This can be explained by their multifunctionality (diluents + binders), their suitability for direct compression (without granulation step), and their availability in commerce. Since they are also regularly detected in our laboratory, focus on this type of substances is certainly justified.

Starch has been often cited in literature, but was actually very seldom observed in our laboratory. It was not regularly sought, but occasional tests showed its presence only a very few times.

Finally, stearates appeared to be interesting as they were often cited, and regularly detected in ecstasy tablets analysed by our laboratory as well. They were also observed in a more negative context, as they are reason for difficulties in extraction of traces of active substances by forming emulsions. Stearates are composed of fatty acids, principally palmitic and stearic acid, which are the acids usually detected by GC-MS analysis. Thus, it seems to be more appropriate to use the term fatty acids from now on.

The following two chapters will contain a description of the physical and chemical properties of carbohydrates and fatty acids, in view of possibilities of chemical analysis in ecstasy tablets.

2.3 CARBOHYDRATES

It has to be reminded that carbohydrates, as well as other excipients, are not produced exclusively for pharmaceutical use, but are taken from productions for the food industry. Therefore, research has principally been focused on analysis of food additives where carbohydrates are not surprisingly very frequent.

In food analysis the carbohydrates are classified as follows (Southgate, 1991):

Carbohydrates
- Sugars, Dextrins, Starches → Available carbohydrates[1)]
- Pectins, Hemicelluloses, Celluloses, Lignin → Unavailable carbohydrates ≡ dietary fibre[2)]

1) carbohydrates used and metabolised as carbohydrate by the organism
2) are considerably broken-down, but not completely, by symbiotic bacteria yielding fatty acids and thus do not provide carbohydrates to the organism

2.3.1 Generalities[7]

The empirical chemical formula for carbohydrates is $C_nH_{2m}O_m$. Monosaccharides (MS) correspond to the carbohydrates consisting in one entity which cannot split further by hydrolysis. They are classified by the number of carbons (e.g. 5C = pentose, 6C = hexose) and by the position of their carbonyl group (e.g. aldose, ketoses). Monosaccharides are the building blocks for oligosaccharides (2 – 7 MS) and polysaccharides (> 7 MS).

Monosaccharides	Hexoses – Glucose, Fructose, Galactose
Disaccharides	Sucrose, Maltose, Lactose
Polysaccharides	Cellulose, Starch
Polyalcohols / Polyols	Sorbitol, Inositol, Mannitol, Xylitol

Examples of the chemical structure of some carbohydrates are represented in Appendix III with their complete chemical name.

[7] (Southgate, 1991; Belitz et al, 2004; MSDA, 2001)

Disaccharides are furthermore distinguished between *reducers* and *non-reducers* :

- reducer : free hemiacetal or hemicetal function,
- non-reducer : the glycosidic bond blocks hemiacetal functions.

The most common food sweetener in the industrialized world – sucrose – is a non-reducer and therefore generally analysed by different ways than other sugars. For comparison lactose and sucrose molecules are shown in *Fig 10*.

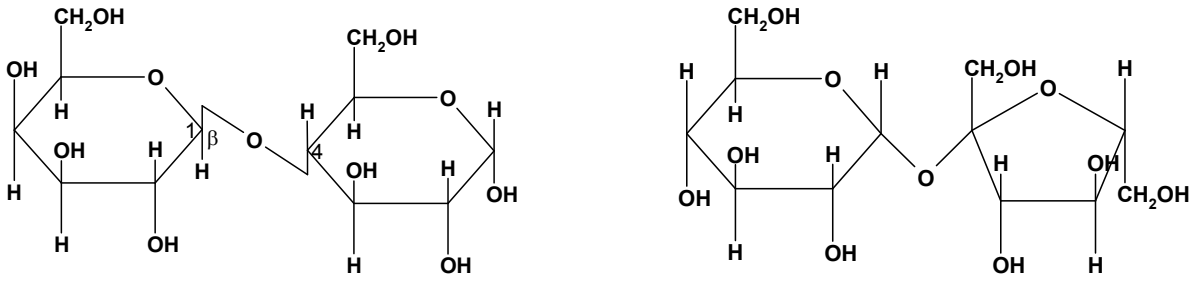


Fig 10 – Lactose on the left – Sucrose on the right.

Polyols as well are to be considered apart. They are generally formed by the corresponding mono- or oligosaccharides by hydrogenation (glucose → sorbitol; fructose → sorbitol and mannitol; xylose → xylitol). The aldo and keto groups of the saccharides are reduced to hydroxy groups and thus form polyols. Hence, they do not have any reductive properties anymore.

As for the polysaccharides, only cellulose will be mentioned, while starch will not be studied further. Cellulose was certainly first known in paper production, but is now also a widely used excipient in pharmaceutical technology. It is used in the form of powdered cellulose which is a white, odourless and tasteless powder of various particle sizes. It is produced by purification and mechanical size reduction of α-cellulose obtained as a pulp from fibrous plant materials. The other form often used is microcrystalline cellulose which is a purified, partially depolymerised cellulose that occurs as a white-coloured, odourless, tasteless, crystalline powder composed of porous particles. It is manufactured by controlled hydrolysis, with dilute mineral acid solution, of α-cellulose. (Kibbe, 2000)

As might have been noticed different terms can be used with regard to carbohydrates. In general, the terms are used as follows:

- *sugars* – all mono-, di- and oligosaccharides,
- *polysaccharides* – by definition,
- *polyols* – polyalcohols, or sugar-alcohols.

(Sturgeon, 2000; Peris-Tortajada, 2000)

2.3.2 Solubility[8]

In general, all sugars and polyols are soluble in water, some of them even extremely soluble. The solubility is reduced in some alcohols (ethanol or methanol), but not anymore when using heated aqueous alcohol solutions. Their solubility in organic non-polar solvents such as ether, chloroform and benzene is insignificant, exceptions can be observed with pyridine.

As for the polysaccharides, the situation changes completely. Celluloses are practically insoluble in water, dilute acids and most organic solvents although it disperses in most liquids. Some polysaccharides have good swelling power and solubility in hot and cold water (e.g. starch, guaran gum). They are slightly soluble in 5% sodium hydroxide solution, and under colloidal form also in water. Substituted cellulose, e.g. methylcellulose, may however show dissolution in water with solubility depending on the grade of substitution.

2.3.3 Chemistry[9]

As already mentioned, the reductive function is rather important for carbohydrates. It is the most used property in sugar analysis. Free components with this function will reduce alkaline solutions of metallic salts in oxides or free metals. Factors influencing this reaction are heating speed, alkalinity and reagent strength. it should be noticed that the non-reducing sugar do not have the free lactol group therefore lack the ability to react with alcohols and amines.

Another chemical property of carbohydrate is the methyl ether formation which is important for structure analyses of polysaccharides, but also for the analysis of mixed mono- and disaccharides. The interest in ether formation lies in the preparation of volatile compounds for gas chromatographic analysis.

Finally, carbohydrates can form complexes. Optical rotations of carbohydrates are improved when put in solution with boric acid. The carbohydrates can form borate complexes, which are used for their separation by electrophoretic analysis.

2.3.4 Analytical Methods

The choice of an appropriate analytical method depends among others on the qualitative composition of the sample, and the requirements of the analysis. There are a number of options available from a simple condensation reaction to the separation and analysis of individual components. When it comes to quantitative analysis of mixtures, a need in instrumental methods becomes evident, as they are more precise and accurate. (Southgate, 1991)

[8] (Southgate, 1991; Kibbe, 2000; Belitz et al, 2004)
[9] (Southgate, 1991; Belitz et al, 2004)

2.3.4.1 Thin-Layer Chromatography (TLC)

Thin-Layer Chromatography (Sturgeon, 2000; Blackwood and Chaplin, 2000) is a sensitive and economic analytical method. It is principally used for qualitative analysis, but recent developments in automatisation of spot deposition made it interesting for quantitative analysis as well. Additionally, the arrival of HPTLC plates (High Performance Thin Layer Chromatography) allowed better resolution and reproducibility.

For quantitative analysis the TLC plate is placed under a scanner which measures the absorption or fluorescence at one or more wavelengths. The result is a sort of chromatogram presenting peaks corresponding to the spots on the plate, and is then treated as such. Calibration curve is obtained by standards deposited on the same plate.

Although analysis time is very long compared to the instrumental techniques, this is caught up by the possibility of eluting up to 20 samples simultaneously. Additionally, requirements in material are low and the procedure is simple.

2.3.4.2 Gas chromatography (GC)

Gas chromatography (Sturgeon, 2000; Southgate, 1991) is a widely used analytical method for a long time. It provides effective separation and analysis and has been applied to carbohydrate analysis before the arrival of other methods like HPLC or Capillary Electrophoresis. However, carbohydrates are polar and not sufficiently volatile to be analysed directly and need to be derivatised before GC analysis. It is therefore very important to study derivatisation possibilities with regard to the aim of the analysis. There exist several types of derivatisation – methyl formation has already been mentioned, other possibilities are the formation of aldol acetates or silylation, which is the method used in our laboratory for routine analysis.

Some derivatisation methods, such as silylation, may produce derivatives corresponding to the equilibrium mixture of the individual sugars. Monosaccharides, for instance, exist in their aldose and ketose forms in aqueous solution which form an equilibrium between the two ring (pyranose and furanose) and acyclic forms. Therefore, it is possible to obtain up to four peaks for one sugar. This may result in rather complex chromatograms and presents a disadvantage in routine analysis. However, this can be avoided by applying other derivatisation methods and additionally, these secondary peaks may also help identifying sugars, as their mass spectra are very similar.

2.3.4.3 High Performance Liquid Chromatography (HPLC)

HPLC (Peris-Tortajada, 2000) has been applied to carbohydrate analysis since the 1970's and ever since performance of columns has constantly improved so that it is possible now to obtain complete separation

of a mono- and disaccharides mixture in most foodstuff. However, a certain investment for the instrumentation is required and detection as well as quantification of carbohydrates can be difficult.

Several separation systems have been developed and tested. Ion exchange chromatography, including anion- and cation-exchange resins, has been used for a long time in carbohydrate analysis. However, this technique has recently been superseded by the use of partition systems, mainly because the former involves long analysis times as well as the need to operate the column at high temperatures. Progress in column quality, on the other side, made the HPAEC (High Performance Anion-Exchange Chromatography) system the most frequently used for separation of monosaccharides and polyols.

Refractive index detection is still the most widely used method for carbohydrates, in spite of its lack of sensitivity. It has the advantage of being universal and some of its drawbacks can be overcome by appropriate sample preparation. However, new detection methods, such as pulsed amperometric detection and mass detection, gain increasing popularity in reason of their high performance.

2.3.4.4 Capillary electrophoresis (CE)

Capillary electrophoresis (Blackwood and Chaplin, 2000; Cancalon, 2000) is relatively new as its major progresses have only been achieved in the 1990's. Compared to the other techniques, publications about carbohydrate analysis are less frequent. However, the technique allows fast and efficient separation, it functions with long-lasting and economic capillaries and requires only small sample volumes and little solvent.

In general, separation of carbohydrates can be realised in two ways. The first procedure, also the most frequently used, works with normal polarity (positive) and uses neutral or basic buffers. The second applies negative polarity and acid buffers are used. The major difference between the two is that in the first method the electro-osmotic flow largely influences the separation of the compounds, whereas in the second the flow is almost eliminated.

Since the carbohydrates are not charged, methods have to be adapted. Good migration can be obtained by ionisation of the hydroxyl groups at high pH, by complexation with borate or alkaline earth metal ions, by derivatisation with reagents containing ionisable functions and by partitioning into micelles using a pseudo-stationary phase (MEKC). However, the last one cited seems not to be appropriate according to (Xu et al, 1995), since the high hydrophilic property of sugars prevents the use of micellar separation systems.

Like in HPLC detection has long been problematic. Several methods exist including fluorescence, absorption in the UV/Vis region, electrochemical detection, mass spectrometry and immunologic essays.

2.3.5 Synthesis

Comparing these different techniques, it immediately appears that TLC is the only non-instrumental technique, a feature which makes it attractive when no other means are available. However, separation of carbohydrate mixtures is not as effective as with the other techniques and visualisation of polyols can be problematic.

HPLC and CE are rather similar techniques and absolutely comparable in terms of quality. However, after long years of studies HPLC is the technique of choice for carbohydrate analysis and CE has still to prove its value. Thus, the most applied techniques these days are HPLC and GC, as they are providing efficient analyses with high precision and sensitivity.

In more recent years, HPLC has probably replaced GC as the most commonly used technique, at least as witnessed by the relative volume of publications in this area. However, although HPLC presents several advantages in carbohydrate analysis, GC instrumentation and methods have evolved and both techniques are comparable now. This is demonstrated in *Table 2*.

HPLC	**GC**
More suitable for the determination of medium- and high-molecular-weight polysaccharides	Far more sensitive
Shorter (50%) analysis time	Allows separation of α and β anomers [a)]
Higher recoveries with more accuracy	Preferable for monosaccharides
Directly applicable to sugar samples	Derivatisation required

a) this can be an advantage as much as a drawback – it depends on the purpose of the analysis

Table 2 – Comparison between HPLC and GC in carbohydrate analysis (Peris-Tortajada, 2000)

It must be added that all three instrumental techniques present autosampling devices, which means that between forty (CE) and over a hundred (GC) samples can be analysed in sequence without the need of the analyst's presence.

In conclusion, it is impossible to determine which technique is the best for carbohydrate analysis. The choice entirely depends on the type of carbohydrates to analyse, on the purpose of the analysis and the means available. In this research project, all techniques but HPLC have been tested.

2.4 FATTY ACIDS[10]

Fatty acids are the components of lipids or fat. The lipids form a complex class of compounds which can be defined as being insoluble in water and soluble in organic solvents. In the past they have been defined as fatty acid derivatives. Their can be either of plant origin (fruits, grains), or animal origin (deposit in animal tissue, milk, and aquatic fauna).

Lipids are often separated in three groups – the fatty acids, the simple lipids and the complex lipids. Major constituents of lipids are the triglycerides which are triesters of glycerol with three fatty acid chains (long-chain aliphatic monocarboxylic acid). They are part of the simple lipids beside other compounds formed by fatty acids and alcohols (sterol or longer alcohols). Free fatty acids (FFA) also are part of this group.

2.4.1 Generalities

There exist more than forty fatty acids in nature. In general, they are carboxylic monoacids which contain an even number of carbon atoms (4-24), present straight aliphatic chains that are not ramified or substituted. They are either saturated or unsaturated :

- saturated fatty acids : do not contain any double bonds or other functional groups along the chain
- unsaturated fatty acids : can contain up to six double bonds; mono-unsaturated fatty acids contain one double bond, poly-unsaturated fatty acids more than one; the double bonds can occur in a *cis* or *trans* configuration, but in nature *cis* configuration is the predominant form and double bonds are situated at the 3n carbons with starting n=2 (no conjugated).

A fatty acid is composed of the following two parts :

- a methyl group and its carbon chain which is insoluble in water, but soluble in oil;
- a carboxyl group which is responsible for the acid character of the molecule; this part is soluble in water, but not in oil.

The classification of the fatty acids is based on the length of the alkyl chain and on the number, position and configuration of the double bonds. A more detailed explanation is given in Appendix IV.

2.4.2 Physicochemical Properties

Fatty acids possess a carboxyl group which confers them acidic properties. Thus, most short-chain fatty acids present a pK_a situated between 4 and 5. However, this parameter is influenced by the chemical

[10] (Christie, 1982; Belitz et al, 2004)

structure of the fatty acid, in particular by the chain length and the number of double bonds. Long-chain fatty acids, for instance, have much higher pK_a values than the shorter ones (*Fig 11*).

An explanation of it is that for longer chains, the van der Waals interactions between the chains is higher, bringing the molecules closer to each other. This way the carboxylic acids are also packed closer, shielding the hydrogen atom between the two oxygen atoms. The closer the molecules, the more strongly the hydrogen atom is shielded and consequently the higher is the pK_a. (Kanicky and Shah, 2002)

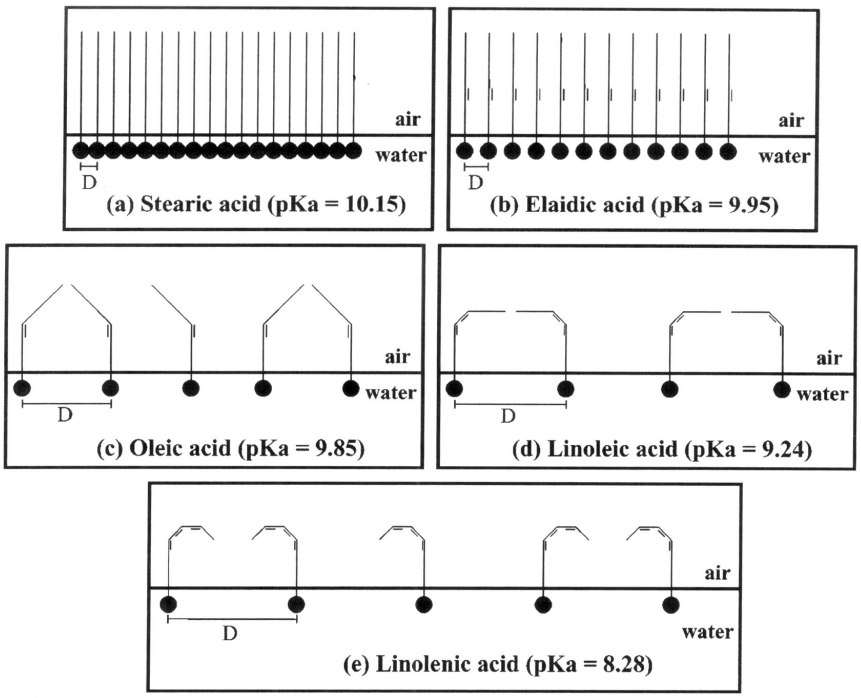


Fig 11 – Schematic representation of C18 fatty acid monolayers at the air/water interface – influence of the insaturation. (Kanicky and Shah, 2001)

It can be guessed from *Fig 11* that solubility is also strongly influenced by this particular chemical structure. Short fatty acids present considerable solubility in water compared to their corresponding hydrocarbons, which is due to the presence of the carboxyl group. An increase in chain length results in diminished solubility in water. However, the carboxyl group has sufficient influence to permit the fatty acids forming a monomolecular layer at the air/water interface with the carboxyl group in water and the chains in air, generally oriented perpendicular to the surface.

As for solubility in organic solvents, it is correlated to the polarity of the solvent. In non-polar solvents, solubility of the fatty acids depends on temperature, a correlation which diminishes by increasing polarity. Again short-chain fatty acids present in general better solubility than longer ones. Completely non-polar solvents are not recommended, but as we will see later are often used for extraction.

To conclude, the melting point depends on the number, the position and configuration of the double bonds. Presence of double bonds will decrease the melting point, and a double bond at the beginning of the chain will decrease it more than one at the end of the chain. Considering the curvature (*Fig 11*) resulting from the presence of double bonds this becomes quite obvious.

2.4.3 Fatty Acid Analysis[11]

Fatty acids are generally analysed by gas chromatography and have therefore to be transformed in volatile compounds – the most common transformation is the formation of Fatty Acid Methyl Ester (FAME). Consequently, studies mostly treat sample preparation rather than analytical methods. Often extraction, or its avoiding, is also investigated, since fatty acids happen to be analysed in food matrix and have therefore to be extracted. The general procedure is represented in *Fig 12*.

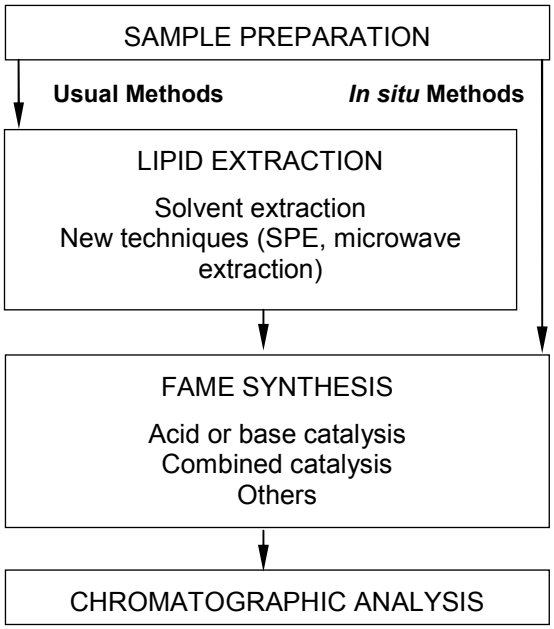


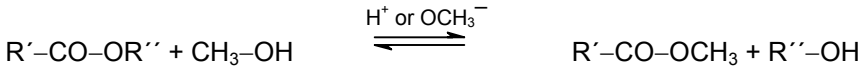
Fig 12 – Procedure for lipid analysis (Carrapiso and Garcia, 2000)

2.4.3.1 Fatty acids derivatisation[12]

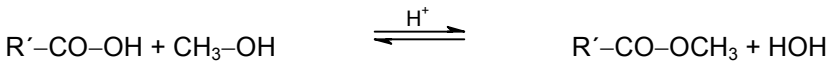
Fatty acids are transformed in FAME, without altering the chemical structure or the geometry of the double bonds. When starting with lipids, the first step consists in saponification, where fatty acids are separated from glycerol, sterol or other, and the second step is their esterification (methylation). As this procedure takes time, alternative methods have been proposed, consisting in direct transesterification –

[11] (Marini, 2000; Shantha and Napolitano, 1992; Eder, 1995; Brondz, 2002; Seppanen-Laakso et al, 2002)
[12] (Marini, 2000; Shantha and Napolitano, 1992; Seppanen-Laakso et al, 2002; Carrapiso and Garcia, 2000)

simultaneous hydrolysis and esterification requiring only one reagent. The reaction can take place in acid or basic media and can be described by the following equation.



When analysing free fatty acids, saponification is not necessary. Methylation, or esterification, only occurs in acid media and can be described by the following equilibrium.



Transesterification appears to be advantageous, but has for long time been associated with problems such as incomplete transformation, modification of original composition of fatty acids, contamination of the GC column by the reagent, incomplete extraction of FAME and loss of short-chain FAME because of their volatility. However, progresses have been made and the method is increasingly applied in food analysis.

Acid esterification

Fatty acids are esterified, or complex lipids transesterified, by heating them in an excess of methanol anhydrous in presence of an acid catalyser. The most common catalysers used in methanol are HCl, H_2SO_4 and BF_3. However, non-polar lipids are not soluble in methanol and another organic solvent has to be added.

Basic transesterification

Basic derivatisation is fast and does not require any heating. Its drawback, however, is that it does not transform free fatty acids, and the saponification of eventually present ester groups. The latter can result in formation of emulsion which can hinder FAME recovery. The procedure is similar to acid reaction, but uses a basic catalyser instead, e.g. sodium methoxide. Comparison with acid reaction is presented in *Table 3*.

	Acid	Basic
Temperature	High	Ambient
Time	Min - h	Sec - min
Esterifying power	medium - high	No
Transesterifying power	Low	High
Risk of saponification	Low	High
Water interference	Low	High

Table 3 – Characteristic of catalytic medium in esterification/transesterification reactions (Carrapiso and Garcia, 2000)

Others

One alternative is the combination of acid and basic reaction, which reduces reaction time and derivatises both, free fatty acids and lipids. This method is recommended by the MSDA (Manuel Suisse des Denrées Alimentaires – Swiss Food Manual, 2001) and applied at the Laboratoire Cantonal of Lausanne. First the extracted fat is reacted with sodium methoxide – basic transesterification – then the acid catalyser is added (BF_3) and finally the FAME are extracted with hexane.

Free fatty acids can also be transformed by exchange with N,N-dimethylformamide dimethyl acetal in pyridine or by use of strong quaternary ammonium salts such as trimethyl(*m*-trifluorotolyl)ammonium hydroxide (TMTFTAH), tetramethylammonium hydroxide (TMAH), trimethylphenylammonium hydroxide (TMPAH), (*m*-trifluoromethylphenyl)trimethylammonium hydroxide or trimethylsulfonium hydroxide (TMSH). Fatty acids form quaternary ammonium salts which decompose during injection in the gas chromatograph. (Butte, 1983; Shantha and Napolitano, 1992; Metcalfe and Wang, 1981; McCreary et al, 1978; Misir et al, 1985)

Another method often cited in literature is using diazomethane (CH_2N_2) for transformation of lipids. Although it seems to be one of the principal procedures, it is also immediately rejected because of its high toxicity, especially when there are alternatives.

Finally, the derivatisation method applied in our laboratory – trimethylsilylation – can also be used for fatty acids. The proof is that fatty acids are actually detected when analysing ecstasy tablets. However, it seems that pyridine is necessary for good derivatisation. It is not often cited in literature, generally only with regard to short-chain fatty acids, and a reason might be that the derivates strongly tend to hydrolyse. (Harris, 1975; Schulte, 1993; Blau and King, 1977)

2.4.4 Synthesis

In the case of fatty acid analysis sample preparation appears to be particularly important. Several methods can be found in literature and there does not exist a true consensus about which one gives the best results. Not surprisingly, this strongly depends on the type of lipids to analyse which largely varies in the

domain of animal and vegetable food. In the case of ecstasy, fatty acids are not very complex and the problematic lies more in the amount available, as a traditional tablet weighs between 200 and 300mg containing 3% of stearate at most.

Samples in food analysis are often larger and generally, transformation takes place directly on the lipid extract, which in itself is already bigger than an ecstasy tablet. Possible derivatisation procedures have therefore to be examined with regard to their application on small amounts of powder containing also other components as fatty acids.

3 METHOD DEVELOPMENT

3.1 INTRODUCTION

Both the excipients chosen – sugars and fatty acids – are detected by routine analysis on a gas chromatograph coupled to a mass spectrometer. However, the method used is a screening method and allows obtaining information about the active substance present and determining any adulterants or sugars eventually present as well.

Sugars might be detected by the screening method, but the separation is not satisfying and identification can be difficult since the mass spectra of sugars are very similar. Additionally, the analysis results in two or more peaks for one sugar, which is inconvenient for quantitative purpose.

Fatty acids are the components of stearates and are present in small quantities in tablet composition (~3%). Considering a chromatogram of a typical ecstasy tablet obtained with the screening method, the two peaks of palmitic (C16) and stearic acid (C18) are very small. This fact and the eventual presence of other fatty acids in smaller quantities lead to the conclusion that extraction followed by concentration is necessary.

Keeping in mind that sugars and fatty acids will be used for drug profiling purpose, a more selective method has to be developed. Therefore an important part of this research was to optimise the analysis of these two types of substances.

3.2 CARBOHYDRATES

3.2.1 Choice of the analytical method

Principal techniques are Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE) and Gas Chromatography (GC). TLC is a good technique because it is inexpensive, simple and allows a simultaneous analysis of up to 20 samples. Especially the launching of HPTLC plates resulted in an improvement of the performance in terms of resolution. However, analysis of 9 sugars[13] found in ecstasy and other illicit drugs showed only incomplete separation. Additionally other problems have been observed with regard to the time of one elution (up to 4 hours) and variation of migration distances. Sugars analysed within a mixture (with other sugars or other substances) would

[13] Glucose, Mannose, Fructose, Lactose, Sucrose, Maltose, Sorbitol, Inositol, Mannitol

present another retention index than the corresponding standard analysed alone. Finally, it has been observed that the analysis of ecstasy tablets containing two sugars resulted in the detection of only one of them. Altogether these difficulties prevented from further investigations in the use of this technique.

Considering the literature about analysis of carbohydrates, HPLC seems to be the technique of choice. Nevertheless, it has not been tested because our institute does not possess such an instrument and other techniques of comparable quality were available. Therefore, research has been focused on capillary electrophoresis first. The technique appeared to be inappropriate for this research and will not be discussed here, but the interested reader can learn about the details in Appendix V. Thus, the technique to be finally applied in this project was gas chromatography coupled to mass spectrometry (GC-MS).

Gas chromatography coupled to mass spectrometry is routinely applied for illicit drug analysis at the IPS (Institut de Police Scientifique). There are methods developed for every type of drug such as cocaine, heroin, ecstasy and a general screening method if the drug type is unknown before analysis. These methods were developed to identify first of all the active substance, but also the possibly present cutting agents or adulterants. Therefore it is important to realise that sugars are already detected by these methods. However, identification is not always easy because mass spectra are almost identical and some sugars present more than one peak of various sizes.

Thus, the aim of the method development for carbohydrates was twofold – firstly to improve the separation and identification of the principal sugars, and secondly to reduce secondary peaks for better quantitation. With this aim in view, the study has focused on the research of an appropriate derivatisation agent and the optimisation of GC conditions.

3.2.2 Preliminary tests

First of all the choice of sugar standards was reviewed and it was decided to take the eight sugars detected in the ecstasy tablets analysed in our laboratory (a database exists since 1996) – sucrose, lactose, mannitol, glucose, xylitol, sorbitol, inositol and maltose. An equal amount of each sugar was taken, mixed together and homogenised in a mortar.

Since sugars are polar and non-volatile they cannot be analysed directly by GC, but have to be derivatised beforehand. Therefore an important part of the work was to investigate which agent was the most appropriate. After a review of relevant literature, a number of methods have been chosen for tests in function of :

- Suitability for the selected sugars
- Preparation time
- Simplicity of preparation
- Amount of ecstasy powder necessary

The resulting preparations to be tested are listed in *Table 4*.

Weigh-in	Preparation method	Reference
4-5mg	500µl $CHCl_3$/Pyridine (5:1) + 100µl MSTFA, 1hour at 80°C	IPS
4-5mg	500µl acetonitrile + 100µl MSTFA/2% TMCS, 30min at 70°C	(Romolo, 2003)
4-5mg	500µl $CHCl_3$/Pyridine (5:1) + 100µl TMSI, 1hour at 80°C	(Romolo, 2003)
4-5mg	500µl Pyridine + 150µl TMSI, 1hour at 80°C	(Cotte et al, 2003)
10mg	1ml Hydroxylamine/aniline (50mg/ml), 10 min at 60°C, 300µl BSTFA, keep 10 min at room temperature	(Rojas-Escudero et al, 2004)
2-3mg	4 drops 1-methylimidazole + 5 drops acetic anhydride, after 5 min add 1ml $CHCl_3$	(Dujourdy, 2004)
2-3mg	1ml pyridine + 200µl HMDS + 100µl TMCS, vortex for 30s, analyse after 5min	(Sweeley et al, 1963)
10mg	40µl pyridine + 50µl BSTFA/1%TMCS, 30min at 80°C	(Selles et al, 2002)
0.5mg	200µl pyridine + 200µl TMSI + 200µl TMCS + 200µl heptane + 400µl H_2O, take 100µl of the upper phase for analysis	(Villamiel et al, 1998)
2-3mg	500µl pyridine + 450µl HMDS + 100µl TFA, vortex for 30s, analyse after 30min	(Brobst, 1972)

MSTFA - *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide, TMCS - Trimethylchlorosilane, TMSI – 1-(Trimethylsilyl)imidazole, BSTFA – *N,O*-bis-(trimethylsilyl)-trifluoroacetamide, HMDS – Hexamethyldisilazane, TFA – Trifluoroacetic Acid

Table 4 – Tested preparation methods and references.

For these first tests, analyses were realised on an Agilent GCD-MS with a DB1 column. Instrumental conditions were applied as noted in the publication corresponding to the derivatisation method as much as possible. If no conditions were given with the derivatisation method, then one of the four routine methods cited before has been applied (the one for cocaine happened to give optimal results in terms of separation and peak quality → method COC) next to a method taken from the publication of (Cotte et al, 2003) (→ method SUGAR).

The chromatograms of the sugar mixture were compared visually by considering :

- if the number of peaks detected corresponded to the number of sugars in the mixture,
- the quality of separation, particularly concerning the separation of mannitol/sorbitol and lactose/sucrose,
- the quality of the peak shapes (symmetry, broadness).

From these comparisons resulted a selection of two derivatisation methods for further investigation :

1) $CHCl_3$/pyridine + TMSI (Romolo, 2003)
2) pyridine + HMDS + TMCS (Sweeley et al, 1963)

It was decided to use both derivatisation agents to determine better instrumental conditions. Additionally to the quality of separation of the eight sugars, time of analysis would have to be considered as well.

3.2.3 Instrumental conditions

Since the beginning of derivatisation tests, our laboratory could acquire two new Agilent gas chromatographs 6890-MS and it was possible to employ one of them for this research. Hence the separation methods had to be tested on the new instrument, which appeared to be much more sensitive.

It has to be emphasized that the routine method for cocaine still gave better results, in terms of sugar peak separation, than the method applied to ecstasy tablets. Indeed when analysing ecstasy containing sorbitol, this one is easily mistaken for mannitol even when comparing with the relevant sugar standard, since retention times and mass spectra are almost identical. The same can be said for lactose and sucrose, but fortunately the second peak of lactose helps in identification. But this is the reason why new or modified methods have been compared to the cocaine method.

Summarizing the situation at the end of the preliminary tests, the method taken from (Cotte et al, 2003) (method SUGAR) certainly achieved a better separation of the eight sugars than the cocaine method (method COC). The latter had, however, the advantage of being less time-consuming and the question appeared if a slightly better separation was worth a 45 min analysis. The same observation was made on the new instrument.

An alternative was found in a publication by (Troyano et al, 1996), in form of a method suitable for trimethylsilyl derivatives of carbohydrates on a similar instrument and same column (DB1). The results were comparable to both the SUGAR and the cocaine method. The separation of the couple mannitol/sorbitol was better, and that of lactose/sucrose slightly worse than with the SUGAR method. As for the analysis time, it was only a few minutes longer than the cocaine method. Even though there was no significant improvement, the method provided a good basis to work on. Thus, it was decided to try out a number of modifications of the temperature program and gas flow, as these were two factors directly affecting separation (Rouessac and Rouessac, 1998).

Temperature Program – Peak retention times depend upon relationships between pressure, flow rate, oven temperature, column dimensions, and stationary phase. The temperature will change the viscosity of the stationary phase inside the column and will influence the partition coefficients of analytes and as a result their separation. The changes undertaken are represented in *Fig 13*.

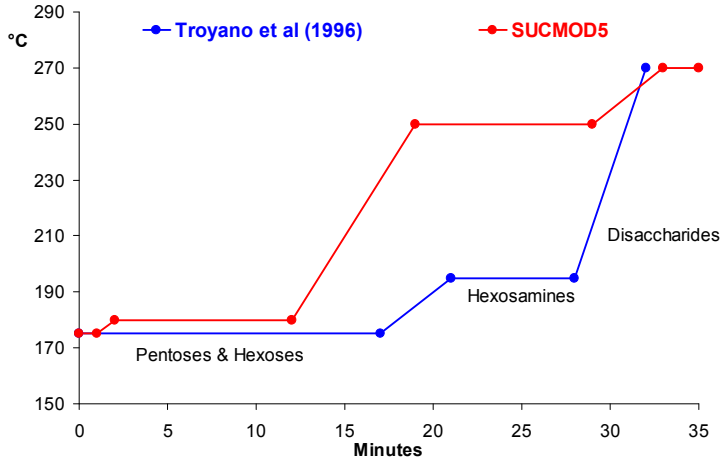


Fig 13 – Initial and modified temperature program for GC analysis of sugars.

Gas Flow – As the name says it, in gas chromatography gas is the mobile phase, but has no interaction with the substances. However, its influence on dispersion in the column is important, and consequently on efficiency and sensitivity of detection as well. The optimal gas flow depends on the internal diameter of the column and on the type of gas. The carrier gas on our instrument was helium and optimal velocities are estimated to be between 30-40cm/s.

After several tests, the gas flow was kept at 1ml/min (average velocity at 38cm/s) as proposed in the original method, but the temperature program was changed and a method could be determined providing a separation of all eight sugars, a reduction of secondary peaks and a comparable analysis time to the cocaine method applied at the laboratory (Appendix VI). Details of the chosen method can be seen in *Table 5*.

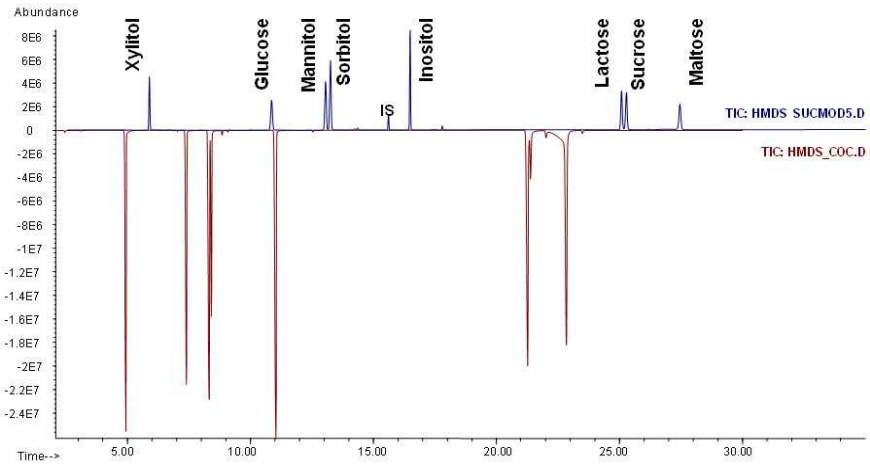


Fig 14 – Mixture of 8 sugars prepared both by HMDS method and analysed by the routine cocaine (red) and the new developed method – Sucmod5 (blue).

The analyses of the same sample preparation (HMDS) of the sugar mixture by the cocaine and the modified carbohydrate method are represented in *Fig 14*. It can be seen that separation is better with the modified method, particularly for the couples mannitol/sorbitol and lactose/sucrose.

The difference in abundance can be explained by the fact that the two preparations were not analysed the same day, but in an interval of two months and weigh-ins were very different (factor of almost 2.5). It has also to be mentioned that the preparation analysed by the cocaine method does not contain any Internal Standard, as this was only a punctual qualitative analysis to verify separation.

Method	**Sucmod5**
Sample introduction	
– Split	20 : 1
– Volume	1µl
– Total flow	24ml/min
– Injector T	275°C
Gas saver	15ml/min after 2min
Carrier gas	Helium
Column	
– Type	DB1MS, 30m(L) x 0.25mm (i.d.) x 0.25µm (film)
– Mode	Constant flow
– Average velocity	38cm/sec
Oven T-program	175°C (1min), 5°C/min, 180°C (10min), 10°C/min, 250°C (10min), 5°C/min, 270°C (2min)
GC-MS interface T	275°C
MS information	
– Solvent delay	2.5 min
– Mass range	50 – 550 a.m.u
– Sample rate	3, A/D samples 8
– MS quad T	150°C
– MS source T	230°C

Table 5 **–** Instrumental conditions for the sugar analysis by GC-MS.

3.2.4 Determination of the derivatisation agent

Now that the analytical method for the analysis of sugars was fixed, it was possible to compare the two derivatisation methods obtained after the preliminary tests. As until now they have only been applied to a standard mixture of carbohydrates, it was decided to test them on real ecstasy tablets. It was important to examine if the matrix would have an influence on the analytical result. For further comparison, derivatisations were investigated with regard to linearity and amount of yield.

3.2.4.1 Ecstasy

Six large ecstasy seizures were chosen in order to have enough tablets available for method testing. Their composition was obtained with the routine method applied to ecstasy street samples and is presented in *Table 6*.

Logo	Internal No	Sample Size	Active Substance (%)	Diluent
Peace and Love	L1635	19	MDMA (44%)	Lactose
Flying Bird, Peace	L989	17	MDMA (38%)	Lactose
"S"	L995B	18	MDMA (35%)	Lactose (B)
Rolling Stone's	L985C	18	MDMA (29%)	Sorbitol
Elephant	L985A	19	MDMA (21%)	Sorbitol
Sparrow	Z9	15	Amphetamine (7%)	Lactose

Table 6 – Ecstasy test samples and their general composition.

The additional "B" next to lactose for sample L995B is used in our database to indicate that the second peak of lactose is of the same size or higher than the first one. This phenomenon will be investigated in another chapter as it is due to the α- and β-form of lactose and both forms are used as excipients (cf. Ch 7 – Additional Studies). In this project, this second peak will always be referred to as lactose2.

For the comparison of the derivatisation methods, five tablets were taken from every seizure, then crushed and homogenised in an agate mortar. Two weigh-ins per tablet were taken and analysed in order to obtain 10 measures per seizure and derivatisation method. The obtained peak areas were then normalised with regard to the sum of areas and the relative standard variation (RSD) was calculated for every peak.

$$RSD = \frac{STDEVA}{Mean} \times 100$$

Both methods worked well on ecstasy street samples and produced chromatograms with reproducible retention times. For a better comparison, the RSD values obtained for every ecstasy sample were averaged per peak and method. The result is represented in the *Fig 15*. Mannitol was taken into account as well for it is regularly detected together with sorbitol. The latter is not shown in *Fig 15* because of its very low values for both preparation methods. Lactose is always present under the form of two peaks and therefore the two would be used.

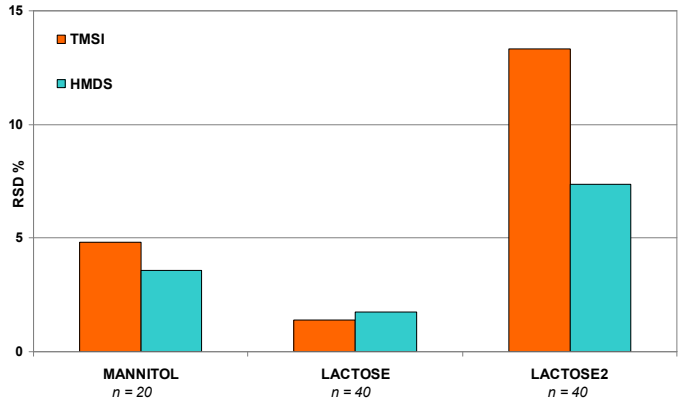


Fig 15 – Comparison of RSD values between TMSI and HMDS preparations.

As expected the RSD values would be higher for smaller peaks and very low for the principal peaks. But when comparing the two derivatisations, HMDS appears to perform slightly better – except for the principal peak of lactose where its RSD value is a little higher than the one obtained with TMSI.

3.2.4.2 Yield

Three sugar standards were chosen with regard to their frequency in ecstasy tablets, which is established through statistical evaluation of the ecstasy database created by a collaboration of the IPS and the WD Zürich (Wissenschaftlicher Dienst Zürich). The corresponding evaluation is represented in the graph below (*Fig 16*).

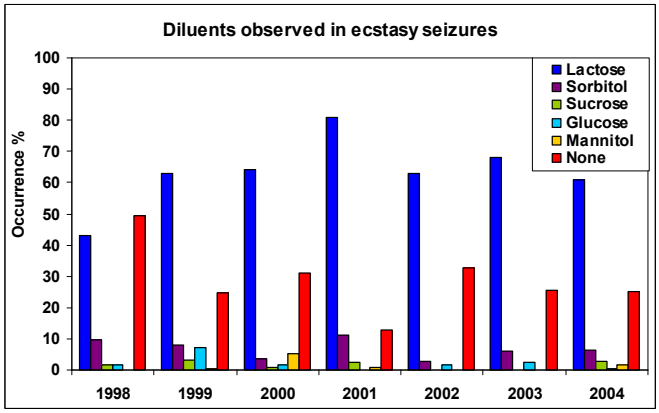


Fig 16 – Extract of statistical evaluation of the ecstasy database of the IPS-WD with regard to diluents

In *Fig 16* it can be seen that lactose is by far the most frequently observed diluent, followed by sorbitol. Sucrose and glucose have been regularly detected, but are altogether rather rare. The frequency of mannitol is principally due to a large seizure in 2000. Considering these statistics, it was decided to take lactose, sorbitol and sucrose (to have one of the rare sugars as well) for the task.

Mixtures of MDMA and the three selected sugars, respectively, were prepared at four different concentrations. The sugar content – 50, 60, 70 and 80% - was fixed with regard to commonly observed MDMA concentrations. As can be seen in *Fig 17* below, the mean value is situated between 25 and 35 %, with a standard deviation giving a range from 10 to 50%.

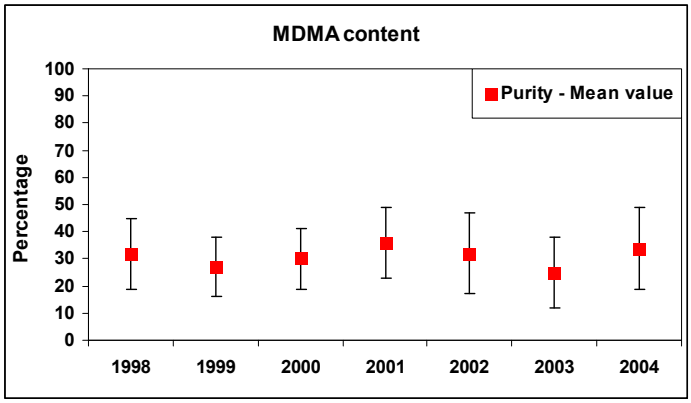


Fig 17 – Representation of mean MDMA concentrations, evaluated from the ecstasy database of the IPS-WD.

The mixtures were homogenised in an agate mortar, derivatised by both methods to investigate and then two weigh-ins per mixture were analysed. In order to be able to compare the two derivatisation methods, the sugar peak areas were first normalised to the concentration (of the mixture in the solution) and then to the internal standard (Heneicosane). The first one was necessary because of the relatively high difference in volume between the TMSI preparation – 0.6ml – and the HMDS preparation – 1.3ml. The second is done to avoid instrumental errors and/or variations and it is commonly applied in comparison between chromatograms. The results are presented in the *Fig 18* to *Fig 20*.

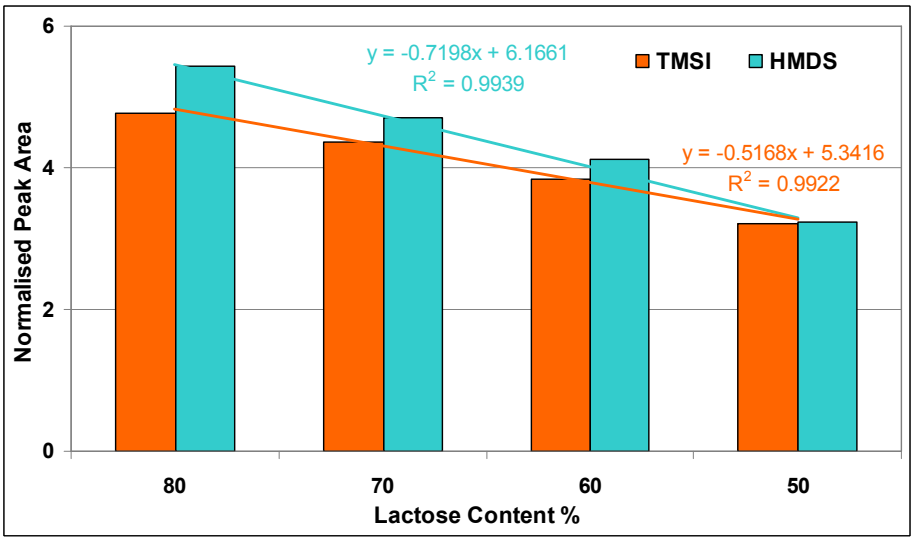


Fig 18 – Comparison for lactose/MDMA mixtures.

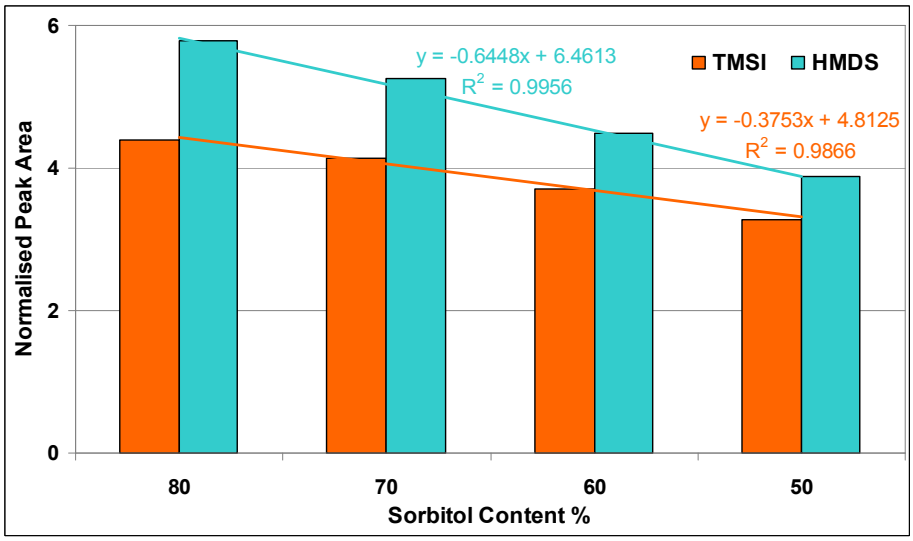


Fig 19 – Comparison for sorbitol/MDMA mixtures.

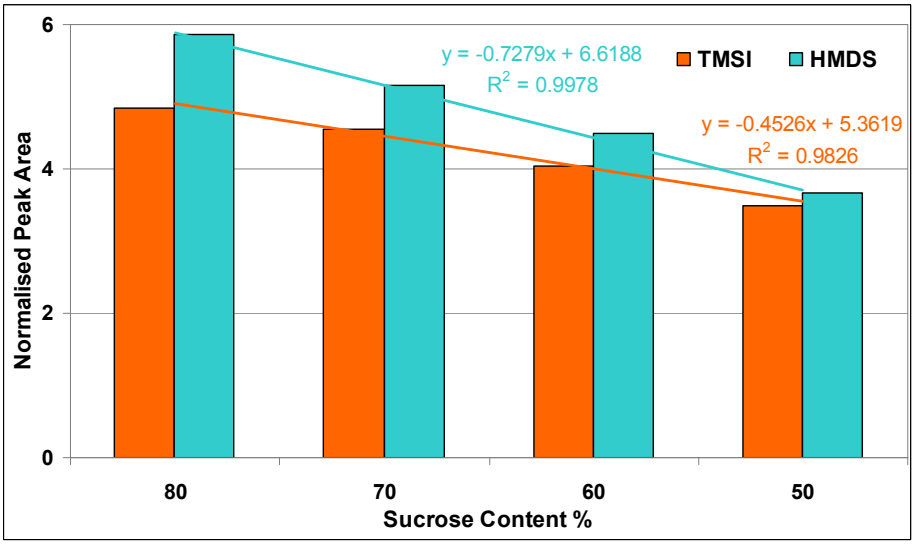


Fig 20 – Comparison for sucrose/MDMA mixtures.

From the graphs above (*Fig 18* to *Fig 20*) it can be seen that the responses are linear for both methods, which indicates that they both work well. But additionally, it appears that the yield obtained with the HMDS preparation is higher for each sugar used.

3.2.5 Conclusion

Considering the results of the two tasks realised for the determination of the derivatisation agent, the method taken from (Sweeley et al, 1963), HMDS combined with TMCS, appears to give optimal results. The relative standard deviation (RSD) for the areas of all the sugar peaks in the examined ecstasy tablets is similar or better than with TMSI derivatisation. Furthermore, yield appeared to be higher as well.

Additionally, the preparation is as easy as for TMSI derivatisation, but much shorter in time since no heating is necessary. It will therefore be applied for the analysis of the ecstasy samples.

2-3mg +	1ml pyridine + 200µl HMDS + 100µl TMCS, 30s vortex, analyse after 5min	(Sweeley et al, 1963)

The instrumental method used with this derivatisation is summarized in *Table 7*

Method	**Sucmod5**
Sample introduction	
– Split	20 : 1
– Volume	1µl
– Total flow	24ml/min
– Injector T	275°C
Gas saver	15ml/min after 2min
Carrier gas	Helium
Column	
– Type	DB1MS, 30m(L) x 0.25mm (i.d.) x 0.25µm (film)
– Mode	Constant flow
– Average velocity	38cm/sec
Oven T-program	175°C (1min), 5°C/min, 180°C (10min), 10°C/min, 250°C (10min), 5°C/min, 270°C (2min)
GC-MS interface T	275°C
MS information	
– Solvent delay	2.5 min
– Mass range	50 – 550 a.m.u
– Sample rate	3, A/D samples 8
– MS quad T	150°C
– MS source T	230°C

Table 7 – Instrumental conditions for the sugar analysis by GC-MS.

3.3 FATTY ACIDS

3.3.1 Introduction

Fatty acids are present in nearly all ecstasy tablets analyzed in our laboratory, so it was only natural to focus research on these substances. But, as it has already been mentioned, their concentration in the tablet mixture is very small, which becomes obvious when considering the chromatogram in *Fig 21*. It is difficult to exploit two peaks of this size – error will increase compared to the high peaks, and other fatty acids entering in the composition of stearates are not detected. Thus, method development has been focused on extraction and eventual concentration of fatty acids.

3.3.2 Choice of the analytical method

The choice of the analytical method has been much easier than in the case of carbohydrates, because there is a sort of consensus that gas chromatography is the technique of choice. However, in general the detector cited in references is the flame ionization detector (FID), whereas in this project a mass spectrometer has been used. This can be explained by the fact that for long time the FID has been much more sensitive and therefore more appropriate for fatty acid analysis. But technical advances made the new generation of mass spectrometers as sensitive.

Fatty acid separation can be realized on non-polar, polar and strongly polar columns. However, it is recommended to use polar columns for better separation of unsaturated fatty acids. Since stearates, used in ecstasy production, are principally composed of saturated fatty acids this point becomes less important and there is no reason not to use non-polar columns such as those employed in our laboratory (DB1).

Instrumental conditions have been taken from (Butte, 1983) as they appeared to give good separation, detecting fatty acids ranging from six to twenty carbons (C8 to C20 - caprylic to arachidic acid) and giving reproducible retention times. Additionally, analysis time is rather short.

3.3.3 Extraction and derivatisation technique

Extraction and derivatisation have to be considered together as preparation methods for fatty acids in generally include both. They are either applied in sequence or simultaneously (in situ).

3.3.3.1 Test sample

Unlike in the method development for carbohydrates no standards have been used in the case of fatty acids. Commercially available standard mixtures appeared to be too expensive for our purpose and were only used for peak identification. Additionally, standards would be inappropriate for extraction tests.

Finally, pure fatty acid standards, which would have been necessary to produce blends similar to ecstasy tablets, tend to be an oily substance making it very difficult to make precise weigh-ins.

In the beginning it was not clear yet how much powder would be needed for effective extraction and research for a test sample aimed at a large ecstasy seizure. A suitable sample was found in a seizure containing thirty tablets, all already scraped or broken for analytical purpose. They were all ground and mixed together resulting in ~7g available "ecstasy powder". The composition is as follows:

Logo	Internal No	Active Substance (%)	Diluent
Hammer & Sickle, "CCCP"	L267	MDEA (45%)	Lactose

An analysis with the screening method for ecstasy proofed that the seizure actually contained fatty acids, presenting the typical peaks of palmitic (C16) and stearic acid (C18) (*Fig 21*).

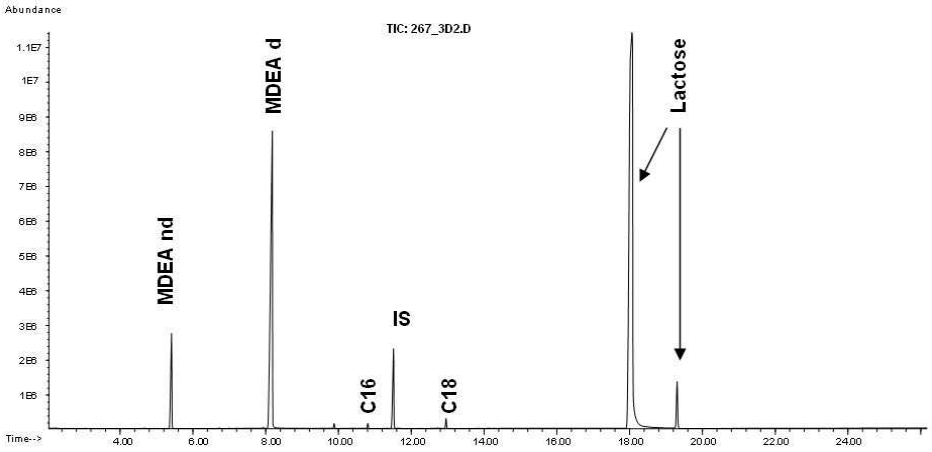


Fig 21 – Test sample 267 analyzed by routine method for ecstasy tablets; *MDEA nd* – MDEA non derivatised, *MDEA d* – MDEA derivatised.

3.3.3.2 Extraction followed by derivatisation

The approach was to proceed to a liquid/liquid extraction of the fatty acids first and then to derivatise with MSTFA – a derivatisation agent used for routine analysis in our laboratory. The procedure generally applied goes as follows :

The powder was put in solution with 1ml of acid buffer (NaH_2PO_4 / H_3PO_4; 0.1 M). After dissolution a volume of 200µl of organic solvent was added, shaken and let to rest. The organic phase was removed and evaporated under a nitrogen stream. Then 200µl of a chloroform/pyridine (5:1) solution and 100µl of MSTFA were added. The solution was heated for 60min at 85°C before analysis to have derivatisation take place.

Solvents tested

According to the solubility of fatty acids and the solubility of the solvent in water, three have been tested: hexane, heptane and dichloromethane. In the beginning they were simply tested for their aptitude to dissolve magnesium stearate and stearic acid standards, and their inaptitude to dissolve MDMA standard (the only one available in sufficient quantity) by putting a few mg of relevant standard in about 1ml organic solvent.

Since all three appeared to be appropriate, they were tested for an extraction of the test sample according to the procedure described above. Hexane and heptane are very similar from a chemical point of view and consequently gave similar results. However, it was of no use to keep them both and therefore heptane has been rejected because of its longer evaporation time.

Extraction with dichloromethane gave good results as well, although its evaporation was also longer. But giving a good extraction yield and being a chemical alternative to hexane, it was kept for further investigation.

Acid / base extraction

The major part of the performed extraction tests have been realized with an acid buffer at pH 2.34 (NaH_2PO_4 / H_3PO_4; 0.1 M). The acid buffer seemed to be appropriate because stearates are composed of acids (cf. Ch 2 – Theoretical Part), and the major substances (active substance and sugars) should dissolve too, since the buffer is aqueous and regularly used for ecstasy quantification. The results were indeed very satisfying because extraction of fatty acids alone could be achieved and their detection by GC improved.

However, the alternative of basic extraction was tested and the procedure was taken from (Palhol et al, 2002). Even though the paper deals with impurity profiling of MDMA, the very good detection of fatty acids in their chromatograms made us try their method on the test sample. The buffer used in this test was a carbonate buffer of pH 10 (K_2CO_3 / $KHCO_3$; 0.5M). Both solvents, hexane and dichloromethane, were applied for this extraction. An example of the resulting chromatograms is shown in *Fig 22*.

The basic extraction was tested three times – twice using the amount of powder recommended in the paper (150mg), and the third time using only 25mg. The chromatograms shown below represent the result of the first test, as indeed for the other two extractions only traces of fatty acids could be detected. There is no explanation why the second and the third time, the extraction did not work. However, even considering the first result alone, extraction might have worked very well, but the MDEA peaks are terribly large and fatty acids could be hidden by these or other peaks of impurities/by-products. Hence the basic extraction has been abandoned.

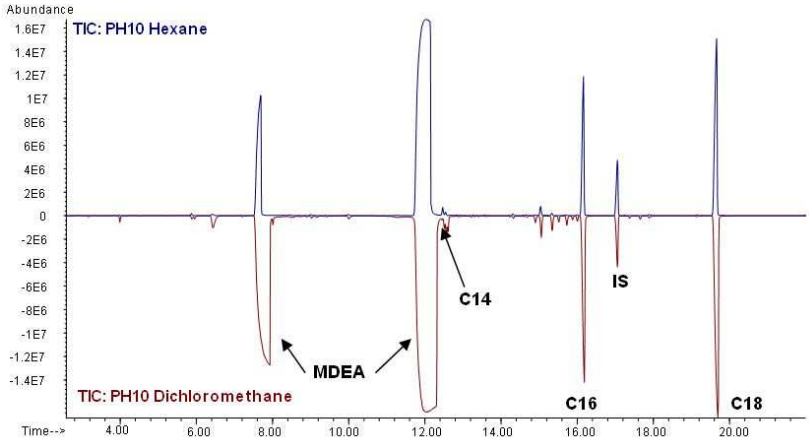


Fig 22 – Chromatograms after hexane (blue) and dichloromethane (red) extraction in alkaline conditions.

Sample amount

For the first tests an amount of 25mg of test sample was judged to be sufficient – considering that the usual amount of stearates in ecstasy mixtures is about 3%, the final concentration would be of more than 1mg/ml which is sufficient to be detected by GC analysis.

However it was decided to test higher quantities of powder in order to verify if new peaks would result. Additionally, linearity of responses could be checked. Thus, extractions have been performed with amounts of 25, 50 and 100mg.

No new peaks have been detected compared to the analysis with the amount of 25mg. As for linearity of the responses, the peak areas of palmitic and stearic acids have been normalized to the sum of areas and the results are represented in the *Fig 23*.

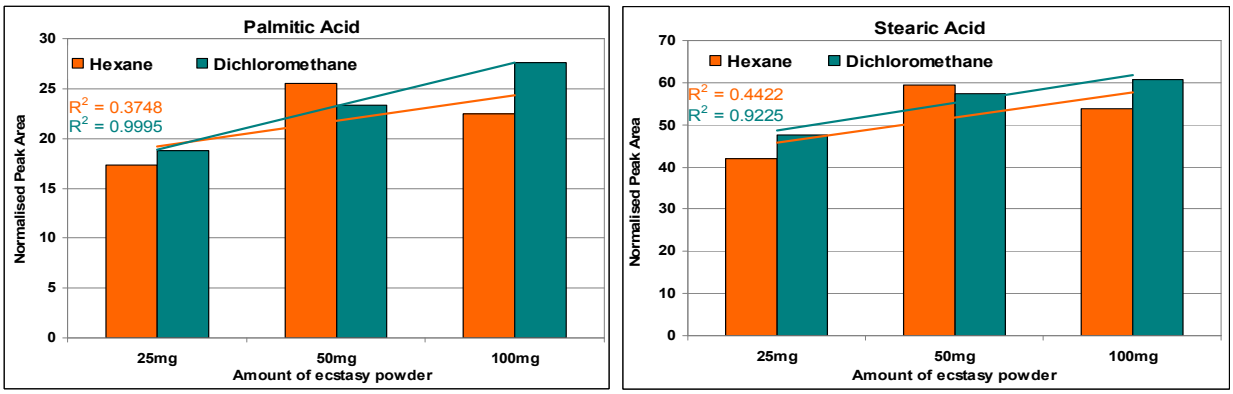


Fig 23 – Graphic representation of C16 (palmitic acid) and C18 (Stearic acid) responses in function of the amount of ecstasy powder for hexane and dichloromethane preparations.

Obviously, there is an irregular response for hexane extraction, whereas dichloromethane extraction is almost linear and gives, as it seems for the amount of 25 and 100mg, also a higher yield.

Thus, the first conclusion to draw from these observations is that an amount of 25mg seems to be sufficient for the extraction of fatty acids, and this for both solvents tested. But then, irregularities have been observed with regard to hexane extraction and yield seems to be better with dichloromethane as well.

Elimination of evaporation step

Every additional step in an analytical method will increase the error in the results. In the procedure presented in the beginning, the aim of the evaporation was to remove the whole organic phase in order to obtain maximum yield of fatty acids. However, the removal of the organic phase is never complete and this is a certain source of error.

As in the end a standardization of the procedure in view of quantitative application was aimed at, this source of error had to be minimized. One possibility was to fix the volume organic phase to remove, to skip evaporation and to add the derivatisation agent directly to the removed solvent. For the dichloromethane extraction this modification was also desirable because the evaporation time is much longer than with hexane.

For comparison of the results before and after the modification of the procedure, three replicas have been extracted and analyzed. The peak areas of palmitic and stearic acid have been normalized to the sum of areas, including the internal standard, and the RSD has been calculated and is represented in *Fig 24*.

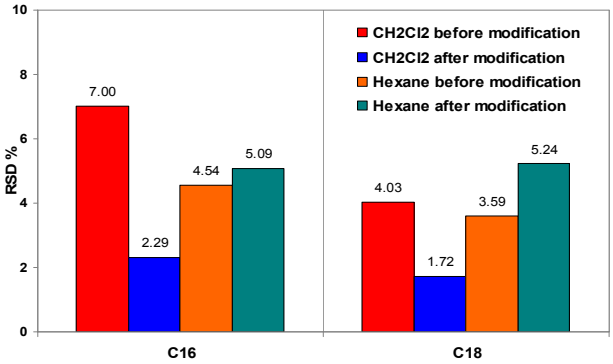


Fig 24 – Comparison of RSD values for hexane and dichloromethane preparations before and after modification of the procedure.

It can be seen that the improvement for the dichloromethane extraction has been significant. On the other hand, for hexane the results were similar or worse. An explanation could be that hexane forms with the

acid buffer two neatly separated phases, where it is much easier to remove the whole volume of organic phase without producing a great error. The separation of the dichloromethane and aqueous phase is by far not as neat and the organic solvent showed tendency of forming bubbles. Additionally, the evaporation was not only long but maybe also incomplete as the appearance after evaporation was very different from that of hexane after evaporation and seemed to still contain solvent. Thus the realized modification would have much more influence on the dichloromethane extraction.

The fact of adding the derivatisation agent directly to the extraction solvent did not have a negative influence on the results and took place just as with the chloroform/pyridine solution.

Preliminary conclusion

The application of liquid/liquid extraction followed by derivatisation is a suitable preparation method for fatty acid analysis in ecstasy tablets. A chromatogram after dichloromethane extraction is shown in *Fig 25*.

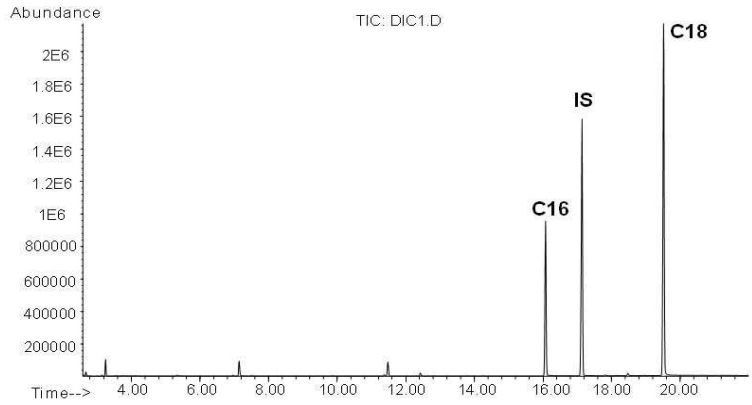


Fig 25 – Chromatogram of the test sample after dichloromethane preparation.

The various observations resulting from the realized experiments are represented in *Table 8*.

Solvent	**Advantages**	**Disadvantages**
Dichloromethane	- good yield - small RSD - works without evaporation step	- organic phase is under aqueous phase and has tendency of forming bubbles - leaks from pipette - traces of MDEA impurities
Hexane	- very good separation of the two phases - fast evaporation - extracts exclusively fatty acids	- yield slightly lower - higher RSD (though in acceptable limits) - performs worse without evaporation step

Table 8 – Advantages and drawbacks of dichloromethane and hexane preparation.

Considering the various points noted in the table above, preference was given to dichloromethane extraction. Its advantages with regard to yield, reduced RSD and decrease of preparation steps were valued as more important than the advantages for hexane. And the negative observations made with dichloromethane were acceptable as they did not seem to significantly influence the performance of the extraction.

3.3.3.3 Derivatisation followed by extraction

The principle is based on the in-situ transesterification, which means that saponification (separation from the glycerides) and esterification (formation of FAME – Fatty Acid Methyl Ester) take place in one solution. The FAMEs have to be extracted afterwards for GC analysis.

Official method

The author had the possibility to dispose of the infrastructure and knowledge of the Laboratoire Cantonal of Lausanne. This laboratory is in charge of food control in the canton of Vaud and has been accredited. The aim of the stay there was to test their dosage of fatty acids by applying it to ecstasy tablets.

The procedure is based on the base-catalyzed transesterification. Lipids are heated with a solution of sodium methoxide for a few minutes. When no fat is visible anymore bortrifluoride is added and heated for another minute. Finally, the FAMEs are extracted with hexane after having added some sodium chloride and analyzed on a GC-FID.

The tests have been realized with 150mg of ecstasy powder per sample and with standard solutions. Unfortunately, results were incoherent in both cases. First, no peaks at all were detected, and then the standard solutions were tested and gave incoherent results as well, as not all standards were observed. Results got better after a few tests, but were still very irregular in terms of peak height. The person in charge in the laboratory suggested that the procedure is appropriate for glyceride analysis only, in the form of which fatty acids are usually present in food (*Fig 26*). Thus, it might not be suitable for ecstasy where the fatty acids are present as salts, in form of stearates (*Fig 26*).

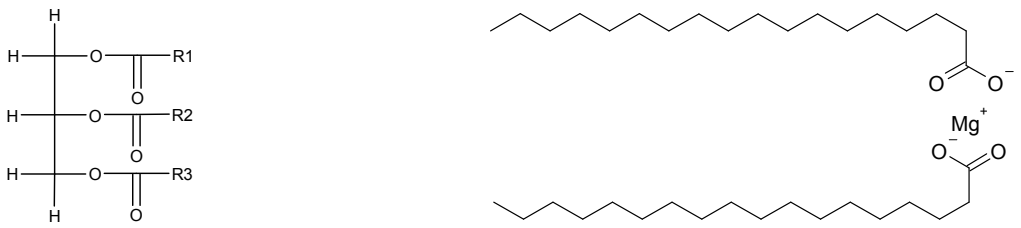


Fig 26 – On the left the schematic structure of a triglyceride, on the right that of Mg stearate is shown, with R1, R2 and R3 representing the alkyl chains.

Indeed, confirmation has been found in literature where often it is pointed out that base-catalyzed transesterification methods might be less suitable for free fatty acids. (Shantha and Napolitano, 1992; Eder, 1995; Carrapiso and Garcia, 2000; Brondz, 2002)

Alternative method

After some research in the relevant literature a method has been found in a study about harbor seals by (Iverson et al, 1997) and has been tested on the test sample. The procedure applied in the study is described hereafter.

The sample is mixed with 1.5ml bortrifluoride (8%) in methanol and 1.5ml hexane, capped under nitrogen and heated for 1h at 100°C. The FAMEs are extracted into hexane, concentrated and analyzed. For the test on the ecstasy sample only one modification has been undertaken – the concentration of bortrifluoride in the used solution was of 14% instead of 8%. The very first test using an 8% solution gave very bad results and so when it was tested again in parallel with another method (Park and Goins, 1994) designed with a 14% BF_3 solution, this one was used for both. Since the results appeared to be much better than expected the concentration of 14% was kept for the ongoing studies. The sample weigh-in proposed in the paper was of over 100mg of lipids. This is a rather high amount, thus smaller weigh-ins of ecstasy powder have been tested – 100, 50 and 25mg.

The results of these first tests were very promising. Indeed, the quality of the chromatograms in terms of yield and number of peaks was very similar to those obtained with the dichloromethane extraction. Additionally, the method appeared to be especially robust – the chromatograms were of good quality even for samples where problems had been encountered during preparation (partial evaporation of hexane in the oven, spilling out of some solution when passing under nitrogen) and one would expect failure of analysis. Therefore it was decided to further investigate this method and to optimize it.

Optimization

Despite the apparent robustness of the method, potential sources of errors had to be minimized. The greatest risk in the procedure consisted in the evaporation of the solvent mixture in the oven. Thus, the material had to be adapted and stabilized (the vials tended to jump out of the rack).

To reduce the risk of evaporation, crimp seal vials (*Fig 27*) have been tried as they are routinely used with volatile solvents in sample preparations and they are known to withstand heat. Unfortunately, only the volume of 1.5ml was available and tests with adapted volume sizes did not give satisfying results. Another problem was the fact that the crimp seals had to be removed for the continuation of the procedure and this is a frequent source of accidents.

Fig 27 – On the left: Crimp Seals with PTFE/Rubber Septa; On the right : green phenolic PTFE liner screw caps.

Therefore, research of appropriate material focused on screw top vials of sufficient size (4ml). It was known that our Fire Investigation group used this type of vials (but with a smaller volume) for their CS_2 desorption procedure – these vials and caps are therefore especially adapted for very volatile solvents and to withstand heat. The same sort of vials and caps could be found with sufficient volume size and were tested – caps are shown in *Fig 27* on the right. The results were much more satisfying and these vials and corresponding caps were chosen for further work.

Care was taken when closing the vials before putting them in the oven and the fixation between two racks assured their stability during heating. Now that the more technical problems were fixed, the extraction procedure had to be standardized in view of quantitative application. The hexane phase containing the FAME consisted in more than 1ml and would have to be completely removed and evaporated under nitrogen. The remaining FAMEs were recovered with 200µl of hexane also containing an internal standard.

No further problems have been observed when applying the procedure after the modifications, and besides the good yield, additional peaks of fatty acids could be detected (myristic and arachidic acid).

Sample amount

Like for the liquid/liquid extraction procedure the necessary sample amount was verified, particularly in view of the smaller peaks detected. Linearity of results had already been observed with the first tests applying the bortrifluoride procedure, but it was checked again after some modifications. Analyses have been performed with sample amounts of 10, 20, 30, 40 and 50 mg of ecstasy powder. For a better representation of the results, peak areas have been normalized to the sum of areas of all detected fatty acids. And the ratios of myristic and arachidic acid have been multiplied by 100 because their peaks are much smaller than those of palmitic and stearic acid.

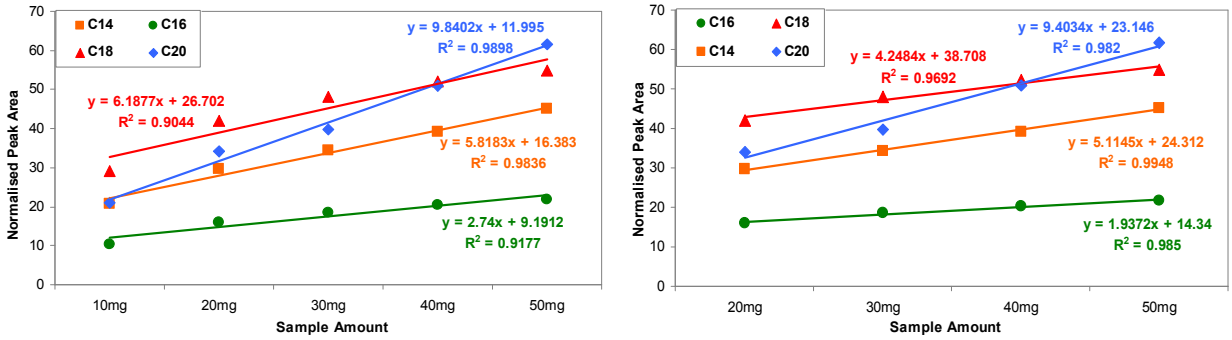


Fig 28 – Responses of myristic (C14), palmitic (C16), stearic (C18) and arachidic (C20) acids in function of the sample amount analyzed.

All four peaks have been detected from a sample amount of 10mg. However, linearity is not as good as expected especially for palmitic and stearic acid – the higher peaks. Surprisingly the responses of the smaller peaks were more linear. On the other side, it can be seen on the graph (*Fig 28*) that it is the response for 10mg which has a negative influence on the linearity (excepted for arachidic acid). Indeed, when removing these responses linearity is improved (*Fig 28*).

Considering these results a sample amount below 20mg does not seem appropriate. It has to be pointed out as well that with an amount of 30mg margaric acid started to be detected and with 50mg even traces of oleic acids have been observed. However, the aim was to avoid high sample amounts such as 50mg. But to have these peaks being significant, even higher weigh-ins would be necessary. Thus, it was decided to keep the sample amount low and to assure the detection of the four detected acids. A sample amount of 25mg would be applied for the continuation.

3.3.3.4 Comparison of the two resulting methods

Both types of procedure gave surprisingly good results with the test sample, but only one would be applied on a greater selection of ecstasy samples. To have an additional element for comparison, the procedures were carried out with a small selection of ecstasy tablets. The same seizures than for carbohydrates were used, plus two more which did not contain any sugars.

Logo	Internal No	Sample Size	Active Substance (%)	Diluent
Superman	L1310A	18	MDMA (29%), MDEA (3%)	
Elephant	L985B	19	MDMA (26%)	
Peace and Love	L1635	19	MDMA (44%)	Lactose
Flying Bird, Peace	L989	17	MDMA (38%)	Lactose
"S"	L995B	18	MDMA (35%)	Lactose (B)
Rolling Stone's	L985C	18	MDMA (29%)	Sorbitol
Tasmanian Devil	L985A	19	MDMA (21%)	Sorbitol
Sparrow	Z9	15	Amphetamine (7%)	Lactose

One last modification was realized before starting the experiments – the internal standard, heneicosane, was replaced by eicosane. The problem was that with the bortrifluoride method heneicosane used to elute right before stearic acid. Thus, it was aimed to find an alcane eluting between palmitic and stearic acid, but at a certain distance. And eicosane was found to fulfill this purpose for both preparation methods, except that with the dichloromethane extraction it was detected before and not between the two peaks.

Similarly to carbohydrates five tablets have been crushed and homogenized, and two weigh-ins were taken per tablet. After having completed all analyses, results were compared considering the number of fatty acids detected with each method and by evaluating the relative standard deviation for relevant peaks. They were regarded as such when appearing in the major part of the ten analyses. In *Table 9* are listed the RSD values of all detected peaks for both methods.

	Dichloromethane							Bortrifluoride						
	C12	**C14**	**C15**	**C16**	**C17**	**C18**	**C20**	**C12**	**C14**	**C15**	**C16**	**C17**	**C18**	**C20**
L1310A				1.11		0.71		41.33	11.39		5.15	21.97	5.76	3.72
L985B				3.36		4.90		22.99	6.21		5.50	22.95	5.47	8.08
L1635	6.76	5.06		0.87	11.77	0.72		7.86	4.63		1.16	3.14	1.30	6.83
L989		9.04		5.42		5.59			2.89		7.11		6.73	
L995B		11.14		5.75		5.95			5.19	4.44	1.72	0.99	0.81	1.64
L985C		6.67		1.36	7.22	1.90			1.74	5.08	0.81	2.27	0.60	3.69
L985A		7.09		0.76	4.09	0.30		16.32	3.54	4.51	3.09	8.03	1.72	2.56
Z9		12.86		2.59		1.56		11.35	3.68	9.28	3.08	15.99	1.09	1.92

Table 9 – RSD values (%) for all detected peaks after dichloromethane and bortrifluoride preparation.

The first observation which can be made is that the number of peaks detected with the bortrifluoride preparation is much higher. Indeed, when comparing the chromatograms of the same sample prepared by the two methods, bortrifluoride appears to result in a better response.

Additionally, after dichloromethane extraction traces of impurities are visible on the chromatogram and might be mistaken for fatty acids since retention times are sometimes close. This can be seen on *Fig 29* where the analysis of the first weigh-in of ecstasy sample L985A for both methods is represented.

On the other side, it has to be pointed out that the RSD values for some peaks (C12 and C17) are rather high so that the usefulness of these peaks has to be questioned. The error seems to depend strongly on the sample character. This is particularly visible in the case of margaric acid (C17), where for some samples the RSD value is very low and for others very high.

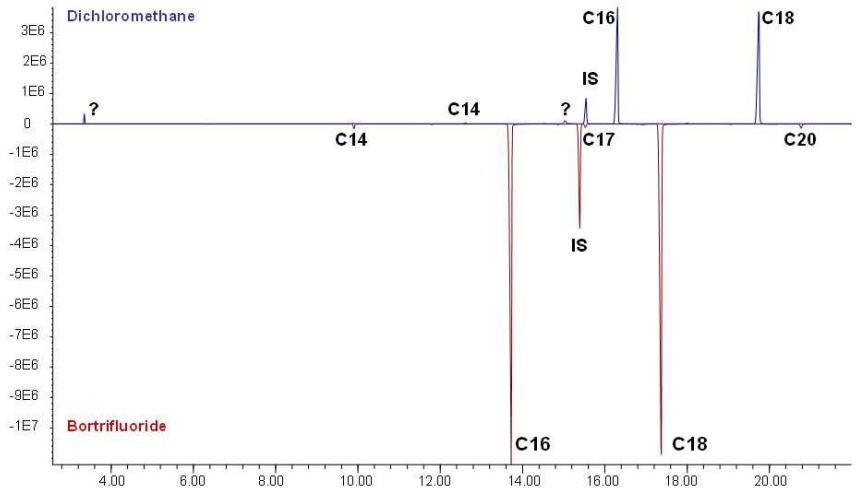


Fig 29 – Chromatograms of the same sample (L985A) after dichloromethane and bortrifluoride preparation.

Comparison of the RSD between the two methods can only be done by considering peaks which are present in both cases. Hence, the fatty acids C14, C16 and C18 have been selected and the means of their RSD values are represented in *Fig 30*.

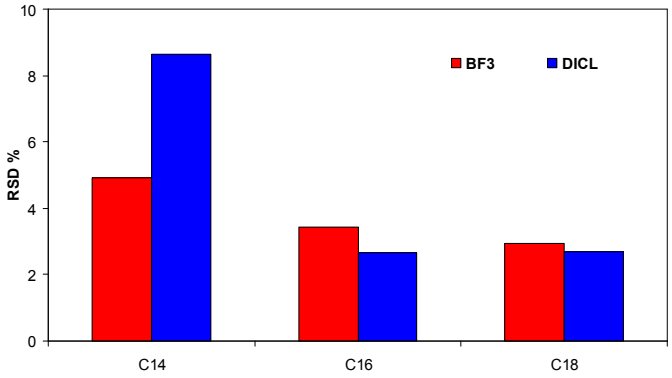


Fig 30 – RSD values (%) for myristic (C14), palmitic (C16) and stearic (C18) acid after dichloromethane and bortrifluoride preparation.

Comparing these RSD values, none of the two methods is clearly better than the other – the values for the bortrifluoride preparation are all below 5% and seem more homogenous, whereas the values for the dichloromethane method are more irregular and that for C14 is much higher than that for bortrifluoride. However, dichloromethane gives slightly better RSD values for palmitic and stearic acid.

Not mentioned until now, it becomes however important to emphasize technical considerations, because these were significant in the determination of the final method to apply. Both procedures are comparable in terms of complexity and duration. In both cases, problems have been met during preparation. But where bortrifluoride showed robustness, dichloromethane appeared to be much more problematic.

The organic phase is removed in the dichloromethane procedure by turning over the vial and piercing the septa of the crimp seal with a syringe. This, in itself, is not practical but can be handled. But it has sometimes been observed that derivatisation did not work, which could only be noticed after GC analysis. The reason for this problem is supposed to be the removal of traces of aqueous phase next to the organic phase.

The dichloromethane phase tends to form bubbles, which impedes this particular preparation step. This tendency could partly be avoided by centrifugation, but for some ecstasy samples it was impossible to get one organic phase instead of bubbles. The result is that out of eighty preparations realized with the dichloromethane method, fourteen could not be used because derivatisation did not work.

3.3.4 Conclusion

Therefore, summarizing the advantages and disadvantages of each method (*Table 10*) it was decided to continue with the bortrifluoride preparation.

Method	**Advantages**	**Disadvantages**
Dichloromethane	- RSD slightly better for C16 and C18 - works without evaporation step	- organic phase is under aqueous phase and has tendency of forming bubbles → derivatisation problem - slightly leaks from syringe - traces of impurities
Bortrifluoride	- very good separation of the two phases - robustness - high yield - more fatty acids detected - extracts exclusively fatty acids	- higher RSD (though in acceptable limits)

Table 10 – Advantages and drawbacks of dichloromethane and bortrifluoride preparations.

The variation of the RSD depending on the peak and ecstasy sample would have to be taken into account. It certainly influences the procedure to adopt and the following exploitation of obtained data. But altogether, the bortrifluoride method for fatty acid derivatisation appears to be an appropriate preparation method for ecstasy analysis.

4 PRACTICAL PART

The optimisation of the analytical method for carbohydrate and fatty acid analysis was an important part of this research. Both methods have to be applied to a greater number of ecstasy tablets. The first reason is the verification of the functionality and suitability of both methods and if the quality of the results remains constant independently of the sample character.

Applied to a larger sample will allow checking of variability between ecstasy tablets (inter-variability) and, because every tablet will be analysed more than once, also the intra-variability. Thus, the resulting observations should allow the assessment of the quality of the analytical method and the characterisation of the ecstasy samples.

A new gas chromatograph was acquired by our laboratory and thus resulted in a change of the quality of the chromatograms, which means, for instance, a more sensitive response for routine ecstasy analysis. Ecstasy tablets analysed on the new GC instrument using the same method than before showed more and higher peaks than on the *old* GC instrument. Therefore, the situation changed with regard to the beginning of the research – fatty acid peaks were not necessarily so small anymore and new peaks of synthetic impurities were detected. This means that the routine method had to be investigated as well. It was important to find out if the increased sensitivity also resulted in useful information suitable for drug profiling.

Therefore it was decided to apply the routine ecstasy method next to the methods developed for carbohydrate and fatty acid analysis. After having analysed the selected ecstasy samples by all three methods, the obtained data would be grouped in data obtained from the ecstasy method and data obtained from the developed methods. The resulting two groups were then compared in terms of quality of information relevant for ecstasy classification.

4.1 SAMPLING

At least a hundred ecstasy seizures – with the eight seizures already used for the method development, were taken for a final selection of 109 seizures. This selection was made considering several criteria which will be explained hereafter.

The most important factor was certainly that the tablets were representative for the illicit drug market, particularly in Switzerland. Representative tablets were first determined by the visual appearance, such as size, colour and logo. This first consideration resulted in the exclusion of all *Thai* tablets (*Fig 31*), known to

be particular as they always present the same small size, characteristic colour (orange and green) and contain methamphetamine adulterated with caffeine and ethyl vanillin (Zingg, 2005).

Fig 31 – Three examples for Thai pills – the two on the left present the very frequent "wy" logo, whereas the one on the right, also making part of this group, presents a "Y", but is for all other characteristics similar.

The regular statistics obtained from the ecstasy database gives a good idea of how a typical ecstasy tablet looks like :

- it is round and the diameter is principally situated around 8mm (*Fig 32*), and weighs between 200 and 300mg (*Fig 32*),

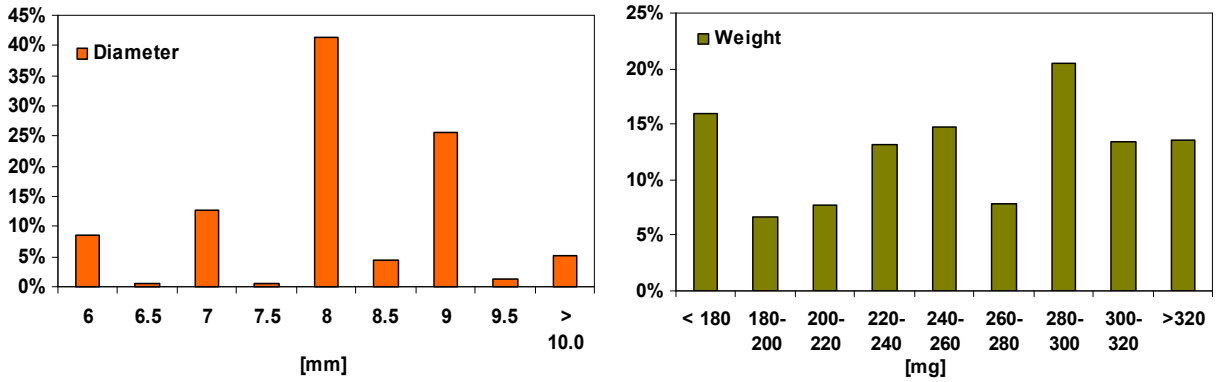


Fig 32 – Statistical evaluation of diameter and weight from ecstasy seizures between 1998 and 2005.

- the colour is variable, but it is not as frequent to observe bright colours as would be expected, a great part of the tablets being white or cream (*Fig 33*),

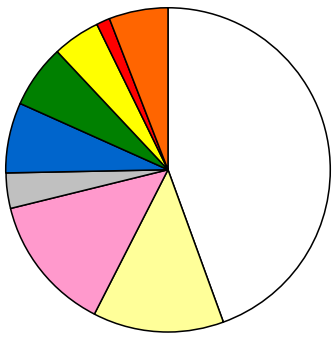


Fig 33 – Colour distribution in ecstasy seizures between 1998 and 2005 – colours correspond to the evaluated categories.

- the logo is very variable as well, but there are a few very popular logos,
- the principal illicit substance is MDMA and the principal diluent is lactose – adulterants are either observed with amphetamine or methamphetamine, but seldom with MDMA. (*Fig 34* and *Fig 35*)

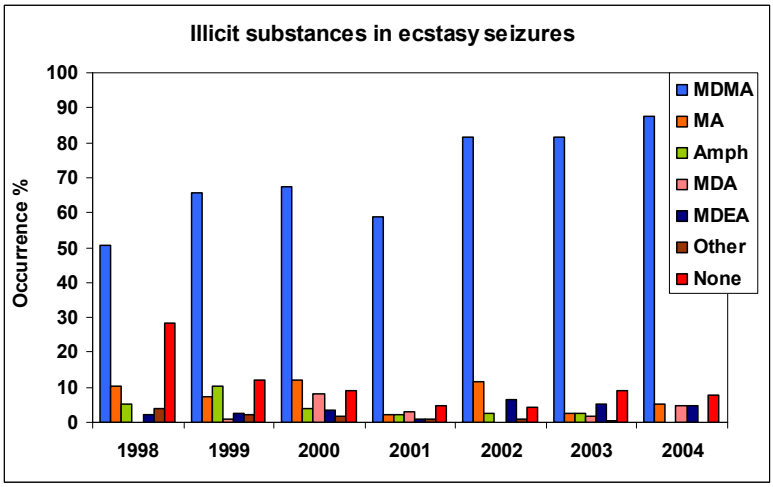


Fig 34 – Graphical representation of the occurrence of illicit substance observed in ecstasy tablets – Ecstasy database (IPS-WD).

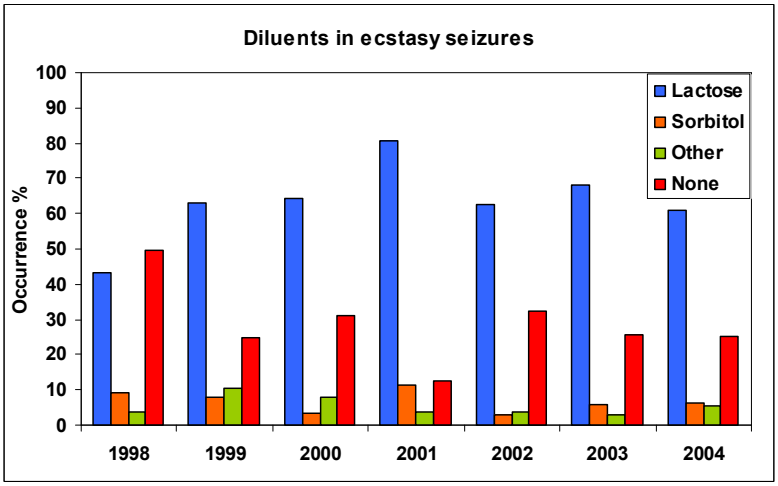


Fig 35 – Graphical representation of the occurrence of diluents in ecstasy tablets – Ecstasy database (IPS-WD).

Thus, it appears that the major part of ecstasy tablets have a diameter of ~8mm, are composed of MDMA and lactose, which is reflected in the selection for this research. Additionally, it was aimed at obtaining groups of several seizures, but being visually similar. This could consist in collecting seizures making part of the same class – a class is composed of tablets having similar physical and chemical characteristics. Otherwise, a popular logo has been chosen, such as Mitsubishi or Superman, and a more important number of seizures was selected by taking care to have similar and dissimilar tablets.

Finally, the last criterion taken into account, but nonetheless important, was the number of tablets available. The possible list was already reduced by the before cited criteria. Additionally, tablets seized

before 1998 were excluded because they were often damaged or incomplete, or simply to avoid the factor of degradation. Furthermore, it was decided only to consider the seizures being composed of at least four tablets. The idea was to use one tablet, but to have fallback possibilities in case problems appeared or additional analyses were necessary, and also to leave some for other ongoing studies at the IPS.

The resulting selection of ecstasy seizures is presented with the relevant details in Appendix VII. It has to be mentioned that the list has been updated during the project, to correct apparent mistakes in the database content.

4.2 SAMPLE PREPARATION AND APPLIED METHODS

One tablet per ecstasy seizure is analysed. The reason is that for the major part of selected ecstasy seizures only a few tablets were available. Another important factor was the time available for analysis, since in average 5 seizures could be analysed per week applying all three methods.

The tablet is ground and homogenised in an agate mortar. Three weigh-ins were taken for each type of analysis and each weigh-in was analysed twice. Thus, six measures were obtained per sample and method. Analyses were performed on an Agilent 6890 gas chromatograph coupled to an Agilent 5973 inert mass spectrometer.

1) Carbohydrate analysis

Sample preparation

About 2mg of ground and homogenised ecstasy powder are dissolved in 1ml pyridine. Then, 200µl of hexamethyldisilazane (HMDS) and 100µl of trimethylchlorosilane (TMCS) are added and the mixture is vortexed for 30 seconds. The solution can be analysed after having rested for 5 minutes.

Instrumental method - The method is called Sucmod5 and is described in *Table 11*.

2) Fatty acid analysis

Sample preparation

About 25mg of the ground and homogenized ecstasy sample are mixed with 1.5ml bortrifluoride (14%) in methanol and 1.5ml hexane and then quickly flushed with nitrogen. The vials are closed tightly and put into the oven for 1h at 100°C. After having taken the samples out of the oven every cap should be

tightened again, as the sudden temperature change seems to loosen them slightly and solvent can easily evaporate.

The hexane phase is entirely transferred into a 1.5ml vial and evaporated under nitrogen. The remaining FAME are recovered with 200µl of hexane containing the internal standard. A 100µl is transferred into a vial with an insert and analysed.

Instrumental method - The method is called AcGras and is described in *Table 11*.

3) Routine analysis

About 2mg of ground and homogenised ecstasy powder are dissolved in 500µl of a chloroform / pyridine (5 :1) solution. Then, 100µl of *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) are added. The vials are sealed and put into the oven for 1h at 85°C. The samples can be analysed after having cooled down to room temperature.

Instrumental method - The method is called XTCIB and is described in *Table 11*.

Method	Sucmod5	AcGras	XTCIB
Sample introduction			
– Split	20 : 1	50 : 1	20 : 1
– Volume	1µl	2µl	2µl
– Total flow	24ml/min	60ml/min	24ml/min
– Injector T	275°C	250°C	290°C
Gas saver	15ml/min after 2min	15ml/min after 2min	15ml/min after 2min
Carrier gas	Helium	Helium	Helium
Column			
– Type	DB1MS, 30m(L) x 0.25mm (i.d.) x 0.25µm (film)	DB1MS, 30m(L) x 0.25mm (i.d.) x 0.25µm (film)	DB1MS, 30m(L) x 0.25mm (i.d.) x 0.25µm (film)
– Mode	Constant flow	Constant flow	Constant flow
– Average velocity	38cm/sec	40cm/sec	38cm/sec
Oven T-program	175°C (1min), 5°C/min, 180°C (10min), 10°C/min, 250°C (10min), 5°C/min, 270°C (2min)	140°C (2min), 5°C/min, 230°C (2min)	150°C, 8°C/min, 250°C, 6°C/min, 320°C
GC-MS interface T	275°C	280°C	280°C
MS information			
– Solvent delay	2.5 min	2.5 min	2.5 min
– Mass range	50 – 550 a.m.u	50 – 550 a.m.u	50 – 550 a.m.u
– Sample rate	3, A/D samples 8	3, A/D samples 8	3, A/D samples 8
– MS quad T	150°C	150°C	150°C
– MS source T	230°C	230°C	230°C

Table 11 – Instrumental conditions of the three methods applied to the sample

5 DATA ANALYSIS

5.1 INTRODUCTION

The aim of this research project is to evaluate the potential of the two types of excipients in drug profiling and to attempt a classification of ecstasy tablets. This could be done by visually comparing the chromatograms. However, it would be unsuitable for the comparison of several dozen or hundreds of samples. Especially, when the results to compare are of a multivariate nature an oversight is all too easy.

The development of advanced chemical instruments and processes has led to a need for advanced methods to analyse the resulting data. (Hopke, 2003) Chemometrics has been developed for this purpose. It is the application of statistical and mathematical methods to chemical problems to permit maximal collection and extraction of useful information.

Data analysis by means of chemometric methods helps to evaluate large and complex data. It uses a characteristic of the samples (e.g. peak heights or areas of the chromatogram) for comparison to other samples. The process can be automated by using macros edited on Visual Basic Editor® and/or particular software. Additionally, data analysis may allow visualising similarities and differences between samples, necessary for sample characterisation and classification.

5.1.1 Purpose

Data analysis will not only allow the characterisation of the samples, followed by their classification, but also the comparison of the potential of the two excipients with the information resulting from routine ecstasy analysis. To do so, comparison procedures were evaluated in function of these two data sets, which will be called XTC and FA/sugars. A suitable comparison method was determined for each data set and was used to form groups of *linked* ecstasy seizures. The notion of *link* would have to be explained at that moment according to the information available (cf. Ch - 6). The groups were compared to the already existing classes defined by Zingg, and then to each other (XTC versus FA/sugars).

The results of sugar and fatty acid analysis were put together, as this was easier to handle. Considering that sugar analysis results mainly in one large peak often accompanied by one very small peak (lactose and lactose2, sorbitol and mannitol), this data was considered to be insufficient for appropriate interpretation on its own and might give an erroneous characterisation. The use of so few variables was estimated to be risky, since, in general, data analysis starts with the maximum of variables which can be reduced later. The grouping of the two types of data was judged to be safe as they are situated on the

same level of analysis, being qualitative and semi-quantitative and with the resulting information of the same nature.

5.1.2 Procedure

For each data set an optimal comparison method of samples has been determined, which would allow a good characterisation of linked samples and a good separation of linked and non-linked samples. Thus, for the determination of the comparison method it was important to define what was considered as linked or non-linked.

Linked samples are supposed to have the same origin – ideally this would mean the same compression batch. But this could also mean the same producer and not necessarily same compression batch if the same excipient blend was used for different compressions. Therefore, linked would in our case signify same origin at the level of compression of the tablets. Consequently, non-linked is situated at the same level, but does not necessarily mean different producers. The same producer could use different blends and produce different tablets.

However, this sort of considerations is too specific when it comes to define data which will be used for the determination of the comparison method. The information necessary to make groups of linked samples and non-linked samples is simply not available in our case, and generally seldom known when dealing with illicit products. The major problem in this research was the absence of large amounts of ecstasy tablets whose common origin was proven. Only small seizures were available and their origin was not necessarily clear. Therefore, other ways had to be found to define a linked and a non-linked sample group.

Finally, similarity was measured between the linked samples and dissimilarity between the non-linked samples by various combinations of pre-treatments and comparison methods. These combinations were then compared by considering several criteria such as false positives and negatives, overlapping between the two groups, etc. In the end, two combinations were determined for the comparison of samples analysed by the XTC method, and also two for those analysed by the FA/sugar methods.

5.1.2.1 Sampling

For the linked sample group, the ten measures obtained from five tablets for each of the eight seizures used for the method development and the six replicas of the remaining 101 seizures (three weigh-ins analysed twice) were taken. This was also a way to check the homogeneity of the ecstasy tablets. However, it has to be emphasised that although some tablets presented considerable variations, the so defined linked group is an ideal case, as the resulting similarity values rather represent homogeneity than intra-variability. In reality the variation inside one compression batch would certainly be higher.

Criteria had to be defined to decide on non-linked ecstasy samples. The problem was that it was impossible to be sure if two samples were really of different origin or not for the same reason expressed above (illicit products – absence of control). This could result in an arbitrary choice but the applied logic seems to guarantee a reasonable selection of probably non-linked samples.

For data analysis it was necessary to have as much similarity values as possible in order to obtain a good representation of the discrimination power of the several comparison methods. A large selection of non-linked samples is very difficult in our case. The information available for the major part of the samples were administrative data, physical and chemical characteristics. All tablets being round, the samples were first sorted out in function of the diameter and the logo. Therefore, the diameter certainly was an important criterion because it is a variable showing very small variation for one press and small differences can already be significant. Concerning the logo it has to be added that in fact the whole visual appearance has been considered – picture, defaults, colour, stains, breakline – as these were, when observed all together, peculiar to a compression batch.

Another very important criterion was the chemical composition – from previous screening analyses (confirmed by the actual XTC method) the active substance and its percentage was known, as well as the presence of sugars. This allowed further differentiation in subgroups. Finally, there were cases where the thickness made the difference by being particularly great and in other doubtful cases it was the date and place of seizure giving helpful information. Considering two seizures made at an interval of 2-3 years in two different cities, the chance that they have a common origin is much smaller than for two seizures being made in the same year in the same city. This is an added subjective appreciation only taken into account after all other criteria.

On the basis of these several characteristics considered together it was possible to select 43 ecstasy tablets constituting the non-linked sample group. The sample list containing all details can be found in Appendix VIII.

5.1.2.2 Data preparation

This task requires some more detailed explanation since it had a strong influence on the determination of the comparison method. In the beginning, the whole procedure – from pre-treatment selection until evaluation of similarity measures by histogram representation – has been carried out with all sample areas such as they were obtained after analysis. But the final visual representation of the results made problems appear with regard to the used data (*Fig 36*).

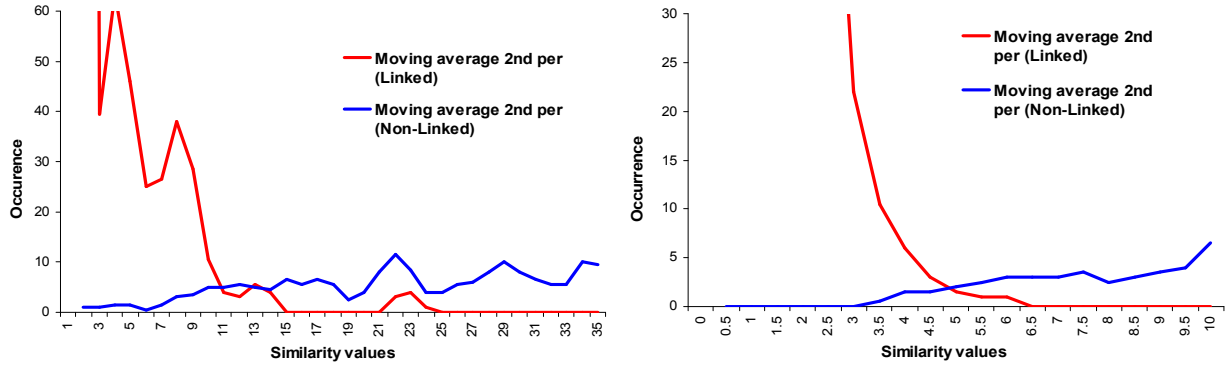


Fig 36 – Histogram representation of the final results before data cleaning (left) and after (right).

Histogram representations showed large overlapping of linked and non-linked samples with values for linked samples that were unusually high. Curve shapes, such as represented in *Fig 36* on the left, were often observed. The irregular shape of the red curve is a strong indicator for erroneous data, particularly the fact that it crosses twice or more the blue curve. After these observations were made, skimming through individual sample values made appear sometimes very high standard deviations. Data cleaning was required, as the sample size is not large enough that such variations could be considered as representation of a real situation, and most of the variations were due to analytical problems.

Thus, it was decided to have a closer look at all samples and to detect those with high standard deviation. If the latter could be explained by some technical reason (instrumental problem, incorrect integration, preparation problem, etc.) the concerned measure has been discarded. If no explanation could be found for the high variation, then it was attributed to the inhomogeneity and the sample was kept.

To make this data verification a bit easier, the pre-treated variables of the questionable samples have been visualised by using the software The Unscrambler©. If observing some irregularities due to a particular measure, the observation has been compared to the results obtained after similarity measures, and the chromatograms of the six replicas have been checked. The decision about discarding a measure or not was taken after having considered all three. An example is given in *Fig 37* and *Fig 38* with the results of sample n° 1668, where it clearly appears that the high variation is caused by the third weigh-in.

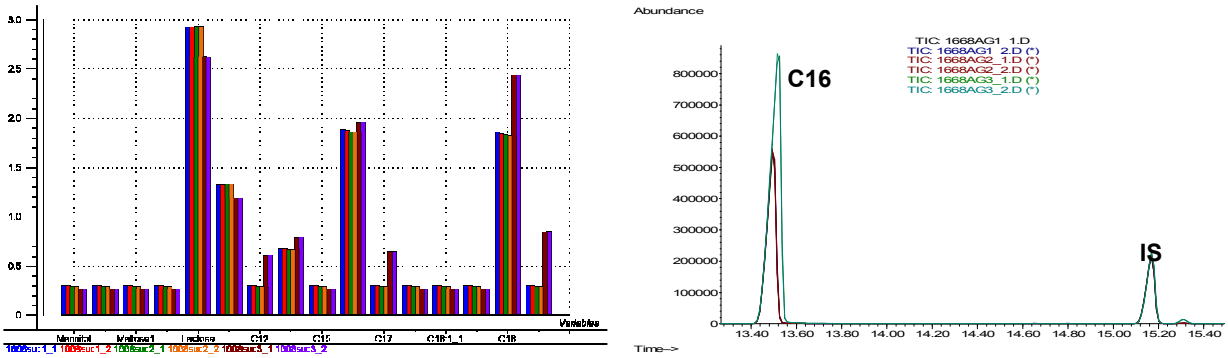


Fig 37 – Visualisation of the pre-treated data of sample 1668 by The Unscrambler© (left) – extract of the fatty acid chromatogram (right).

	1668suc1_1	1668suc1_2	1668suc2_1	1668suc2_2	1668suc3_1	1668suc3_2
1668suc1_1	0	0.00129625	0.0059858	0.00762398	4.6425668	4.70293298
1668suc1_2	16	0	0.00198779	0.00281249	4.72900353	4.79010334
1668suc2_1	16	16	0	0.00021559	4.85309284	4.91526105
1668suc2_2	16	16	16	0	4.87944101	4.94191159
1668suc3_1	16	16	16	16	0	0.00052426
1668suc3_2	16	16	16	16	16	0

Fig 38 – Correlation values obtained with the squared cosine function.

Additionally, the number of variables has been reduced in both data sets. For the XTC method five peaks (the non-derivatised MDMA, MDEA and MDA, a second unknown peak, and myristic acid) and for the FA/sugar method two peaks (two maltose peaks) have been discarded. All these peaks were only present in trace levels or in irregular quantities and it appeared that they induced a great variation in the intravariability values.

The consequence was that the whole procedure had to be started again with the two cleaned data sets in order to obtain coherent results. The drawback is naturally that the latter will represent an ideal situation, which can be very different from reality.

5.2 DETERMINATION OF A COMPARISON METHOD

All comparisons of data will be realised with the relevant peak areas obtained by the three analytical methods. The applied procedure was largely inspired by the work realised in the SMT project about amphetamine profiling (SMT, 2003; Lock, 2005), since it gives a good overview of commonly used math treatments for the comparison of analytical results. The same pre-treatments and comparison methods have been tested. Additionally, profiling methods applied in our laboratory and projects for heroin and cocaine sample comparison were considered (INTERREG, 2006; Esseiva and Guéniat, 2005; Esseiva, 2004).

5.2.1 Theoretical introduction to the tested treatments

5.2.1.1 Pre-treatments

Raw data may have a distribution that is not optimal for analysis. Meaningful information might be hidden or affected by noise, instrumental influences, preparation variances, and so on. Samples analysed in the same sequence can eventually be analysed by using the raw data – but those analysed at different times may not, even if preparation is identical and nothing has been changed on the instrumental level.

The pre-treatments are a sort of preparation of the data to make them comparable and reduce noise information and external influences (external to the ecstasy sample). The following four pre-treatments have been tested.

Normalisation

The peak areas were normalised by dividing each one by the sum of areas. For the ecstasy routine method this signifies to divide every target peak by the sum of target peaks in the chromatogram. For the FA/sugar method this means dividing the target peak by the sum of all fatty acid and sugar peaks for one sample.

$$N_i = \frac{x_i}{\sum x}$$

with x_i = Area of peak i

Normalisation is used to get all data in approximately the same scale and to remove variation due to varying sample amounts or instrument detector sensitivity. However, high peaks may reduce influence of the smaller peaks. This can be avoided by using an additional pre-treatment, e.g. standardisation before or the logarithm or fourth square root after normalisation. (Sauve and Speed, 2004; Lock, 2005)

Standardisation

Data standardisation consists in the division of the target peak area by the standard deviation calculated for the whole sample set for the same peak.

$$S_i = \frac{x_i}{SD_i} \qquad \text{with} \qquad SD_i = \sqrt{\frac{n\sum x_i^2 - \left(\sum x_i\right)^2}{n(n-1)}}$$

with x_i = area of peak i

n = number of samples

SD_i = standard deviation of peak i

Great differences in ranges are eliminated through standardisation and variables are put on the same scale. Therefore, influence is given to all variables (peaks), supposed that the standard deviation is similar for the various peaks. It has to be mentioned that smaller peaks generally produce a higher error in peak areas than the higher peaks, which influences the standard deviation as well.

Logarithm

The logarithm is applied to every peak area. Care has to be taken with missing peaks, as they might be represented by "0" whose logarithm is infinite.

$$L_i = \log x_i$$

with x_i = area of peak i

However, the logarithm is a widely used data transformation and is especially useful to make skewed variables more symmetrical. It reduces the influence of the larger peaks while giving them a higher importance than smaller peaks.

Fourth square root

The fourth square root is calculated for every peak area. It serves a similar purpose as the logarithm and is appropriate in cases of slight asymmetry.

$$R_i = \sqrt[4]{x_i}$$

with x_i = area of peak i

It therefore represents a good alternative to the logarithm in case of data containing zeros, although the influence of higher peaks is not reduced as much as with the logarithm. However, the reduction of the peak's influence might deteriorate the discriminating aspect of the analytical method.

5.2.1.2 Methods for data comparison

In the methods available to compare analytical data, we can distinguish two types of comparison – there are methods which calculate the correlation between data, and there are others which calculate a distance between data. In this research three of each have been tested. The correlation methods used were the Pearson correlation, the Squared Cosine function and the Similarity Index. For the distance methods, the Euclidean distance, the Canberra Index and the Manhattan distance have been tested. Additionally, it appears that the correlation methods highlight the similarities between samples, whereas the distance methods rather highlight the differences.

Pearson correlation

The Pearson correlation coefficient r, is generally a value between -1 and 1 reflecting the degree of correlation between two data sets. A coefficient of 1 would correspond to very strong correlation, a value near 0 to absence of correlation and a coefficient of -1 to negative (inverse) correlation.

$$r_{kl} = \frac{\sum_{j=1}^{n} \left(x_{kj} - \bar{x}_k\right)\left(x_{lj} - \bar{x}_l\right)}{\sqrt{\sum_{j=1}^{n} \left(x_{kj} - \bar{x}_k\right)^2 \sum_{j=1}^{n} \left(x_{lj} - \bar{x}_l\right)^2}}$$

where x_{kj} and x_{lj} are the j[th] peak areas of data sets k and l, respectively, and $\bar{x}_k$ and $\bar{x}_l$ the means of the peaks in data sets k and l. For a better comparison of the different methods, the correlation coefficient has been transformed in order to obtain values between 0 and 100, with 0 corresponding to strong correlation, around 50 to absence of correlation and 100 to negative correlation. (Lock, 2005)

$$Pearson = \frac{(1-r)}{2} \times 100$$

Squared cosine function

The squared cosine function derives from the angle calculation between two vectors determined by the variables (peaks) of two data sets. By developing the equation of the scalar product of the two vectors, the squared cosine of the angle between them can be determined. The correlation value that derives from there is given by the following equation.

$$C = 100 \times \left[\frac{(a_1 b_1 + a_2 b_2 + \ldots + a_n b_n)^2}{(a_1^2 + a_2^2 + \ldots + a_n^2) \times (b_1^2 + b_2^2 + \ldots + b_n^2)} \right]$$

where $a_1, a_2, \ldots a_n$ correspond to the values of the variables 1 to n of chromatogram A and $b_1, b_2, \ldots b_n$ to those of chromatogram B. The correlation value is a number without dimension and auto-normalising. The values obtained are situated between 0 and 100. (Keto, 1989; Esseiva, 2004)

Similarity Index

The similarity index (Karkkainen et al, 1994; Eerola and Lehtonen, 1988) is a metric defined by the following equation

$$SI = \frac{100 \times m}{n} \times \sum_{i=1}^{n} \left(\left(w \times \frac{x_i}{y_i} - k_2 \right)^k - 1 + m \right)^{-1}$$

where k, k_2, m and w are constants and n the number of peaks. The x_i is chosen as the bigger of x_i and y_i which gives $x_i/y_i \geq 1$. The constants were determined by (Eerola and Lehtonen, 1988) and are given the values 6, 0.25, 50 and 1, respectively. The resulting equation is

$$SI = \frac{5000}{n} \times \sum_{i=1}^{n} \left(\left(\frac{x_i}{y_i} - 0.25 \right)^6 - 49 \right)^{-1}$$

The highest value that can be obtained from this formula is 101.67 and is used for the conversion of the distances in values between 0 and 100.

$$D = 100 - \frac{SI}{1.0167}$$

Euclidean and Manhattan Distance

These two distances are popular similarity measures for vector distances (Tanaka et al, 1994; Smolinski et al, 2002; Perkal et al, 1994; Janzen et al, 1992; Avramenko and Kraslawski, 2006). They are special cases of the Minkowski distance which is defined as

$$d_{kl} = \left[\sum_{j=1}^{n} \left| x_{kj} - x_{lj} \right|^{q} \right]^{\frac{1}{q}}$$

where x_{kj} and x_{lj} are the j[th] peak areas of the data sets (or vectors) K and L. If q = 2, then d_{kl} represents the Euclidean distance, whereas for q = 1, it represents the Manhattan distance. The obtained similarity values can range from 0 to infinite. Therefore they are transformed by dividing each value by the highest value obtained and then multiplying with 100.

Canberra Index

Canberra index is a distance where the difference in each variable is weighed by the sum of the two values of the variable. This means that the contribution from each variable / target peak cannot be larger than 1/n. The equation is given below

$$CanberraIndex = \frac{1}{n} \sum_{i=1}^{n} \frac{\left| x_i - y_i \right|}{\left| x_i + y_i \right|}$$

where x_i and y_i are the i[th] peak areas of the data sets X and Y, and n is the number of peaks. In order to get distances between 0 and 100 the obtained values are multiplied by 100. (Lock, 2005)

5.2.2 Selection of pre-treatments

Considering the several pre-treatments and comparison methods presented above, it becomes clear that there are numerous possibilities of combinations which could be tested. This, however, would take considerable time and it has been decided to realise first tests with a smaller sample group in order to limit the number of pre-treatments to use for the whole sample set.

5.2.2.1 Sampling

Linked samples

Concerning the XTC method, eight ecstasy tablets with seven to ten replicas were taken which resulted in 285 similarity values. As for the FA/sugars method, fifteen ecstasy tablets with four to ten replicas were chosen which resulted in 250 similarity values.

The differences of sample size and number can be explained by the fact that some ecstasy tablets presented unacceptable variations. All analytical measures were verified and the data cleaned as sometimes variations were due to analytical problems. This could mean that some analyses did not work well, or peak areas were not correctly integrated, or baseline problems were observed, etc. Some samples showed a high variation that could not be explained by technical problems and was attributed to the inhomogeneity of the tablets. These samples were not taken into account for the determination of the comparison method.

Non-linked samples

The values for the non-linked sample group were calculated by using the means of the replicas of fifteen ecstasy samples considered to be different. In this way, 105 values could be obtained. For the FA/sugar method the same fifteen ecstasy samples than above were used and for the XTC method the eight ecstasies were completed by seven additional tablets so that in the end the same ecstasies were used for both methods.

5.2.2.2 Missing peaks

Considering the nature of the variables, it was important to find a solution to the problem of the missing peaks. The peaks taken into account for the two data sets are presented in *Table 12*.

XTC method	FA/sugar method
MDMA	Mannitol
MDEA	Sorbitol
Amphetamine	Lactose
MDA	Lactose2
Unknown Peak	C12 (Lauric acid)
Caffeine	C14 (Myrsitic acid)
Mannitol	C15 (Pentadecanoic acid)
Sorbitol	C16 (Palmitic acid)
Lactose	C17 (Margaric acid)
Lactose2	C18_2 (Linoelic acid)
C16 (Palmitic acid)	1C18_1 (Oleic acid)
C18 (Stearic acid)	2C18_2 (Oleic acid)
	C18 (Stearic acid)
	C20 (Arachidic acid)

Table 12 – Peaks used for sample comparison.

It appears from *Table 12* that in each sample there will necessarily be some missing peaks, because no ecstasy tablet contains all of the listed substances, at least in the case of the XTC method. When importing the data with an excel macro the missing peaks are simply replaced by 1. In the SMT project

replacements of missing peaks by 0 and by 200 were compared. It was regarded as pointless to use replacements by 1 and by 0, as both were very low compared to a peak area of five numbers upwards. In order to observe influence from missing peaks resulting from replacements, key values from the whole scale were chosen – a zero, an intermediate and a high value. The latter was determined in function of the minimal area integrated by both analytical methods. Thus, the replacement values tested were :

XTC method	− 0, 200 and 1E5
FA/sugars method	− 0, 200, 1E4 and 6E4

Two high values were used for the FA/sugars method because when this part of data analysis was begun the value of 1E4 was considered to be low enough for not mixing with the smallest integrated peaks. Later it was discovered that the areas of the latter were much higher and that 1E4 did not at all constitute an upper limit of noise signal. Of more than 4500 peak areas about ten were below 1E5. Therefore a higher value was added.

5.2.2.3 Tested pre-treatements

Six combinations of pre-treatments have been tested (*Table 13*). Taking into account the replacement of the missing peaks this resulted in the application of sixteen pre-treatments to the XTC data set and twenty to the FA/sugar data set.

Combination	**Given short Name**	**Missing peak replacement**
Normalisation + Fourth square root	N4R	All
Normalisation + Standardisation	NS	All
Normalisation + Logarithm	NL	All, except 0
Standardisation + Normalisation	SN	All
Standardisation + Normalisation + Logarithm	SNL	All, except 0
Standardisation + Normalisation + Fourth square root	SN4R	All

Table 13 – Tested combinations of pre-treatments.

5.2.2.4 Evaluation of the pre-treatments

All six comparison methods have been applied to the various combinations of pre-treatments in order to compare them. The considered criterions were the evaluation of the false positive, the estimate of discrimination and confirmation by PCA analysis. The idea is the reduction of combinations to test on the whole data set and these criteria allow a first selection. They will reveal a tendency of combinations which seem to be appropriate for the application on the given data sets.

False positive

For each combination of pre-treatment and comparison method the highest distance value in the linked sample group has been determined and was set as threshold value. The percentage of false positives has been calculated by counting all values in the non-linked sample group that were below this threshold. The basis for this calculation is given by the notion that a distance between two non-linked samples which is smaller than the distance between two linked samples is a false positive. The results are represented in *Table 14* and *Table 15*. Because the majority of the results was very good and it was decided to highlight the less good results for better visibility.

0	**Pearson**	**Squared Cosinus**	**SI**	**Manhattan**	**Canberra**	**Euclid Dist**
N+4R	2.86	na	>10	>10	>10	>10
N+S	>10	na	>10	>10	>10	>10
S+N	>10	na	>10	>10	>10	>10
S+N+4R	9.52	na	>10	>10	>10	>10
200						
N+4R	0.00	0.00	0.00	0.00	0.00	0.00
N+S	5.71	5.71	1.90	15.24	0.00	20.00
N+L	0.00	0.00	12.38	0.00	na	0.00
S+N	5.71	5.71	1.90	6.67	0.00	9.52
S+N+L	0.00	0.00	0.95	0.00	na	0.00
S+N+4R	0.00	0.00	0.00	0.95	0.00	1.90
1E5						
N+4R	0.00	0.00	0.00	0.00	0.00	0.00
N+S	5.71	5.71	1.90	15.24	0.00	20.00
N+L	0.00	0.00	22.86	0.00	na	0.00
S+N	5.71	5.71	1.90	6.67	0.00	9.52
S+N+L	0.00	0.00	7.62	0.00	na	0.00
S+N+4R	0.00	0.00	0.00	0.00	0.00	0.95

Table 14 – Percentages of false positives for the XTC data set; na – not available (calculation problems due to the value 0).

0	Pearson	Squared Cosine	SI	Manhattan	Canberra	Euclid Dist
N+4R	8.57	na	>10	>10	7.62	5.71
N+S	>10	na	9.52	>10	9.52	>10
S+N	>10	na	9.52	>10	9.52	>10
S+N+4R	>10	na	>10	>10	7.62	9.52
200						
N+4R	0.00	0.00	0.00	0.00	0.00	0.00
N+S	0.00	0.00	0.00	0.00	0.00	0.00
N+L	0.00	0.00	0.00	0.00	na	0.00
S+N	0.00	0.00	0.00	0.00	0.00	0.00
S+N+L	0.95	0.00	2.86	0.00	na	0.00
S+N+4R	0.00	0.00	0.00	0.00	0.00	0.00
1E4						
N+4R	0.00	0.00	0.00	0.00	0.00	0.00
N+S	0.95	0.00	0.00	0.00	0.00	0.95
N+L	0.00	0.00	0.95	0.00	na	0.00
S+N	0.95	0.00	0.00	0.00	0.00	0.00
S+N+L	0.00	0.00	0.00	0.00	na	0.00
S+N+4R	0.00	0.00	0.00	0.00	0.00	0.00
6E4						
N+4R	0.00	0.00	0.00	0.00	0.00	0.00
N+S	0.95	0.00	0.00	0.00	0.00	1.90
N+L	nd	nd	nd	nd	nd	nd
S+N	0.95	0.00	0.00	0.00	0.00	1.90
S+N+L	nd	nd	nd	nd	nd	nd
S+N+4R	0.00	0.00	0.00	0.00	0.00	0.00

Table 15 – Percentages of false positives for the FA/sugars data set; na – not available (calculation problems due to the value 0), nd – not done.

Both methods show relatively similar results. A difference between the two data sets appears in the number of false positives. There are more with the XTC data set than with the FA/sugars data set. However, both show many combinations having a percentage of false positives of 0, which is actually perfect. On the other side, the replacement value of 0 gives very bad results and can be discarded in the two data sets. The pre-treatment combination of N4R happens to be the only one with no false positive at all and therefore represents an interesting choice for further studies.

Finally, the presence of some missing values ("na") has to be explained. The squared cosine function has not been applied with the replacement value 0. Problems have appeared during calculations with this combination and could not be resolved. Therefore, it was decided not to use this correlation method with 0 values.

Problems also appeared when calculating the distance values for the Canberra Index with data that had been pre-treated by the normalisation followed by logarithm. Just for reminding here the basic principle concerning the logarithm :

$$y = \log x \quad \Leftrightarrow \quad 10^y = x$$

It has already been mentioned that the logarithm cannot be applied for $x \leq 0$. It also results from these equations that the logarithm of 1 is 0 and that for $0 < x < 1$ the logarithm will be negative. Thus, if the logarithm is applied to normalised peak areas, generally negative and positive values are obtained. This appeared to be a problem when calculating the Canberra Index with data presenting negative and positive values for the same peak. It has to be noted that the peak areas do not have to be very different – they can be very close, but after normalisation one of them gives a value below and the other above 1, which will result for the first in a negative logarithm and for the second in a positive one.

$$CanberraIndex = \frac{1}{n}\sum_{i=1}^{n}\frac{|x_i - y_i|}{|x_i + y_i|}$$

That may appear to be a small thing, but considering the equation of the Canberra Index, it makes a great difference if x and y are both of the same sign or not. So it happened that for some samples completely aberrant distances have been observed. For that reason, it was decided not to use the Canberra Index with a pre-treatment including the logarithm. And as the values for the missing peak replacement 6E4 were calculated later, the logarithm has not been taken into account at all ("nd").

Estimate of the discrimination

To confirm the results from the evaluation of the false positives, an estimate of the discrimination was calculated for all combinations except those which were already discarded (replacement by 0). The discrimination is calculated with the mean values and the standard deviations of the obtained distances/correlations. A high discrimination value signifies good separation between linked and non-linked samples. (INTERREG, 2006)

$$D = \frac{Mean_{non-linked} - STDEV_{non-linked}}{Mean_{linked} + STDEV_{linked}}$$

The calculation can be represented graphically as shown in *Fig 39*. The higher the value D, the better the separation between the linked and the non-linked zone.

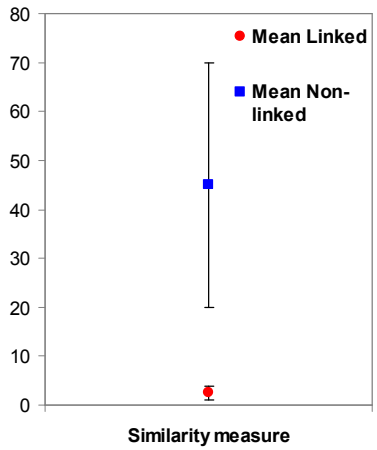


Fig 39 – Graphical representation of the estimate of the discrimination.

The results are represented in *Table 16* and *Table 17* below.

200	Pearson	Squared Cosine	SI	Manhattan	Canberra	Euclid Dist
N+4R	167.77	155.99	112.25	12.07	12.51	12.41
N+S	11.57	11.50	21.52	2.33	6.57	1.69
N+L	759.67	570.29	2.59	17.23	na	21.84
S+N	11.57	11.50	21.52	3.07	6.57	2.24
S+N+L	825.18	589.78	48.43	17.23	na	21.84
S+N+4R	69.99	77.84	112.25	8.78	12.51	8.36
1E5						
N+4R	136.67	121.19	108.49	11.31	10.79	10.98
N+S	11.55	11.42	21.52	2.33	6.57	1.69
N+L	229.87	181.84	1.92	12.07	na	12.74
S+N	11.55	11.42	21.52	3.08	6.57	2.23
S+N+L	259.84	181.20	5.09	12.07	na	12.74
S+N+4R	62.15	56.73	108.53	8.68	10.79	7.57

Table 16 – Discrimination factor for the XTC data set; na – not available (calculation problems due to the value 0).

200	Pearson	Squared Cosine	SI	Manhattan	Canberra	Euclid Dist
N+4R	162.88	139.93	17.84	19.35	14.84	17.06
N+S	45.55	37.55	27.29	8.31	10.12	6.19
N+L	24.37	42.47	10.74	16.31	na	9.29
S+N	45.55	37.55	27.29	8.53	10.12	6.35
S+N+L	14.26	20.66	3.88	16.31	na	9.29
S+N+4R	38.26	25.96	17.84	16.30	14.84	11.63
1E4						
N+4R	257.54	219.32	42.34	17.92	14.47	17.85
N+S	44.76	35.60	26.99	8.31	9.96	6.08
N+L	53.22	93.18	8.86	15.43	na	11.10
S+N	44.76	35.60	26.99	8.53	9.96	6.24
S+N+L	27.12	23.19	10.92	15.43	na	11.10
S+N+4R	57.28	45.22	42.34	14.27	14.47	10.91
6E4						
N+4R	303.22	265.50	126.77	16.94	13.44	18.08
N+S	41.57	31.67	37.71	7.35	9.57	5.30
N+L	nd	nd	nd	nd	nd	nd
S+N	41.57	31.67	37.71	7.55	9.57	5.43
S+N+L	nd	nd	nd	nd	nd	nd
S+N+4R	81.45	64.31	126.77	12.68	13.44	10.50

Table 17 – Discrimination factor for the FA/sugars data set; na – not available (calculation problems due to the value 0), nd – not done.

All values above 100, which is a very good result, were highlighted and marked in red. In the case of the FA/sugar method the results are similar to those obtained with the false positives. For each similarity measure the pre-treatment combination N4R always gives the best results, sometimes equalled by SN4R. For the XTC method the result is not that clear. Discriminations obtained with N4R and SN4R are certainly very good, but the pre-treatments with the logarithm give exceptionally high values for Pearson and Squared cosine correlations and slightly higher values for Manhattan and Euclidean distance.

For better comparison of the XTC and FA/sugars data sets, it was aimed at applying the same pre-treatment to both. The estimate of the discrimination did confirm the results obtained by the false positive evaluation in that sense that the pre-treatment N4R gave altogether very good results for both calculations. Concerning the logarithm, it was considered that the very high discrimination values for the XTC method did not compensate the fact that this pre-treatment could not be applied to one of the similarity measures.

Therefore, preference was given to the pre-treatment N4R and it was decided to evaluate the pre-treatment SN4R with the highest missing peak replacement as well, because it gives very good results for the false positives.

Evaluation by PCA

Principal Component Analysis (PCA) was applied to verify the distribution of the data in function of the pre-treatment and thus to control if the latter gave satisfying results. The principles of PCA are fully explained in a following chapter where its use was of higher importance (cf. Additional Studies - NIR). In short, PCA is a projection method that helps visualise all the information contained in a data table. Here it was applied to the data pre-treated by N4R and SN4R. Results are shown in *Fig 40* and *Fig 41*.

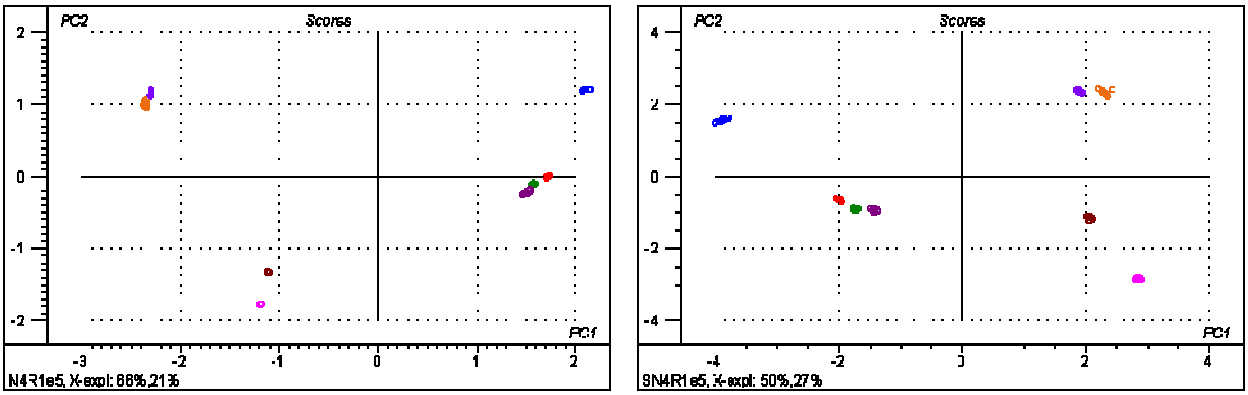


Fig 40 – PCA analysis with the XTC data set pre-treated by N4R_1E5 (left) and SN4R_1E5 (right).

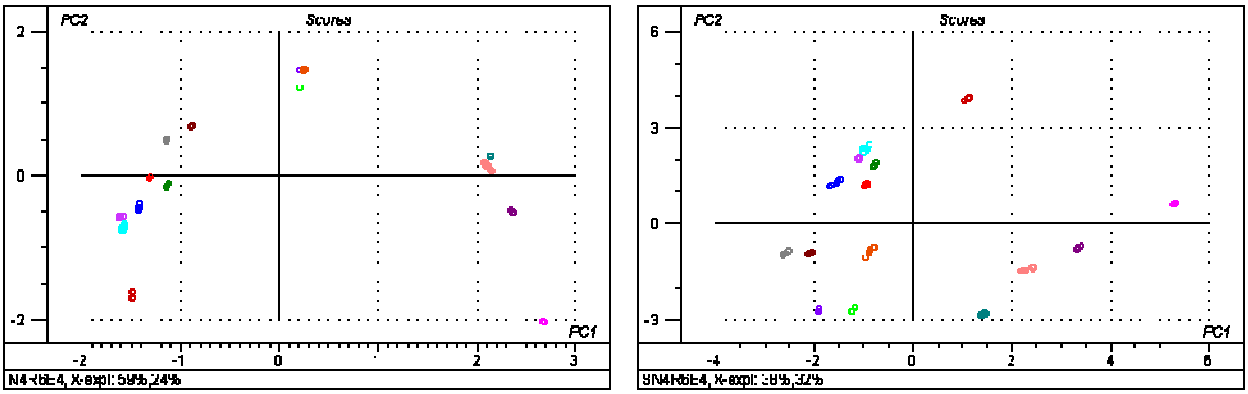


Fig 41 – PCA analysis with the FA/sugars data set pre-treated by N4R_6E4 (left) and SN4R_6E4 (right).

Every colour corresponds to one ecstasy tablet and its replicas meaning eight colours for the XTC data set and fifteen colours for the FA/sugars data set. Therefore, circles of the same colour should be grouped together and be separated of those of a different colour.

The PCA analysis was applied to have visual representation of the good functioning of the chosen pre-treatments and it can be observed that this is the case in all four figures, as sample separation is almost complete.

5.2.2.5 Choice of pre-treatment

Therefore, the pre-treatments that would be applied in combination with the six similarity measures on a larger sample set are the following :

	Pre-treatment	Missing peak replacement
XTC data set	N4R SN4R	200 and 1E5 1E5
FA/sugars data set	N4R SN4R	200, 1E4 and 6E4 6E4

5.2.3 Selection of a comparison method

Now that a selection of pre-treatments has been made, the six available methods for similarity measures had to be compared. The similarity values for linked samples should be obtained from the replicas of all samples analysed (109) and those for the non-linked samples from a maximum selection of ecstasy tablets considered to be of different origin (43).

5.2.3.1 Evaluation of the comparison methods

The selected pre-treatments have each been applied together with the six similarity measures to all samples in order to obtain similarity values for the linked sample group. They have as well been applied to the mean areas of the replicas of the selected non-linked sample group. The number of similarity values obtained is shown *Table 18*.

	Linked Samples		Non-linked
	XTC method	FA/sugars method	samples
Number of similarity values	1642	1422	903

Table 18 – Number of similarity values obtained for each sample set.

The results have been evaluated by representing all values on histograms (Tanaka et al, 1994) and then for each combination the following characteristics have been determined:

- Minima value of the non-linked sample group - Min_{NL}
- Maxima value of the linked sample group - Max_{L}
- Standard deviation of the linked sample group
- False positives and negatives
- Estimate of the discrimination
- Percentage of linked samples below the minima value of the non-linked sample group

The histograms constitute the basis for the comparison because many of these values are just the numerical form of what is represented in the graph. (*Fig 42*)

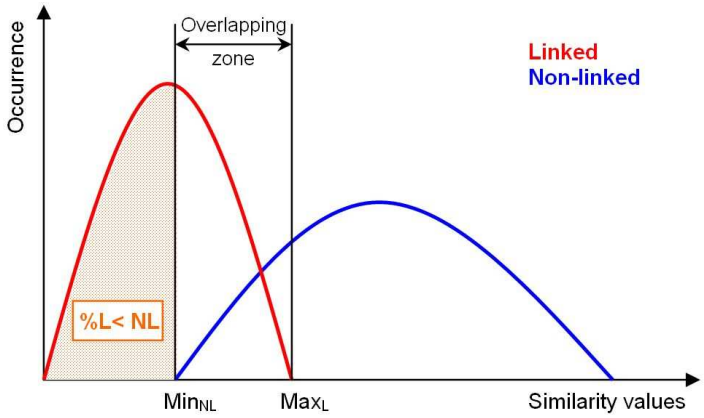


Fig 42 – Schema of a histogram with some comparison criterions.

Concerning the false positives and negatives, two slightly different approaches have been found. The first one has been taken from the SMT project (SMT, 2003) and is represented in *Fig 43* :

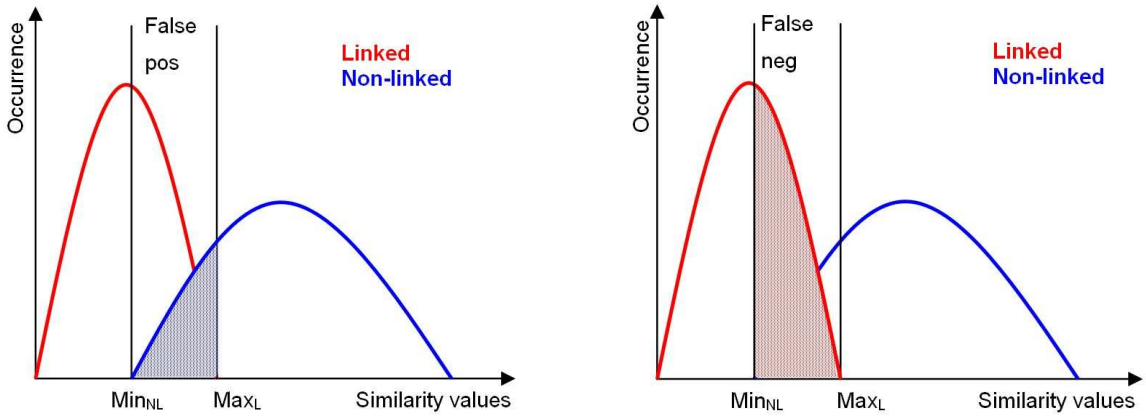


Fig 43 – Calculation of false positives and negatives according to the SMT project.

Considering these graphs the false positives correspond to all non-linked values below the maxima of the linked similarity values, and the false negatives to all linked values above the minima of the non-linked similarity values.

The second approach has been applied in the INTERREG project (INTERREG, 2006) and is represented in *Fig 44*.

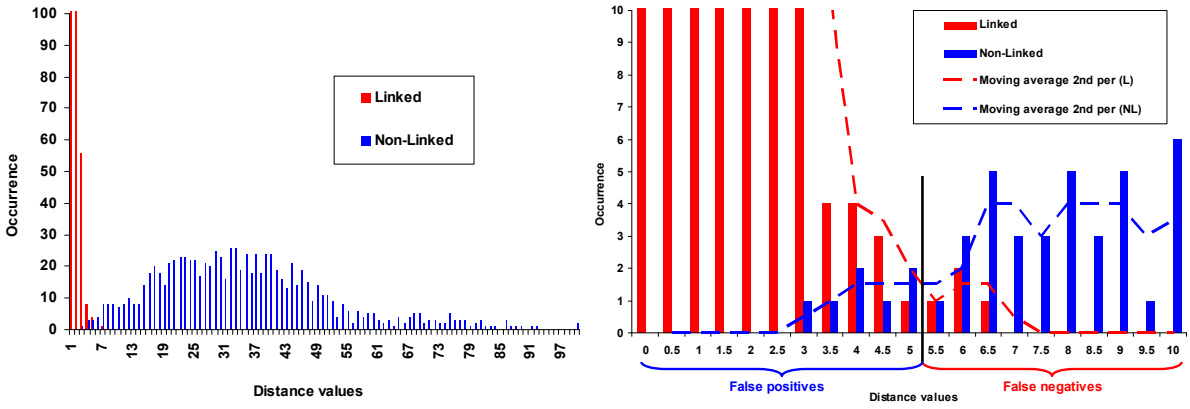


Fig 44 - Histogram for FA/sugars data set obtained after N4R (200) and Manhattan distance.

In this approach, the moving average curves are drawn for linked and non-linked data sets and the similarity value of their intersection is determined. The false positives correspond to all non-linked values below the similarity value of the intersection, and the false negatives to all linked values above the similarity value of the intersection.

It appears that the first approach takes into account the whole overlapping zone whereas in the second approach a cross-value is determined at the intersection of the moving average curves of linked and non-linked samples. Both approaches have been applied in this research and no significant differences have been noticed, except for the false negatives whose level is regularly higher with the first approach.

The final evaluation of the comparison method has been carried out by considering these various criterions under numerical form, represented on the two following pages in *Table 19* and *Table 20*, and by visually comparing the appearance of the histograms, especially the overlapping zone. The latter should be small, not to say absent, and come close to a regular triangular shape. All histograms can be found in Appendix IX.

Concerning the evaluation of the other criteria – the maxima value of the linked sample group (Max_L), the standard deviation of the linked sample group ($STDEV_L$) and the false positives (%FP) and negatives (%FN) should be small. Whereas the minima value of the non-linked sample group (Min_{NL}), the estimate of discrimination (D) and the percentage of linked samples below the minima value of the non-linked sample group (%L<NL) should be high.

Considering the different nature of the similarity measures it was decided to choose one correlation and one distance method per data set. The selected methods are highlighted in the two tables below (*Table 19* and *Table 20*).

Method	Pre-treatment	MinNL	Inter-section	MaxL	STDEV L	SMT		INTERREG		%L<NL	D
						% FP	% FN	% FP	% FN		
Pearson	N4R 200	0.015	0.400	0.364	0.038	1.883	24.361	2.104	0.000	80.88	122.95
	N4R 1E5	0.013	0.450	0.443	0.046	1.772	29.233	2.215	0.000	76.31	92.95
	SN4R 1E5	0.068	0.800	0.950	0.074	1.993	10.840	1.661	0.061	92.45	82.21
Squared Cosine	N4R 200	0.048	0.900	0.845	0.088	1.550	100.000	1.772	0.000	85.93	146.81
	N4R 1E5	0.092	0.900	0.825	0.086	1.772	13.398	1.772	0.000	92.39	100.86
	SN4R 1E5	0.218	1.000	1.371	0.099	2.547	3.837	1.772	0.122	96.41	82.58
Similarity Index	N4R 200	0.155	0.300	0.294	0.036	0.554	2.253	0.775	0.000	97.99	151.62
	N4R 1E5	**0.155**	**0.300**	**0.294**	**0.036**	**0.554**	**2.253**	**0.775**	**0.000**	**97.99**	**138.44**
	SN4R 1E5	0.155	0.300	0.294	0.036	0.554	2.253	0.775	0.000	97.99	138.44
Euclidean Distance	N4R 200	1.353	7.500	7.097	1.014	1.883	31.669	2.215	0.000	78.87	14.74
	N4R 1E5	2.211	8.000	8.010	1.128	1.993	13.642	1.993	0.061	88.86	12.49
	SN4R 1E5	4.104	8.500	10.804	1.282	2.658	4.324	1.218	0.305	95.74	11.04
Manhattan	N4R 200	0.827	4.000	4.357	0.596	1.661	42.875	1.218	0.061	75.64	15.93
	N4R 1E5	2.444	4.500	5.168	0.726	1.329	7.125	0.886	0.183	95.86	13.72
	SN4R 1E5	3.337	5.500	6.531	0.810	1.772	2.862	0.664	0.183	97.81	12.22
Canberra	**N4R 200**	**1.846**	**3.000**	**2.623**	**0.455**	**0.443**	**5.055**	**0.664**	**0.000**	**98.17**	**16.16**
	N4R 1E5	1.846	3.000	2.623	0.454	0.443	5.055	0.664	0.000	98.17	12.99
	SN4R 1E5	1.846	3.000	2.623	0.454	0.443	5.055	0.664	0.000	98.17	12.99

Table 19 – Evaluation of the comparison methods applied to the XTC data set.

Method	Pre-treatment	MinNL	Inter-section	MaxL	STDEV L	SMT		INTERREG		%L<NL	D
						% FP	% FN	% FP	% FN		
Pearson	N4R 200	0.007	0.550	0.481	0.065	0.664	94.726	0.775	0.000	69.06	58.12
	N4R 1E4	0.000	0.350	0.285	0.031	0.554	94.726	0.664	0.000	32.12	105.30
	N4R 6E4	0.013	0.200	0.208	0.019	0.775	94.726	0.775	0.070	76.72	154.07
	SN4R 6E4	0.903	1.600	1.525	0.143	0.664	0.633	0.664	0.000	99.37	65.06
Squared Cosine	N4R 200	0.315	1.400	1.275	0.164	0.664	4.641	0.775	0.000	95.36	65.71
	N4R 1E4	0.313	0.800	0.614	0.067	0.443	2.250	0.443	0.000	98.38	106.93
	N4R 6E4	**0.230**	**0.400**	**0.389**	**0.035**	**0.332**	**0.625**	**0.332**	**0.000**	**99.58**	**140.62**
	SN4R 6E4	0.497	0.900	0.795	0.087	0.443	1.195	0.554	0.000	99.30	59.61
Similarity Index	N4R 200	0.208	10.000	14.396	1.634	7.863	5.556	5.094	0.281	94.59	10.89
	N4R 1E4	0.208	4.000	5.603	0.511	3.987	5.556	1.993	0.281	94.59	20.89
	N4R 6E4	0.208	0.600	0.890	0.054	1.440	1.758	0.664	0.070	98.45	64.57
	SN4R 6E4	0.208	0.600	0.890	0.054	1.440	1.758	0.664	0.070	98.45	64.57
Euclidean Distance	N4R 200	4.304	9.500	8.975	1.411	0.775	4.782	0.886	0.000	95.22	11.80
	N4R 1E4	4.725	7.500	7.577	1.075	0.776	2.180	0.554	0.070	98.31	13.38
	N4R 6E4	**4.567**	**6.500**	**6.756**	**0.900**	**0.664**	**0.555**	**0.443**	**0.139**	**99.51**	**14.45**
	SN4R 6E4	2.798	6.500	6.620	0.848	1.329	5.485	0.997	0.070	95.85	6.94
Manhattan	N4R 200	2.755	5.000	6.132	0.691	1.772	2.321	0.775	0.281	98.59	13.31
	N4R 1E4	3.285	4.500	5.805	0.648	1.330	0.985	0.887	0.211	99.23	13.18
	N4R 6E4	3.453	4.500	5.447	0.637	0.998	1.195	0.443	0.141	99.37	13.21
	SN4R 6E4	2.119	4.500	5.674	0.620	1.883	4.008	0.997	0.141	96.84	7.43
Canberra	N4R 200	2.296	7.500	11.756	1.243	1.440	6.048	0.886	0.141	94.51	10.88
	N4R 1E4	2.296	6.000	6.776	0.736	1.661	6.329	0.997	0.141	94.73	11.28
	N4R 6E4	2.296	4.000	3.979	0.521	0.775	2.461	0.775	0.000	98.45	11.20
	SN4R 6E4	2.296	4.000	3.979	0.521	0.775	2.461	0.775	0.000	98.45	11.20

Table 20 – Evaluation of the comparison methods applied to the FA/sugars data set.

The choice of the final comparison methods is not necessarily obvious, as there was no method superior to all others. The selection is built on a compromise between the numerical values and the appreciation of the histograms. A general view of the histograms and a zoom of the overlapping zone are shown for the chosen comparison methods in *Fig 45* (XTC data set) and *Fig 46* (FA/sugar data set).

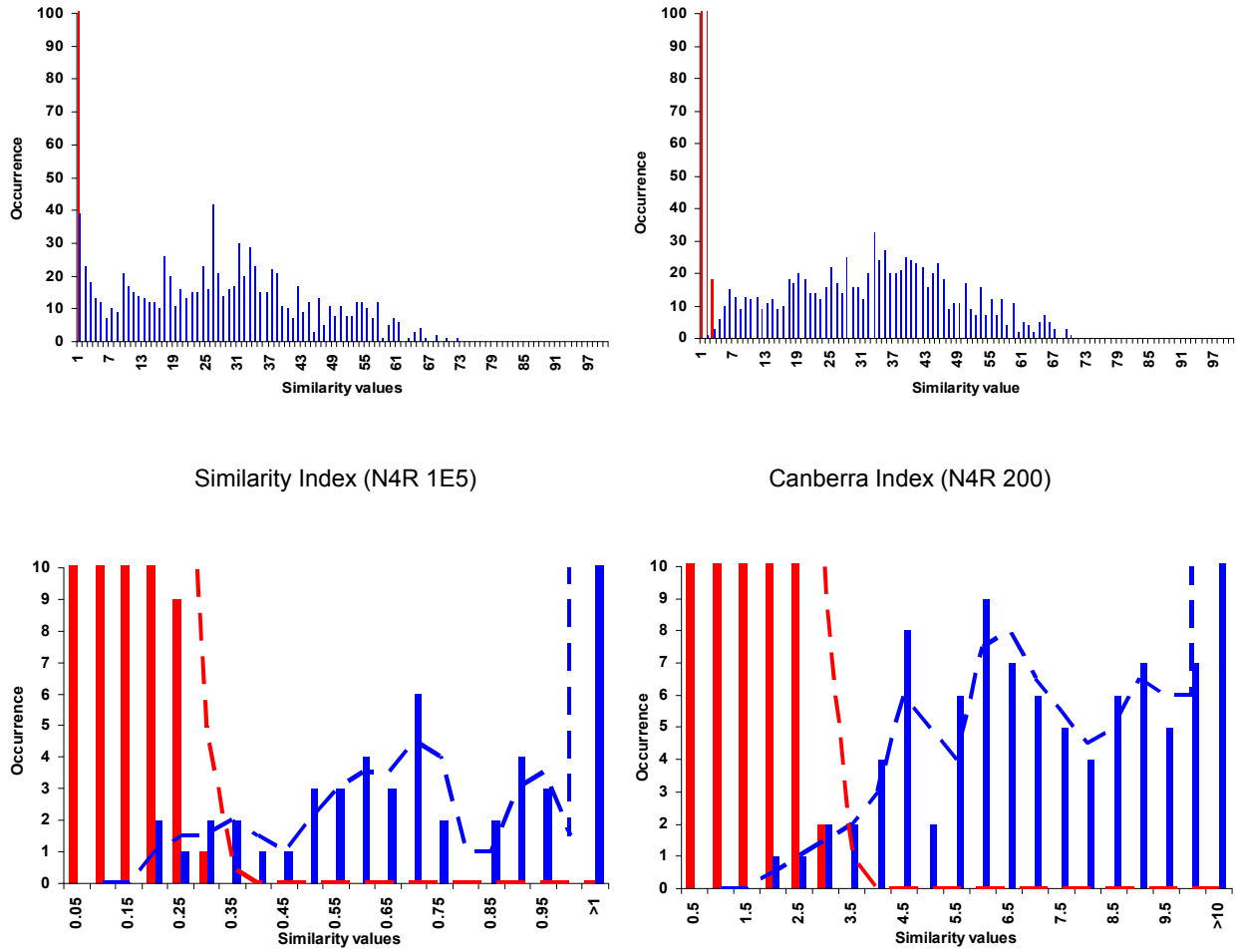


Fig 45 – Histograms of the selected comparison methods for the XTC data set – linked samples are represented in red, non-linked in blue.

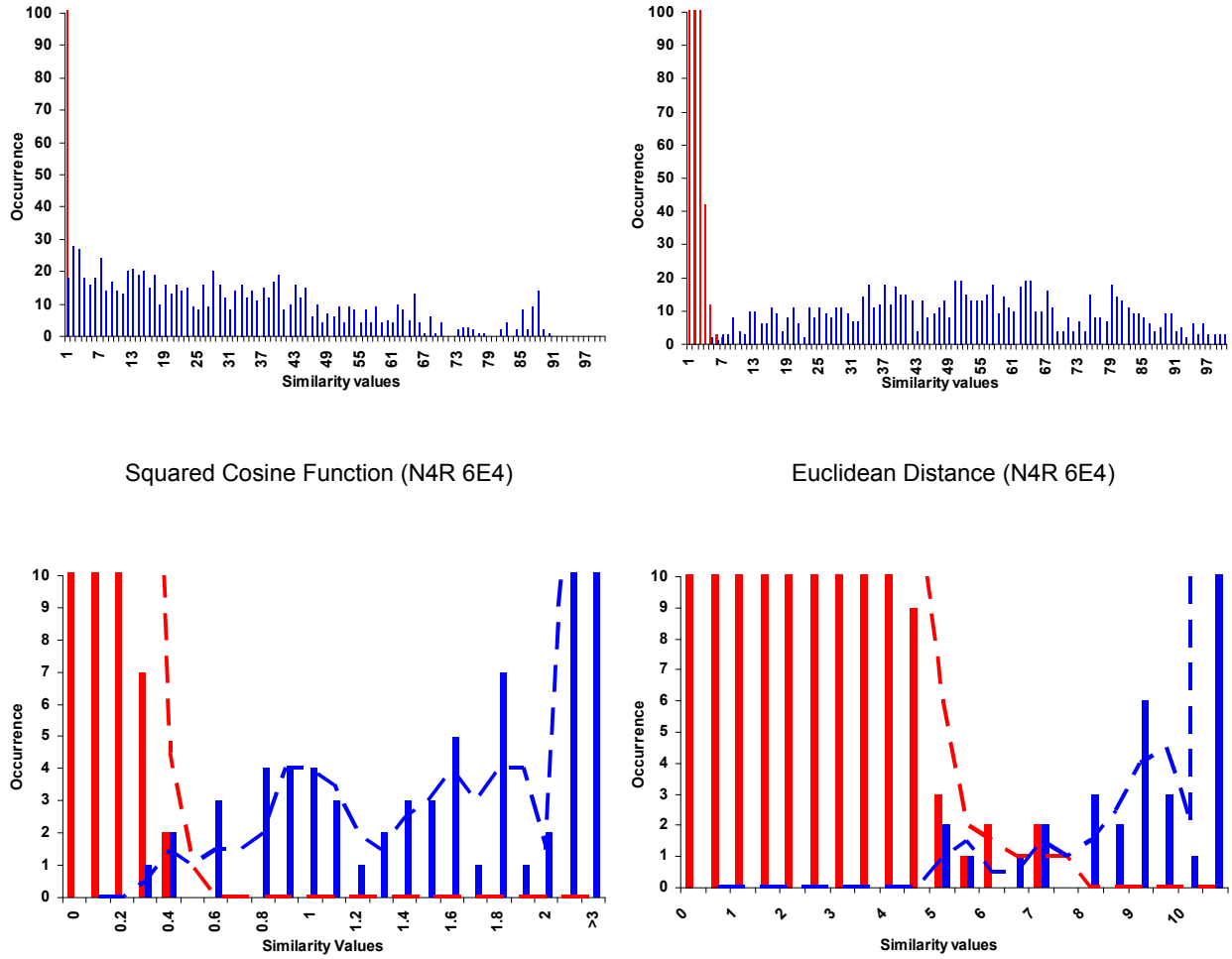


Fig 46 – Histograms of the selected comparison methods for the FA/sugars data set – linked samples are represented in red, non-linked in blue.

5.3 INTERMEDIATE CONCLUSION

Data analysis has been carried out with a sample set of 109 ecstasy seizures, each seizure having been previously analysed by three analytical methods. The routine method – XTC method – was to be compared to the new ones – FA and sugar methods, therefore the results of the latter have been put together in order to have two groups in total.

During this task several problems had to be dealt with. For reasons explained earlier (Ch – 4) the obtained data made intravariability to be calculated mostly by using the replicas of each sample analysed. Analyses of larger sample batches would have allowed a more realistic result. Unknown origin was also a problem when samples had to be determined for the intervariability analysis. They were chosen on the basis of an appreciation of physical and chemical characteristics known for each sample as well as operational data coming from investigation. The small sample set size was also problematic because it required careful

data cleaning arising from variations that might not even have been noticed in a larger data set, but seriously influenced the results here.

However, a comparison method could be determined. It was shown in the selection of the pre-treatment that the overall best results were obtained with pre-treatment containing the normalisation and the fourth square root. Missing peaks were best replaced by either an intermediary value (200), or a higher value near to the noise signal (> 6E4), since the replacement value of 0 not only gave worse results, but also caused some problems. After having compared several similarity measures, one correlation and one distance method can be proposed for each of the two data sets.

	Missing peak	**Pre-treatment**	**Similarity measure**
XTC method	200 1E5	N4R	Canberra Index Similarity Index
FA/sugars method	6E4	N4R	Squared Cosine Euclidean Distance

These combinations were applied on the mean areas of the replicas of all 109 ecstasy samples in order to verify if groups can be determined, and if so, to compare the groups obtained from the data set after XTC routine analysis with those obtained after fatty acid and sugar analysis.

6 EVALUATION OF THE ECSTASY DATA ANALYSIS

6.1 EVALUATION OF COMPARISON METHODS

One distance and one correlation method have been applied to the pre-treated mean values of all selected 109 ecstasy samples. Thus, for each data set – XTC and FA/sugar – two tables were obtained. They contain the similarity values of every ecstasy sample compared to all others, the table being thus of the size 109 x 109 cells. In this first part, both will be compared in order to verify if results are coherent for each data set and to evaluate differences between the similarity measures.

6.1.1 Excipient data set – Squared Cosine function vs. Euclidean Distance

6.1.1.1 Graphical representation

The FA/sugar data set has been treated by the Squared cosine function and the Euclidean distance. Visual comparison is almost impossible due to the big size of the resulting tables and they can not be represented here as such. However, the tables could be imported by The Unscrambler© software, Camo, and be visualised as matrix plots. The latter allows getting an overview of the distribution of the similarity measures by creating five differently coloured segments according to the listed values. The resulting plots are represented in *Fig 47* and *Fig 48*.

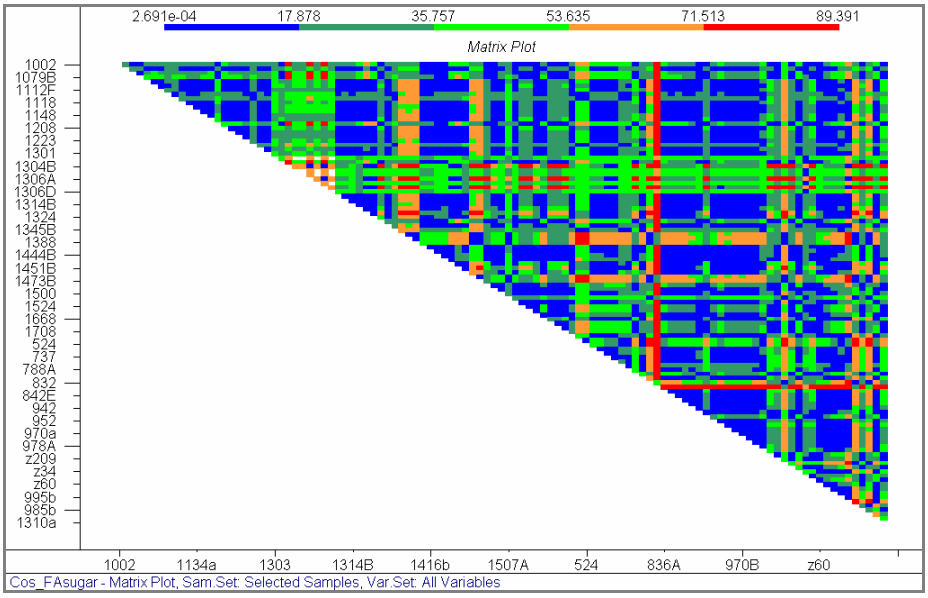


Fig 47 – Matrix plot of the table obtained after application of the Squared cosine function.

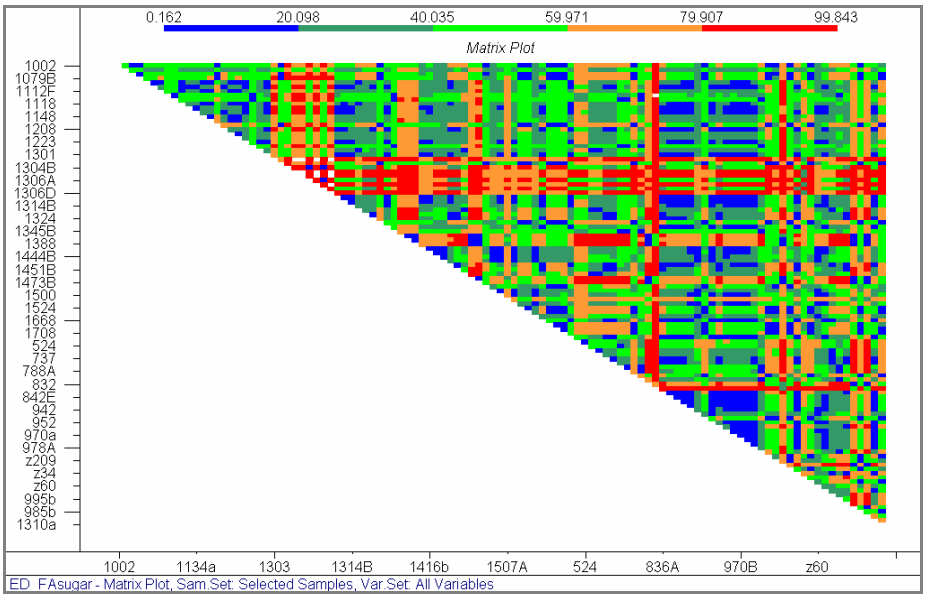


Fig 48 – Matrix plot of the table obtained after application of the Euclidean distance.

Considering the two matrix plots above, immediately two observations can be made. One is the similar distribution of colours, or at least a similar tendency, indicating also a similar attribution in distance values. This similarity can only be considered as a tendency since there is an obvious difference in coloration between the two methods. Indeed, it has been observed during data analysis that the values for distance methods are regularly higher than for correlation methods, resulting in less blue and more orange or red colours. Knowing this, the distribution for both methods can be regarded as similar.

6.1.1.2 Use of threshold values

In order to proceed to further comparison of the two methods, potentially possibly linked samples had to be determined. This does not mean a complete evaluation of similarity. Sample pairs were listed according to two threshold values. The highlighted ecstasy pairs for the two applied similarity measures were then compared in order to verify if differences existed and if so, how they could be explained.

The two threshold values simply corresponded to the MinNL (minima non-linked sample group) and the MaxL (maxima linked group) determined in the previous chapter and are shown in *Table 21* for reminder. The resulting list contained in the first column all ecstasy pairs presenting a similarity value below MinNL, and in the second all ecstasy pairs with a similarity value between MinNL and MaxL.

Similarity measure	MinNL	MaxL
Squared cosine function	0.23	0.39
Euclidean distance	4.57	6.76

Table 21 – Threshold values for both applied similarity measures.

The two similarity measures resulted in an almost equal number of pairs for the values below MinNL (*Fig 49*). Additionally, the pairs were found to be the same. The one additional pair found with the Euclidean distance was attributed to the second group with the Squared cosine function.

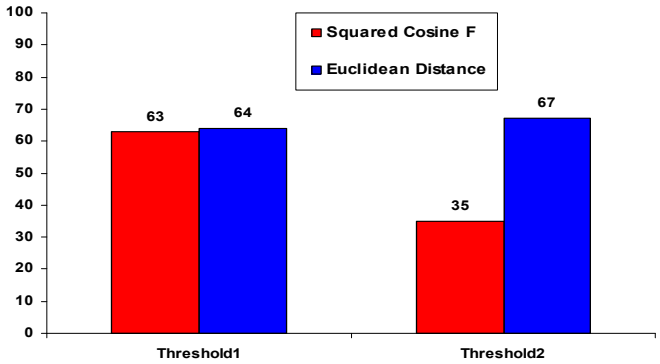


Fig 49 – Number of ecstasy pairs below MinNL (Threshold1) and between MinNL and MaxL (Threshold2).

A greater difference was observed when considering the values between MinNL and MaxL (Threshold2 in *Fig 49*). These values represent the overlapping zone between linked and non-linked samples in the histogram representation used in the previous chapter. Thus, a higher number of them indicates a greater overlapping. The Euclidean distance method resulted in almost twice as much pairs (67) than the Squared cosine function (35). All of the observed pairs resulting from the Squared cosine method are also found in those resulting from the Euclidean distance method. The overlapping zone should be small, if possible even inexistent, therefore the correlation method can be considered to be more appropriate.

6.1.1.3 Conclusion

From the previous considerations it can be concluded that both methods present similar results without contradiction. The observed differences could be explained and did not represent any incoherence, but rather a logical consequence of the type of calculation. Therefore, it was not regarded necessary to use both methods for the ongoing evaluation of data analysis. Only the results obtained with the Squared cosine function would be used for the evaluation of similarity between ecstasy tablets.

6.1.2 XTC data set – Similarity Index vs. Canberra Index

6.1.2.1 Graphical representation

The XTC data set has been treated with the Similarity index and Canberra index. The obtained tables were also imported by The Unscrambler© in order to visualise them in form of matrix plots which are represented in *Fig 50* and *Fig 51*.

The same observations as for the FA/sugar data set can be made. Again, a similar distribution of colours can be observed and the difference between correlation and distance method can be seen. However, the latter is not marked as much as for the precedent data set. No significant difference can be detected for the two methods applied to the XTC data set.

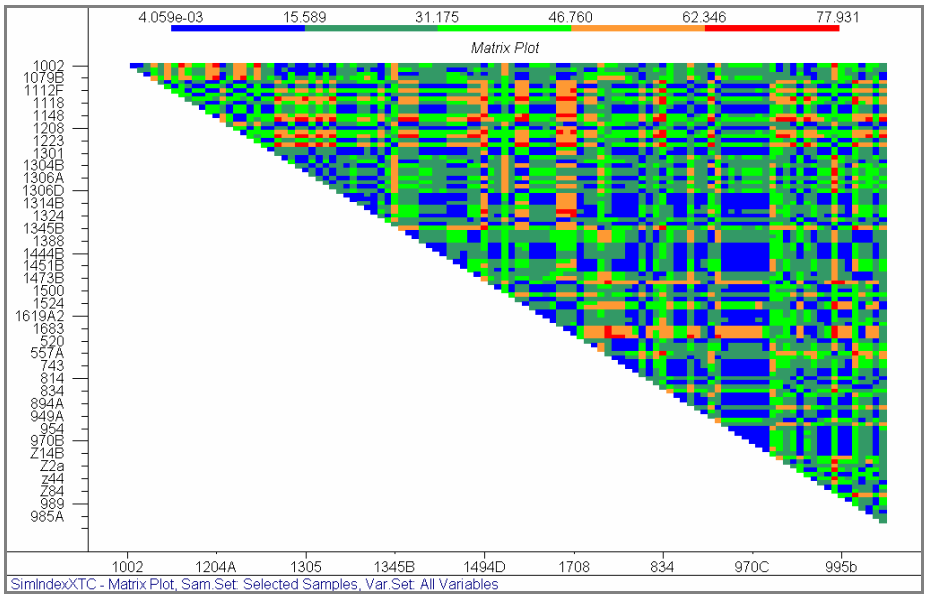


Fig 50 – Matrix plot of the table obtained after application of the Similarity index.

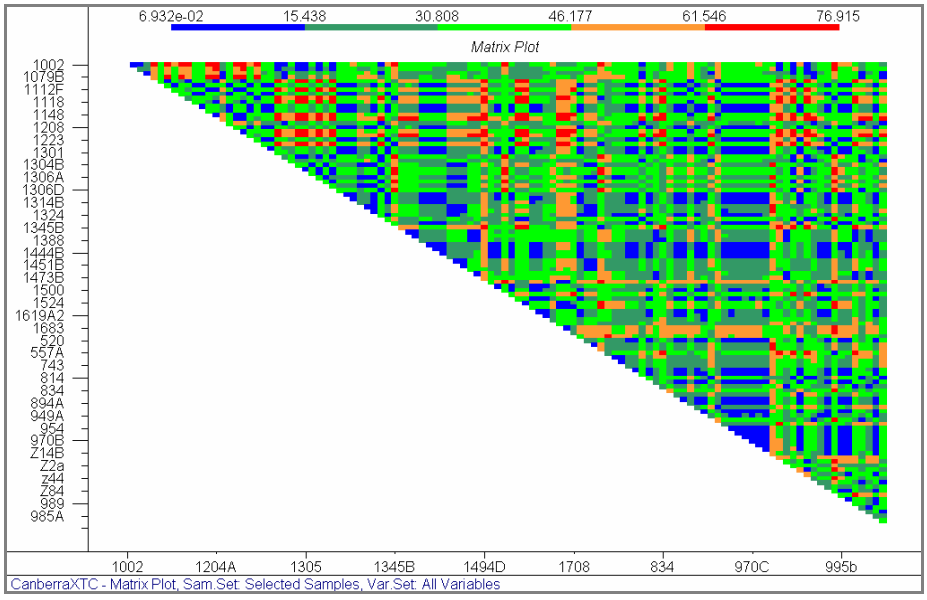


Fig 51 – Matrix plot of the table obtained after application of the Canberra index.

6.1.2.2 *Use of threshold values*

The lists of similar ecstasy pairs have been established considering the MinNL and MaxL values for the two selected methods, which are reminded in *Table 22*.

Similarity measure	MinNL	MaxL
Similarity Index	0.16	0.29
Canberra Index	1.85	2.62

Table 22 – Threshold values for both applied similarity measures.

The resulting lists give a similar picture to the one obtained with the FA/sugar data set (*Fig 52*). The difference here is that the distance method seems to be more appropriate, and not the correlation method. When applying the same reasoning than above, the overlapping zone should be smaller with the Canberra Index than with the Similarity Index.

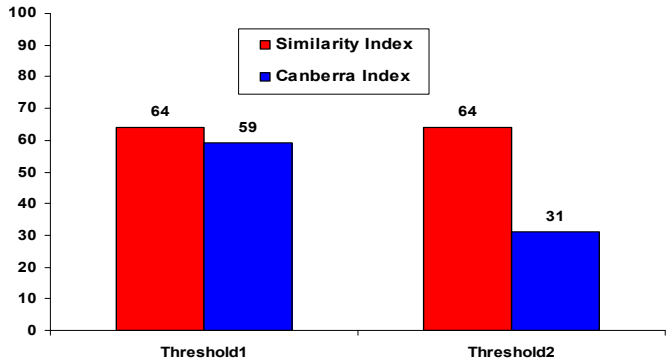


Fig 52 – Number of ecstasy pairs below MinNL (Threshold1) and between MinNL and MaxL (Threshold2).

6.1.2.3 *Conclusion*

The same conclusion can be drawn here compared to that for the FA/sugar data set – the results of the two methods seem to be similar and no inexplicable differences were observed. Therefore, only the Canberra Index was chosen for further data analysis.

6.2 EVALUATION OF THE ECSTASY TABLET COMPARISON

Similarity values are calculated in order to express the closeness of two samples. More specifically, it is used as a means for the search of eventual links. Is the value small ? The presence of a link becomes very probable – is it high ? The absence of a link is more likely. However, depending on the sample type the meaning of a link has to be defined.

In illicit tablet production links can be found at the following levels:

- Synthesis
- Tablet producer
- Blending
- Compression

The first considers the active substance and how it could be characterised, generally through trace analysis of by-products, precursors, etc. It will not be considered here.

The level of producer has been introduced because a tablet producer may have a personal recipe differentiating him from others. Additionally, he may possess more than one press resulting in visually different tablets, but being made of the same blend. The level of the producer is simply less specific with regard to the excipients content than the level of the blend. A link at this level would signify that the same active substance and the same excipients in very similar proportions can be observed in two tablets.

The link at the level of the blending would be very specific as it considers two tablets originating from the same mixture prepared at a certain moment and probably being compressed in the same run. They do not necessarily present the same physical characteristics (except the diameter) if they were for example produced on a rotary machine with various pairs of punches. However, their chemical composition would be identical.

The tablets presenting the same physical characteristics are considered as linked through compression: they are supposed to come from the same press. Therefore, they do not necessarily have the same chemical composition. However, this definition of link requires specific knowledge about physical characteristics which are not available in this project.

It results from these considerations that information obtained by the excipients particularly deals with the second and third level – tablet producer or blend. The following chapter will evaluate how much information is obtained from excipient analysis and what type of links can be expected.

6.2.1 Results obtained with fatty acid and sugar analysis

6.2.1.1 Evaluation of the excipient distribution

One of the aims of this research was to show the variety in excipients used by different illicit producers and furthermore to classify ecstasy tablets with regard to their excipient content. These two aims are necessarily strongly correlated because the classification presupposes presence of variety in excipient composition. Therefore, by showing that ecstasy samples can indeed be grouped according to their excipients both hypotheses will be confirmed.

The number of different sugars detected in all 109 ecstasy samples is limited to four, which are lactose monohydrate, anhydrous lactose, sorbitol and mannitol, the latter having been observed only in traces. For the differentiation of lactose monohydrate and anhydrous lactose, the reader is referred to Chapter 7 (Additional studies). Mixtures of two sugars were rare and they were not blended in equal proportions. Actually, they seemed to have been mixed rather accidentally since the second sugar was always present in very low quantities. Concerning the stearate, up to ten different fatty acids could be observed, with palmitic (C16) and stearic (C18) acid being the principal ones.

In order to get an idea of the importance of each of the variables, statistics were run on the data (peak areas) obtained for the whole data set. The data was mean normalised beforehand because of great range differences. *Fig 53* shows the distribution of peak areas in form of box plots for each variable and it appears clearly that lactose, palmitic (C16) and stearic acid (C18) would be the main factors in ecstasy comparison.

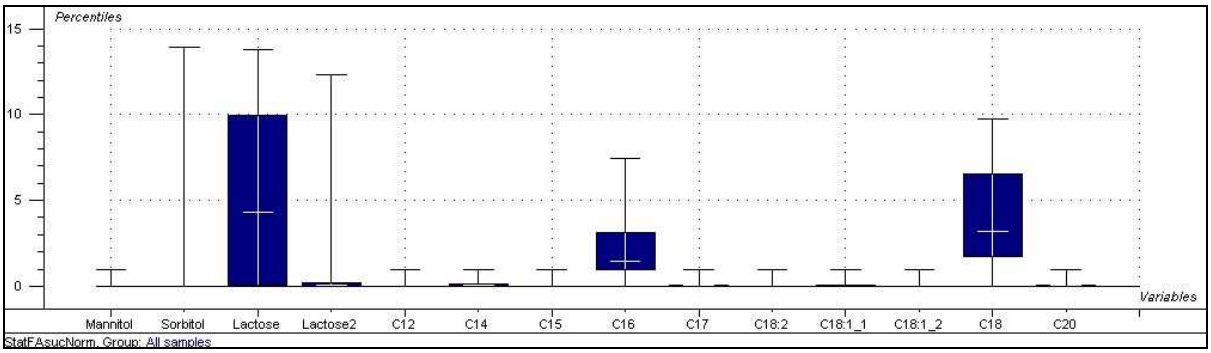


Fig 53 – Distribution of peak areas of all ecstasy samples for each excipient variable.

This seemed to be few factors for sample grouping and it was decided to have a closer look at their variation inside the sample set. A histogram in *Fig 54* shows a more detailed view of the peak area distribution for these three variables. It appears that all three variables present a large distribution in the sample set and might therefore result in useful variety between tablets, especially since the other peaks would also be taken into account.

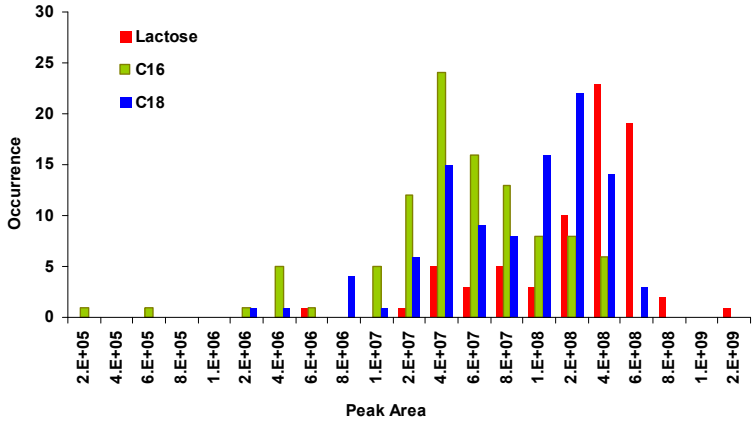


Fig 54 – Peak Area distribution for lactose (peak1), palmitic (C16) and stearic acid (C18).

6.2.1.2 Sample grouping

In order to evaluate the signification of the obtained correlation values after excipient analysis, it was necessary to investigate how far the ecstasy tablets could be differentiated by only using the excipient peak areas. It was therefore decided to compare the data of all samples visually according to the following three criteria, going from the general to the more specific ones :

1) The first one is the qualitative criterion. Samples were grouped according to their active substance. It was decided to use this additional information for a quite obvious reason. An analyst would never start with the analysis of excipients before at least making a screening test to verify if and what illicit substance is present. The identity of the latter is necessarily known because it is inherent to the chemical composition and as such to the blending to be compressed. Therefore, it was taken into account. However, no quantitative data was used.

   Samples were furthermore grouped according to the type of sugar. A semi-qualitative criterion could be defined for the fatty acids. There appeared to be three distinct types of palmitic and stearic acid proportions, which could be clearly differentiated : C16 < C18, C16 ≈ C18 and C16 > C18 (*Fig 55*).

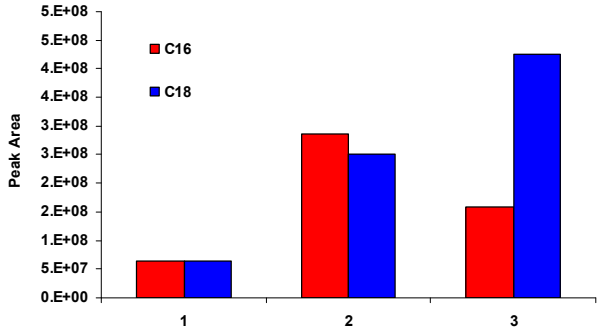


Fig 55 – The three different types of C16/C18 distribution.

2) The second criterion took into account the proportions of the two or three main peaks which were the principal sugar peak and the palmitic and stearic acid. If substantial differences in the relevant peak areas appeared which would result in further differentiation, this was taken into consideration. Small differences were not taken into account as no quantitative analysis has been performed.

3) Finally, the distribution of the small peaks was observed concerning particularly the eight other possible fatty acids apart from palmitic and stearic acid. It can be seen in the example shown in *Fig 56* that the *profile* does not change for the six replicas of the same ecstasy sample. To demonstrate the variation that can be observed, the distribution of the eight fatty acids in two different samples is shown in *Fig 56* as well.

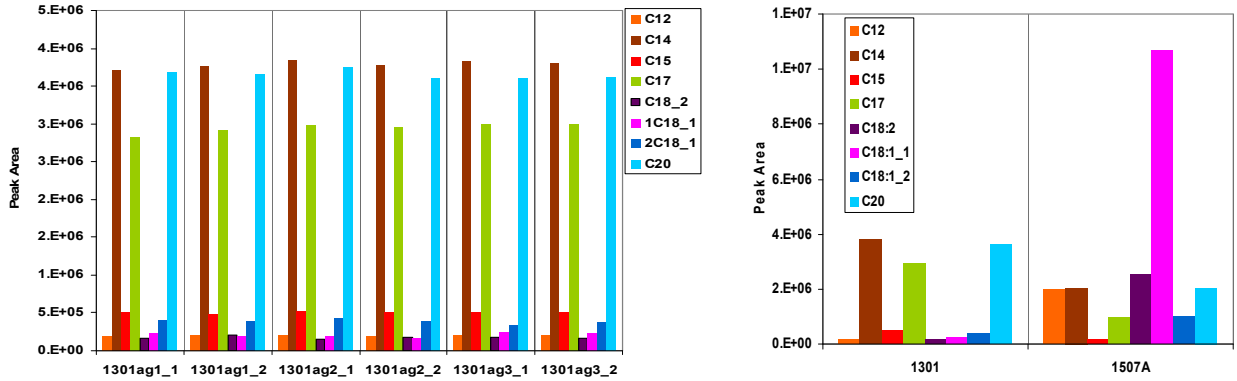


Fig 56 – Fatty acid distribution for six replica of ecstasy sample 1301 on the left – for sample 1301 and 1507A on the right.

Physical properties of the tablets have not been taken into account for the sample grouping as they were not directly related to the blending. The colour might have been considered but it appeared as not reliable, since about 75% of the tablet were attributed the colours beige, white or dirty white. Additionally, the visual attribution of colour being very subjective and the photographs being taken in varying conditions, the information is not necessarily trustworthy.

The examined data set could be divided into 67 groups, with 43 of them containing only one sample. Eight groups included samples that had to be separated into subgroups. This means that the concerned samples could not be separated according to the above criteria, but they appeared to present slight differences, generally quantitatively. The groups are listed in Appendix X together with physical characteristics and quantitative data.

6.2.1.3 Examination of the groups

The correlation values obtained by the Squared cosine function were noted for all groups in order to verify if the groups, formed in a sort of manual way, were confirmed by the calculated similarity values, or in other words, if the similarity measure resulted in the same groups. Additionally, it would be seen if the threshold value determined in the previous chapter (MinNL) was reliable. *Fig 57* shows the distribution of the values obtained with the cosine function and which were below "1". Although the overlapping zone for the squared cosine function was determined as being situated between 0.23 (MinNL) and 0.39 (MaxL), it was decided to extend it up to 1 in order to insure the collection of all similar sample pairs.

It has to be specified that the values for the subgroups are also present in those for a group and were simply taken apart to have a view of the values for the smallest entities determined. It is true that in this representation the term subgroup is somehow fallacious because this term grouped values with all samples that could not be differentiated further. Therefore, it also contains the values of groups with identical samples. Obviously, it does not make much sense to call them subgroup, as there is none.

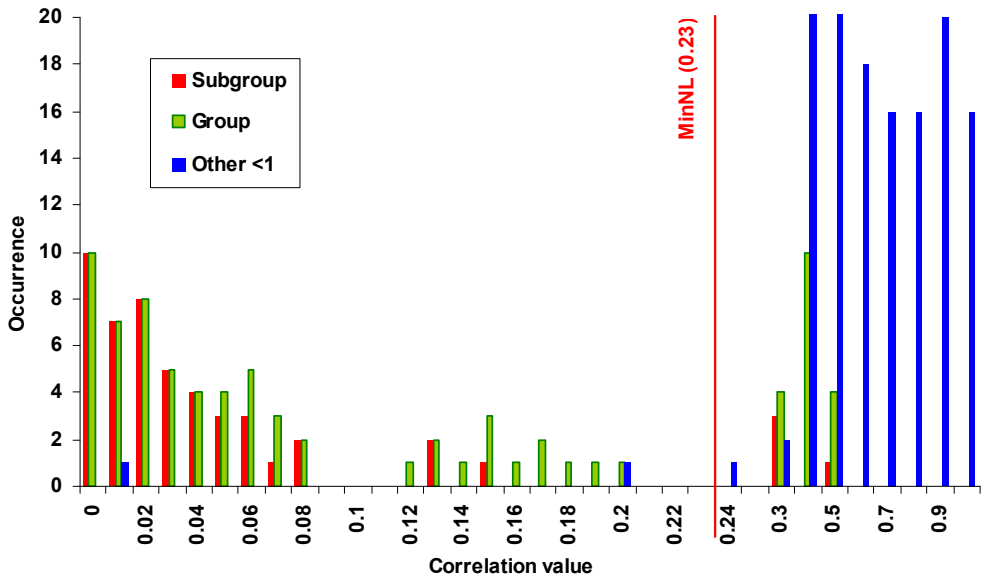


Fig 57 – Correlation values below 1, with those for samples of a group in green, for samples inside a subgroup in red and all others in blue.

It appears from *Fig 57* that the overlapping zone might indeed be bigger than determined earlier, but this was expected since more samples were analysed and some of them were slightly inhomogeneous which may have caused some higher values. On the other side, the highest correlation value, and therefore the lowest correlation, for a subgroup was 0.43 which is not that far from the initial value (0.39). Additionally, the second threshold value, and maybe the more important one, separates the linked from the non-linked population and seemed to be appropriate. Nearly 80% from the group similarity values and more than 90% of the subgroup values were below the MinNL (0.23). However, there were two values below 0.23 from tablets that were not grouped together, the reason being that they do not contain the same illicit substance. These two cases will be considered further.

The lower value of 0.009 was obtained with the two ecstasy samples Z209 and 1494D, the former containing a blend of MDMA and MDEA, and the latter just MDMA. The remaining characteristics can be found in *Table 23* below.

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Illicit Substance	Qty [%]	Diluent
Z209	23.02.04	8.01	4.22	239.10	"STAR DUST"	dirty white	Yes	MDMA, MDEA		
1494D	19.12.01	8.02	5.22	296.95	Elephant	white	Yes strong	MDMA	29.46	

Table 23 – Summary of the main characteristics of the samples Z209 and 1494D.

These two samples present a rather particular case, inasmuch that they do not only contain no sugar, but also that only palmitic and stearic acid were present in very small quantities. Consequently, they are identical because there is no data to show any variation. It thus appears that when no excipient can be

detected, much care has to be taken in the comparison of the results. Consequently, all other available information is to be considered when evaluating the similarity. In this case we have to do with very common tablets – presenting a frequently observed form (round and flat), the same for the diameter, a very common colour and frequent logos. The only particular characteristic is the presence of two active substances in seizure Z209 which speaks against a link between the two seizures. Considering the interval of almost 2½ years between the two seizures it also tends to indicate a non-link. But altogether there is so few significant information available, that no conclusion can be drawn.

On the other side, the absence of excipients can also be considered as a significant characteristic as it is rather rare to find nothing except the illicit substance (absence of excipients meaning no excipients detected by GC). This was for example the case with ecstasy samples 1303, 1304C, 1305, 1306B and D (*Table 24*). These were the only tablets were no trace of any excipient has been observed. As they appear to show no difference, the absence of sugar and fatty acids can be considered significant in confirming their linkage.

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Illicit Substance	Qty [%]	Diluent
1306D	17.05.00	8.10	3.36	219.71	Star	dirty white	None	MDMA	36.84	
1306B	17.05.00	8.09	3.41	221.59	Star	dirty white	None	MDMA	36.28	
1303	17.05.00	8.11	3.44	222.48	Star	beige	None	MDMA	41.83	
1305	17.05.00	8.09	3.45	220.93	Star	dirty white	None	MDMA	37.29	
1304C	17.05.00	8.10	3.48	225.19	Star	dirty white	None	MDMA	40.49	

Table 24 – Summary of the main characteristics of the samples 1303, 1304C, 1305, 1306B and D.

However, this is a particular case. It serves to demonstrate the importance of the main variables – one sugar and two fatty acid peaks. The loss of one or more of them requires much caution in the evaluation of any similarity. It has been observed that in the case of absence of a principal sugar, it was important to have some additional fatty acids next to C16 and C18 for the grouping of the samples. These smaller fatty acid peaks have always been considered as a whole and never separately, the first reason being certainly the very small size of the peak, and then they were expected to be correlated. The correlation of the variables has been calculated and is represented in *Table 25*.

Not surprisingly, correlation can be observed between some fatty acids, the highest value being observed with C15 and C17. Being odd-numbered they are rare and considering the nature of fatty acids, it seems logical that they are correlated. Even for the more common fatty acids there is no such thing as observing one fatty acid on its own. Depending on the substance (animal, plant, or other) a varying number of characteristic fatty acids can be detected, but generally more than two. Knowing this, the correlation values presented above are actually better than might be expected.

	Mtol	Stol	Lac1	Lac2	C12	C14	C15	C16	C17	C18:2	1C18:1	2C18:1	C18	C20
Mtol	1													
Stol	0.16	1												
Lac1	0.16	-0.28	1											
Lac2	-0.09	-0.10	-0.06	1										
C12	-0.03	0.27	-0.04	-0.05	1									
C14	0.04	0.31	0.03	0.38	0.41	1								
C15	0.00	0.41	0.02	0.01	0.41	0.78	1							
C16	0.06	0.46	0.17	0.09	0.40	0.53	0.55	1						
C17	0.06	0.36	0.07	0.06	0.33	0.78	**0.97**	0.49	1					
C18:2	0.40	0.10	0.29	-0.07	0.32	0.12	0.12	0.04	0.16	1				
1C18:1	0.56	-0.06	0.36	-0.10	0.18	-0.05	-0.12	-0.02	-0.05	0.89	1			
2C18:1	-0.04	-0.05	0.34	-0.04	-0.03	-0.06	-0.07	0.01	-0.05	0.57	0.51	1		
C18	0.05	0.34	0.19	0.27	0.47	0.78	0.76	0.83	0.77	0.13	0.01	0.02	1	
C20	0.14	0.17	0.16	0.24	0.31	0.65	0.73	0.50	0.81	0.21	0.10	-0.04	0.82	1

Table 25 – Correlation of the fourteen variables obtained with the data of 109 ecstasy samples.

It results that the number of fatty acids to be used for sample comparison can be reduced and the same variation can be observed. This might be examined in future work. However, for this research the maximum of information has been taken into account.

A second case has been observed with two ecstasy tablets containing different illicit substances (ecstasy seizure 952 and 960), but presenting a high similarity through their excipients. The similarity value being this time 0.19, it is situated at the upper limit of the linked population zone. Thus, the simplest reaction would be to reduce the threshold value of 0.23 to e.g. 0.18 in order to avoid these bad surprises. Observing the distribution of the similarity values in *Fig 57*, this would not change anything for the subgroup values. However, it would be a blind reaction. The two samples in question do indeed present a very similar distribution of sugar and fatty acids and it is imperative to consider eventual explanations. This brings us to the interpretation of the similarity values, essential especially for cases like this one.

6.2.1.4 Signification of the groups

As already mentioned, the similarity value is calculated in order to give information about the presence of a link between two seizures of ecstasy tablets. A low value indicates a high correlation and vice versa. Such a low value like that observed for the two samples above would intuitively lead to the conclusion that the two tablets are the same – they are linked. Nevertheless, they have been classed in two different groups, so there is actually some difference. Thus, it has to be evaluated where the high similarity or the link comes from. Did the tablets come out of the same press a few minutes one after the other ? Or were they produced in the same clandestine laboratory ? Or do they just happen to show identical properties ? It is very difficult to answer these questions for one particular reason.

No information is available about illicit production, except for the synthesis of the active substances. Better knowledge about production habits would be of great use and research should be done in order to answer questions with regard to a clandestine laboratory, such as :

- What type of press can be found ?
- How many presses can be found ?
- How many different logos are used ?
- Are there more than one type of excipients ?
- Are there one or more dyes ?
- Is there a preference for a particular active substance ? Etc.

The answer to these questions would at least give an idea of how production works, of what can be expected, but as the situation stands at the moment only assumptions can be made. And these are based on the observations made on ecstasy seizures.

However, when considering the basic production steps presented in *Fig 58*, it appears that the excipients are necessarily related to the blend before compression. It was decided that the term blend included the active substance as it is most probable that someone prepares a mixture according to a recipe from A to Z. Preparing a bulk quantity of excipient blend and using it for different illicit substances, and thus different compression blends, appears to be improbable particularly with regard to the stearates which are added just before compression.

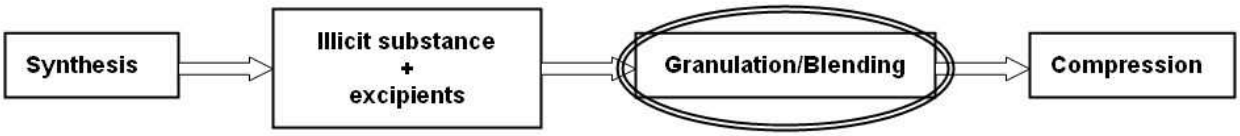


Fig 58 – Basic production steps for ecstasy tablets.

The most general assumption which can be made is that a low similarity value indicates that the blends used for the two concerned tablets contained the same excipient, except in the previously mentioned case where excipients were lacking. With regard to the sugars, this might not be a very selective indication since we have seen in ecstasy statistics that there is a clear preference for lactose (cf. Chapter 2). However, the realised analyses showed that only half of the tablets contained lactose monohydrate, the other half contained no sugar at all, or sorbitol, anhydrous lactose, or combinations. Additionally, variations in proportions of the two lactose peaks were observed. This can be an indication for different lactose products, particularly in the case of anhydrous lactose. This will be shown in Chapter 7 (Additional Studies). Thus, the information obtained from sugars can be more specific than simply its identity.

The fatty acids are the ingredients of metallic stearates used as lubricant. Despite the name, stearates also contain palmitates and other fatty acid salts. The US pharmacopoeia states that stearate and palmitate together should account for not less than 90% of the fatty acid content. This still leaves a considerable range of materials to be supplied as stearate and it would be expected to observe variations in the fatty acid content. Indeed, this can be confirmed by the various number and proportions of fatty acids observed in the analysed ecstasy samples. Additionally, it is recommended to use a single supplier for a given formulation because a stearate from a new supplier might have other effects on compaction and dissolution.

Another interesting point concerning the presence of stearates is its hydrophobic character. It has been shown that it might reduce dissolution rate and bioavailability of several tablet formulations. Even an illicit ecstasy producer should be worried about optimal effects of the illicit substance to take place. In order to minimise this behaviour, the stearates should be the last excipient added to the blend and only be mixed for a short time before compression. (Davies, 2001) The fatty acid content thus appears to be characteristic for a blend.

A low similarity index would therefore not only imply the use of the same excipients, but even indicate a link towards the same producer. That two producers use the same excipients is absolutely conceivable. However, the likelihood that two producers use excipients with identical chemical properties (coming from the same supplier) in identical proportions is much smaller. Considering the variety observed during sample grouping it might even be conceived that a low similarity value signifies a link to the same blend. There are indeed groups with one or two tablets showing very slight differences in quantities but still visible enough to distinguish them (*Fig 59*). Considering the distribution of the peak areas in these groups, there simply seems to be a little less/more sugar or fatty acids so that the proportions between the two very slightly changes (*Fig 60*).

1345A	Heart (Punchmark)
1130	Heart (Punchmark)
1134A	Heart (Punchmark)
1314B	Heart (Punchmark)

1204A	Double lightning
1148	Diamond
1209	Diamond
1112F	Diamond
1218A	Double lightning

1388	"FF"
1380A	"FF"
1473A	"FF"

1693	Heart
1683	Heart
1708	Heart

Fig 59 – Groups with one tablet being very slightly different.

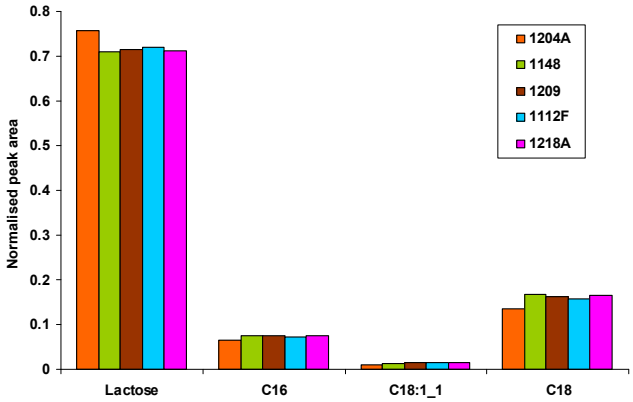


Fig 60 – Group *Diamond/Double lightning* – sample 1204A is distinguished by its lactose and fatty acid content.

This small change could be explained for example by the fact that they were produced in consecutive batches. However, nothing is known about the homogeneity inside one batch which is typically one of the problems addressed in pharmaceutical tablet production. And therefore not enough data is available to make a statement with regard to a link at the level of a single blend. However, since variation in the excipient content has been proven, a low similarity value does rather indicate a link with regard to the producer.

Although focussing on the chemical composition of the ecstasy tablets, their physical characteristics need to be considered as well, as they are also related to the producer. All determined groups are composed of tablets presenting identical physical characteristics. There was only one exception where two different logos were observed (*Diamond* + *Double lightning*). The concerned tablets are however linked on the basis of the physical characteristics (Zingg 2005), presenting a particular tablet shape and a strong colour (*Fig 61*).

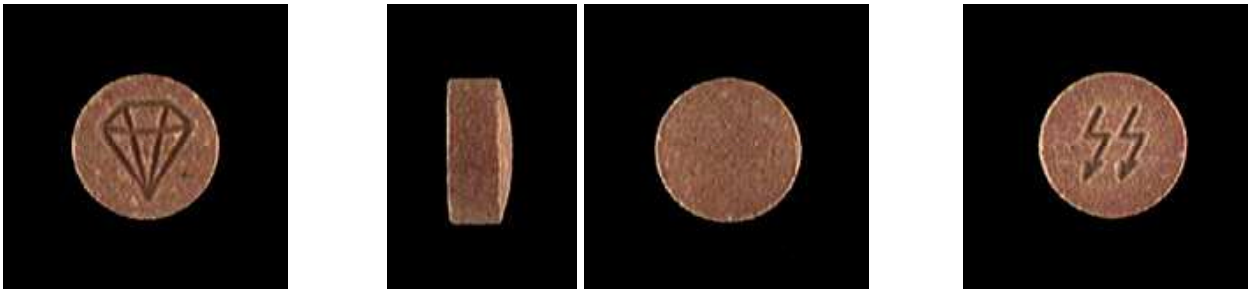


Fig 61 – Front view of sample 1209 on the very left and of sample 1204A on the very right; side and back view being the same for both they are shown once in the middle.

The physical correspondence was therefore confirmed by the excipient composition. Nonetheless, there were several tablets showing identical physical properties and as a result were sometimes attributed to the same class, but were differentiated by their excipients. In these cases care should be taken before making any classes and the significance of the various characteristics should be discussed.

In this context it is interesting to notice that a sort of fashion effect has been observed. A large group of somehow similar but not identical tablets happen to correspond to very common logos as well (*Ferrari, Mitsubishi*). Additionally, the above mentioned tablets – similar in physical characteristics, different in excipient content – also had very popular logos, such as *Mitsubishi* or *Superman*. Therefore, when dealing with tablets presenting popular logos and no particular colour (white, beige), a low similarity value should be considered with caution. The similarity in excipient content does not necessarily prevail over physical characteristics and vice versa, but they have to be considered together. It seems that with these *fashionable* imprints, similarities may easily appear at any level which might simply be due to their high frequency. There must be an enormous amount of tablets with these imprints on the illicit market, and it would be interesting to study the excipient content on a large sample of tablets seized at different places to learn more about the variation inside batches and in-between batches.

Finally, something should be said about the colour of the tablets, because intuitively it was attributed to the blend as well. The fact is that it was difficult to include it in the criteria for the grouping. The colour is not at all reliable when dealing with white, beige or dirty white tablets which concerned however over half of the sample set. It was not included in the grouping criteria because dyes are not essential excipients and may be added whenever to the blend. Their significance was not clear enough to rely on it for the sample grouping. However, when evaluating the groups it sometimes was a very helpful indicator and was in that sense also mentioned in the previous paragraphs.

6.2.1.5 Conclusion

If we get back to the initial hypotheses, they have been confirmed through the precedent paragraphs. Variation in excipient content could be shown and as consequence of this, it was possible to form groups as well. The variation observed between these groups was confirmed by the similarity values obtained. The evaluation of these values resulted in the conclusion that a high correlation in excipient content (therefore a low similarity value) indicates a link towards the tablet producer.

However, it also has been shown that it is important to consider all available information about the remaining characteristics of the tablets and particularly be careful with popular logos. This is even imperative when dealing with tablets where no excipient has been detected by GC-MS analysis.

6.2.2 Results obtained with the routine method – Comparison with FA/sugar method

The particular circumstances of the application of this method have to be reminded. The ecstasy sample set has been analysed by the routine method usually applied for the screening and quantification of ecstasy tablets, because the new GC-MS instruments appeared to be more sensitive than those used before. Small peaks after analyses on the old instrument were accentuated on the new one (e.g. palmitic

and stearic acid) and new peaks were detected. Therefore, it was considered interesting to evaluate this new information and to compare it with the FA/sugar method. No changes or optimisation have been applied to the routine method and as such it is an accessory study carried out in parallel with the excipient analysis. The same procedure with regard to data analysis has been applied in order to be able to compare the results with those obtained from the excipient data set. However, interpretation will be limited to this comparison.

6.2.2.1 Evaluation of the variables

Similarly to the excipient analysis the applied variables will shortly be evaluated in order to see which ones have the strongest influence, how they interact and if variation can be observed. Contrarily to excipient analysis, the substances detected by the routine method are of different kinds. They are composed of the active substance(s), traces of synthetic impurities, adulterants and excipients (fatty acids and sugars). Altogether, twelve peaks have been used for data analysis. The obtained data has been exported to the software The Unscrambler© and statistics have been run on the raw peak areas. The result is shown in form of box-plots in *Fig 62*.

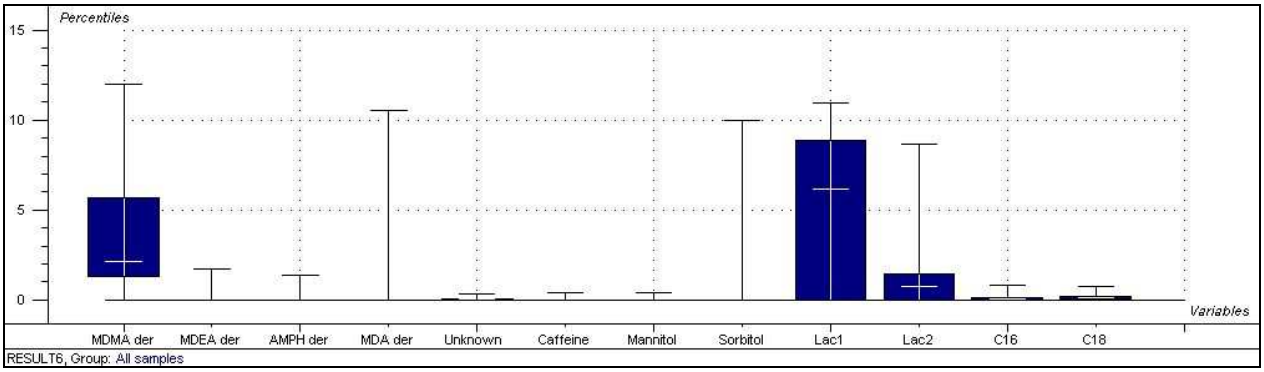


Fig 62 – Distribution of peak areas of all ecstasy samples for each variable.

The result is similar to the excipient analysis inasmuch that again three major variables are observed – MDMA, and the two lactose peaks. However, when considering the area distribution of the three peaks in detail differences appear (*Fig 63*). It is interesting to observe that the peak areas obtained for MDMA show a relatively small distribution indicating a less important variation.

Therefore, the principal source of variation seems to be the sugar content. However, the other variables may have a stronger influence than appears first. The variables used in the routine analysis are, by their nature, supposed to be less correlated than were the fatty acids in the excipient analysis. And, indeed, much less correlations are observed in *Table 26*.

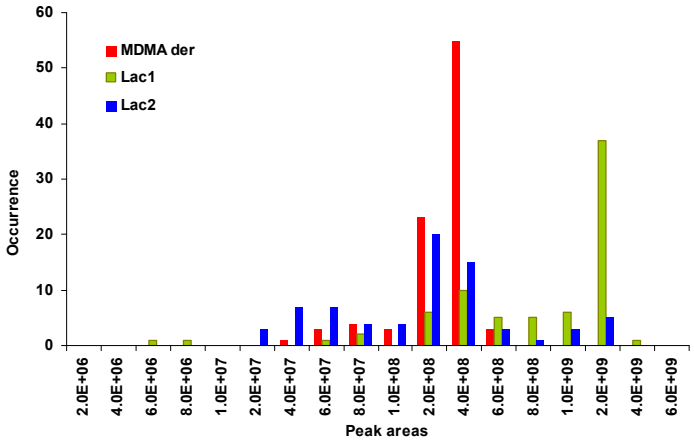


Fig 63 – Peak area distribution of the three main peaks MDMA, Lac1 and Lac2.

	MDMA	MDEA	Amph	MDA	Unknown	Caffeine	Mtol	Stol	Lac1	Lac2	C16	C18
MDMA	1											
MDEA	-0.11	1										
Amph	-0.31	-0.04	1									
MDA	-0.50	-0.06	-0.06	1								
Unknown	0.85	-0.10	-0.28	-0.46	1							
Caffeine	-0.39	-0.05	0.84	0.22	-0.36	1						
Mtol	-0.33	-0.05	-0.04	0.71	-0.31	0.32	1					
Stol	0.17	-0.01	-0.06	-0.10	0.11	-0.08	-0.03	1				
Lac1	-0.08	0.02	0.02	0.25	-0.03	0.10	0.25	-0.32	1			
Lac2	-0.08	-0.05	0.66	-0.07	-0.01	0.55	-0.03	-0.17	0.17	1		
C16	0.09	-0.07	0.15	-0.07	0.15	0.10	-0.02	0.47	0.26	0.19	1	
C18	0.07	-0.08	0.26	-0.06	0.10	0.20	-0.02	0.28	0.31	0.43	0.84	1

Table 26 - Correlation of the twelve variables obtained with the data of 109 ecstasy samples.

The observed correlations next to the fatty acids are not surprising since the unknown peak has only been detected when MDMA was present and similarly for caffeine when amphetamine was present.

6.2.2.2 Sample grouping

The similarity values obtained with the XTC data set were evaluated in the same way than with the excipient data set. Samples were visually compared according to similar criteria than for the sugars and fatty acids, only that here the quantitative analysis of MDMA, performed after the seizure of the tablets, was intended to be taken into account as it is part of the routine analysis. However, a substantial problem has been discovered. The MDMA percentages taken from the ecstasy database (IPS-WD) were not correlated to the obtained peak areas in this research. When plotting the raw peak areas against the percentages no tendency can be observed (*Fig 64*).

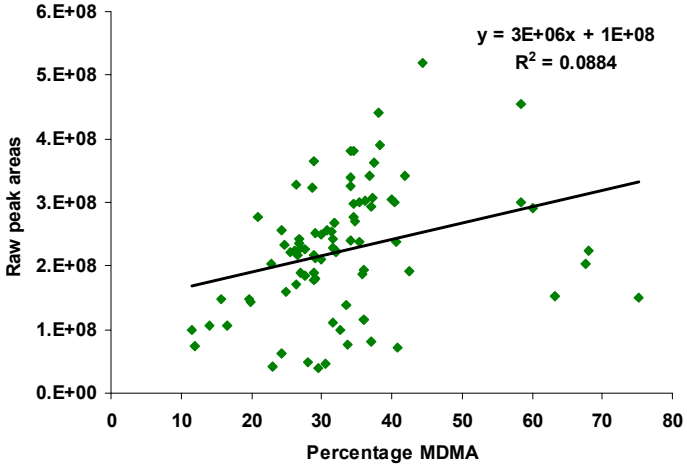


Fig 64 – Raw peak areas plotted against the MDMA percentages.

The quantitative analyses have been made over more than five years and on another instrument (Capillary Electrophoresis). This explains to some extent the absence of correlation. Consequently, the quantitative data was not taken into account for the sample grouping.

Therefore, the criteria used for the sample grouping were the following :

1) The first was again the qualitative composition. The samples were sorted according to their active substance, type of sugar, adulterant and fatty acids. It appears that for the fatty acids the same criterion could be used than for the excipient sample grouping, inasmuch as the same kind of relationship between palmitic and stearic acid could be observed.

2) Secondly the relationship between the major peaks have been considered and difference in proportions resulted in differentiation.

3) Finally, differences in quantity were considered and if significant lead to differentiation.

The sample grouping appeared to be rather difficult. Variation was not as great as expected and differences were often small. A particular problem was that differences in proportions were not always very obvious and reliable. When comparing with the similarity values, samples could be observed that were separated because of variation in proportions, but have been given a very good similarity value. Another problem was that differences in fatty acid proportions were visible as such but have not been taken into account by automatic comparison. Finally, a great number of samples looked very similar, presenting MDMA and sugar content in similar ranges, and were therefore difficult to differentiate.

In the beginning this was thought to be caused by the use of the raw peak areas and the grouping has been repeated with the normalised peak areas (values after the applied pre-treatment). It appeared not to

be much easier and the obtained results were even worse in terms of similarity value distribution, because more not-grouped sample pairs below the MinNL limit were observed. Altogether, the sample grouping seemed to be rather coincidental and it would not be surprising if a slightly different grouping would be found when carried out another day.

This observation is very different from the sample grouping with the excipients. For the latter, the analytical methods have been adapted to sugar and fatty acid detection and their peaks were therefore considered as reliable. This cannot be assured for the routine analysis which is a screening method and it was difficult to appreciate as how reliable the peaks could be considered, and consequently how reliable the similarity values were.

6.2.2.3 Evaluation of the groups

For further comparison with the excipient method, the sample groups obtained with the raw peak areas have been used. As mentioned above, the similarity values obtained with the Canberra Index have been attributed to the determined sample groups and their distribution was compared with that of the values below the upper limit fixed at three which could not be attributed. The result is presented in *Fig 65*.

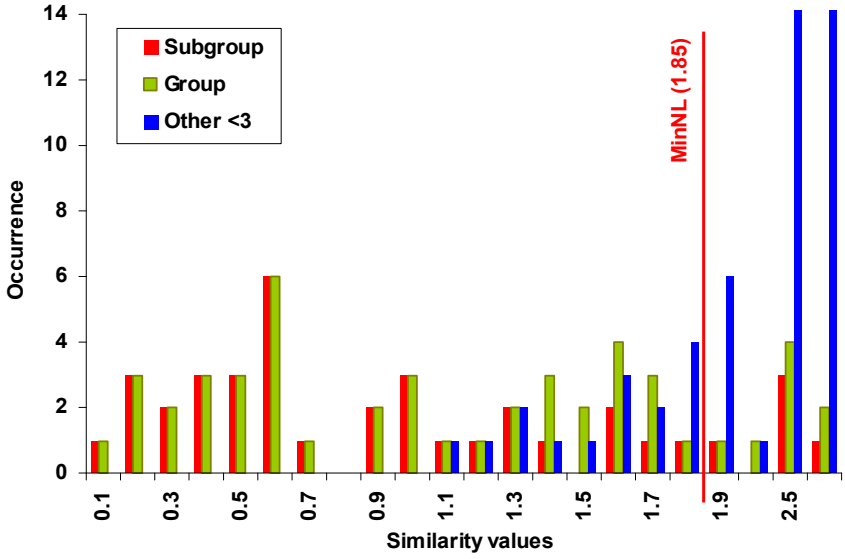


Fig 65 – Correlation values below 3, with those for samples of a group in green, for samples inside a subgroup in red and all others in blue.

The first remark to make with regard to *Fig 65* is that the overlapping zone appears to be much bigger than previously determined. The highest value for non-differentiated samples is 7.6, much higher than the MaxL value – 2.6 – determined in the previous chapter (Data analysis). And as can be seen in the above graph, the values for sample pairs which were not grouped together goes down to 1.

Against what was said for the FA/sugar grouping, an adaptation of the threshold value (MinNL) seems to be appropriate here. But this would not resolve the problems in making groups and a more thorough study of the screening method in the context of ecstasy comparison would be advisable.

However, groups have been made in order to compare them to those obtained with the excipient analysis. A number of 72 groups have been determined, of which 46 were composed of a single sample and 7 contained subgroups.

The kind of information that should be obtained with the global ecstasy analysis is similar to that obtained with the excipient. Additional knowledge is gained in the form of the peak area of the active substance and potentially present adulterants. On the other side, some specificity is lost with regard to the excipients, since only two fatty acids are detected. Nonetheless, both data sets are related to the blend before compression and give information about the chemical composition of the whole tablet mixture.

The ecstasy screening method appears to be more general because it deals with qualitative and semi-quantitative aspects. In other words, samples are differentiated because of the present substances and their relative quantities. However, this combined information results in enough variation to form groups. In the excipient analysis, the detected lactose and much more the stearate is characterised by its peak proportion for the first and its fatty acid content for the second, which appears to distinguish different types of lactose or stearates. Therefore, the qualitative criteria go further than that obtained with the screening method. This might be the reason why the sample grouping appeared easier because greater variation seemed to have been observed.

The groups determined by the two methods are not contradictory, but slightly different. A first general remark is that there are four groups in the XTC data set composed of samples which do not correspond in all physical characteristics. It has been explained in the evaluation of the excipient groups that it is conceivable to have tablets with identical composition and different physical characteristics. However, the correspondence in chemical composition should be certain, something which cannot be assured in this case. The four groups were not formed within the excipient data set. The concerned samples were differentiated either because of proportional differences in sugar and fatty acid peaks, or because of substantial quantitative differences. This rather speaks against a link based on chemical composition.

Concerning the other differences observed, in ten cases, samples which have been grouped in the excipient data set were separated in the XTC data set. Possibly, the active substance content made the difference. However, this is considered as a loss of information since the links highlighted by the excipients were not seen anymore.

One case remains where samples separated by the excipients were found to be grouped in the XTC data set. This is due to the greater specificity of the excipient analysis. The samples were separated because of small proportional differences in the fatty acid content, something which would not be detected by the ecstasy screening analysis. Additionally, they do correspond in physical appearance, except for the colour

(*Fig 66*). Thus, they might be considered as linked, but being made at different moments, which would explain the difference in fatty acids and colour.

Fig 66 – Front view of the samples 1451A on the left and 1451B on the right.

All groups are listed in Appendix XI and the samples being differently grouped with regard to the excipient data set (Appendix X) are highlighted in red.

6.2.2.4 Conclusion

The screening method for ecstasy analysis is an interesting alternative as it gives information about the several substances possibly present in an ecstasy tablet and detectable by GC-MS in one time. However, problems have been encountered. First of all, the quantification of the active substance should be verified in order to get reliable results which could be used in sample comparison. Then, it has been seen that variation seemed to be less obvious than with the excipient analysis which appeared to be more specific. Groups formed by both methods were to a great deal similar, but several differences were observed which in many cases signified a loss of interesting information, because samples considered as linked by the excipient method were separated by the routine method.

Altogether, the screening method does not appear to be an appropriate method for ecstasy sample comparison because similarity values seemed to be less reliable than for the excipient method and incoherencies have been detected between calculated similarity and observed variation.

7 ADDITIONAL STUDIES

7.1 THE PARTICULAR CASE OF LACTOSE

7.1.1 Introduction

It has soon appeared from statistical evaluation of our ecstasy database and from research in relevant literature that lactose is one of the most, if not the most, frequently used excipients in ecstasy tablets. Since the seized tablets have been analysed by GC-MS, lactose is easily recognised on the chromatogram with its two peaks – one big and one small – at the end of the analysis.

The reason for our further interest in this substance is that sometimes the second, normally smaller peak happens to be bigger or equal to the first one, and that this is not the result of an instrumental error, preparation or degradation. With the method developed for sugar analysis, this inversion of size seemed to be even accentuated. Just as for the "normal" case the smaller peak is even more reduced in size.

It is known that lactose exists under two anomer forms – α- and β-lactose. The standard usually applied for test analyses and during the method development for sugar analysis was α-lactose monohydrate, because this is the most common form. Thus, faced with this phenomenon of inversed peaks, it was decided to analyse a standard of β-lactose. And indeed, the standard happened to produce two peaks with the second one being higher.

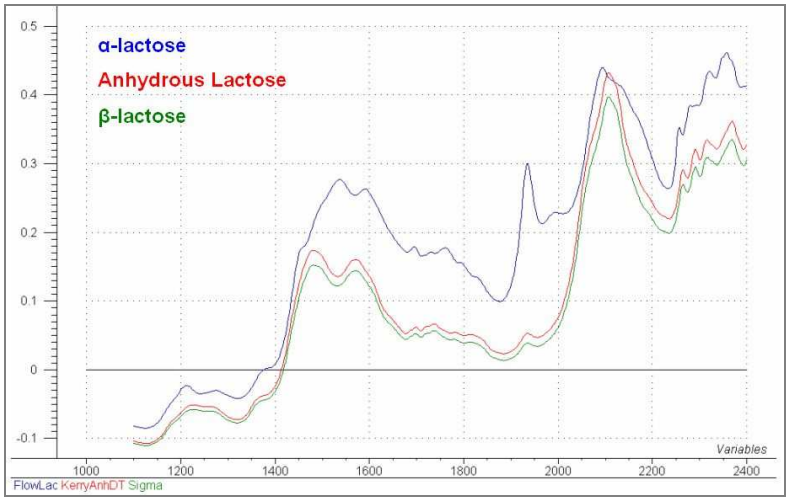


Fig 67 – NIR spectra of a β-lactose standard with samples of anhydrous (Kerry) and α- (Meggle) lactose.

The answer seemed to have been found. However, it remained unclear why β-lactose has never been mentioned in any publication read for this research. It is not rare to observe this form of lactose. In the ecstasy tablets analysed in our laboratory the β-lactose form appears to be as frequent as sorbitol.

Things became clearer when another project using NIR spectroscopy required the collection of a high number of cellulose and lactose samples. Another common excipient next to α-lactose monohydrate is anhydrous lactose and was therefore also obtained for the NIR project. During this project it was observed that β-lactose is the principal component of anhydrous lactose. And although the latter can contain α-lactose to a minor extent, the spectra of an anhydrous lactose sample and the β-lactose standard were almost identical (*Fig 67*).

7.1.2 Lactose blends

The origin of this second high lactose peak could now be explained and some further investigations appeared worthwhile. According to the pharmacopoeia and other publications about excipients (Ph.Helv., 1997; Kibbe, 2000; DMV, 2005), anhydrous lactose is seldom composed of pure β-lactose, but generally blended to some extent with α-lactose.

Thus, four 100mg blends were made from α- and β-lactose standards at different proportions (20/80, 40/60, 60/40 and 80/20) to be analysed on the GC-MS by the routine ecstasy method and the developed sugar method. The purpose was first, to compare both methods with regard to linearity between the two lactose peaks and the α- and β-lactose content, and second, to characterise the anhydrous lactose samples in function of this content.

7.1.2.1 Linearity

All data were represented on graphs by plotting the respective quantity of α- and β-lactose against the area of the first peak (Peak1) and of the second peak (Peak2). The data obtained for the areas were either used untreated, or normalised. It appears that normalisation of the areas has no influence on linearity. Regression coefficients are the same in both cases. Therefore, only one graph per method is represented below in *Fig 68*.

Considering *Fig 68* it can be seen that less points were used for the ecstasy routine method. This is due to analytical problems, a part of the analyses showing secondary peaks next to the two principal ones whose peak areas were strongly influenced by this presence. The problematic measures were therefore discarded. These secondary peaks are supposed to be caused by degradation, although exact reasons are unknown.

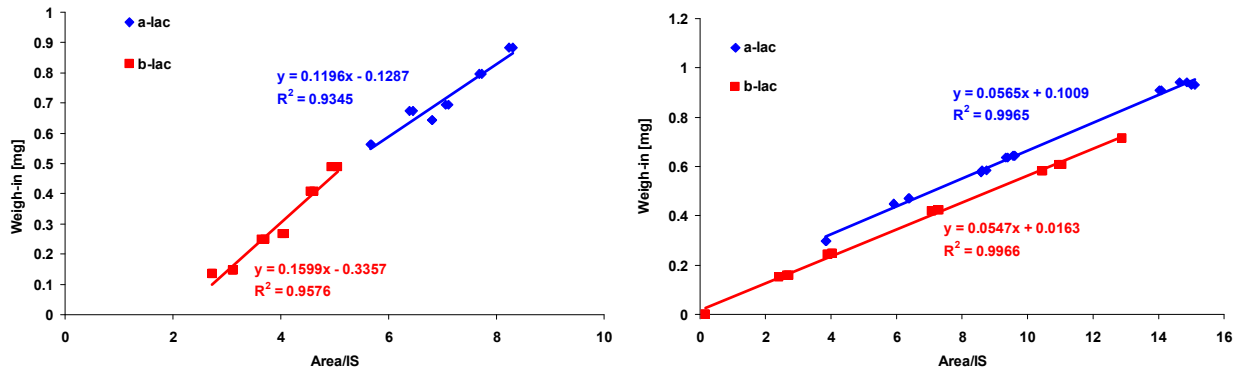


Fig 68 - α- and β-lactose content in function of the normalised areas of peak1 and 2, respectively, analysed by the routine ecstasy method (left) and the sugar method (right).

Although both methods gave good linearity, regression shows that the sugar method gave significantly better results. These graphs (*Fig 68*) were realised with data obtained in Scan mode, which means that a pre-determined scan range of mass/charge ratios is used for the detection and the peak observed in the chromatogram corresponds to all mass ratios detected at that moment.

Another possibility of detection is the SIM mode, which integrates only the area of selected target ions – in our case these would be the characteristic ions for α- and β-lactose (*Fig 69*). This mode of detection is generally much more sensitive and has therefore been tried on the lactose blends. Considering the mass spectra below it is obvious that they are very close. The difference lies in the abundance of the ions 191, 217 and 361.

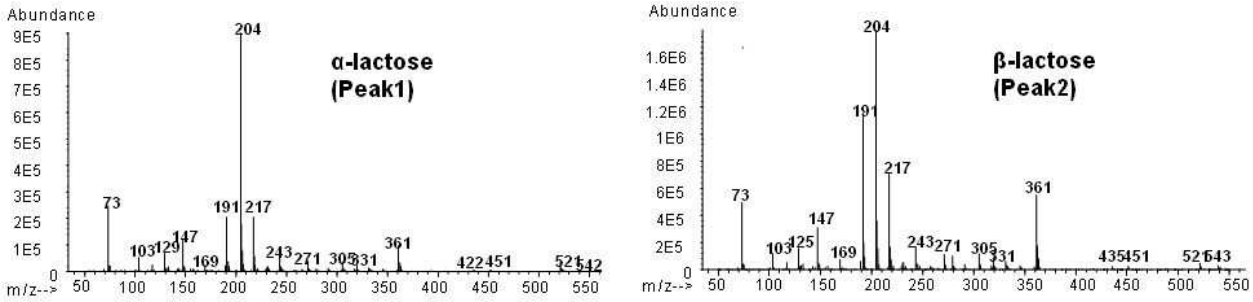


Fig 69 – Mass spectra of lactose peak1 and 2 after analysis of α- and β-lactose standards by the sugar method.

The results are shown in *Fig 70*. Like above normalisation was not necessary in the case of the sugar method. The results of the ecstasy routine method, however, were slightly improved by normalisation. For the sugar method it resulted in a nearly perfect correlation between sample amount and peak area. However, concerning the sugar method the scan mode gave already very good results and might be sufficient when the SIM mode is not available.

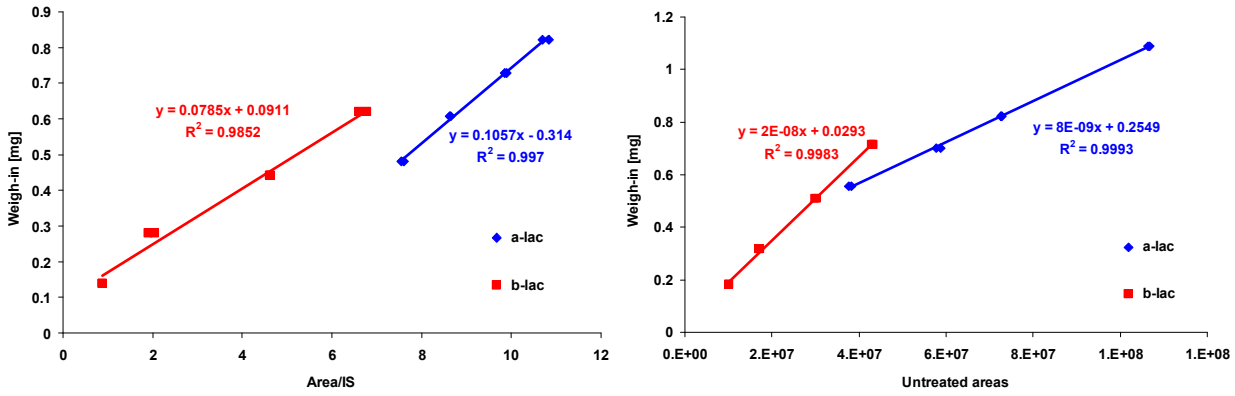


Fig 70 - α- and β-lactose content in function of the normalised areas (XTC method - left) / untreated areas (sugar method - right) of peak1 and 2, respectively – data analysis in SIM mode.

7.1.2.2 Anhydrous lactose

The five anhydrous lactose samples which have been analysed together with a β-lactose standard are of three different origins. It has to be mentioned that the β-lactose standard is not pure and has an α-lactose content of about thirty percent.

In continuation of what was shown above, the purpose of these analyses was to estimate the proportions of α- and β-lactose content and then to see if differences between the samples appeared as this might indicate a possible differentiation of origin. Since the peak areas have been proven to be proportional to the respective lactose anomer content, the analysed samples are represented by their normalised peak1 and peak2 areas, corresponding to α- and β-lactose, respectively (*Fig 71*).

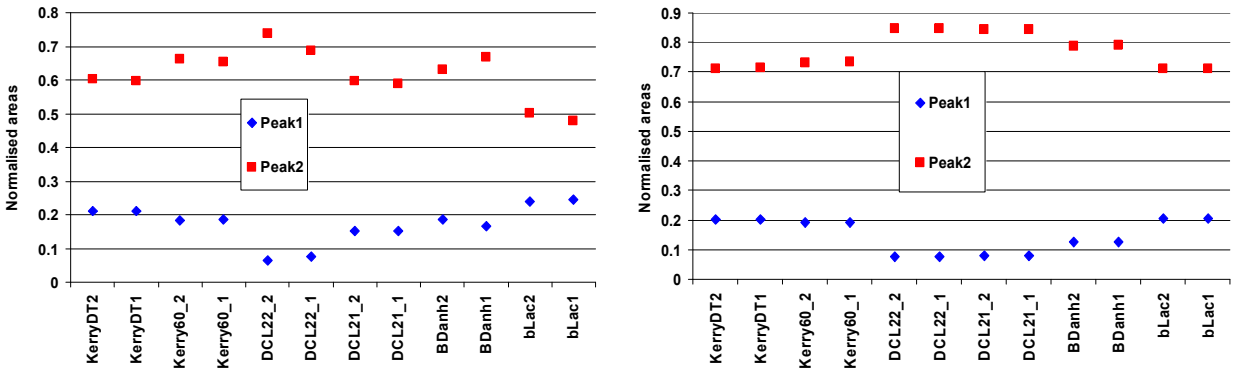


Fig 71 – Normalised peak areas for anhydrous lactose samples analysed by the ecstasy method (on the left) and the sugar method (on the right).

First of all, it can be seen that the results obtained with the sugar method are more regular than those obtained with the ecstasy method. Additionally, or rather as a consequence of it, the three origins can be detected – Kerry, DCL (DMV International) and BD (Borculo Domo) – on the graph on the right.

Using the regression equations from the first graphs it was tried to evaluate the β-lactose content for the DCL 21, 22 (DMV) and standard (b-lac – Sigma) samples as these were the only ones where the content was known. Even though this was not the usual procedure – normally the calibration samples should be analysed in the same sequence than the unknown samples – the results were rather good and near the real amount (*Table 27*).

	Real β-lactose	**Measured β-lactose content**	
Sample	**content**	**Ecstasy routine method**	**Sugar method**
DCL21	~84%	89%	84%
DCL22	~80%	100%	85%
β-lactose Sigma	~70%	72%	72%

Table 27 – Comparison of real β-lactose content with measured values for both methods.

7.1.3 Conclusions

Both methods give linear results for both peaks in function of the α- and β-lactose content and are therefore suitable for quantitative evaluation of lactose. However, considering the results above, the sugar method appears to result in better linearity than the routine ecstasy method. Since the method has been developed for sugar analysis, the results therefore confirm an improvement of performance due to analytical optimisation. Thus, this observation is not something negative, but just a logical consequence of the efforts realised. Therefore, these results indicate that if quantitative evaluation of lactose or its anomers is required, the sugar method is certainly more appropriate.

The sugar method also seemed to be more appropriate for the analysis of anhydrous lactose samples; the results appeared to be more regular and quantitative evaluation of the β-lactose content was better as well. Although, only a few samples could be analysed, these first observations were interesting. They seemed to indicate that there are differences in the α- and β-lactose content between the three producers. To determine if this could be used for origin characterisation, analysis of a bigger sample is however necessary.

On the other hand, this approach could also be used in the case of α-lactose monohydrate. However, the problem here is that the second peak is particularly small and its use could result in erroneous amounts. It appears that further investigations are needed in order to obtain a significant conclusion with regard to the origin of lactose samples. This, however, is beyond the scope of this research project.

7.2 NEAR INFRARED SPECTROSCOPY

7.2.1 Introduction

Near Infrared Spectroscopy (NIR) has gained great importance in pharmaceutical technology. Its applications are wide ranging over on-line measurement in pharmaceutical manufacturing (Herkert et al, 2001; Rantanen et al, 2000), moisture measurement (Gupta et al, 2005; Rantanen et al, 2000), verification of quality and identity of products (Yoon et al, 2004; Clarke et al, 2001), measuring of polymorphism or degree of crystallinity (Gombas et al, 2003; Buckton et al, 1998), study of physical properties due to manufacturing (e.g. particle size) (Roggo et al, 2005), identification and comparison of excipients (Krämer and Ebel, 2000; Yoon et al, 1998; Langkilde and Svantesson, 1995), and many more (Chalus et al, 2005; Reich, 2005; Bakeev, 2003). The great advantage of this technique is that it doesn't require any sample preparation and the analysis is done in less than a minute.

In this project NIR was applied for the study of lactose and cellulose – the aim being the determination of the different chemical forms of these two substances, as well as the differentiation of their origin (producer). Cellulose could not be analysed before and it was suspected to be present in ecstasy tablets where no or only a little amount of sugars was detected. Therefore the technique was to be applied to real ecstasy tablets as well. Additionally, mixtures of amphetamine and chosen cellulose and lactose standards were prepared in order to test quantification.

7.2.2 Basic Principles[27]

7.2.2.1 Absorption

On the electromagnetic spectra the infrared region is situated between the microwaves and visible light. It is divided in three zones : the near infrared (NIR) from 800 to 2500nm, the middle infrared (MIR) from 2500nm to 40µm and the far infrared (FIR) from 40 to 1000µm. (*Fig 72*)

A molecule hit by energy in form of electromagnetic radiation whose frequency of radiation matches the vibrational frequency of the molecule will show radiation absorption and the energy of the radiation will change. After the absorption of energy two types of vibrations can be observed – stretching (symmetric and asymmetric) and bending (scissoring, rocking, wagging and twisting). The result in the NIR spectra will be bands of different orders of overtones (harmonics) and combinations (with decreasing intensity) of the fundamental vibrations, which are visible in the MIR region. *Fig 73* gives an overview of the absorption bands of the most common functional groups.

[27] (Reich, 2005; Schneider, 2002; Röseler, 2004)

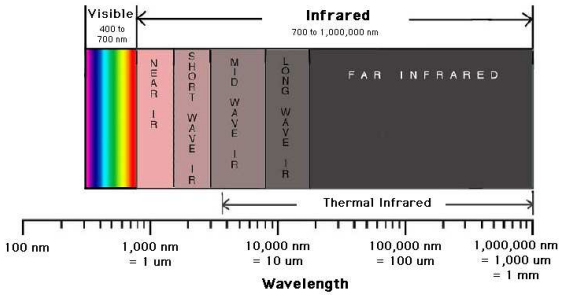


Fig 72 – Infrared wavelength region in the electromagnetic spectrum (taken from www.mvh.sr.unh.edu).

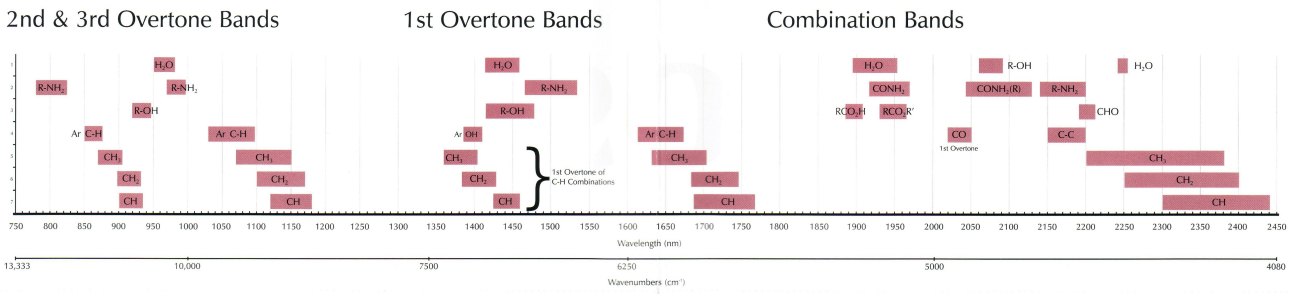


Fig 73 – Position of the absorption bands of the most common functional groups in the NIR region (taken from Clairet Scientific Ltd).

Near infrared spectra are characterised by broad and badly separated bands and a raising baseline. Assignment of bands to specific vibration is not possible because of overlapping between overtones and combinations. Distinct bands correspond principally to molecules of low atomic masses with strong chemical bonds, such as C-H, O-H, N-H or S-H. Since these functional groups can be found in almost all organic substances, NIR can be universally used. Although the single bands are not specific for structural elements, the spectra as a whole is characteristic for the compound analysed. And the low intensity of the oscillation has also its advantage, because the sample can be analysed without further preparation, e.g. in form of dilution or extraction etc.

NIR spectra do not only describe the chemical composition of a sample, but also its physical parameters. The spectra can be quite complex because they represent a multitude of information. Therefore, it is necessary to treat the spectra with chemometric methods in order to obtain a proper characterisation of a sample.

7.2.2.2 Instrumental details

The NIR system composed of a light source, a monochromator, a detector and a computer for the mathematical treatments of the spectra. The light source is in general a tungsten halogen lamp, which shows high intensity in the NIR region. Semi-conductive photo-electronic elements made off Ge, Si, PbS,

InSb or InGaAs (increasing sensitivity) are used for the detection. The detectors are chosen according to the wavelength region to scan, and their sensitivity. The reference signal is made on ceramic standard. The measurement can be done in reflection or transmission mode.

Only the reflection mode has been used in this project, and transmission will not be further detailed. In the diffuse reflection mode the detectors are placed at an angle of 45° towards the sample surface in order to detect exclusively diffuse reflected radiation, so called remission, because only this one interacts with the sample material and therefore contains substantial information. In diffuse reflection the incident wavelength is supposed to be much smaller than the sample thickness and no transmission should take place. Depending on the particle size and the compactness of the sample it is estimated that light penetrates up to a depth of 5mm. Common materials analysed in the reflection mode are solid substances, such as powders or tablets.

7.2.2.3 Data treatment

Interfering spectral parameters, such as light scattering, path length variation and random noise, resulting from physical sample properties (particle size, inhomogeneous distribution, etc) or instrumental effects, require treatment of the spectra. In the beginning it was tried to add correcting factors, but during practice it became clear that classification and prediction models worked better after reduction or even elimination of negative influences. For that reason usual software products offer a range of possible data treatments for the extraction of relevant information. In most cases, meaningful multivariate analysis of NIR measurements becomes only possible after having transformed the data by the means of math-treatments. Some of these will be presented hereafter.

Raw Spectra

Reflectance spectra are transformed by a logarithmic function (log 1/R) based on the Lambert-Beer law. The so-called absorbance spectra are preferred to the reflectance spectra and also recommended by the European Pharmacopeia. It also allows the establishment of a nearly linear relationship between the concentration of the analyte and absorption. After having taken the measure of a sample, the transformation is directly carried out by the software and spectra are displayed as absorbance spectra.

1/X Transformation

An spectra can be displayed as function of the wavelength (λ, nm) or the wave number (ν, cm^{-1}). This depends on the monochromator used in the spectrometer. In NIR spectroscopy the use of the wavelength has been established and is standard now. However, it is the wave number which is directly proportional to the absorption energy. The 1/x transformation consists in a conversion of all data points in wavelength

spectra into data points in wave number spectra, which is a more realistic presentation. In the λ presentation longer wavelengths have more statistical weight, which influences the interpretation of the data. In ν presentation all data points have the same weight according to their energy.

Standard Normal Variate (SNV)

The method has been developed to compensate scatter-induced baseline offsets. It consists in a standardisation of the spectra according to the following equation :

$$A_i^{SNV} = \frac{A_i - \overline{A}}{\sqrt{\frac{\sum_{i=1}^{n}(A_i - \overline{A})^2}{n-1}}}$$

with A_i absorbance at point i
$\overline{A}$ mean value of the spectra
n number of data points in the spectra

Each spectrum is mean centred and then divided at every data point by the standard deviation of the absorbance values of the entire spectrum. Every spectrum is treated separately and does not require normal distribution such as is necessary for MSC (see below). However, SNV and MSC give similar results.

Multiplicative Scatter Correction (MSC)

The method was developed for the same purpose than SNV, but here the treatment is based on a data-set and not on an individual spectrum. This is to eliminate scatter-induced effects on the spectra by linearization on an "ideal" spectrum. This "ideal" spectrum is in fact the mean spectrum calculated from all the spectra in the set. The mean spectrum is used to calculate two correcting factors, offset and slope, which are established through a Least Squares Regression. The offset value is subtracted from every data point in the spectrum and then divided by the slope value.

$$A_i^{MSC} = \frac{(A_i - b_i)}{m_i}$$

with A_i absorbance at point i
b_i offset value
m_i slope value

MSC requires a large data-set, more likely to produce a true mean spectrum.

Derivatives

Derivatives can be applied to improve the resolution and to reduce baseline offsets. The most commonly used derivatives in NIR are the 1st and the 2nd derivative. The first corrects baseline offsets and the second describes the parabolic curvature of the spectrum. The principle change of the spectrum is shown in *Fig 74*.

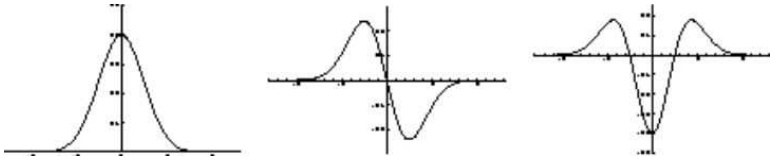


Fig 74 – Curve change by derivation from left to right – original curve, first and second derivative.

Since derivation also amplifies spectral noise, it is often combined with a Savitzky-Golay algorithm which is both a smoothing and derivative algorithm. It relies on the least squares fit of a polynomial to a specified segment. Each segment is derived by also considering the adjacent segments. Therefore the amplification of spectral noise provoked by the derivation is reduced by the smoothing through the adjacent segments. The difficulty in derivation is the choice of the spectral segment size. If the segment is too small the smoothing will have nearly no effect. If it is too big smoothing will be too strong and spectral information gets lost and artefacts are created.

7.2.2.4 Multivariate Analysis[28]

Principal Component Analysis - PCA

Principal Component Analysis is a descriptive method which permits to model a single data set, so as to find the underlying structure of the data, study the correlation between variables, describe the objects according to summary variables, detect outliers or extreme observations, etc. Sample patterns, such as particular groupings, can be observed and useful information identified – as opposed to noise or meaningless variation.

Every sample in a data set defined by *n* variables can be represented as a point in a *n*-dimensional space. Thus, the data set is a swarm of data-points in this space and presents a general shape (*Fig 75*, ①). The idea of PCA is to reduce the *n* variables to a few number of principal components (PC) containing the maximum variation (which is information) characterising the data set. First, the origin of the variable space is translated to the centre of the swarm of points (the average of each variable X_i is subtracted from the value of X_i for each observation). The first PC is then chosen by drawing a line through the cloud of data-

[28] (Reich, 2005; Schneider, 2002; Röseler, 2004; CAMO, 2001; Massart, 1988)

points in such a way that variation is maximum along the line and minimum around it (*Fig 75*, ②). This means that the line is directed more or less the maximal elongation of the cloud of points.

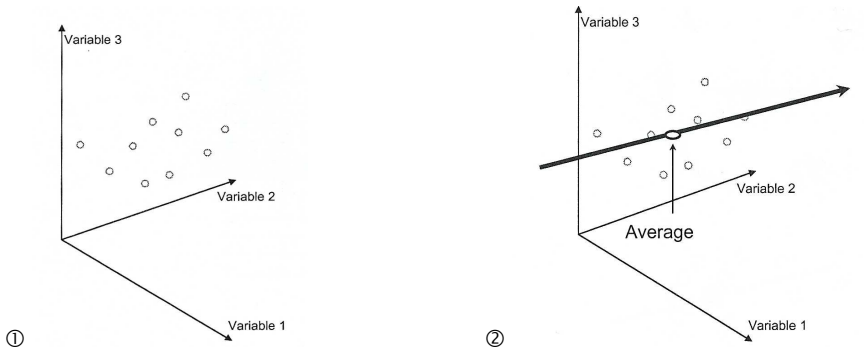


Fig 75 – Data set described by three variables and determination of the first PC. (CAMO, 2005)

The second PC is chosen in the same way, by considering the plane orthogonal to PC1 and choosing the direction with maximum spread around it (*Fig 76*, ③).

Every new PC is representing the maximum variance of the residual information (not taken into account by the previous PCs – *Fig 76*, ④). This can go on until as many PCs have been computed as there are variables in the data table. It is important to find a compromise in the number of PCs since only the first ones contain useful information and the latter ones most likely describe noise. A model with a high number of PCs is often more complex and less robust.

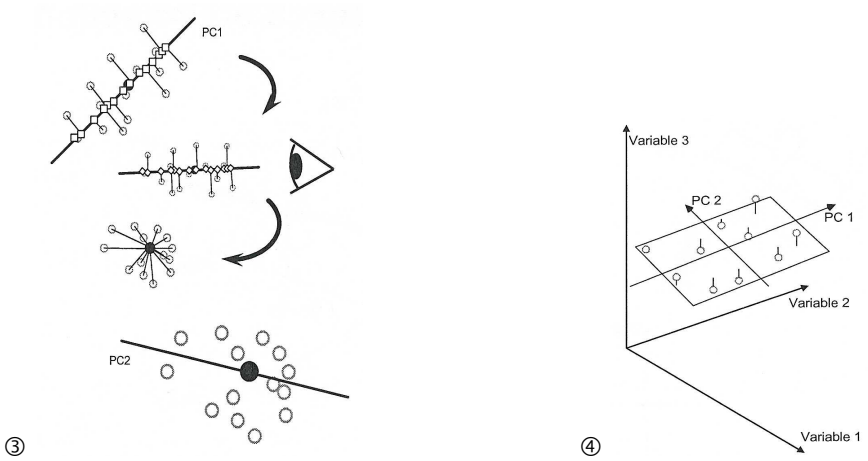


Fig 76 – Determination of the second and third PC. (CAMO, 2005)

Each component of a PCA model is characterised by three complementary sets of attributes :

- *Scores* show how the samples are distributed, any specific patterns in the data, and which objects are close to each other.
- *Loadings* reflect how the original variables are taken into account on each projection (PC). The loading plot shows which variables are important and how variables are correlated.
- *Variances* are error measures and show how much of the information contained in the data set is actually taken into account by the model.

Partial Least Squares - PLS

PLS is a regression method, a term for all methods attempting to fit a model to observed data in order to quantify the relationship between two groups of variables. The purpose of the method is

1) to describe the relationship between a set of predictors (X-matrix) and a set of responses (Y-matrix). The mathematical form of this relationship is called a model.

2) to predict new values, for which only the X-values are known.

Just like PCA the PLS regression is based on projection principles. Partial Least Squares – or Projection of Latent Structures – models both the X- and Y-matrix simultaneously to find the latent variables in X that will predict the latent variables in Y the best. The objective is to maximise the covariance between X and Y. The PLS components are similar to principal components and are therefore referred to as PCs.

There are two versions of PLS algorithms – PLS1 only deals with one response variable at a time, whereas in PLS 2 several response variables are modelled together. The PLS2 is a bit like a double PCA, with the additional constraint that the t- and u-scores – summarising sample differences in X and Y spaces – should be as correlated as possible. Since the PLS has to handle two matrices scores, loadings and variances will be in double. There are two additional attributes to consider :

- *Loading weights* show how much each X-variable contributes to explain the Y-variations, and therefore exists only for the X-variables.
- *B-coefficients* are specific to regression methods and are also called *regression coefficients*. The PLS model can be written in an equation that summarises how each response variable is related to the set of predictors.

$$Y = B_0 + B_1X_1 + B_2X_2 + \ldots + B_NX_N$$

7.2.3 Practical Work

7.2.3.1 Instrumentation

Analyses were realized on a FOSS SmartProbe™ Analyzer 6500, equipped with a monochromator for 400 - 2500nm coverage and a SmartProbe module with integrated detector and a 3 meter optic interactance fibre bundle. The spectra were recorded in diffuse reflection mode in the full range coverage (400 – 2500nm) and at 2nm intervals. Individual spectra were determined as averages of 32 scans. A ceramic standard served as reference.

7.2.3.2 Procedure

a) *Cellulose/lactose standards* – Free samples could be obtained from 22 suppliers. The 25 cellulose and 23 lactose samples are listed below (*Table 28* and *Table 29*). In the beginning all samples were analysed as powders directly in their containers. Then some samples were selected for compression in order to verify if there would be a great difference in the NIR spectra. As this turned out to be the case, all samples were compressed and then measured partly as whole tablet (10 measures on every side), partly as crushed powder (10 measures).

Supplier	Product name	Composition
Acros		Carboxymethyl cellulose Sodium salt Ethyl cellulose Hydroxypropyl cellulose Methyl cellulose
Asahi Kasei	Ceolus	Microcrystalline cellulose
Blanver	Microcel MC-101	Microcrystalline cellulose
CFF	Sanacel Pharma 90 Qualicel 90	powdered cellulose Cellulose
Colorcon	Methocel E-50 Methocel A15	Hydroxypropylmethylcellulose Methyl cellulose
DMV	Primellose	Na cross-linked carboxymethyl cellulose
FMC	Avicel 102	Microcrystalline cellulose
Fluka	Avicel 101	Microcrystalline cellulose
Merck*		Microcrystalline cellulose
JRS Pharma	Vivapur101	Microcrystalline cellulose
Nisso	Nisso-HPC	Hydroxypropyl celllulose
Noviant	ZSB 16 Nymcel ZSX	Sodium Carboxymethylcellulose Crosslinked Sodium Carboxymethylcellulose
NP Pharm	Ethispheres 250	Microcrystalline cellulose
Shin-Etsu	LHPC11 LHPCb1 Metolose60SH MetoloseSM Pharmacoat	Low-Substituted Hydroxypropyl Cellulose Low-Substituted Hydroxypropyl Cellulose Hydroxypropylmethylcellulose Methyl cellulose Hydroxypropylmethylcellulose
Wolff Cellulosics	Walocel CRT 15000 PPA	Sodium Carboxymethylcellulose

*the sample was not sent by Merck but obtained in a local drugstore

Table 28 – List of obtained cellulose samples with their commercial and chemical name.

Beforehand, measuring conditions had to be tuned in order to get reproducible results. Test measures included varying pressure, amount of powder, as well as measures on whole tablets and crushed tablets. Pressure did not have much influence, but so did quantity and stability of the probe. Thus, stable conditions were fixed for the continuation of the project.

Supplier	Product Name	Composition
Acros		Lactose monohydrate
BASF	Ludipress Ludipress LCE	Lactose Mh (93%), povidone (3.5%), crospovidone (3.5%) Lactose Mh (96.5%) and povidone (3.5%)
Borculo Domo	Lactopress Spray-Dried Lactopress Anhydrous Lactochem Lactochem Crystals	Lactose Monohydrate + 15% amorphous lactose Anhydrous lactose Lactose Monohydrate Lactose Monohydrate
DMV	Pharmatose DCL 14 Pharmatose DCL 21 Pharmatose DCL 22	Lactose Monohydrate spray-dried Anhydrous lactose Anhydrous lactose
Fisher		Lactose monohydrate
JT Baker - Mallinkrodt Baker	Lactose	Lactose monohydrate
Lactose New Zealand	Super-Tab Spray-Dried	Lactose Monohydrate
Meggle	GranuLac 70 Tablettose 80 FlowLac 100 Cellactose 80 MicroceLac 100 StarLac	Lactose monohydrate Lactose monohydrate Lactose monohydrate 75% lactose monohydrate + 25% cellulose 75% lactose monohydrate + 25% microcrystalline cellulose 85% lactose monohydrate + 15% maize starch
Kerry Bio-Science	Impalpable Lactose Anhydrous 60M Lactose Lactose, Anhyd, DT NF	Lactose monohydrate Anhydrous lactose Anhydrous lactose
Sigma		Beta-lactose

Table 29 – List of obtained lactose samples with their commercial and chemical name.

b) *Amphetamine blends* – In order to study the possibilities of NIR technique to analyse substances such as found in illicit tablets, six cellulose and seven lactose standards were chosen to make mixtures with amphetamine at three purities (5, 10 and 15%). Amphetamine has been chosen as it was available in large quantity. The selected standards are listed in *Table 30*.

All the mixtures were homogenised in an agate mortar before analysis. Four mixtures were taken for compression – 3, 6, 10 and 11 – and analysed as solid form and crushed.

1 - CFF Sanacel 90	7 - Borculo Domo Lactopress Anhydrous
2 - Colorcon Methylcellulose	8 - Borculo Domo Lactopress Spray-Dried
3 - FMC Avicel 102	9 - DMV Pharmatose DCL 14
4 - Blanver Microcel MC-101	10 - DMV Pharmatose DCL 21
5 - Shin-Etsu Metolose SM	11 - Meggle FlowLac 100
6 - JRS Vivapur 101	12 - Kerry Anhydrous Lactose Direct Compression
	13 - Lactose New Zealand

Table 30 – Selected cellulose (1-6) and lactose (7-13) samples for amphetamine blends.

c) *Ecstasy* – 39 ecstasy tablets from 35 seizures were analysed with the aim to determine the excipient and to check them against each other. The configuration of the instrument did not allow analysing them in their solid form, the surface of the tablets being smaller than the surface of the probe. Thus, they were homogenised in agate mortar before analysis. Five measures were taken for every tablet and care was taken that the powder was regularly shaken in order to vary particle disposition.

7.2.3.3 Software

Vision©

Vision© is a software package specifically designed for use with the FOSS NIRSystems and is delivered with the NIR instrument. It was developed for NIR spectral acquisition, method development and routine analysis for the pharmaceutical and chemical application. Thus, it controls the instrument and allows further treatment of the spectra. Several math treatments have been included for the construction of a database and characterization / identification of analysed substances. It was used for the acquisition of the spectra and first observations after having applied pre-treatments, such as SNV and derivations.

The Unscrambler©

The Unscrambler© is a software developed by Camo, Norway, and is designed for multivariate analysis. It offers a selection of common pre-treatments, which can be followed by explorative analysis (PCA, Descriptive statistics). Quantitative models can be built by different types of regression analysis (PLS1, PLS2, PCR, MLR) and used for prediction of responses of new samples. Furthermore, it proposes methods for classification (SIMCA) and experimental design (ANOVA, Response surface analysis). Data could be directly imported from Vision software by using NSAS files. All treatments of spectra have been realised with The Unscrambler©.

7.2.4 Results

It has to be kept in mind that this project was an additional research, completing the main work about sugar and fatty acids, and therefore data analysis has not gone that far as it would have in a more consequent study. What will be presented hereafter is not only the answer to the exposed questions, but is also meant to give an idea of what can be done in the given context.

a) Cellulose / Lactose standards

Differentiation of chemical form

In the case of cellulose, seven different forms have been analysed by NIR. The raw spectra are shown in *Fig 77*. Since the names of the different types are rather long, abbreviations have been used and will also be applied here in this report. The abbreviations used were the following :

Cell = Microcrystalline cellulose	**LHPC** = Low-Substituted Hydroxypropyl Cellulose
CMC = Carboxylmethylcellulose	**HPMC** = Hydroxypropylmethylcellulose
EC = Ethylcellulose	**MC** = Methylcellulose
HPC = Hydroxypropylcellulose	

They can be visually differentiated, but show in some cases a similar shape. The spectra of HPMC and MC, for example, are very close and it would be impossible to determine the type when seeing the spectrum of each alone.

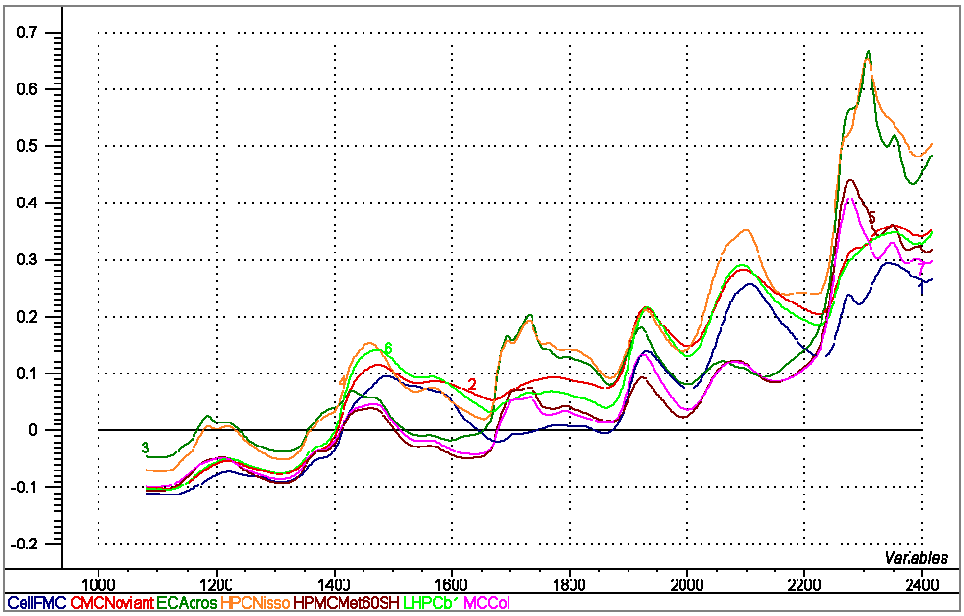


Fig 77 – Chemical forms of cellulose – Blue (1)=microcrystalline cellulose, Red (2) = Carboxylmethylcellulose, Dark Green (3) = Ethylcellulose, Orange (4) = Hydroxypropylcellulose, Brown (5) = Hydroxypropylmethylcellulose, Clear Green (6) = Low-Substituted Hydroxypropyl Cellulose, Pink (7) = Methylcellulose.

However, it has to be evaluated to what extent variations observed on NIR spectra are significant. Visually this can be done by considering in the beginning the variation for one standard. For that purpose thirty measures per sample have been taken and it can be seen on the two pictures below (*Fig 78*) that the variation can be very different depending on the sample.

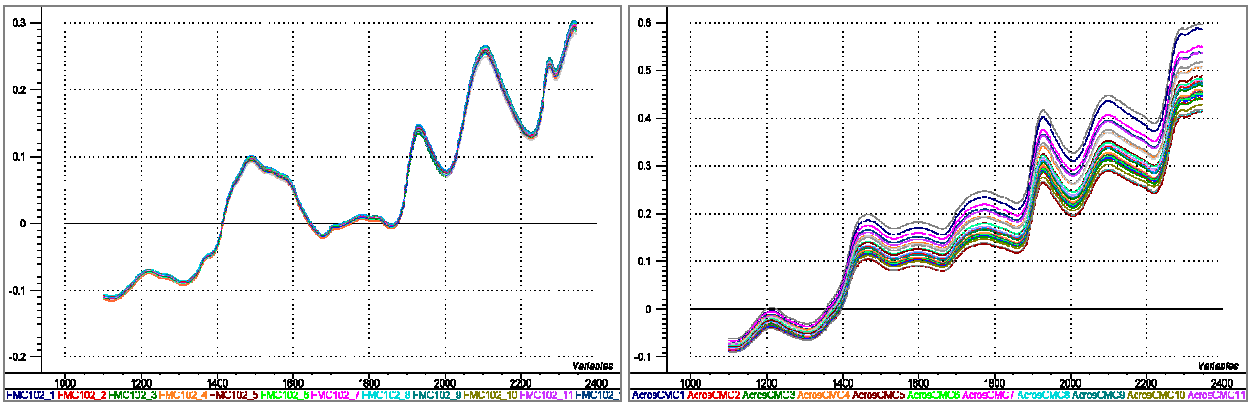


Fig 78 – 30 measures of standard FMC microcrystalline cellulose (on the left) and Acros CMC (on the right).

But it has to be said that the example on the right (Acros) was more an exception, and generally the spectra for one standard were very close already on the raw spectra. Additionally, when considering the spectra as such, it is recommended to apply a noice-reducing pre-processing method, such as SNV. The importance of the treatments of spectra has been explained earlier in this chapter, but it was realised during the observation of these standards that a variation detected after e.g. SNV may indicate a real difference between samples. *Fig 79* is showing an example of the SNV treated thirty spectra of microcrystalline cellulose produced by FMC and one originating from NPPharm – the difference is well visible.

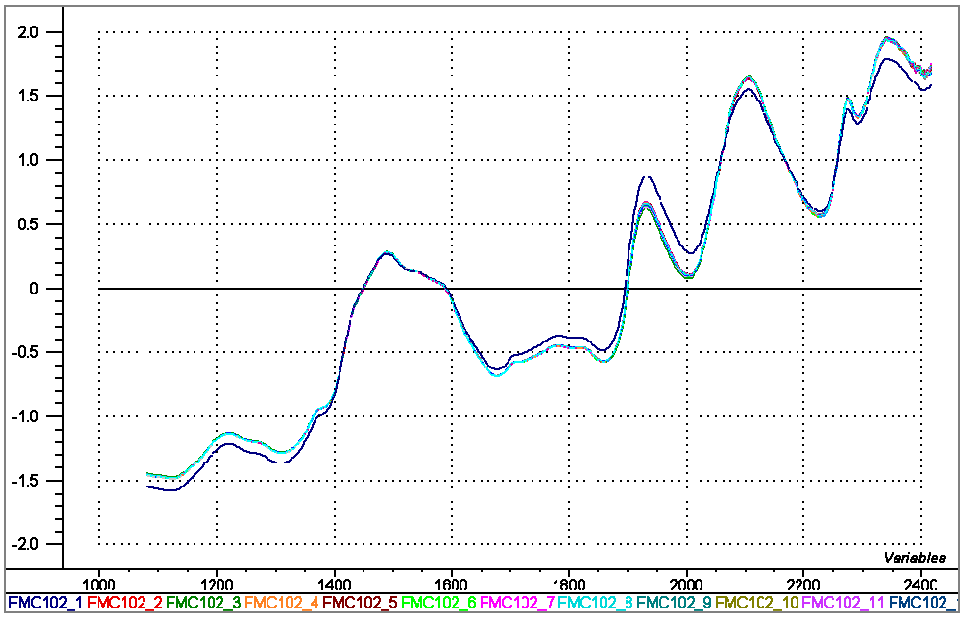


Fig 79 – FMC cellulose standard (30 measures) with one measure of the NPPharm cellulose (dark blue).

Later, we will see that the differentiation between producers is not that easy. One of the reasons is that what appears here as a visible difference is principally due to varying intensities – the general curve shape (minima, maximum, slopes) is very similar.

Altogether, the visual comparison of the spectra is good and necessary in the beginning, but very tiresome when the mass of data is getting larger. And as the Vision© software was also designed to create databases, which would then be applied in routine to quickly identify the analysed substance, it was decided to test the automatic recognition of our samples. The work was started on Vision©, but due to software problems, all data treatments have been done with The Unscrambler©.

The first aim was to determine a data pre-processing method and appropriate wavelength selection that produces a PCA model separating the seven types of cellulose. The mean spectra of all powdered and compressed standards have been imported to The Unscrambler© and various pre-processing methods (Yoon et al, 2004; Schneider, 2002; Röseler, 2004; Roggo et al, 2005; Krämer and Ebel, 2000; Chalus et al, 2005; Brigger et al, 2000) have been tested – they are all listed in Appendix XII. In general, all PCA analyses were first applied on the whole spectrum range (1100 to 2300nm), but often a wavelength selection was applied as well. The principal reason was to avoid the influence of water which has strong overtones in the 1450 and 1930nm region (Yoon et al, 2004; Schneider, 2002; Rantanen et al, 2000; Bakeev, 2003). Later it would also be used to select a region that seems particular for a substance.

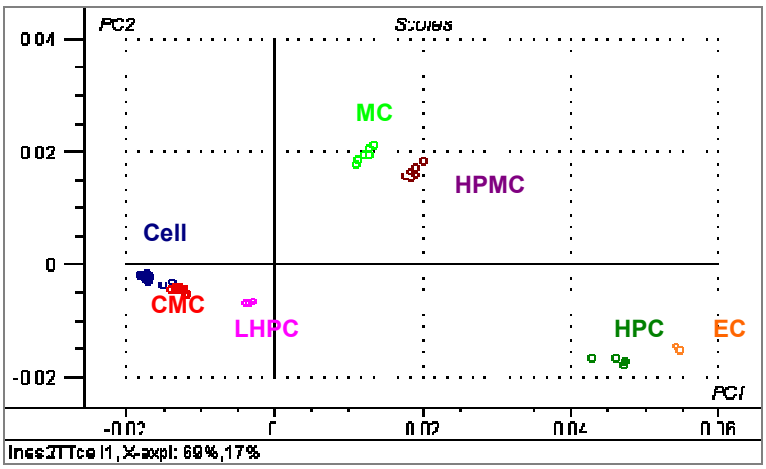


Fig 80 – PCA after pre-processing method New2 (Appendix XII) of the seven cellulose groups.

It appeared that complete separation was made difficult by two couples – HPMC/MC as expected, but also CMC and Cell. It can be seen on the figure above that the respective cellulose types are close (*Fig 80*). But as it seemed to be possible to differentiate the seven groups, the pre-processing method was used to create a PLS1 model. The x-variables were the chosen wavelengths of the standard spectra and the y-variable to predict was a series of numbers standing for the corresponding type of cellulose. Only four of the seven cellulose forms were used for this work – Cell, CMC, HPMC and MC – because only a few samples were available for the others. The regression plot of the created model is shown in *Fig 81*. The model was tested with 21 randomly chosen spectra which have been all correctly identified.

	Y
Microcrystalline C.	1
CMC	2
HPMC	3
MC	4

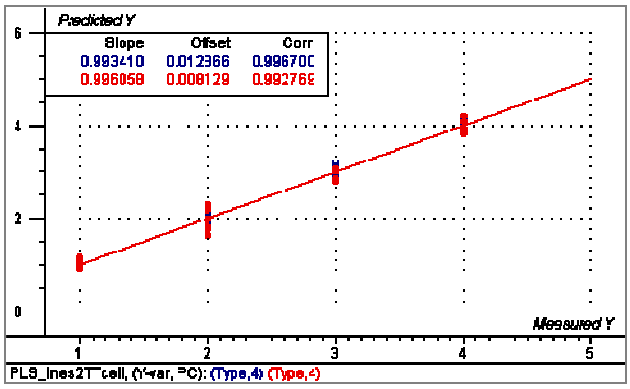


Fig 81 – Predicted vs measured regression plot for the prediction of the cellulose types.

It has been mentioned sooner that the data analysis has been started on Vision©. Indeed, a database for the cellulose standards could be created and several mathematical treatments have been tested. To verify the quality of the database, samples were analysed just like in routine in order to see if they were correctly identified - most of them were not. The obtained spectra of these 28 samples were taken now to test the PLS model and the result is presented in *Fig 82*.

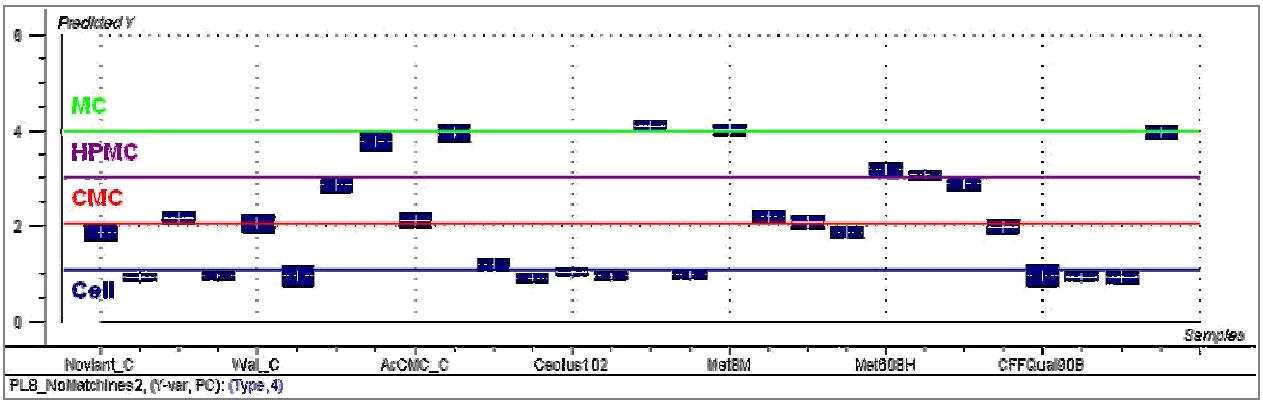


Fig 82 – Prediction results for samples non-identified by the Vision© database.

All spectra were correctly predicted. For each sample a deviation is given which expresses how similar the prediction sample is to the calibration samples used when building the model. The more similar, the smaller the deviation. The average deviation for these samples is 0.13.

Concerning the lactose samples, only three types had to be differentiated – lactose monohydrate, anhydrous lactose and the blends (Ludipress, LudipressLCE, Cellactose, MicroceLac, StarLac). The raw spectra of three standards are shown in *Fig 83*. The same observations than those for cellulose are valid concerning the comparison of the spectra and will not be detailed again.

Abbreviations have also been used for the lactose samples and will be applied here :

LacMh = lactose monohydrate **AnhLac** = anhydrous lactose

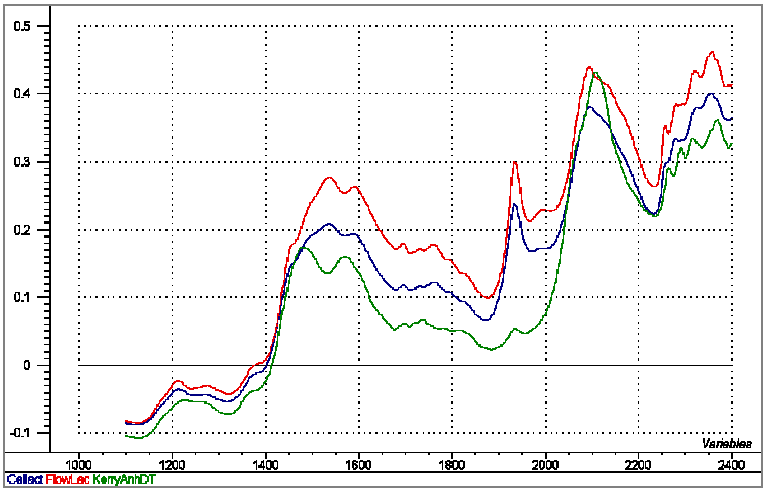


Fig 83 – Chemical forms of lactose – Red = Lactose monohydrate, Green = Anhydrous lactose and Blue = CelLactose (a blend of cellulose and lactose monohydrate).

Even though the spectra are not very different they could be easily separated. The spectra have also been treated for PCA analysis and lactose monohydrate and anhydrous lactose were already separated by using the raw spectra. However, to differentiate the blends from the lactose monohydrate pre-processing of the data and wavelength selection was necessary.

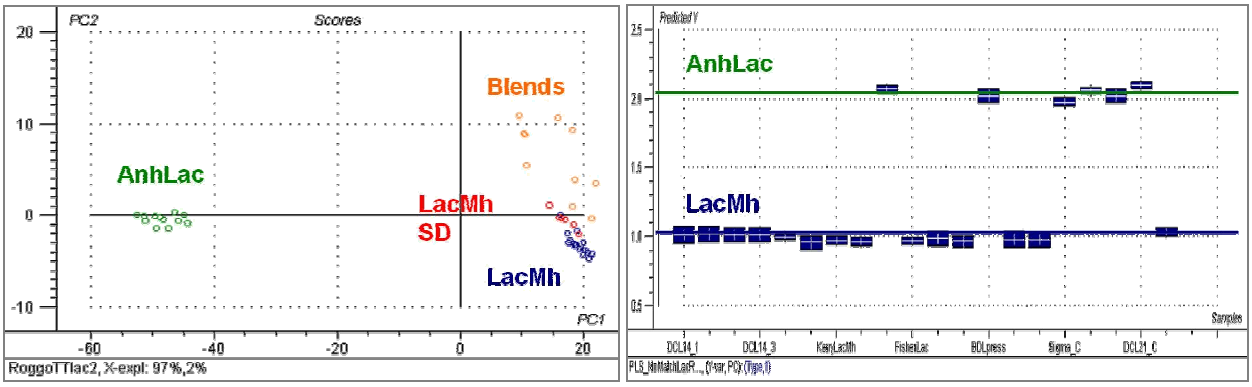


Fig 84 – On the left : PCA after pre-processing method Roggo (Annexe XII) – on the right : prediction results for non-identified lactose samples applying a PLS1 model after pre-processing method Roggo.

It can be seen in *Fig 84* that PCA analysis achieves separation of the three groups. The blends are not surprisingly a little dispersed and those with the highest lactose monohydrate content are very close to the pure standards. The few red spots at one side of the lactose monohydrate group are samples being spray-dried. Thus, it is the same compound (Lac Mh) but produced differently. Prediction of the non-identified samples by Vision© was attempted as well, using the same pre-processing method than for the PCA analysis. The result, at the right in the figure above, shows a clear separation between the two types of lactose and spectra were also correctly identified with an average deviation of 0.04.

Differentiation of producers

This part was carried out with spectra from cellulose, powdered and microcrystalline cellulose (Abbreviation - Cell), because this is the chemical form the most often used as excipient and the most likely to be found in ecstasy tablets. It can be seen on *Fig 85* that the samples can not be distinguished visually apart from the two powdered cellulose standards (CFFSan and CFFQual).

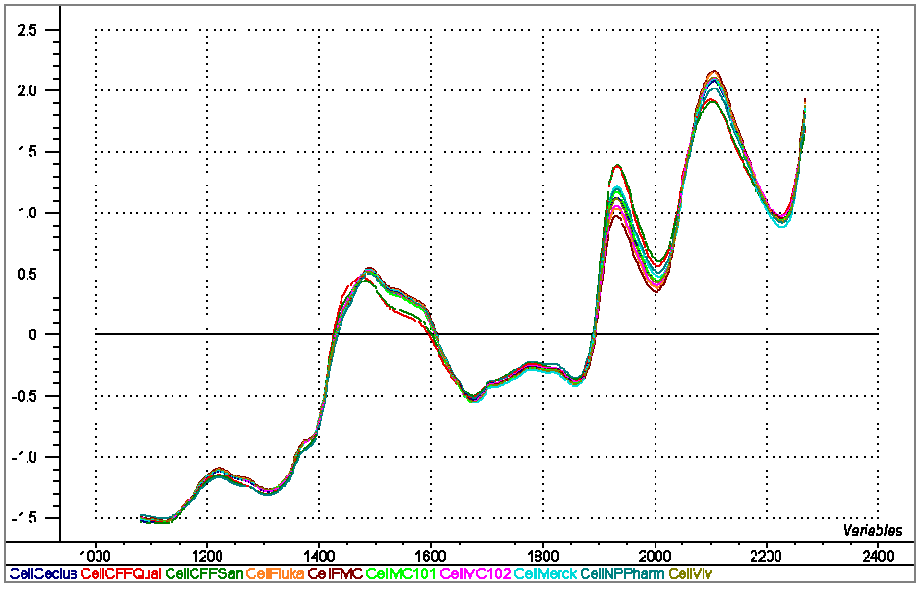


Fig 85 – SNV treated spectra of the ten cellulose standards.

For each standard, compressed and powdered, fifteen spectra were imported to the software in order to test PCA analysis preceded by the various pre-processing methods (Annexe XII). The result appeared to be rather complex – the spectra being almost identical very small differences would make out the grouping by PCA. Thus, the first observation was that each standard formed little subgroups and the reason for that was not clear. One factor having certainly a great influence was the compression. For the same standard the measures after compression were differentiated from those before compression.

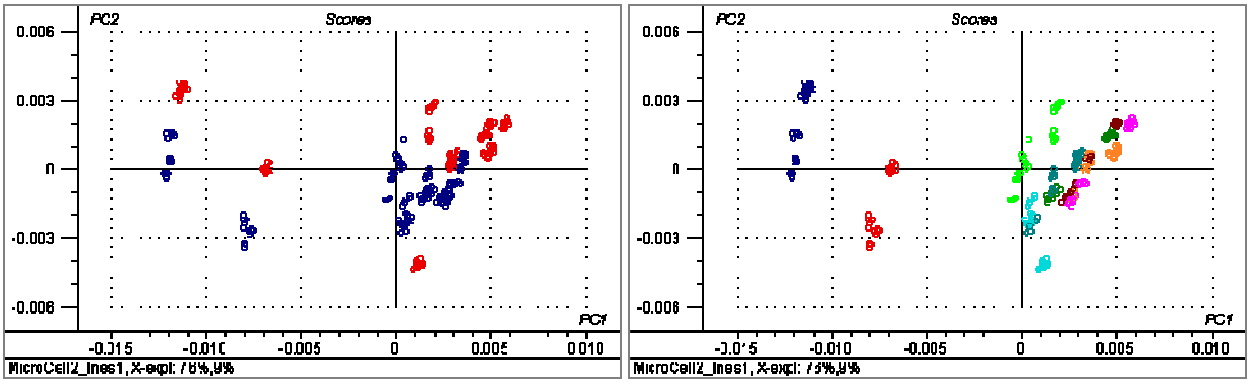


Fig 86 – PCA of Cell standards after pre-processing method New2.

The two pictures above (*Fig 86*) show the same PCA – on the left samples have been coloured in function of compressed / non-compressed, on the right side the sample identity is highlighted. It was then decided to consider the compressed and non-compressed spectra separately and it appeared that different producers could indeed be differentiated, but only using the measures of the powdered samples (*Fig 87*, right).

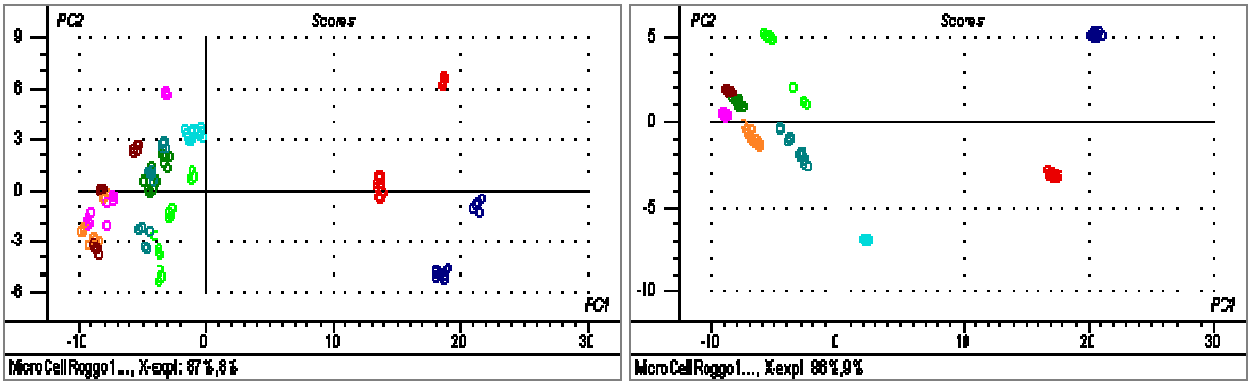


Fig 87 – PCA of Cell Standards after pre-processing method Roggo – on the left PCA of compressed standards, on the right PCA of non-compressed standards.

Since this is a sort of exploratory research in the context of ecstasy analysis, differentiation after compression was much more important. Considering the PCA of the measures taken from tablets (*Fig 87*, left), the situation is similar to the beginning in the sense that again subgroups are present. It appeared that these could be explained by the different way of measuring – on the tablet surface or the crushed powder. The result is that many small groups of 4-5 measures are formed and superpose. An important conclusion that can be drawn is the importance of being coherent in sample measuring and processing. If the same PCA would be carried out but with spectra produced in exactly the same conditions the groups would separated, as can be seen in *Fig 88* .

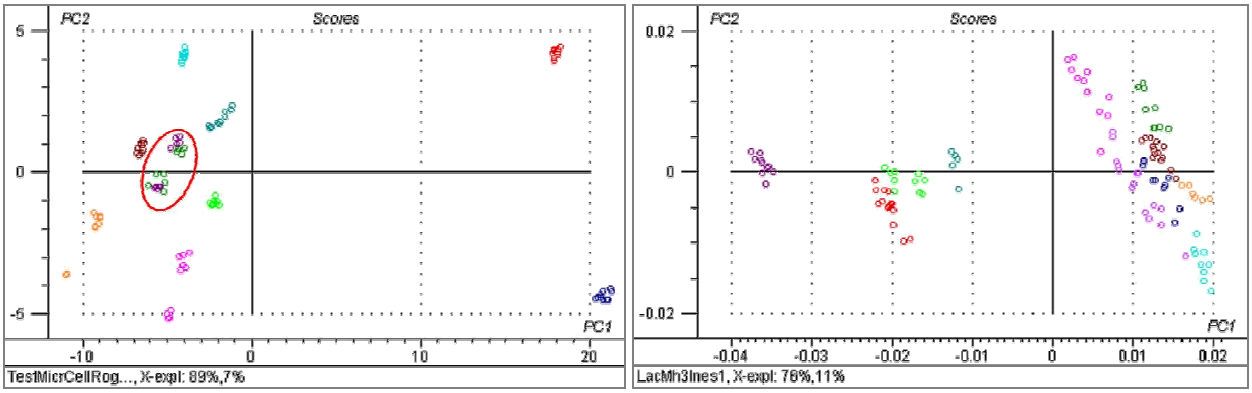


Fig 88 – Left : PCA of compressed Cell standards after pre-processing method Roggo; Right : PCA of compressed LacMh standards after pre-processing method New2.

Only measures of crushed tablets have been taken for this PCA. Spectra could almost all be separated in function of their producer, except Ceolus and Vivapur (surrounded by the red circle – *Fig 88*). The samples that still form subgroups are those where measures have been made on two crushed tablets. The same kind of observations was made with the lactose monohydrate standards where it was also attempted to separate the different origins. Again, a test with measures taken in the same conditions did not show such a neat separation than in the case of cellulose, but the different groups are not overlapping (*Fig 88*, on the right).

The data analysis of the cellulose and lactose has been stopped here. It has been shown that differentiation of the producers is possible, however under very strict conditions. It has to be reminded that the work was carried out with standard samples directly obtained from the producer – a situation far from "ecstasy reality". Ecstasies are compressed blends, maybe not very complex blends, but still complex enough not to allow such a specific differentiation. For the determination of the chemical form of the excipient alone it would be necessary to proceed to an extraction. But the results are still very interesting as they show that small differences can be detected.

b) Amphetamine blends

Five spectra per concentration have been imported for the evaluation of the amphetamine blends – this means fifteen spectra per excipient resulting in 255 spectra altogether. Before starting any data analysis spectra have been compared in order to see if the change in concentration could be detected, if it was regular and in what wavelength region it could be observed. In *Fig 89* the three blends of CFFSanacel are shown in the wavelength section where the changes were the most obvious. The three groups can be easily distinguished. Changes due to the concentration could also be observed in other wavelength regions, such as around 1140nm and 2200nm, but were less visible.

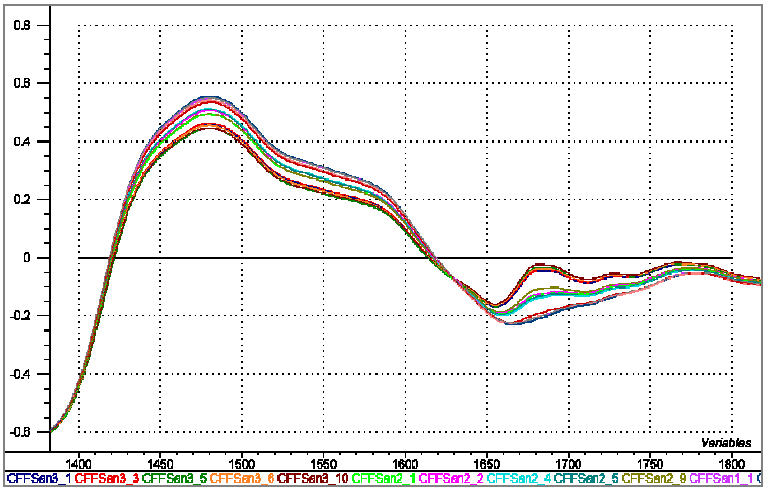


Fig 89 – Amphetamine blends with CFFSanacel in the region between 1400 and 1800nm (after SNV treatment).

Considering these encouraging observations it was decided to test PCA analysis and even PLS prediction. The question of prediction of illicit substance content in ecstasy has been thoroughly studied by Sondermann (1999) and (Schneider, 2002), who have shown that good prediction is possible. It was therefore tested on our amphetamine blends. Additionally, the identification of the excipient was attempted as well.

The various pre-processing methods were tested for PCA classification to insure good separation of the four types of excipients used and simultaneously of the three concentrations prepared. This was achieved after wavelength selection of the three interesting regions and the results are shown in *Fig 90*. The four different types of excipients – microcrystalline cellulose, methylcellulose, lactose monohydrate and anhydrous lactose – are very well separated. And inside of each group the three prepared concentrations can be distinguished.

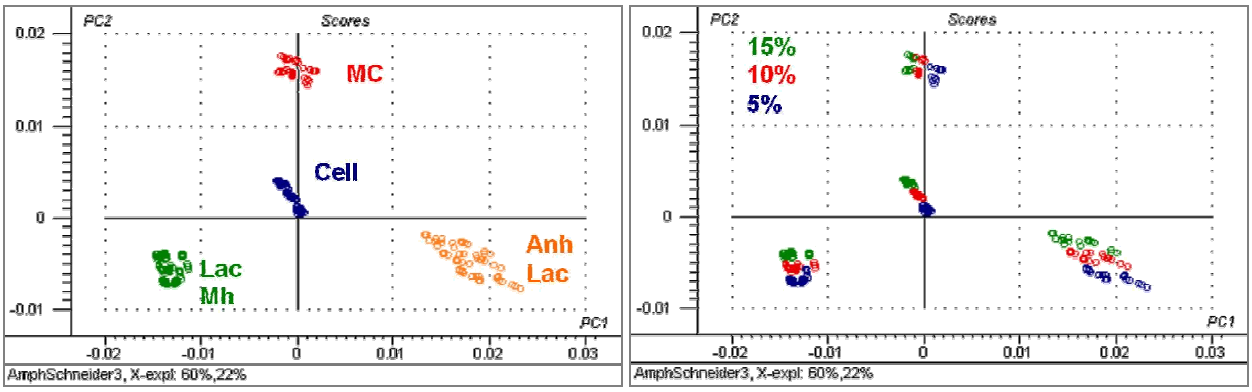


Fig 90 – PCA of amphetamine blends after pre-processing method New2 – highlighting the type of excipient on the left, and the concentration on the right.

The pre-processing method applied for the PCA analyses above and three others giving similar results have been tested for the creation of a PLS2 model. Additionally, the sample set was completed by remaining spectra not imported in the beginning, so that 390 spectra could be used for this model. Again, for the x-variables the selected wavelengths have been chosen (1120-1160, 1458-1740 and 2100-2300nm), and for the y-variables a series of numbers standing for the corresponding excipient and the amphetamine concentrations expressed as percentage.

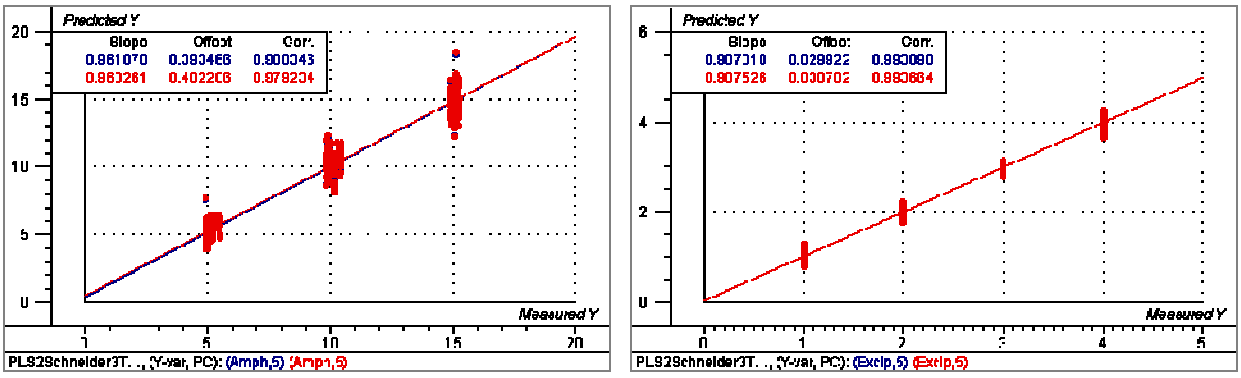


Fig 91 – PLS2 after pre-processing method New2 – Predicted vs measured regression plot for the amphetamine concentration on the left and the excipient on the right.

In the figure above the predicted values for the amphetamine concentration and the identity of the excipient are plotted against the measured values (*Fig 91*). Slope and correlation should be near 1. As they were very high for both y-variables it was decided to test the model on a set of selected spectra representing the whole data set used for the construction of the model. The cross-validation mode was used for this prediction test. The results are represented in *Table 31*.

	Amphetamine concentration			Excipient			
Sample	**Predicted**	**Deviation**	**Reference**	**Predicted**	**Deviation**	**Reference**	
BDLactp3_5	15.96	0.78	15.11	3.99	0.12	4.00	Anh Lac
BDLactp2_3	9.70	0.60	10.01	4.07	0.09	4.00	Anh Lac
BDLactp1_1	4.89	0.80	5.00	4.14	0.13	4.00	Anh Lac
BDLactpSD1	5.11	0.72	5.10	2.94	0.11	3.00	Lac Mh
BDLactpSD3	14.32	0.70	15.00	3.00	0.11	3.00	Lac Mh
BDLactpSD2	10.28	0.53	10.36	2.96	0.08	3.00	Lac Mh
CFFSan90_3	12.37	1.43	15.09	1.28	0.22	1.00	Cellulose
CFFSan90_2	9.92	1.24	10.17	1.19	0.19	1.00	Cellulose
CFFSan90_1	4.18	1.54	4.91	1.16	0.24	1.00	Cellulose
ColMC3_2	15.23	1.31	14.93	1.78	0.21	2.00	Methylcell
ColMC2_3	10.29	0.64	9.87	1.93	0.10	2.00	Methylcell
ColMC1_8	6.03	0.84	4.92	2.14	0.13	2.00	Methylcell
DCL21_1_13C	4.03	1.47	4.99	4.19	0.23	4.00	Anh Lac
DCL21_2_21C	9.28	1.43	10.45	4.11	0.22	4.00	Anh Lac
DCL21_3_2C	14.66	1.27	14.93	3.91	0.20	4.00	Anh Lac
FMC1_29C	5.00	0.87	5.14	0.85	0.14	1.00	Cellulose
FMC2_15C	9.69	0.84	9.90	0.92	0.13	1.00	Cellulose
FMC3_11C	14.63	0.90	14.92	0.99	0.14	1.00	Cellulose
FlowLac1_8C	5.04	0.76	5.18	2.86	0.12	3.00	Lac Mh
FlowLac2_23C	8.81	0.81	9.96	2.87	0.13	3.00	Lac Mh
FlowLac3_15C	12.97	1.13	15.06	2.90	0.18	3.00	Lac Mh

Table 31 – Prediction results for the concentration of amphetamine and the type of excipients in amphetamine blends.

All data were correctly attributed to the corresponding sample. However, the average deviation for amphetamine concentration is 0.98 and higher than for the prediction of the excipient which is 0.15. Thus, prediction may not be perfect yet, but rather good for a first attempt and without further optimisation. The tests were certainly carried out with simple mixtures – real tablets contain more than the active substance and an excipient – but the results are promising. To obtain a more reliable prediction model, blends with more components and a wider range of concentrations should used. Additionally, data processing can surely be optimised. In conclusion, the results were good enough to show the potential of NIR analysis of tablets in combination with an appropriate data processing tool. It is an interesting alternative to routine GC analysis considering that the technique does not require any sample preparation and results are obtained very quickly.

c) Ecstasy

Excipient identification

In the beginning the ecstasy tablets have been analysed by NIR in order to verify if they contain cellulose. Therefore only seizures with no or little excipient detected by GC-MS have been used for this work. It appeared that the identification of the excipient was not that easy. Considering the principle of NIR analysis we know that the spectrum reflects the absorbance in function of the various functional groups (*Fig 73*, schema overtones), which means that substances with similar chemical structures will absorb in similar wavelength regions. Blends will therefore present spectra of superposing absorptions and compounds might only be recognised if they have a particular functional group.

That explains why MDMA is generally recognised in our tablets, however, no comparison could be made to similar substances such as MDE, or MDA. On the other hand, chemical structures of cellulose and sugars are the same. In our case we knew beforehand if sugars were present, which was very useful in the evaluation of the spectra. The example shown in *Fig 92* was an easy case: the shape of the curve was very similar to the cellulose standard.

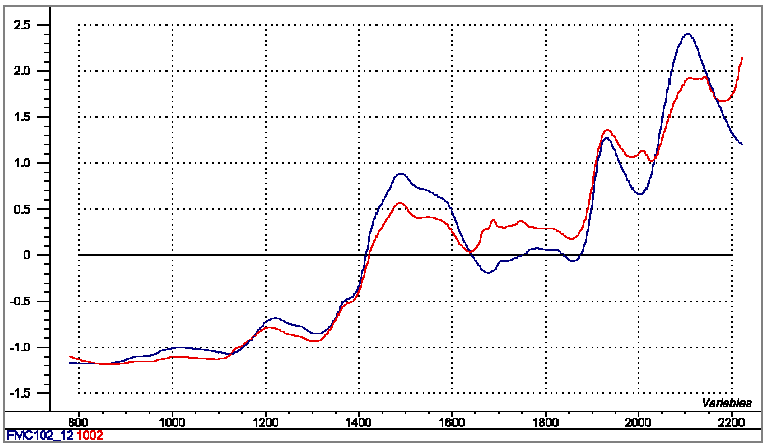


Fig 92 – SNV treated spectra of ecstasy sample 1002 (red) and a standard of microcrystalline cellulose (FMC – blue).

But another example shows a more complicated picture (*Fig 93*). When considering the spectrum without knowing the result of GC-MS analysis, the curve shape would make think of anhydrous lactose rather than cellulose.

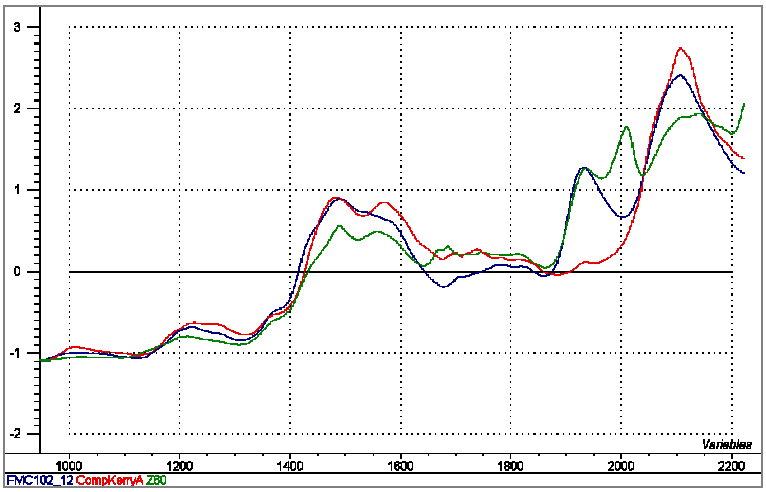


Fig 93 – SNV treated spectra of ecstasy sample Z60 (green), a standard of anhydrous lactose (KerryDT – red) and a standard of microcrystalline cellulose (FMC – blue).

Thus, what appeared to be the problem was to decide if there was one excipient or a mixture. And as was explained before, curve shapes in mixtures are different from that of a substance alone. And no tests could be made to verify how the mixture spectrum changes in function of the excipient content (cellulose alone, cellulose/lactose, lactose alone). From the results of GC-MS analysis it appeared that no anhydrous lactose was present in that tablet, but lactose monohydrate and therefore the additional presence of cellulose was very likely. (*Fig 94*)

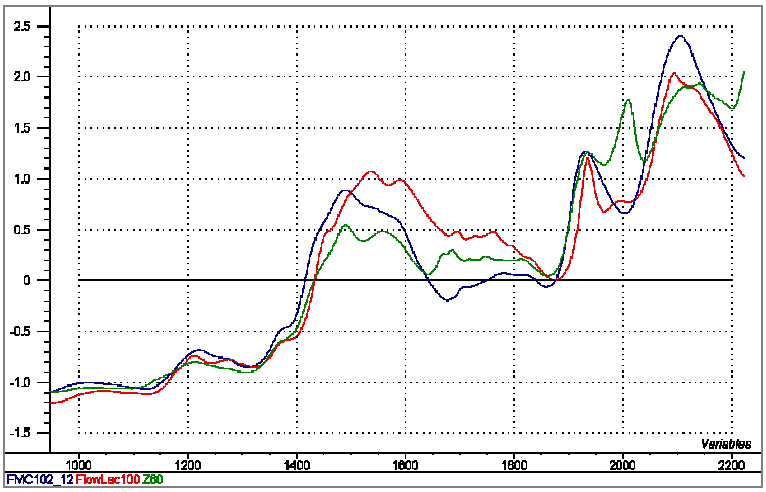


Fig 94 – SNV treated spectra of ecstasy sample Z60 (green), a standard of lactose monohydrate (FlowLac – red) and a standard of microcrystalline cellulose (FMC – blue).

However, in the cases where anhydrous lactose was detected by GC-MS, nothing could be said about the eventual presence of cellulose since the curve does not permit any conclusions. Finally, some cases were observed where nothing at all could be said. That means, no excipient could be determined apart from a likely presence of talc. (*Fig 95*)

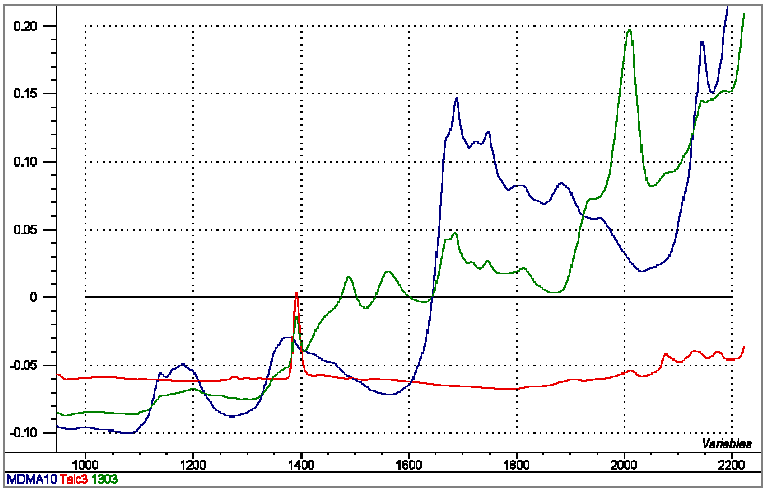


Fig 95 – Raw spectra of ecstasy sample 1303 (green), a standard of talc (red) and a standard of MDMA (blue).

No good explanation could be found for the absorbance in the section between 1450 and 1650nm. The curve shape reminds the blend of lactose monohydrate and cellulose seen before, the presence of sugars could however be excluded, and cellulose alone would present another form. Inorganic excipients might be present because the absorbance in this region is not high and these do not present notable absorbance. Starch could be excluded, due to the curve shape that did not correspond. The MDMA content of the concerned tablets is either 40, or 60%. Therefore, another substance must be present next to talc, as its absorbance does not seem high enough to explain 40-60% content in each tablet. Unfortunately, it was impossible to determine what compound that could be.

The excipient composition determined after GC-MS and NIR analysis are presented in *Table 32*. In the NIR column those substances detected in addition to those noted under GC-MS are mentioned. Lactose monohydrate was pointed out in cases such as presented in *Fig 94* (Z60).

Sample	MDMA content	GC-MS	NIR
1002	26%	Mg Stearate	Cellulose
1003	29%	Lac Mh, Mg Stearate	Cellulose
1061	27%	Mg Stearate	Cellulose
1079B	29%	Mg Stearate	Cellulose
1112G	38%	Anh Lac, Mg Stearate	Talc
1207	59%	Mg Stearate	Talc
1303	42%		Talc
1304A	28%	Mg Stearate	Cellulose
1304C	41%		Talc
1305	37%		Talc
1306B	36%		Talc
1306C	34%	Lac Mh	Talc
1306D	37%		Talc
1310A	29%	Mg Stearate	Cellulose
1327	27%	Mg Stearate	Cellulose
1473A	36%	Sorbitol, Mg Stearate	Sorbitol
1494D	30%	Mg Stearate	Cellulose
1498	41%	Lac Mh, Mg Stearate	Cellulose, Lac Mh
1524	37%	Mg Stearate	Cellulose
1528	34%	Mg Stearate	Cellulose
1619A	43%	Lac Mh, Mg Stearate	Cellulose, Lac Mh
1683	31%	Mg Stearate	Cellulose
1693	28%	Mg Stearate	Cellulose
760	32%	Anh Lac, Mg Stearate	
788	36%	Anh Lac, Mg Stearate	
814	32%	Mg Stearate	Cellulose
831	32%	Mg Stearate	
834	41%	Sorbitol	Talc
949A		Lac Mh, Mg Stearate	Cellulose
949B			Cellulose
954		Lac Mh, Mg Stearate	
985B	26%	Mg Stearate	Cellulose
985C	29%	Sorbitol, Mg Stearate	
Z14B	34%	Anh Lac, Mg Stearate	
Z197		Mg Stearate	Cellulose
Z209		Mg Stearate	Cellulose
Z30	30%	Mg Stearate	Cellulose
Z44	34%	Mg Stearate	Cellulose
Z4A	36%	Mg Stearate	Cellulose
Z60	27%	Lac Mh, Mg Stearate	Cellulose, Lac Mh

Table 32 – Identification of excipients after GC-MS and NIR analysis.

Comparison of ecstasy samples

The spectra of the different tablets were also compared simultaneously with each other in order to check out similarities. The spectra have been observed after SNV treatment and those that were identical or very similar were noted. They were grouped together and can be seen in Annexe XIII. An example is shown in *Fig 96* of two tablets presenting different logos, but an identical spectrum.

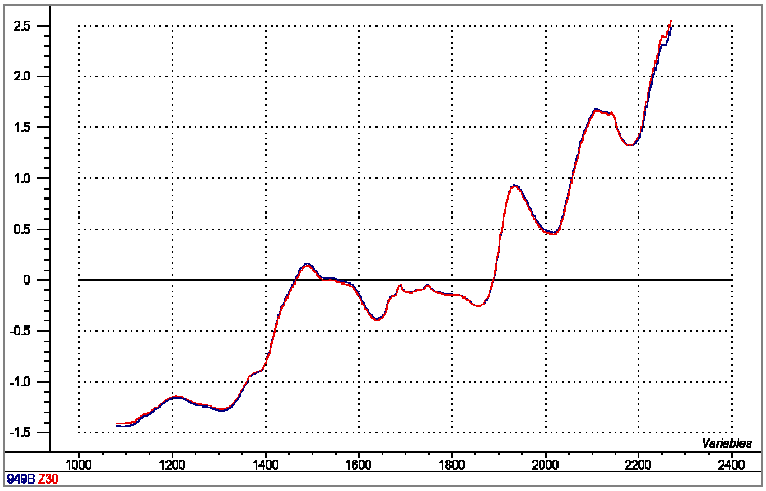


Fig 96 – SNV treated spectra of ecstasy sample 949B (blue) and Z30 (red).

However, this visual comparison is tedious and difficult, since there is too much information to compare. Additional mathematical treatments are necessary to extract latent information. Therefore, the data has been imported to The Unscrambler© in order to test PCA analysis and to verify the groups formed after having applied pre-processing methods.

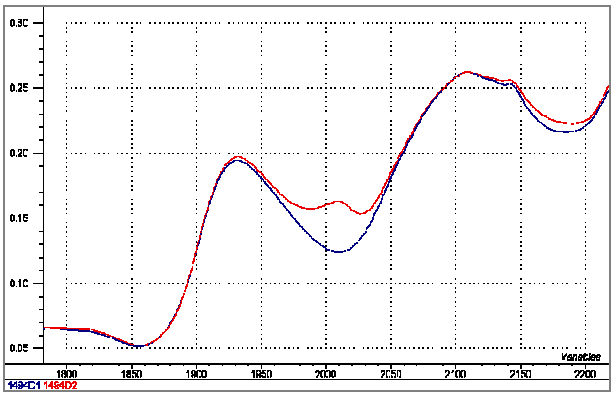


Fig 97 – Extract of raw spectra of ecstasy sample 1494D – in blue the first measure and in red the second, two months later.

A particular mention has to be made for the wavelength selection. During spectra comparison a maximum has been observed at 2010nm in about 75% of the ecstasy tablets. Not only this maximum could not be explained by any of the standards, but additionally in two tablets, which were analysed twice in an interval of 2 months, it happened to appear only after the second analysis (*Fig 97*). This was not due to measuring conditions which were the same for all tablets and no apparent link existed with the date of analysis.

Contacts at Bayer Healthcare suggested appearance of polymorphism often observed in excipients. However, in this case it was certainly not due to the excipients since the maximum was observed in tablets having different compositions. The only apparent common denominator for all concerned tablets was that they contained MDMA. Unfortunately, nothing is known about potential polymorphism of MDMA. However, substances show polymorphism under particular circumstances and the polymorphic forms are often not stable. Additionally, it does not seem to explain the sudden emergence in the two tablets analysed twice.

Another suggestion was made by Prof Tony Moffat from the School of Pharmacy (University of London). He proposed either oxidation or hydrolysis of one of the compounds contained in the tablets to give a new compound that absorbs around 2010 which is where amines and amides absorb, or a loss of a solvent that reduces the background to give the appearance of an increase at 2010nm. The latter seems unlikely since the peak for some tablets was very high, too high in our opinion to be explained by a loss of solvent. The first suggestion cannot be verified, but might be possible. In any case, there seems to be a link to the concentration of MDMA as tablets with high content also show a very high peak, whereas tablets with lesser content present a smaller peak. This relationship is however not linear and only indicates a tendency.

In conclusion, it was impossible to find an explanation for this maximum. And as it appeared to vary over the time and has therefore to be considered as unreliable for our purpose, the corresponding wavelength section has been excluded for the PCA analysis together with those corresponding to moisture. The various pre-processing methods applied also resulted in some variations with regard to the formed groups, but showed altogether the same tendency. Therefore only one PCA will be shown here in *Fig 98*.

The groups formed by PCA are similar to those observed by visual comparison (Annexe XIII). However, nothing can be said about the significance of these similarities because there is not enough information available for a proper interpretation of the group formation. An interesting point is that most of the correspondences are observed in samples presenting the same physical and general chemical characteristics. Nevertheless no further conclusion about any tendency should be drawn from that, because of the very small sample size.

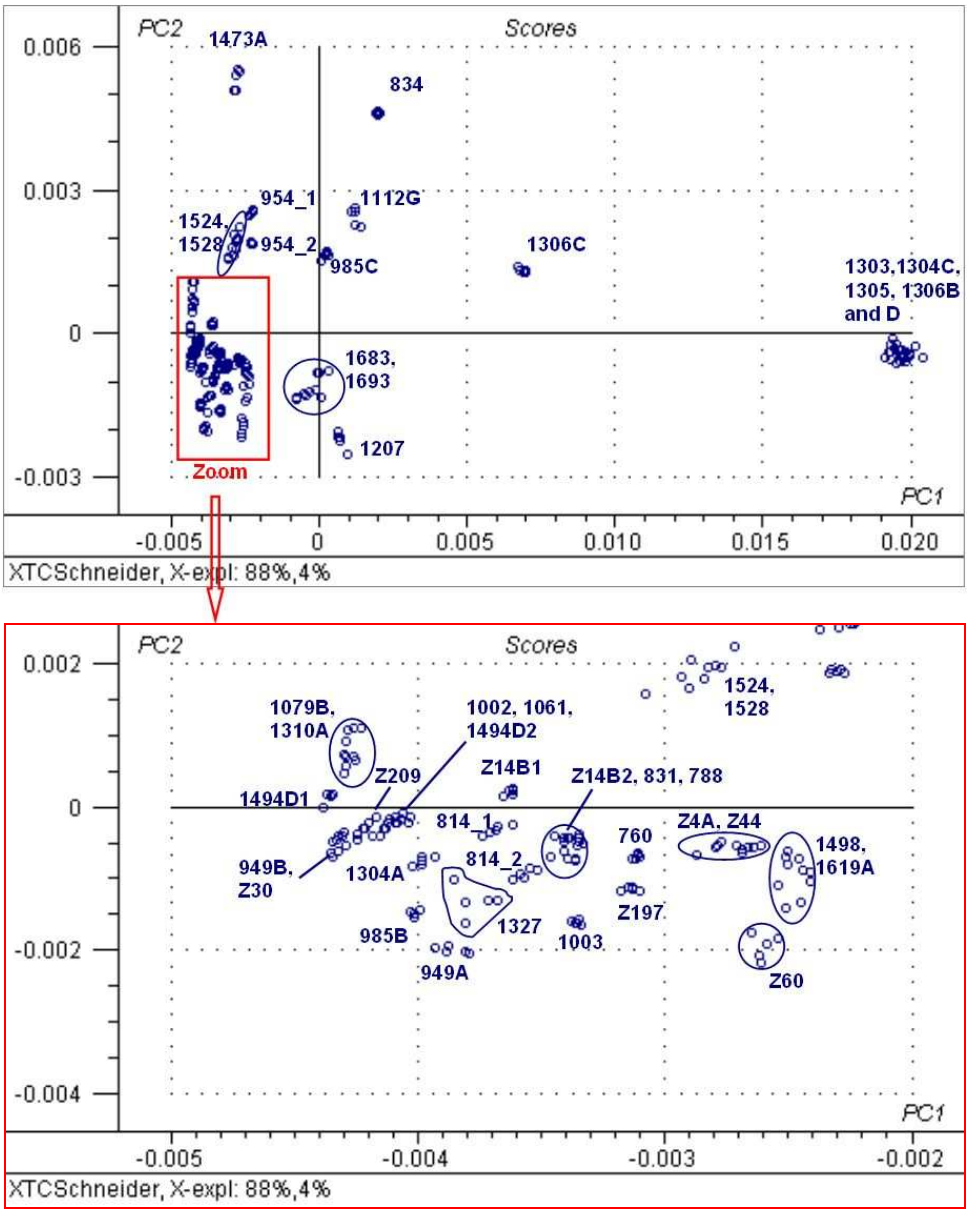


Fig 98 – PCA after pre-processing method Schneider, with at the top an overview and than below a zoom of a dense area.

It was seen in the cellulose and lactose standard evaluation that already small differences might indicate difference in origin as well. However, ecstasy tablets are far from being standards. It can be supposed that the NIR spectra for tablets coming from one compression batch are rather variable. Nothing is known about this intravariability, therefore it is very difficult to judge how much spectral variance may allow deciding whether samples are linked or not. When comparing similarities obtained by NIR to the results obtained after GC-MS analysis, they were not so different. The problem in this comparison is that the three methods consider something different. The XTC method gives us information about the general composition, but does not take into account substances such as cellulose. The FA/sugar method focuses on two excipients and might therefore be considered as more specific than the XTC method. And finally, NIR analysis results in spectra representing the tablet blend as a whole taking into account all absorbing compounds. This results in complex spectra but is more representative for the tablet blend.

From this point of view NIR analysis seems to be an appropriate method for ecstasy profiling, but at the same time it is also the method where the least is known about intra- and intervariability of compression batches. Additionally, spectral changes would have to be studied thoroughly when working with blends. But it has to be reminded that profiling was not the purpose of this additional project and was therefore never considered when planning the work to realise. The possibility of profiling using NIR analysis only appeared when comparing the ecstasy spectra and discovering similarities to results obtained by GC-MS analysis. And it should therefore be mentioned here that this technique represents an interesting issue in the profiling problematic.

8 FINAL REMARKS

8.1 RESULTS OBTAINED BY EXCIPIENT ANALYSIS

The purpose of this research was to study how the excipients present in ecstasy tablets could be exploited and how useful they would be in the establishment of links between seizures. To evaluate if the purpose has been fulfilled, the initial hypotheses should be considered.

1) Similarities with pharmaceutical production

The verification of this hypothesis was principally carried out by studying the relevant literature and evaluating qualitative data collected in the ecstasy database (IPS / WD Zürich). The knowledge acquired during this study is reported in Chapter 2 and Appendix I and II. It became obvious that considering the manufacture of tablets, even if simple, a minimum number of excipients are required and that consequently they should be present in all tablets. Furthermore, it was shown that the substances regularly found in illicit tablets correspond to commonly used excipients in pharmaceutical production. Finally, the schema, represented in *Fig 99*, elaborated by Europol after evaluation of data from seized clandestine laboratories shows us that illicit production is indeed very similar to pharmaceutical production.

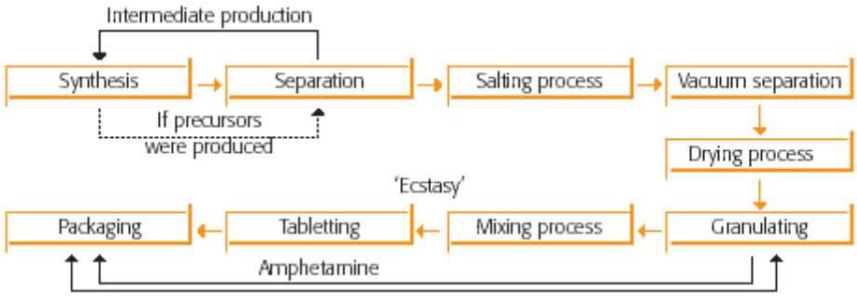


Fig 99 – Detailed procedure of ecstasy production (Europol, 2004).

Tablets are solid products, a blend of various substances being compressed to a solid form which will not be modified anymore. Therefore, their composition does not change between the time of production and the seizure by the police. In the context of illicit tablet comparison, they appeared to present an obvious potential. Unfortunately, the study realised by Europol was not as extensive as to give us any information

about the use of excipients in illicit tablet production. The absence of this kind of information appeared to be problematic for the evaluation of the following hypotheses.

2) Variation between producers

It was shown in the theoretical part (cf. Ch – 2) that a recipe for an excipient mixture resulting in tablets of good quality is not so easily modified. Thus, producers will probably stick to one once they have found it. Consequently, compression batches for one producer should not considerably vary for the same recipe. However, we have seen that not very much is known about production habits in illicit tablet manufacture. Do different producers collaborate ? Do they exchange recipes ? Does everybody find out for himself ? etc. Probably, all of these situations can be observed in reality and it had to be evaluated what a variation detected by analytical means signifies and how significant it would be.

It was shown that excipients can be of very different chemical nature and thus research has been focussed on two types of excipients, carbohydrates and stearates, which were known to be present in a great percentage of ecstasy tablets in our database (IPS-WD). Analytical methods were developed in order to obtain optimal information with regard to these two types of substances and applied to 109 ecstasy samples.

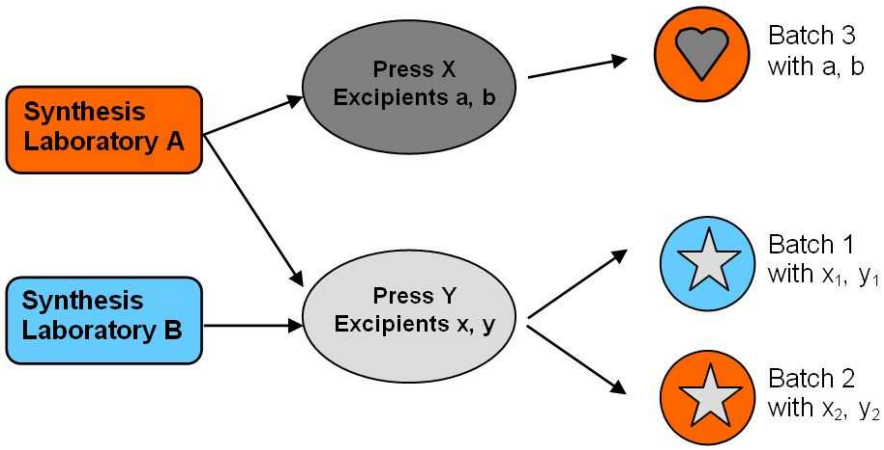


Fig 100 – Schema of illicit ecstasy production.

Considering the schema above *Fig 100*, already presented in the initial hypotheses (cf. Ch - 1), we can observe three levels of possible variation between illicit tablet producers by using information provided by excipients: the nature of excipients (qualitative – a, b, x, y), their quantity (x_1, y_1, x_2, y_2) and their origin (a, x). Similarly to these three levels, the obtained data has been evaluated according to qualitative and semi-quantitative criteria. Variation could be obtained with regard to the first two levels (the third not being intentionally considered), and the formation of groups of similar ecstasy samples was achieved. The variation obtained by excipient analysis was such that differentiation of illicit producers appeared to be

possible. However, some research about clandestine laboratories, as was specified in Chapter 6.2.1.4., and verification of homogeneity inside large compression batches should be carried out.

Furthermore, the confirmation of variation between producers requires a larger sample set, if possible originating from large batches coming from known producers. This would be particularly interesting in producer countries such as the Netherlands. If such research was undertaken, a particular interest should be given to very frequent logos, such as Mitsubishi, Superman, Ferrari, etc. Data comparison showed that tablets with these logos often seem to generate a sort of standard composition profile showing little variation. This means that they often presented a similar composition, but still showed differences, big enough to differentiate them, but not so small to definitely group them. That might be due to the frequency of the logo. Being very popular it might be conceivable to find a common production procedure going around among producers. For a better appreciation of this kind of possibilities, knowledge about the variability inside a production batch and between different batches of these popular imprints would be very useful.

Concerning the third level, the origin, fatty acid analysis resulted in such specific information that characteristic profiles could be observed; indicating that differentiation of stearate origin might be possible. However, this information is based on analysis of seized ecstasy tablets – it would be very interesting to investigate the fatty acid composition of stearates provided by different suppliers, and also that of other ecstasy batches.

3) Possibility of classification by the use of excipients

The hypothesis is not that much different from the second one – it goes a step further. The observed variation is used to form classes / or groups. It will be expressed by calculated intra- and inter-variability – the former meaning the variation inside a sample batch, and the latter that between sample batches. Classification will be achieved through similarity measures. In other words, the manual sample to sample comparison is replaced by automated comparison of every analysed sample with all others in the database. According to the result of the similarity measure, a sample will be attributed / or not to a class.

This procedure implies the use of mathematical treatments and comparison methods. Several combinations have been tested in order to determine a comparison method giving a good separation between linked sample populations and non-linked sample populations. When this was achieved, the method has been applied to the whole data set and correlation values were obtained for all possible sample pairs. These values were evaluated by comparison to the groups determined as explained under the previous hypothesis and could be confirmed. This means that the same similarities were observed, and thus similar groups were formed. Consequently, the determined comparison method is applicable in this context with this type of data.

However, a great limitation to the result has to be mentioned inasmuch that this study was rather exploratory and the determined comparison method is only indicative, the sample set not being representative in terms of sample size. In no way it represents a final result, but can be strengthened or modified with extensive sample. Larger ecstasy batches should be analysed which would give a more realistic view of the intra- and inter-variability.

Despite these limitations interesting information could be obtained. The samples being grouped together were evaluated according to their chemical similarities and the additional data from the database (physical characteristics and data coming from police investigations). The tablets grouped by the excipient content appeared to be very similar with regard to the physical characteristics as well. Thus, similar physical properties are indeed a first indicator of a possible relationship between two tablets. However, there also have been cases of physically similar tablets, but differentiated by their excipients. Therefore, no classes should be created on the basis of physical characteristics only, except when having the means to find a relationship towards the used press. Indeed, there is a type of comparison allowing the possible establishment of a link between an illicit tablet and the press used for its compression. The required visual comparison between the tablet surface and the suspected punches is at present exclusively carried out at the Bundeskriminalamt in Germany. (Dahlenburg, 2002b) It therefore represents an exception and this kind of information is normally not available (*Fig 101*).

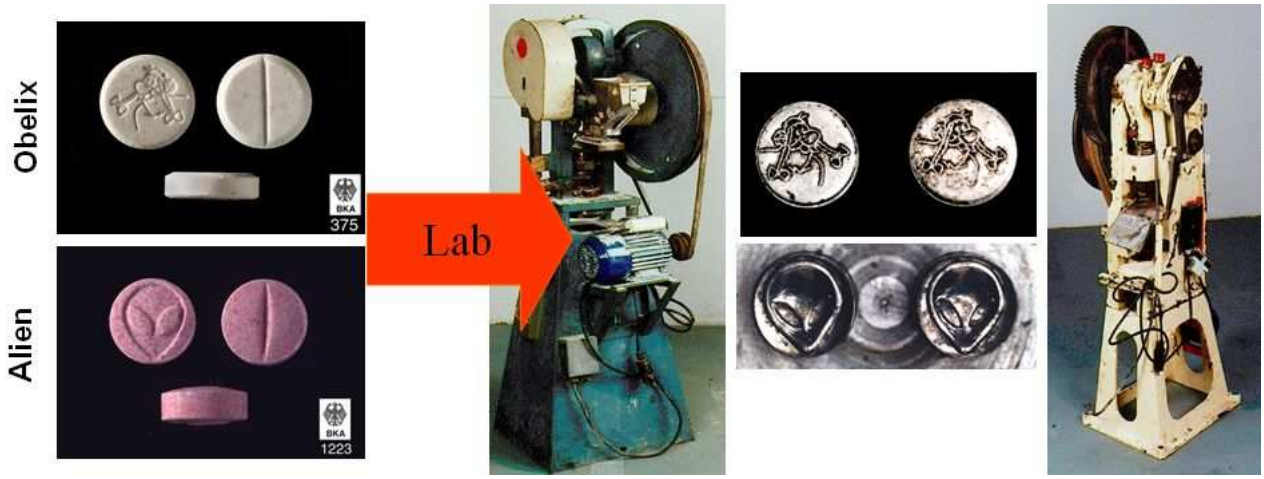


Fig 101 – Comparison of external tablet characteristics (picture taken from (Dahlenburg, 2002a)).

Furthermore, it has been shown that particular care should be taken when grouping samples containing no detectable excipients (by GC). The absence of any data to compare makes them consequently identical from a chemical point of view. Thus, any other available information has to be taken into account before declaring them linked.

Altogether, samples were considered as linked when they could not be differentiated by their excipient content and after verification that all other available information did not present any unexplainable

difference. Concerning the signification of this kind of link, theoretically it could be attributed to the blend before compression, as that is the moment when the chemical composition does not change anymore and according to *Fig 100* slight variations between batches may subsist even for the same producer. Although the presence of some results supporting this theory, the lack of quantitative data and of information about homogeneity inside a blend makes such a specific link impossible.

Alternatively, a link of this specificity might actually not be that interesting. Our evaluation of similarities has shown that sample groups formed by their excipient content rather indicate a link with regard to the producer. This is significant inasmuch that it relates tablets to the same clandestine laboratory. When considering *Fig 100* from another point of view, a producer might make several tablet batches with similar excipient content ($x_1 \approx x_2$ and $y_1 \approx y_2$) and could thereby be characterised. And therefore, a link at this level is of high strategic interest.

8.2 ADDITIONAL RESULTS

It was explained earlier that the same samples were also analysed by the routine method, generally used for the screening of ecstasy tablets, as consequence of improvements in analytical sensitivity. The results of the ecstasy screening method appeared to show less variation than expected and should not be applied for sample comparison as such. The method was not modified or optimised; it was therefore difficult to estimate how reliable peak proportions and therefore similarities / differences were. The formed groups were for a good part similar to those formed by excipient analysis, but in many cases samples that were grouped by the excipients have been separated by the screening method which was considered as a loss of useful information. It might be advisable to investigate optimisation of the analytical method for the simultaneous detection of active substance and sugar content giving reliable and reproducible results. Detection of fatty acids might be included in this optimisation, but no such detailed information as obtained with the newly developed method could be obtained for the simple reason of great differences in quantity. For the moment, this can only be achieved through extraction or concentration of the stearates.

Finally, the additional study on a NIR spectroscopy instrument gave very interesting results. It was shown that it was potentially possible to distinguish microcrystalline cellulose and lactose monohydrate standards from different suppliers. Although this was a very important observation, it could not be applied to ecstasy tablets as such, the differences used for the differentiation being so small, that they would probably not be detected after the extraction of the relevant substance. And it was seen that compression alone resulted in separate groups so that other actions such as extraction might result in a slightly different spectrum as well.

In the context of ecstasy comparison, the problem had to be approached differently. The potential of the technique was obvious since already small differences could be detected. Its drawback, however, is the interpretation of spectra made of blends, as could be observed with the analyses of ecstasy tablets. NIR

might not be the most appropriate technique to determine chemical composition, but it could be very useful in comparison of illicit tablets, since it considers the ecstasy blend as a whole just as it comes out of the press.

8.3 FURTHER RESEARCH

The evaluation of the results of the two techniques applied in this project made appear that the obtained information is very complementary. It would be interesting to combine GC-MS and NIR by studying blends of MDMA, being the most frequent illicit substance, and common excipients – such as lactose, cellulose and Mg stearate – provided by different suppliers. The blends would be prepared by varying supplier and concentration of the different compounds. Then, they would be compressed and analysed by GC-MS and NIR. A first interesting point would be to verify if the same information is obtained by the two techniques in terms of similarity between samples and attribution of original supplier. Another approach would be like the one applied for the amphetamine blends analysed by NIR – to use the information from both techniques in order to predict the composition and origin.

Another context which is not so far from the ecstasy problematic here presented is the investigation of counterfeit drugs and illicit trafficking of licensed drugs. The NIR technique is particularly appropriate for this sort of research and has already been applied in that sense. Several publications (Herkert et al, 2001; Yoon et al, 2004; Röseler, 2004; Rodionova et al, 2005; Westenberger et al, 2005) and presentations at the recent IAFS2005 (Moffat, 2005) confirm this interest.

8.4 CONCLUSION

The aim of this research was to investigate how far information obtained by the analysis of the excipients in ecstasy tablets could be useful in the establishment of links between seizures. Two analytical methods have been developed in that sense and give detailed and reproducible results about the sugar and fatty acid content in tablets. It has been shown that variation in excipient content could be obtained, leading to the classification of the samples into groups. The variation observed between the groups was confirmed by the similarity values we obtained. The evaluation of these data gave rise to the conclusion that a high correlation in excipient content (corresponding to a low similarity value) indicates a link towards the tablet producer. However, we furthermore showed that it is important to consider all available information about the remaining characteristics of the tablets. This is even imperative when dealing with tablets where no excipient has been detected by GC-MS analysis.

It has been shown that a link towards a producer can be of strategic importance as it might cover similarities between several compression batches represented by a high number of tablets distributed on the illicit market and all being related to the same production site. However, confirmation of these observations is required by realising further studies with a larger sample set including large seizures, if possible from known production sites.

Furthermore, analysis of fatty acids appeared to give specific profiles which were useful for sample characterisation. It could even be imagined to use these profiles for the determination of suppliers / sources of stearates, after having studied possible differentiation with known products.

Finally, the application of NIR spectroscopy on cellulose and lactose standards resulted in possible differentiation of suppliers for the cellulose standards, however not yet being applicable to ecstasy tablets as such. Then, prediction of amphetamine concentration and excipient type was demonstrated which might be very useful in routine ecstasy analysis. The possibility of illicit drug profiling using NIR analysis appeared during the comparison of ecstasy spectra and the detection of similarities to results obtained by GC-MS analysis. The two techniques being complementary, NIR represents an interesting issue in the profiling problematic.

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APPENDIX I – TABLET MANUFACTURE

PHARMACEUTICAL PRODUCTION[16]

"Tablets are solid preparations. Each tablet contains one dosage of one or more active substances and is obtained by agglomerating through compression a constant volume of particles." (Le Hir, 1997)

The tablet has gained much importance since its launching on the pharmaceutical market and is now the most used pharmaceutical dosage form. This can easily be explained by its advantages which are enumerated hereafter :

- simple use : tablets have a small volume and are solid that makes them easy to handle in conditioning and transport manipulations,
- easy and practical taking,
- precise dosage per taking,
- good conservation by dry and condensed environment,
- particularly interesting dosage form for insoluble active substances,
- low cost price thanks to large industrial production possibility,
- the release of the active substance can largely be regulated or modified with the corresponding production / formulation techniques.

There are few drawbacks, the most important being the tablet perfection. Indeed, if the production is not perfectly studied the tablets risk easy damage or bad disintegration in the alimentary canal.

Tablet production can be divided into two principal phases :

- the first consists in preparing the blend of the various substances which means the active substance/s and part of the excipients,
- in the second the blend is compressed in order to obtain tablets.

The quality of the tablets depends on both phases, but principally on the first one. In galenic two types of blend preparations are known – granulation and direct compression. Generally, granulation is the more widespread preparation. (Kummer, 1998)

[16] (Bauer et al, 1999; Le Hir, 1997; Davies, 2001)

Blend preparation

Granulation

The purpose of this procedure is to modify the texture of the blend in order to increase its density so that it presents good flow properties and reduces presence of air between particles. Important presence of air might hamper the compression. Additionally, granulation helps to optimise surface properties, porosity, solubility and disintegration time with regard to bioavailability. The two granulation modes mostly used are wet granulation and dry granulation.

The principal advantages of granulation over a simple powder blend are :

- a better conservation of homogeneity in the powder blend,
- a higher density,
- a better fluidity,
- and a better aptitude for compression.

Direct compression

It is possible to avoid the granulation step by proceeding to direct compression of powder blends thanks to excipients presenting the required characteristics which are the following :

- a good compressibility,
- a good fluidity,
- disintegration properties,
- lubricant properties,
- reproducibility between batches,
- high dissolving capacities,
- and moderate cost.

Thus, the concerned compounds are at the same time binder, diluent and even disintegrator. The blend has to fulfil three conditions – good plasticity, good flowability and absence of agglomerate formation. Some common excipients used for direct compression are : crystallised lactose (coarse), spray-dried lactose, anhydrous lactose, dextrates, microcrystalline cellulose, powdered cellulose, dicalcium phosphate, pre-gelatinised starch. (Kummer, 1998; Marquis, 2000)

Comparison

The easier and more economic production mode of tablets is the direct compression as it does not require the additional step of granulation. However, manufacture conditions and excipient requirements as described above are difficult to handle. It only needs a small deviation in particle size / form of one of the blend compounds for the final tablets to be mechanically not resistant enough. For this reason, tablet production including granulation is often preferred, and more particularly including wet granulation as it is less problematic than the dry mode.

Compression

In the beginning compression took place on single punch machines. Later, almost exclusively rotary tablet machines were used.

SINGLE PUNCH MACHINES – the most important parts of this machine are :

- *the die* : generally with a vertical cylindrical hole (simplest case) – this part is immovable,
- *two mobile punches* : the movements of the lower and upper punch are adjustable by an eccentric cam in order to determine amplitude and compression strength,
- *hopper* and *feed shoe* : required for the die filling and the ejection of the tablet. The hopper is the powder reservoir. It is attached to the feed shoe which oscillates horizontally.

Compression can be summarized in four principal phases (*Fig 1*):

I. the feed shoe oscillates above the die, filling it with powder and moves away,
II. the upper punch enters the die and compresses the powder,
III. the upper punch is removed, and the lower punch rises in the die to eject the tablet,
IV. ejection of the tablet by the feed shoe and filling of the die.

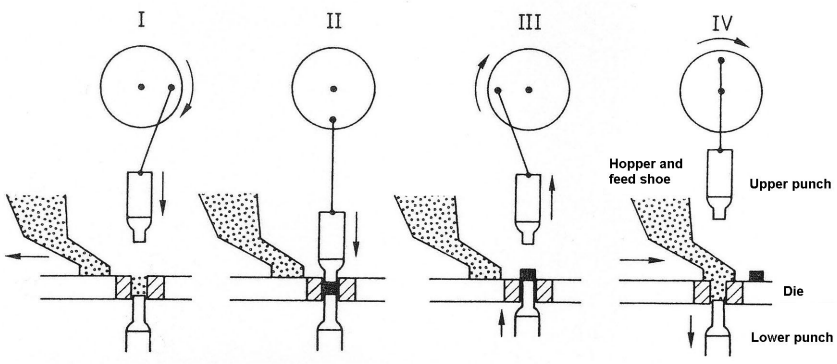


Fig 1 – Compression steps on a single punch machine (Bauer et al, 1999).

For a single punch machine the output per hour is between 1500 to 6000 tablets depending on the machine. For rotary machines the number of tablets is increased per number of punch pairs. Simple rotary machine already reach an output per hour of 20000 to 50000 tablets which can still be increased.

ROTARY TABLET MACHINES – on this machine the hopper and feed system is fixed and the rotating turret, corresponding to the die but with more than one hole and punch pair, is turning horizontally around its axis. The powder hopper is positioned above a feed frame that retains a powder bed above the dies when the lower punch is in the filling position. As the lower punches pass below the feed frame, they descend within the die into their lowest possible position. The powder is filled into the dies by the suction effect caused by their descent and gravity from the feed frame above. Then, the lower punches pass over a weight control cam which causes the punch to rise, ejecting some of the powder which is scraped off by the edge of the feed frame. Compression takes place when the upper and the lower punches pass between compression rollers. During compression the punches move together, in contrast to the single punch machine. Following compression, the upper punches are removed by the upper punch cam track and the lower punches pass over an ejection cam. Tablets are removed from the punch tip by a scraper blade positioned in the edge of the feed frame. The rotary tablet machine is represented schematically in *Fig 2*.

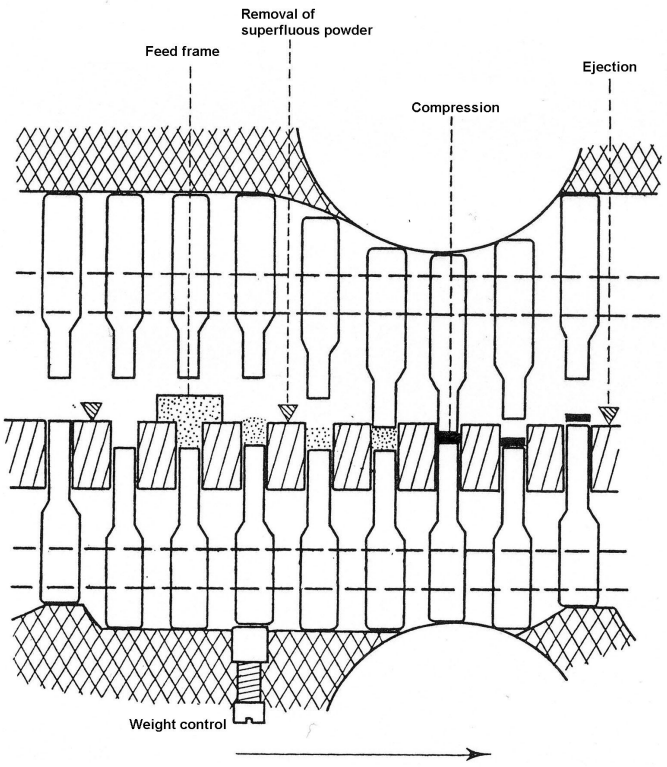


Fig 2 – Tablet compression in a rotary machine (Le Hir, 1997).

Commercial manufacture of tablets is performed almost exclusively on rotary tablet machines due to their higher output. Single punch machines are rarely seen, but might be used for small batches. They are less expensive, have a simpler mechanism and require only small amounts of material to produce tablets.

Appearance

The obtained tablets can be of various shapes, sizes and colours. The size is generally governed by the dosage. As for the shape, the most common is round, but can also be oval, squared, etc. Depending on the punches tablets are flat-faced, flat bevel-edged or concave. They might be given unique identity by marking the surface with a code. This can be achieved by printing or by compressing powder with punches that are embossed with the code, producing tablets with intagliations/logos. Colouring of tablets can be obtained either by incorporating a dye or pigment into the powder prior to compression or by applying a coloured coat to the tablet following compression.

ILLICIT TABLET MANUFACTURE[17]

An illicit tablet is first characterised by its mode of manufacture. The most common method is the one used in pharmaceutical industry – the compression of a powder or granules in a die between two punches. Similarly to licit production, compression can take place on a single punch machine with one pair of punches, or on a rotary machine with several pairs of punches. However, other modes of manufacture exist such as moulding which is an interesting alternative for a clandestine producer as it does not require expensive and heavy machinery.

Moulded Tablets

Moulded tablets are all produced in the same simple way. A blend of the active substance, an appropriate diluent and a small quantity of binder, sufficient to keep the tablet together, is prepared. The required material consists in an upper perforated metal plate and a lower plate with projecting pegs. The blend is moistened and pressed in the holes of the upper plate so that it is flush with the surface of the plate on both sides. The upper plate is then placed on the lower plate and the content of the holes is forced out by the pegs and let to dry. Although variation between moulded tablets is much higher than with machine-compressed tablets, it is possible to produce tablets of identical diameter and thickness by using this technique. The drawback is its low production yield. Moulded tablets are principally used for LSD.

Compressed Tablets

Compressed tablets are not exclusively produced on machines. Simpler manual methods exist requiring little material. The simplest production mode encountered in illicit tablet manufacture is similar to the process used in the very beginning of tablet production. The material consists in a brass plate containing holes 10mm in diameter which is placed on an aluminium base plate. The holes are filled up with the

[17](Gomm and Hughes, 1991)

blend to compress. The powder is tamped down with a steel punch and finally compressed by a hammer blow to the punch.

Another example for hand compression of tablets uses a press that looks a bit like a corkscrew of 23cm length. The production is very slow as compression is obtained via a screw thread, resulting in the press to be dismantled each time to remove the tablet. However, illicit amphetamine and ephedrine tablet have both been produced in this way.

Finally, illicit tablets are also produced with machines which might be of old design, originating from second-hand suppliers. However, a collection made by (Europol, 2004) concerning material found in clandestine laboratories showed that recent powerful machines were found as well. An example of a seized press is shown in *Fig 3*.

Fig 3 – Rotary machine seized in a clandestine laboratory (Europol, 2004).

APPENDIX II – DESCRIPTION OF SOME COMMON EXCIPIENTS[18]

Name	Chemical name, CAS	Chemical Formula	MW	Application	Particularities	Acidity / Alkalinity	Melting Point	Solubility
Calcium carbonate	Carbonic acid, calcium salt [471-34-1]	$CaCO_3$	100.09	Tablet and capsule diluent, Therapeutic agent		pH = 9.0 (10% w/v aqueous dispersion)	Decomposition at 825°C	Nearly insoluble in ethanol (95%) and water; solubility increases in presence of ammonium salts or CO_2 , the presence of alkali hydroxides decreases solubility.
Calcium phosphate, dibasic hydrate	Dibasic calcium phosphate dihydrate [7789-77-7]	$CaHPO_4 \cdot 2H_2O$	172.09	Tablet and capsule diluent	Requires presence of a lubricant; coarse powder is used for direct compression	pH = 7.4 ; (20% slurry of DI-TAB)	Decomposition below 100°C with water loss	Nearly insoluble in ethanol, ether and water; soluble in diluted acids
Calcium phosphate, tribasic	Is not a clearly defined chemical entity, but a blend of Ca phosphates; Calcium hydroxide phosphate [12167-74-7] Tricalcium orthophosphate [7758-87-4]	$Ca_3(PO_4)_2$ $Ca_5(OH)(PO_4)_3$	310.20 502.32	Anticaking agent, glidant, tablet and capsule diluent	Requires the presence of a lubricant and a disintegrator	pH = 6.8 ; (20% slurry of TRI-TAB)	1670°C	Nearly insoluble in acetic acid and alcohols; slightly soluble in water; soluble in diluted mineral acids

[18] (USP25, 2001; Rhodes and Thornton, 1979; Kibbe, 2000)

Name	Chemical name, CAS	Chemical Formula	MW	Application	Particularities	Acidity / Alkalinity	Melting Point	Solubility
Calcium sulphate	Calcium sulphate [7778-18-9] Calcium sulphate dihydrate [10101-41-4]	$CaSO_4$ $CaSO_4 \cdot 2H_2O$	136.14 172.17	Tablet and capsule diluent; anhydrous form is used as drying agent		pH = 7.3 (10% slurry) dihydrate form; pH = 10.4 (10% slurry) anhydrous form	1450°C anhydrous form	For the dihydrate at 20°C : nearly insoluble ethanol (95%) ; 1 in 375 in water ; 1 in 485 in water at 100°C
Calcium stearate	Octadecanoic acid calcium salt [1592-23-0]	$C_{36}H_{70}CaO_4$ USP : Ca compound composed of a blend of solid org. acids obtained from fats; consists in variable proportions of Ca stearate and Ca palmitate	607.03 (pure subst.)	Tablet and capsule lubricant		Acid value : 191-203	149 – 160°C	Nearly insoluble ethanol (95%), ether and water
Dextrin	Dextrin [9004-53-9]	$(C_6H_{10}O_5)_n \cdot xH_2O$	$(162.14)_n$	Suspension agent, tablet binder, tablet and capsule diluent	Dextrin is partially hydrolysed maize or potato starch			Nearly insoluble in chloroform, ethanol (95%); ether and 2-propanol; slowly soluble in cold water, very soluble in boiling water forming a mucilaginous solution.

Name	Chemical name, CAS	Chemical Formula	MW	Application	Particularities	Acidity / Alkalinity	Melting Point	Solubility
Dextrose	D-(+)-Glucose monohydrate [5996-10-1]	$C_6H_{12}O_6 \cdot H_2O$	198.17 (for monohydrate)	Tablet and capsule diluent, therapeutic agent, tonicity agent, sweetening agent	Used as binder and diluent in direct compression, requires more lubricant than other diluents	pH = 3.5-5.5 (20% w/v aqueous solution)	83°C	20°C : nearly insoluble in chloroform and ether, 1 in 60 for ethanol (95%), 1 in 1 for water, soluble in glycerine
Ethyl vanillin	3-ethoxy-4-hydroxybenzaldehyde [121-32-4]	$C_9H_{10}O_3$	166.18	Aromatizing agent			bp : 285°C mp : 76 - 78°C	20°C : 1 in 2 for ethanol (95%); 1 in 250 for water, 1 in 100 for water at 50°C; soluble in alkali hydroxyde solutions, chloroform, ether, glycerine et propylene glycol
Glucose, liquid	D-(+)-Glucose monohydrate [5996-10-1]	Liquid glucose is a mixture of various compounds, principally of dextrose, dextrin and maltose.		Coating agent, sweetening agent, tablet binder				Miscible with water; partially miscible with ethanol (90%)

Name	Chemical name, CAS	Chemical Formula	MW	Application	Particularities	Acidity / Alkalinity	Melting Point	Solubility
Lactose	O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose anhydrous [63-42-3] O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate [64044-51-5]	$C_{12}H_{22}O_{11}$ $C_{12}H_{22}O_{11}•H_2O$	342.30 360.31	Tablet and capsule diluent	Lactose (spray-dried) is used for direct compression. Can be combined with microcrystalline cellulose or starch and requires the presence of a lubricant. Incompatible with amphetamines.		201-202°C for α-lactose monohydrate 223°C for anhydrous α-lactose 252.2°C for anhydrous β-lactose	25°C : nearly insoluble in chloroform, ethanol and ether; 1 in 4.63 for water, 1 in 3.14 for water at 40°C, 1 in 2.04 for water at 50°C, 1 in 1.68 for water at 60°C, 1 in 1.07 for water at 80°C
Magnesium stearate	Octadecanoic acid magnesium salt [557-04-0]	$C_{36}H_{70}MgO_4$ USP : Mg compound composed of a blend of solid org. acids obtained from fats; consists in variable proportions of Mg stearate and Mg palmitate	591.34	Tablet and capsule lubricant	Physical characteristics can vary between different suppliers, as they are influenced by manufacturing conditions.		117-150°C (commercial samples) 126-130°C (very pure Mg stearate)	Nearly insoluble in ethanol (95%), ether and water; slightly soluble in benzene and warm ethanol (95%)

Name	Chemical name, CAS	Chemical Formula	MW	Application	Particularities	Acidity / Alkalinity	Melting Point	Solubility
Maltose	4-O-β-D-galactopyranosyl-β-D-glucopyranose anhydrous [69-79-4] 4-O-β-D-galactopyranosyl-β-D-glucopyranose monohydrate [6363-53-7]	$C_{12}H_{22}O_{11}$ $C_{12}H_{22}O_{11}•H_2O$	342.31 360.31	Sweetening agent, tablet diluent	Crystalline maltose is used in direct compression of tablets.	pH = 4.5 – 6.5 for an aqueous solution of 10%	102 - 103°C with decomposition	Nearly insoluble in ether; very slightly soluble in cold ethanol (95%); very soluble in water
Mannitol	D-Mannitol [69-65-8]	$C_6H_{14}O_6$	182.17	Sweetening agent, tablet and capsule diluent, tonicity agent, vehicle for lyophilised preparations	Mannitol can be used in direct compression. Isomer of sorbitol.		166 - 168°C	20°C : nearly insoluble in ether; 1 in 100 for 2-propanol; 1 in 83 for ethanol (95%); 1 in 18 for glycerine; 1 in 5.5 for water; soluble in alkalis
Sodium bicarbonate	Carbonic acid monosodium salt [144-55-8]	$NaHCO_3$	84.01	Alkalising agent, therapeutic agent		pH = 8.3 for a freshly prepared aqueous solution 0.1M at 25°C	270°C (with decomposition)	20°C : nearly insoluble in ethanol (95%) and ether; 1 in 11 for water, 1 in 12 for water at 18°C, 1 in 10 for water at 10°C, 1 in 4 for water at 100°C

Name	Chemical name, CAS	Chemical Formula	MW	Application	Particularities	Acidity / Alkalinity	Melting Point	Solubility
Sorbitol	D-Glucitol [50-70-4]	$C_6H_{14}O_6$	182.17	Humectant, plasticizer, sweetening agent, tablet and capsule diluent	Sorbitol is used as diluent in tablet preparations after wet granulation or for direct compression.	pH = 4.5 – 7 for an aqueous solution 10% w/v	110 – 112°C for the anhydrous form 97.7°C for the polymorph V	25°C : nearly insoluble in chloroform and ether; slightly soluble methanol; 1 in 25 for ethanol (95%), 1 in 8.3 for ethanol (82%), 1 in 2.1 for ethanol (62%), 1 in 1.4 for ethanol (41%), 1 in 1.2 for ethanol (20%), 1 in 1.14 for ethanol (11%) ; 1 in 0.5 for water
Sucrose	β-D-fructofyranosyl-α-D-glucopyranoside [57-50-1]	$C_{12}H_{22}O_{11}$	342.30	Base for medicated confectionery, granulating agent, sugar coating adjunct, suspending agent, sweetening agent, tablet and capsule diluent, viscosity-increasing agent			160 – 186°C (with decomposition)	20°C : nearly insoluble in chloroform; 1 in 400for ethanol; 1 in 170 for ethanol (95%); 1 in 400 for 2-propanol; 1 in 0.5 for water and 1 in 0.2 for water at 100°C
Zinc stearate	Octadecanoic acid zinc salt [557-05-1]	$C_{36}H_{70}O_4Zn$ USP : Zn compound composed of a blend of solid org. acids obtained from fats; consists in variable proportions of Zn stearate and Zn palmitate	632.33 (pure subst.)	Tablet and capsule lubricant			120 – 122°C	Nearly insoluble in ethanol (95%), ether and water; soluble in benzene

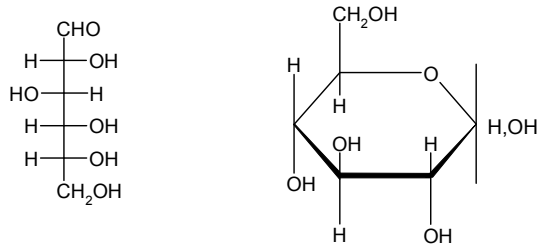
Name	**Melting range**	**Analytical method**
Caffeine	235° - 239°C	LC-UV (ou HPLC)
Mannitol	164° - 169°C	LC-RI (ou HPLC)
Lactose Monohydrate		TLC
Magnesium stearate		Stearic and palmitic acid content : GC-FID
Sorbitol		LC-RI (ou HPLC)
Stearic acid		GC-FID

Name	**Binder**	**Lubricant**	**Disintegrator**	**Diluent**
Calcium carbonate			X	X
Calcium phosphate dibasic			X	X
Calcium phosphate tribasic			X	X
Calcium sulphate				X
Dextrin	X			X
Dextrose	X			X
Glucose liquid	X			
Lactose				X
Maltose				X
Mannitol				X
Mineral salts	X	X		
Sorbitol	X			X
Stearates	X	X		
Sucrose	X			X

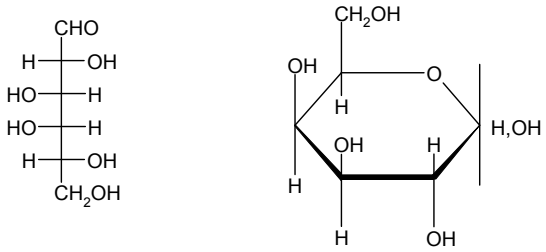
APPENDIX III – CHEMICAL STRUCTURE OF SOME CARBOHYDRATES

MONOSACCHARIDES

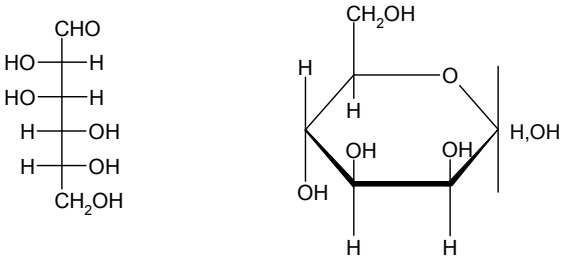
d-Glucose (Hexose)



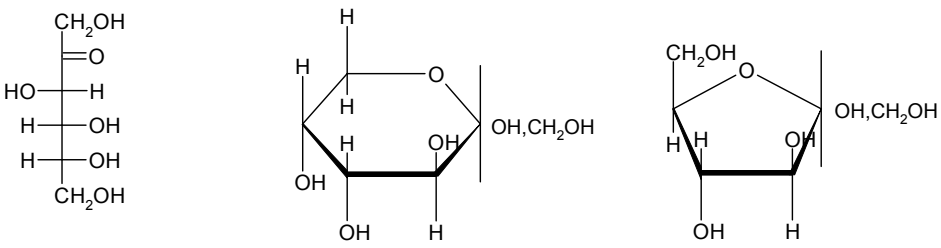
d-Galactose (Hexose)



d-Mannose (Hexose)



d-Fructose (Hexose)

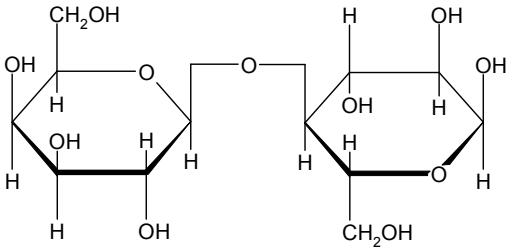


pyranose (6)

furanose (5)

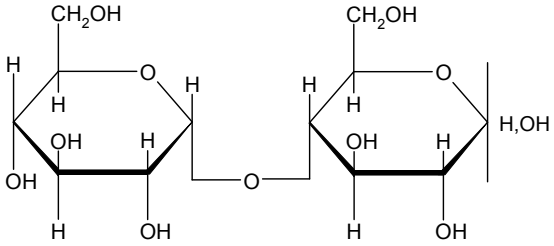
DISACCHARIDES (OLIGOSACCHARIDES)

Lactose



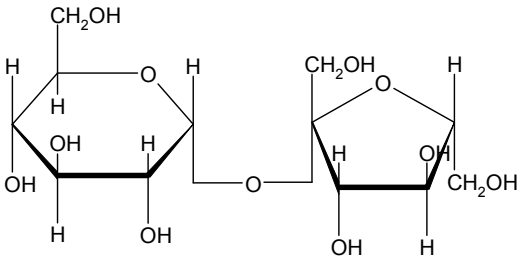
β–D-galactopyranosyl-(1-4)-α–D-glucose

Maltose



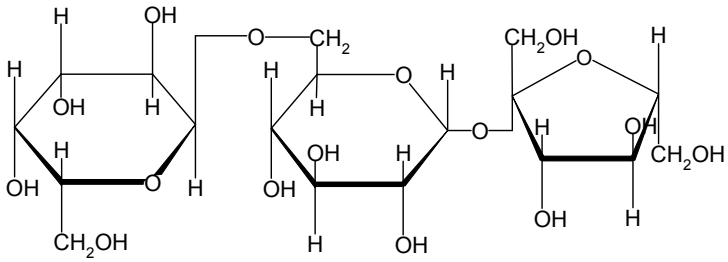
(1-4)α-D-glucopyranosyl-D-glucose

Sucrose (Saccharose)



α–D-glucopyranosyl-(1-2)-β–D-fructofuranose

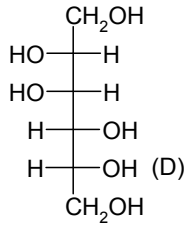
Raffinose (Oligosaccharide)



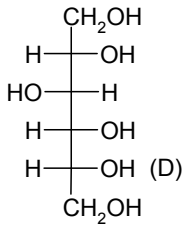
α-D-galactopyranosyl-(1-2)-α-D-glucopyranosyl-(1-2)-β-D-fructofuranose

POLYOLS

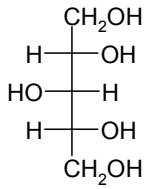
d-Mannitol



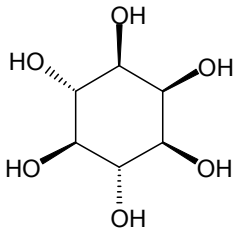
d-Sorbitol



Xylitol



Inositol

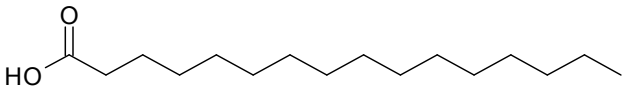


APPENDIX IV – FATTY ACID CLASSIFICATION[19]

NOMENCLATURE

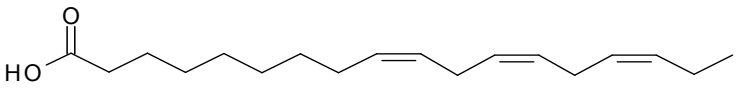
Fatty acids are classified in function of the alkyl chain length and of the number, position and configuration of the double bonds. There are three possible ways to designate them – the IUPAC nomenclature (seldom used for fatty acids), the historical name (common) and the symbolic name (practical, simple, unequivocal). The latter, however, requires a short explanation. The *symbolic* name contains the number of carbons followed by a double point and the number of double bonds.

Example of a saturated fatty acid : palmitic acid – 16 : 0



In the case of unsaturated fatty acids the formula is completed by the indication of the positions and configurations of the double bonds. The numbering of the double bonds starts at the methyl end (CH_3–) of the chain (position n).

Example of an unsaturated fatty acid : [α-] linolenic acid – 18 : 3 cis, cis, cis, n-3, n-6, n-9



However, this nomenclature is generally simplified by considering the natural *cis* configuration and the nonconjugated nature of the fatty acids.

Therefore the same fatty acid than above would result in : 18 : 3 (n-3) [USA: 18 : 3 ω3]

It appears that only the position of the first double bond is added to the formula.

[19] (Christie, 1982; Belitz et al, 2004)

Saturated fatty acids

Generally, they are separated in short-chain fatty acids (≤ 12 carbons) and long-chain fatty acids (≥ 14 carbons), whose principal representatives are palmitic (16 : 0) and stearic acid (18 : 0). The most common are listed in *Table 1*.

IUPAC name	**Historical name**	***Symbolic name***
Butanoic	Butyric	4 : 0
Hexanoic	Caproic	6 : 0
Octanoic	Caprylic	8 : 0
Decanoic	Capric	10 : 0
Dodecanoic	Lauric	12 : 0
Tetradecanoic	Myristic	14 : 0
Hexadecanoic	Palmitic	16 : 0
Octadecanoic	Stearic	18 : 0
Eicosanoic	Arachidic	20 : 0
Dodocosanoic	Behenic	22 : 0
Tetracosanoic	Lignoceric	24 : 0
Pentanoic	Valeric	5 : 0
Heptanoic	Enanthic	7 : 0
Nonanoic	Pelargonic	9 : 0
Pentadecanoic		15 : 0
Heptadecanoic	Margaric	17 : 0

Table 1 – Saturated fatty acids – formula $CH_3(CH_2)_nCOOH$.

Unbranched, straight-chain molecules with an even number of carbon atoms are dominant among the saturated fatty acids. They are normally of animal origin and solid at room temperature like for example margarine, butter or lard. However, there are some vegetables and oleaginous plants which contain high amounts saturated fatty acids as well. These are for example palm fat, coconut, avocado or peanut.

Fatty acids with odd numbers of carbon atoms are present in food only in traces. Some of these are important as food aroma constituents.

Unsaturated fatty acids

Unsaturated fatty acids are the predominant form in lipids and those with 18 carbons are the most important. The five most common are listed below in *Table 2*.

Historical name	***Symbolic name***
Oleic acid	18 : 1, cis, n-9 (ω9)
Linoleic acid	18 : 2, cis, cis, n-6, n-9 (ω6)
[α-] Linolenic acid	18 : 3, cis, cis, cis, n-3, n-6, n-9 (ω3)
Arachidonic acid	20 : 4, cis, cis, cis, cis, n-6, n-9, n-12, n-15 (ω6)
Erucic acid	22 : 1, cis, n-9 (ω9)

Table 2 – Some unsaturated fatty acids.

Unsaturated fatty acids are generally of plant origin, but can also be found in animal products like fish. They are characteristically fluid because they are oils.

Melting point and crystalline structure are strongly correlated in the case of fatty acids. The unsaturated fatty acids, because of their double bonds, are not free to rotate and hence have rigid kinks along the carbon chain. A molecule is less bent by a *trans* than by a *cis* double bond, this one causing a bending of 40°. Hence, the molecular crumpling is increased by an increase of *cis* double bonds.

Odd-numbered and unsaturated fatty acids can not be uniformly packed into a crystalline lattice as can the saturated and even-numbered acids. The consequence of less symmetry within the crystal is that the melting points of even-numbered acids exceeds the melting points of the next higher odd-numbered fatty acids.

APPENDIX V – SUGAR ANALYSIS BY CAPILLARY ELECTROPHORESIS

CAPILLARY ELECTROPHORESIS[20]

Capillary electrophoresis has often been cited as the new revolutionary technique for sugar analysis and several publications can be found presenting various methods. So it was only natural to test this technique for this research. Eleven sugar standards – glucose, galactose, fructose, mannose, sorbitol, mannitol, inositol, xylitol, lactose, sucrose and maltose - have been used to be analysed on a Agilent 3D CE.

The principal of the analysis has been taken from Hofstetter-Kuhn et al (1991) who had achieved a good analysis of carbohydrates by applying borate complexation (in situ complex formation with borate ions). However their method conditions could not be used on the machine available in our laboratory. Thus, variations of methods presented in other publications using borate complexation have been tested (Soga, 1995; Bennani and Fabre, 2001; Altria et al, 1999).

To determine an optimal method to separate the eleven selected sugars, various parameters have been studied : concentration of tetraborate (including variations of running and sample buffer concentrations), pH, voltage and preconditioning.

Buffer & pH

Because the procedure applied here uses borate complexation the buffer used is a borate buffer. It is important to notice that it is the tetrahydroxyborate ion that undergoes complexation, rather than boric acid. At constant sugar concentration the amount of complex increases with the borate concentration as well as with pH due to a higher amount of borate ions. Control of pH is one of the most important means to influence separation of compounds in electrophoresis since the pH enters directly in the equation of the effective electrophoretic mobility. Furthermore, it influences the complex formation between carbohydrates and borate ions with a basic pH being optimal.

Carbohydrates have a very low UV absorbance, but their detection in the low UV region can be significantly enhanced by using borate complexation and alkaline pH.

[20] (Paulus and Klockow, 1996; Hoffstetter-Kuhn et al, 1991; El Rassi and Nashabeh, 1995; Andersson and Hagglund, 2002; Altria et al, 1997)

Voltage

Voltage is determining for the duration of analysis and its quality as well. It should not be too high as that could cause negative side-effects such as incomplete separation and Joule heat. Low voltages however could result in long analysis times. The voltages tested here were between 15 and 19kV.

Preconditioning

In order to obtain consistent results it is important to keep the capillary in a good state. Consequently, three points have to be taken care of when working with CE and particularly when analysing carbohydrates. First of all, a new capillary has to be pre-conditioned before being used for any analysis. This is done by rinsing it for ~20 min with 0.1M NaOH, deionised water and running buffer.

Once it is in use, the inside of the capillary must be allowed to stabilise by applying pre-conditioning before every analysis and by realising blanks at regular intervals. For the former, normally the same solutions are used then cited above, but for a shorter time. The blanks are generally realised with the sample buffer and it has to be tested at whatever interval they have to be performed.

The following parameters were fixed in the beginning and kept unchanged for all tests realised :

Capillary	Length: 56 cm BF3 (extended path), I.D: 50 µm
Temperature	60°C
Injection	50 mbar, 3s
Detection	UV-DAD, 195nm (10nm)/450nm

Run-time varied between 15-18 minutes depending on the voltage applied.

The capillary was chosen because of relatively short length and the presence of the bubble cell BF3, which means that the detection path length is increased by a factor of three and will therefore improve sensitivity.

Temperature has been determined according to references using the same principle for carbohydrate analysis. Generally, high temperatures in CE result in an increase of band widths. For carbohydrates, however, the opposite has been observed. An increase of temperature means that the equation equilibrium of complexation is reached faster, thus resulting in narrower peak shapes. Additionally, the content of the open-chain form of sugars in increased which is better for complexation, and injection volume is increased as well due to a decreased viscosity of the sample.

The detection wavelength is the same than used by Hofstetter-Kuhn (1991) for the reasons mentioned under ***Buffer & pH***. The injection mode has been taken from Altria et al (1999), his method being based on the study of Hofstetter-Kuhn but applied on a similar machine than the one used in our laboratory.

Results

First results were very promising – all sugars could be detected, but separation was not good enough yet. Unfortunately, during the time when optimisation of the parameters was attempted, problems started to appear.

- Optimisation of all three groups together (monosaccharides, disaccharides and polyols) was not possible – if an improvement for one group could be achieved, the result for the other groups would be worse than before. And it appeared impossible to obtain an acceptable compromise.
  For example, disaccharides, monosaccharides and polyols would complex differently for a given borate concentration, the first ones needing a higher amount of borate to form complexes. But a high borate concentration would also result in longer migration times. (Altria et al, 1999)
- Over the time a variation of migration times has been observed. For a same sugar and same conditions they would be different if analysed alone or in a mixture, for different concentrations and before and after maintenance of the machine. These variations were sometimes very important, but even slight differences could not be accepted because the migrations times were very close for certain sugars.
- Furthermore it appeared that there were great differences of peak size between the three types of sugars for the same concentration. Monosaccharides often presented large but not very high peaks, the opposite was observed for polyols (high but not large) and disaccharides showed very small peaks in both ways. This wouldn't be a problem if the migration times were not as close as they were in our case. Additionally, lactose would suddenly present split peaks (probably the α- and β-form) for conditions thought to give better results than before.
- Finally, it was observed that although preconditioning was applied before every analysis the baseline was often irregular. Various attempts to improve its quality were made by changing conditions of preconditioning, filtering the buffer (0.45µm, then 0.2µm) and regular washing of the capillary, without very much success.

As it appeared to be impossible to get satisfying, reproducible separation of the tested sugars, it was decided to abandon this technique for gas chromatography which after only a few tests gave already better results than CE.

APPENDIX VI – INSTRUMENTAL CONDITIONS OF THE COCAINE GC-MS METHOD

Method	**COC**
Sample introduction	
Split	20 : 1
Volume	2µl
Total flow	23 ml/min
Injector T	230°C
Gas saver	15ml/min after 2min
Carrier gas	Helium
Column	
Type	DB1MS, 30m(L) x 0.25mm (i.d.) x 0.25µm (film)
Mode	Constant flow
Average velocity	37cm/sec
Oven T-program	180°C (1min), 4°C/min, 275°C (5.25min)
GC-MS interface T	250°C
MS information	
Solvent delay	2 min
Mass range	50 – 550 a.m.u
Sample rate	3, A/D samples 8
MS quad T	150°C
MS source T	230°C

APPENDIX VII – SAMPLING, ALL SAMPLES

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Illicit Substance	Qty [%]	Diluent
760	19.7.98	9.03	3.56	250.37	None	Rose	Yes	MDMA	32	Lactose (B)
788	7.6.98	9.02	3.38	254.31	None	Rose	Yes	MDMA	36	Lactose (B)
831	26.4.98	9.02	3.58	240.56	None	White	Yes	MDMA	32	Lactose (B)
Z14B		9.08	4.86	273.24	None	White	Yes	MDMA	34	Lactose (B)
985C	14.6.99	9.06	4.61	297.08	Rolling Stones	Beige	None	MDMA	29	Sorbitol
1111B	17.9.00	9.19	3.35	261.11	Ferrari	Beige	Yes	MDMA	34	Lactose
1319A	8.1.01	7.97	4.70	259.80	Ferrari	Yellow	Yes	MDMA	35	Lactose
1451A	16.1.01	7.99	4.38	255.46	Ferrari	Orange dirty	Yes	MDMA	33	Lactose
1451B	16.1.01	7.96	4.68	258.67	Ferrari	Yellow	Yes	MDMA	32	Lactose
1130	24.7.00	9.18	3.91	315.37	Heart (punchmark)	Beige	Yes	MDMA	14	Lactose
1134A	12.8.00	9.16	3.92	315.42	Heart (punchmark)	Beige	Yes	MDMA	17	Lactose
1314B	13.1.01	9.21	3.94	305.77	Heart (punchmark)	Beige	Yes	MDMA	16	Lactose
1345A	29.6.00	9.14	3.87	311.16	Heart (punchmark)	Beige	Yes	MDMA	20	Lactose
1324	4.2.01	9.14	3.89	294.37	Heart	White	Yes	MDMA	24	Lactose
1668	26.12.03	8.07	4.57	262.49	Heart	Rose	Yes	MDMA	23	Lactose
1683	10.6.04	8.17	4.07	248.05	Heart	White dirty	Yes	MDMA	31	
1693	17.2.05	8.20	3.69	216.99	Heart	White dirty	Yes	MDMA, MA	28	
1708	8.4.05	8.20	3.69	220.35	Heart	White dirty	Yes	MDMA, MA	24	
520	21.1.98	9.13	3.95	285.52	Crown	White	None	Amphetamine	30	Lactose
523	12.2.98	9.14	4.01	294.90	Crown	White	None	MDMA	29	Lactose (B)
524	12.2.98	9.14	4.04	300.27	Crown	White	None	MDMA	29	Lactose (B)
1444B	26.12.01	6.06	4.23	139.09	Dolphin	Blue	None	MDMA	68	Lactose
1445	30.12.01	6.06	4.06	131.09	Dolphin	Blue	None	MDMA	68	Lactose
1500	3.2.02	6.05	3.97	128.35	Dolphin	Blue	None	MDMA	63	Lactose
1519		6.02	3.96	128.73	Dolphin	Blue	None	MDMA	75	Lactose
1507A	10.2.02	9.04	4.84	298.25	Tasmanian Devil	Beige	None	MDEA	25	Sorbitol, Lactose
985A	14.6.99	9.08	4.63	294.72	Tasmanian Devil	White dirty	None	MDMA	21	Sorbitol
922	18.9.99	8.30	4.33	243.17	Diamond	Violet	None	MDA	28	Lactose
952	22.10.99	8.07	3.49	214.02	Diamond	Violet	None	MDA	28	Lactose
1091	19.6.00	8.18	3.35	213.55	Diamond	Violet	None	MDA	27	Lactose
1118	19.6.00	8.17	3.30	208.65	Diamond	Violet	None	MDA	26	Lactose
1148	13.8.00	8.21	3.54	224.76	Diamond	Violet	None	MDA	23	Lactose, Mannitol (tr)
1209	24.9.00	8.22	3.58	226.53	Diamond	Violet	None	MDA	24	Lactose, Mannitol (tr)
1112F	17.9.00	8.21	3.52	222.63	Diamond	Violet	None	MDA	24	Lactose, Mannitol (tr)
Z197	2.4.04	8.07	2.79	160.60	Diamond	Violet	None	MDA		
1204A	10.9.00	8.23	4.15	274.46	Double lightning	Violet	None	MDA	22	Lactose, Mannitol (tr)
1218A	31.8.00	8.20	4.16	275.14	Double lightning	Violet	None	MDA	25	Lactose, Mannitol (tr)
1112G	17.9.00	7.04	4.37	218.40	"007"	Orange	None	MDMA	38	Lactose (B)
1388	30.7.01	7.04	4.98	240.08	"FF"	Yellow	None	MDMA	32	Sorbitol
1380A	15.7.01	7.04	4.94	240.81	"FF"	Yellow	None	MDMA	35	Sorbitol

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Illicit Substance	Qty [%]	Diluent
1473A	29.6.01	7.07	5.04	242.37	"FF"	Yellow	None	MDMA	36	Sorbitol
995B	20.12.99	8.06	5.39	361.41	"S"	Green	Yes	MDMA	35	Lactose (B)
Z60	2.1.99	8.03	4.65	278.66	"STAR DUST"	White	Yes	MDMA	27	Lactose
949A	31.1.99	8.01	4.85	294.38	"STAR DUST"	White	Yes	MDMA		Lactose
949B	31.1.99	8.03	4.93	296.41	"STAR DUST"	Beige	Yes	MDMA		
1002	2.1.00	8.04	5.47	335.88	"STAR DUST"	White dirty	Yes	MDMA	26	
1061	3.4.00	8.11	5.69	345.95	"STAR DUST"	Beige	Yes	MDMA	27	
1304A	17.5.00	8.07	5.52	336.74	"STAR DUST"	Beige	Yes	MDMA	28	
Z209	23.2.04	8.01	4.22	239.10	"STAR DUST"	White dirty	Yes	MDMA, MDEA		
1207	16.9.00	7.09	3.80	173.88	"Xhi"	White dirty	Yes	MDMA	59	
1498	2.2.02	7.06	4.89	230.79	"Xhi"	Rose	Yes strong	MDMA	41	Lactose
1619A	13.2.02	7.05	4.86	227.01	"Xhi"	Rose	Yes strong	MDMA	43	Lactose
1301	2.5.00	9.18	3.91	308.16	Ferrari (framed)	White	None	MDMA	31	Lactose
1309	9.6.00	9.15	3.89	307.71	Ferrari (framed)	White	None	MDMA	32	Lactose
942	5.12.99	9.09	3.67	292.18	Ferrari (framed)	White	None	MDMA	25	Lactose
970A	4.2.00	9.09	3.77	292.35	Ferrari (framed)	White	None	MDMA	26	Lactose
970B	4.2.00	9.11	3.74	300.29	Ferrari (framed)	White	None	MDMA	29	Lactose
970C	4.2.00	9.10	3.72	296.43	Ferrari (framed)	White	None	MDMA	28	Lactose
Z2A	22.7.98	8.29	4.80	288.73	Elephant	Beige	Yes	MDMA	25	Sorbitol
Z30	3.10.98	8.06	4.71	291.89	Elephant	Beige	Yes	MDMA	30	
985B	14.6.99	8.03	4.93	303.49	Elephant	White	Yes	MDMA	26	
1494D	19.12.01	8.02	5.22	296.95	Elephant	White	Yes	MDMA	29	
737	2.7.98	11.14	4.92	393.75	Star	Rose	Yes	4-MTA		Lactose
743	15.4.98	11.15	4.94	399.08	Star	Rose	Yes	4-MTA		Lactose
1303	17.5.00	8.11	3.44	222.48	Star	White dirty	None	MDMA	42	
1305	17.5.00	8.09	3.45	220.93	Star	White dirty	None	MDMA	37	
1304B	17.5.00	8.10	4.75	255.17	Star	White dirty	None	MDMA	37	Lactose
1304C	17.5.00	8.10	3.48	225.19	Star	White dirty	None	MDMA	40	
1306A	17.5.00	8.11	4.75	253.18	Star	White dirty	None	MDMA	35	Lactose
1306B	17.5.00	8.09	3.41	221.59	Star	White dirty	None	MDMA	36	
1306C	17.5.00	8.12	4.74	254.54	Star	White dirty	None	MDMA	34	Lactose
1306D	17.5.00	8.10	3.36	219.71	Star	White dirty	None	MDMA	37	
1317	5.1.01	9.06	3.90	298.11	Star	White	Yes	MDMA	40	Lactose
1460	6.4.01	8.09	3.84	297.35	Star	White	Yes	MDMA	36	Lactose
1405	7.9.01	8.02	3.25	201.53	Euro	Orange	None	MDMA	60	Lactose
1416B	1.8.01	8.01	3.24	200.23	Euro	Orange	None	MDMA	58	Lactose
832	29.5.98	9.04	4.76	309.58	Twins	Beige	Yes	MDMA	27	Sorbitol
1380B	15.7.01	9.08	4.40	314.77	Twins	Beige	Yes	MDMA	32	Sorbitol
1473B	29.6.01	9.11	4.41	308.05	Twins	Brown	Yes	MDMA	34	Sorbitol
1524	14.4.02	8.15	4.81	287.90	Marlboro	Blue	Yes	MDMA	37	
1528	4.5.02	8.14	4.86	299.65	Marlboro	Rose	Yes	MDMA	34	
Z44	30.8.98	8.05	4.85	291.04	Mitsubishi	Beige	Yes strong	MDMA	34	
Z4A	8.8.98	8.06	5.05	317.67	Mitsubishi	Beige	Yes	MDMA	36	
834	8.8.98	8.13	5.21	299.51	Mitsubishi	Beige	Yes	MDMA	41	Sorbitol
814	23.8.98	8.16	5.19	300.74	Mitsubishi	Beige	Yes	MDMA	32	
1003	2.1.00	8.65	4.56	320.02	Mitsubishi	White dirty	Yes	MDMA	29	Lactose
1300	28.3.00	9.04	3.83	297.00	Mitsubishi	White	Yes	MDMA	29	Lactose
Z84	14.6.99	9.07	4.65	348.30	Mitsubishi	White	Yes	MDMA	20	Lactose

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Illicit Substance	Qty [%]	Diluent
954	12.8.99	9.08	5.53	390.31	Mitsubishi	White	None	MDMA		Lactose
978A	18.2.00	9.09	3.82	296.44	Mitsubishi	White	None	MDMA	35	Lactose
960	5.12.99	9.09	3.84	295.44	Mitsubishi	White	None	MDMA		Lactose
1223	15.10.00	9.12	4.04	294.13	Mitsubishi	White dirty	Yes	MDMA	26	Lactose
1312	31.7.00	9.14	3.87	302.96	Mitsubishi	Beige	Yes	MDMA	37	Lactose
557A		9.08	3.63	286.42	Sparrow	White	Yes	Amphetamine	5	Lactose
557B		9.09	3.54	287.90	Sparrow	White	Yes	Amphetamine		Lactose
Z9		9.07	3.54	286.42	Sparrow	White	Yes	Amphetamine	7	Lactose
989	21.6.99	8.63	3.40	226.96	Flying bird	Beige	Yes	MDMA	38	Lactose
1635	19.11.02	7.60	4.67	261.15	Peace and love	Violet	Yes	MDMA	44	Lactose
1265	14.12.00	8.05	4.09	255.97	Superman	White	Yes	Amphetamine	24	Lactose (B)
1116A	4.7.00	8.00	4.06	243.02	Superman	White	Yes	Amphetamine	23	Lactose (B)
1345B	29.6.00	8.01	4.01	249.22	Superman	White	Yes	Amphetamine	19	Lactose (B)
1327	17.2.01	8.14	5.06	287.00	Superman	White	Yes	MDMA	27	
842E	2.6.99	9.12	4.25	317.45	Superman	Beige	Yes strong	MDMA	27	Lactose
894A	16.8.99	9.19	4.35	321.66	Superman	White dirty	Yes strong	MDMA	23	Lactose
1208	21.10.00	8.06	3.64	237.26	Superman	White	Yes	MDMA, MDA	35	Lactose
1079B	16.4.00	8.04	5.05	296.82	Superman	White dirty	Yes	MDMA, MDEA	29	
1310A	19.6.00	8.07	5.01	292.45	Superman	Beige	Yes	MDMA, MDEA	29	
836A	6.10.98	9.01	3.19	258.75	Superman	Beige	Yes	MDMA, MDEA	12	Lactose
Z34	4.10.98	9.07	3.22	264.50	Superman	Beige	Yes	MDMA, MDEA	12	Lactose

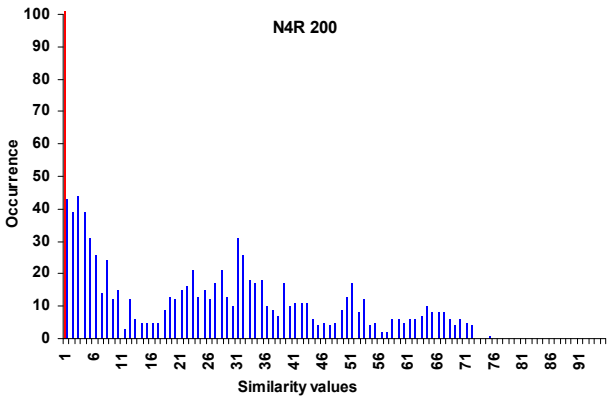
APPENDIX VIII – SAMPLING, NON-LINKED SAMPLES

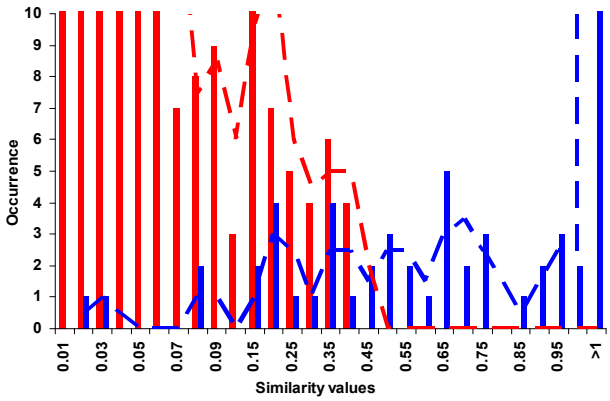
Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Illicit Substance	Qty [%]	Diluent
1112G	17.9.00	7.04	4.37	218.40	"007"	Orange	None	MDMA	38	Lactose (B)
1473A	29.6.01	7.07	5.04	242.37	"FF"	Yellow	None	MDMA	36	Sorbitol
995B	20.12.99	8.06	5.39	361.41	"S"	Green	Yes	MDMA	35	Lactose (B)
1061	3.4.00	8.11	5.69	345.95	"STAR DUST"	Beige	Yes	MDMA	27	
949A	31.1.99	8.01	4.85	294.38	"STAR DUST"	White	Yes	MDMA		Lactose
Z60	2.1.99	8.03	4.65	278.66	"STAR DUST"	White	Yes	MDMA	27	Lactose
1207	16.9.00	7.09	3.80	173.88	"Xhi"	White dirty	Yes	MDMA	59	
1619A	13.2.02	7.05	4.86	227.01	"Xhi"	Rose	Yes strong	MDMA	43	Lactose
523	12.2.98	9.14	4.01	294.90	Crown	White	None	MDMA	29	Lactose (B)
922	18.9.99	8.30	4.33	243.17	Diamond	Violet	None	MDA	28	Lactose
1091	19.6.00	8.18	3.35	213.55	Diamond	Violet	None	MDA	27	Lactose
1112F	17.9.00	8.21	3.52	222.63	Diamond	Violet	None	MDA	24	Lactose, Mannitol (tr)
Z197	2.4.04	8.07	2.79	160.60	Diamond	Violet	None	MDA		
1445	30.12.01	6.06	4.06	131.09	Dolphin	Blue	None	MDMA	68	Lactose
1494D	19.12.01	8.02	5.22	296.95	Elephant	White	Yes	MDMA	29	
Z2A	22.7.98	8.29	4.80	288.73	Elephant	Beige	Yes	MDMA	25	Sorbitol
1416B	1.8.01	8.01	3.24	200.23	Euro	Orange	None	MDMA	58	Lactose
1451A	16.1.01	7.99	4.38	255.46	Ferrari	Orange dirty	Yes	MDMA	33	Lactose
942	5.12.99	9.09	3.67	292.18	Ferrari (framed)	White	None	MDMA	25	Lactose
989	21.6.99	8.63	3.40	226.96	Flying bird	Beige	Yes	MDMA	38	Lactose
1324	4.2.01	9.14	3.89	294.37	Heart	White	Yes	MDMA	24	Lactose
1708	8.4.05	8.20	3.69	220.35	Heart	White dirty	Yes	MDMA, MA	24	
1134A	12.8.00	9.16	3.92	315.42	Heart (punchmark)	Beige	Yes	MDMA	17	Lactose
1524	14.4.02	8.15	4.81	287.90	Marlboro	Blue	Yes	MDMA	37	
834	8.8.98	8.13	5.21	299.51	Mitsubishi	Beige	Yes	MDMA	41	Sorbitol
954	12.8.99	9.08	5.53	390.31	Mitsubishi	White	None	MDMA		Lactose
1003	2.1.00	8.65	4.56	320.02	Mitsubishi	White dirty	Yes	MDMA	29	Lactose
1223	15.10.00	9.12	4.04	294.13	Mitsubishi	White dirty	Yes	MDMA	26	Lactose
Z44	30.8.98	8.05	4.85	291.04	Mitsubishi	Beige	Yes strong	MDMA	34	
760	19.7.98	9.03	3.56	250.37	None	Rose	Yes	MDMA	32	Lactose (B)
831	26.4.98	9.02	3.58	240.56	None	White	Yes	MDMA	32	Lactose (B)
985C	14.6.99	9.06	4.61	297.08	Rolling Stone	Beige	None	MDMA	29	Sorbitol
557A		9.08	3.63	286.42	Sparrow	White	Yes	Amphetamine	5	Lactose
743	15.4.98	11.15	4.94	399.08	Star	Rose	Yes	4-MTA		Lactose
1460	6.4.01	8.09	3.84	297.35	Star	White	Yes	MDMA	36	Lactose
1306A	17.5.00	8.11	4.75	253.18	Star	White dirty	None	MDMA	35	Lactose
1306B	17.5.00	8.09	3.41	221.59	Star	White dirty	None	MDMA	36	
1208	21.10.00	8.06	3.64	237.26	Superman	White	Yes	MDMA, MDA	35	Lactose
1079B	16.4.00	8.04	5.05	296.82	Superman	White dirty	Yes	MDMA, MDEA	29	
1345B	29.6.00	8.01	4.01	249.22	Superman	White	Yes	Amphetamine	19	Lactose (B)
894A	16.8.99	9.19	4.35	321.66	Superman	White dirty	Yes strong	MDMA	23	Lactose
Z34	4.10.98	9.07	3.22	264.50	Superman	Beige	Yes	MDMA, MDEA	12	Lactose
832	29.5.98	9.04	4.76	309.58	Twins	Beige	Yes	MDMA	27	Sorbitol

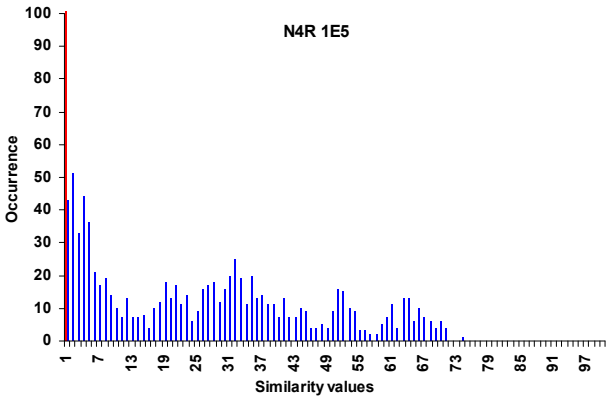
APPENDIX IX – HISTOGRAMS

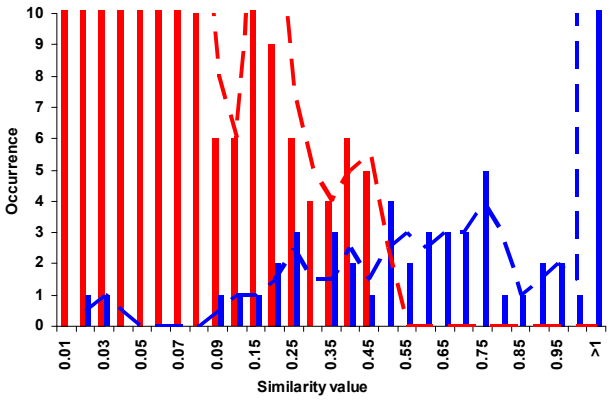
HISTOGRAMS FROM THE XTC DATA SET

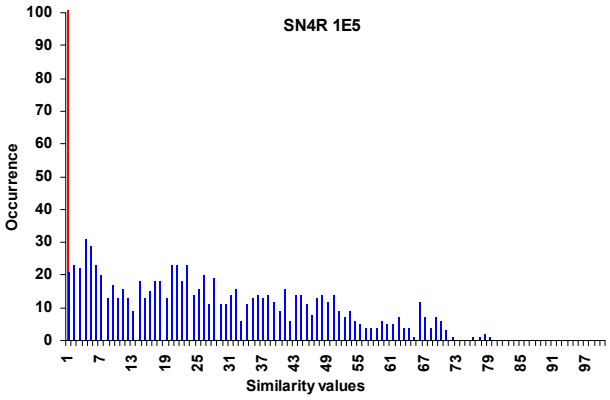
Pearson correlation

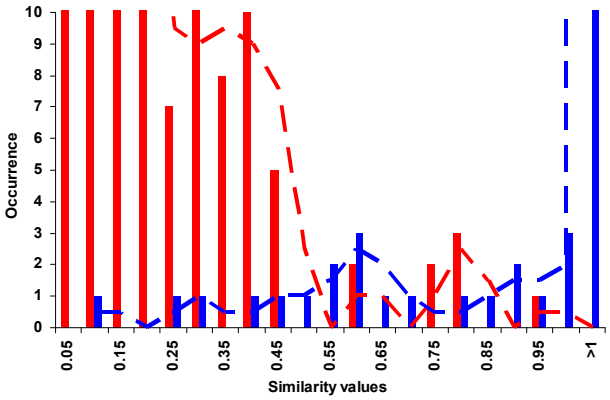












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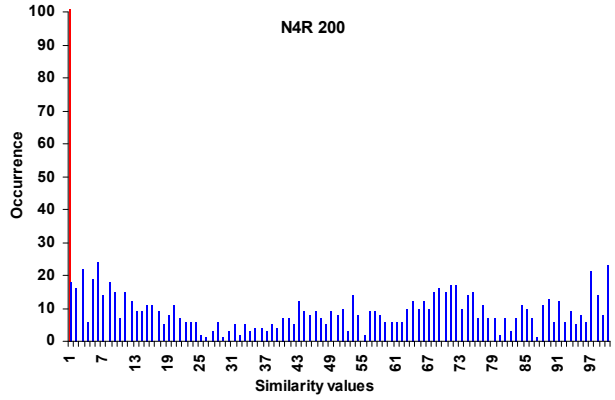

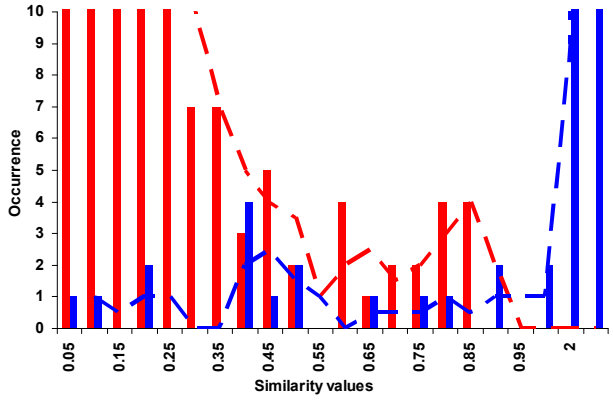

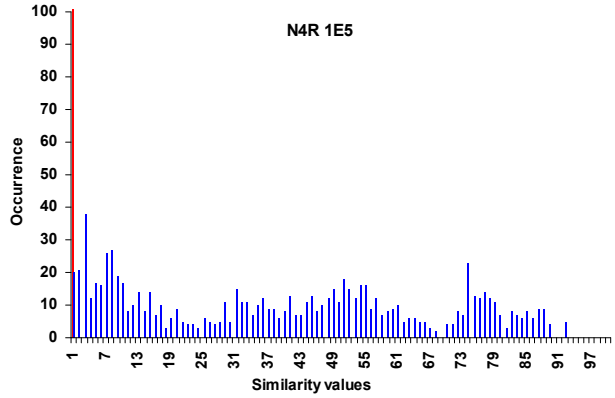

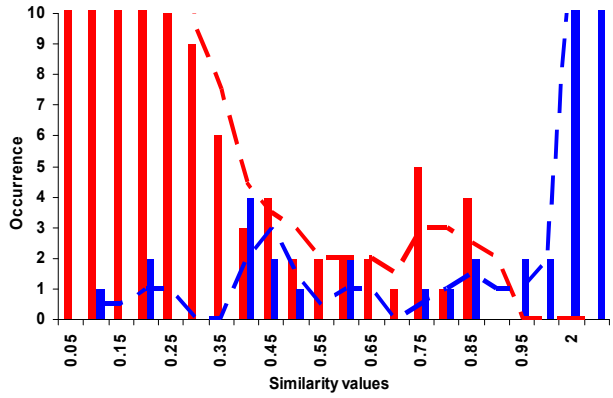

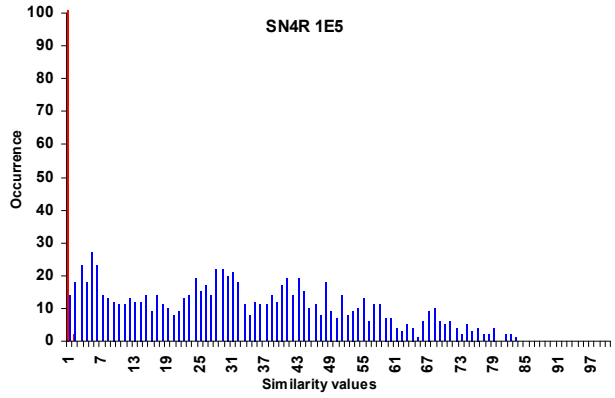

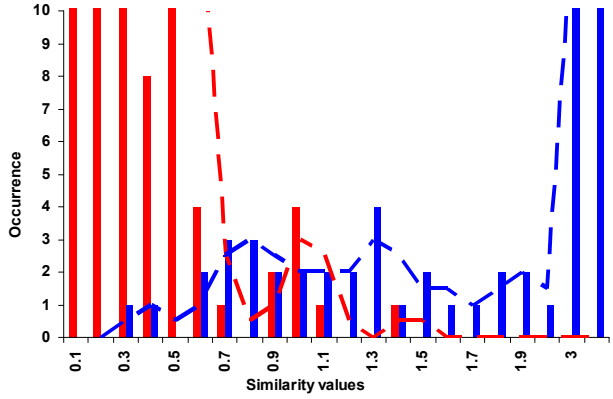

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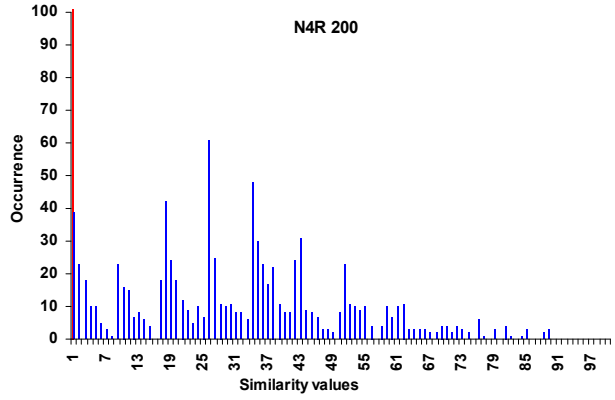

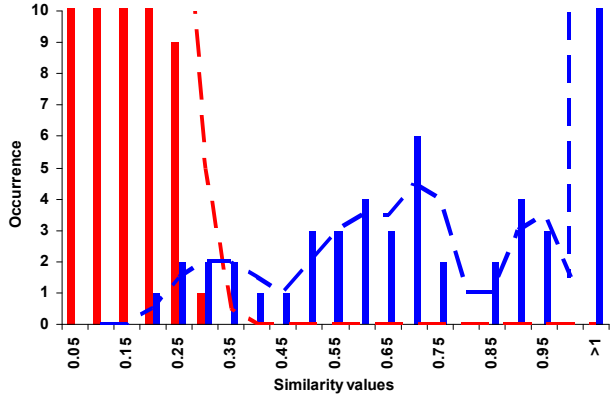

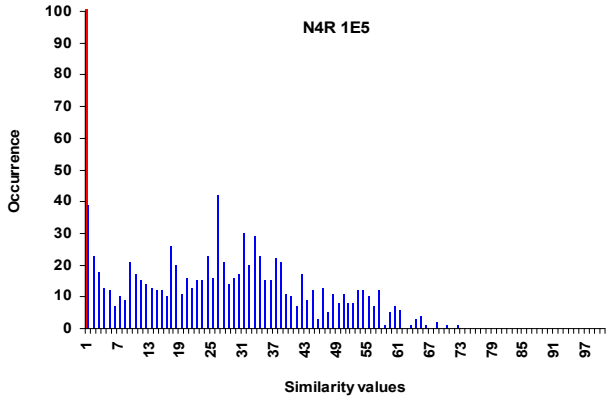

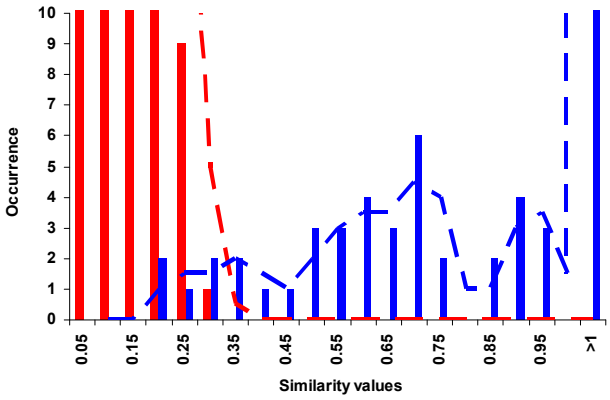

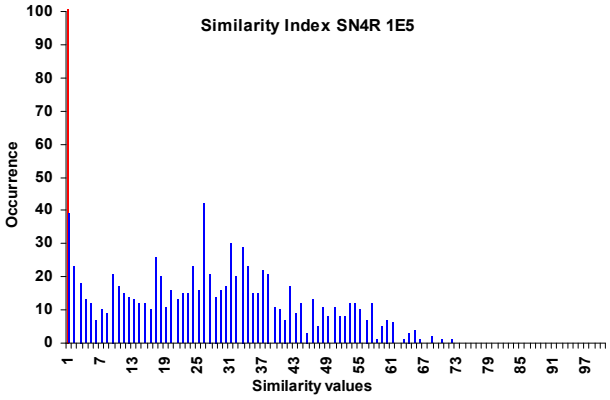

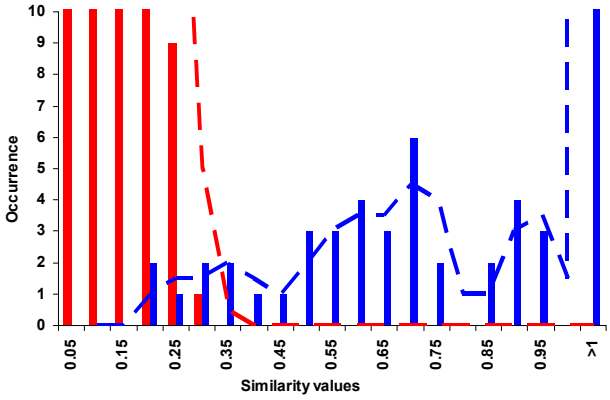

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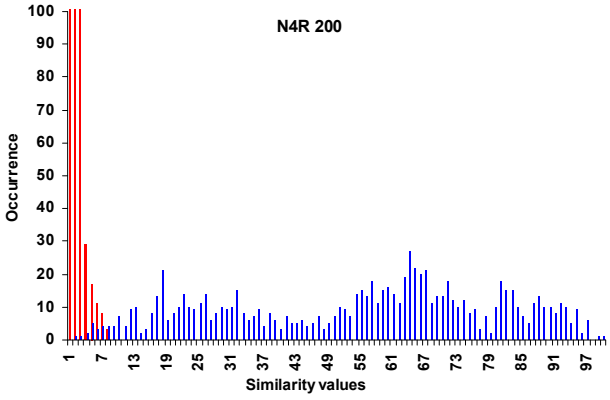
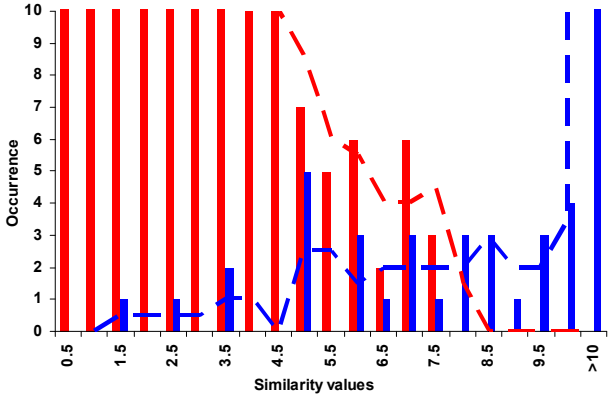
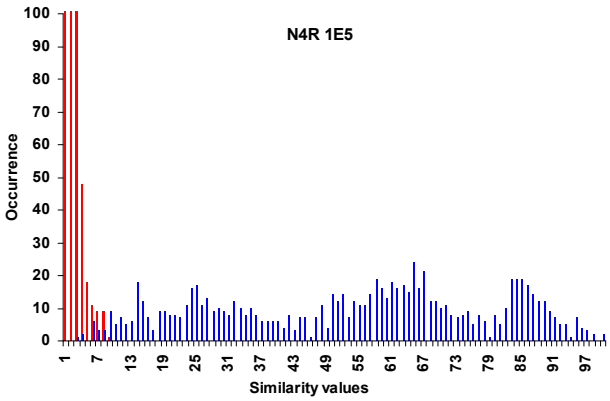
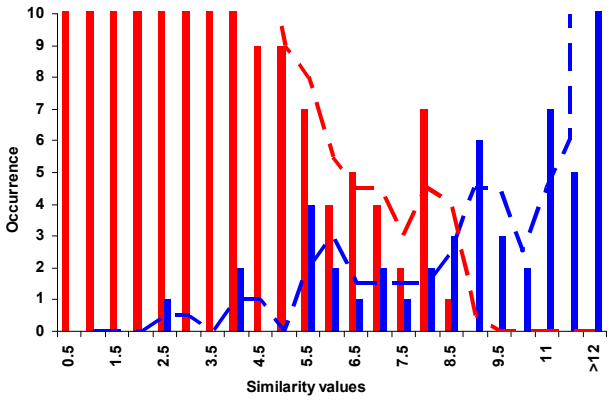
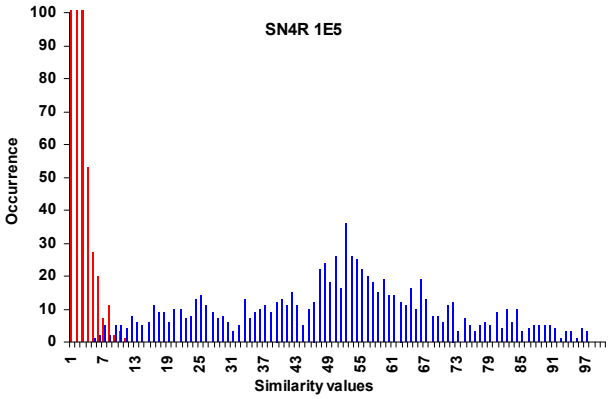
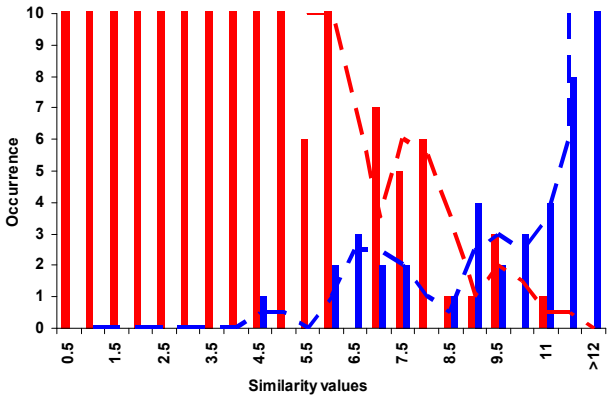




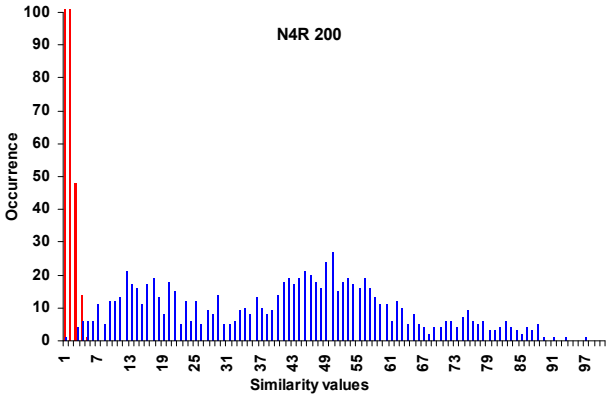


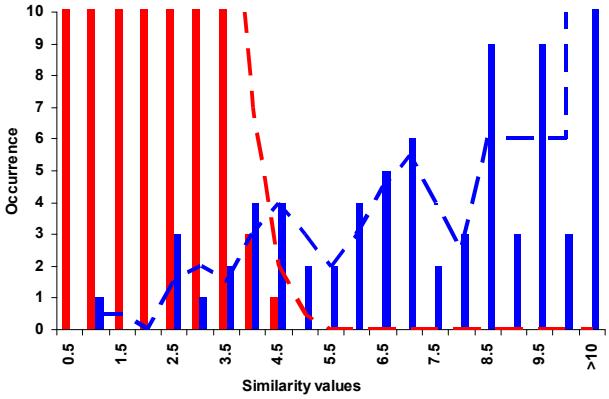


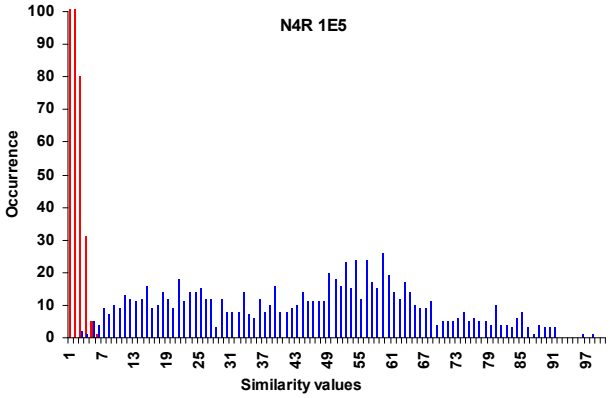


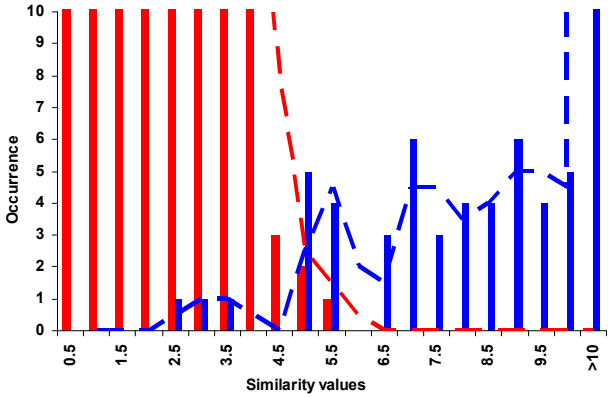


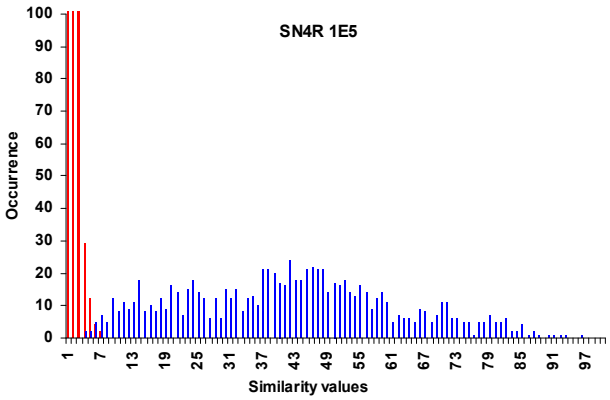
Manhattan Distance

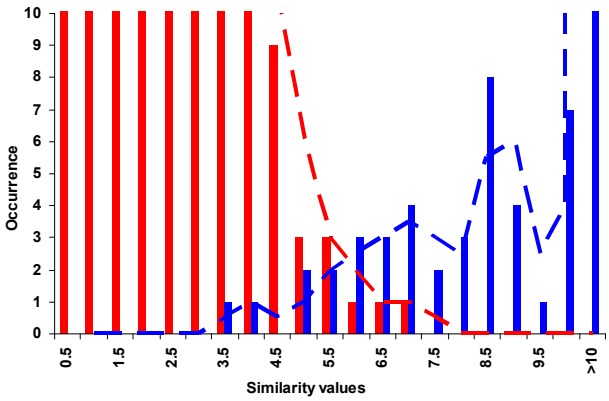




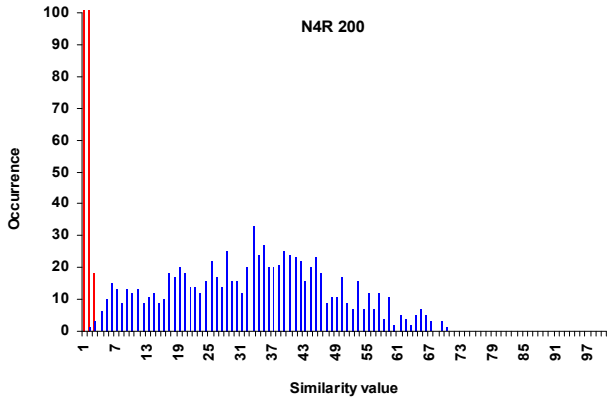


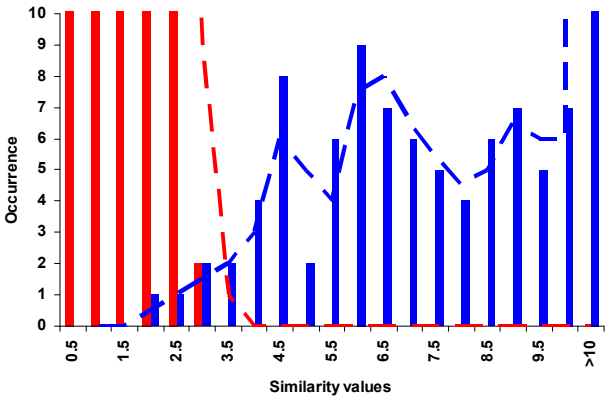


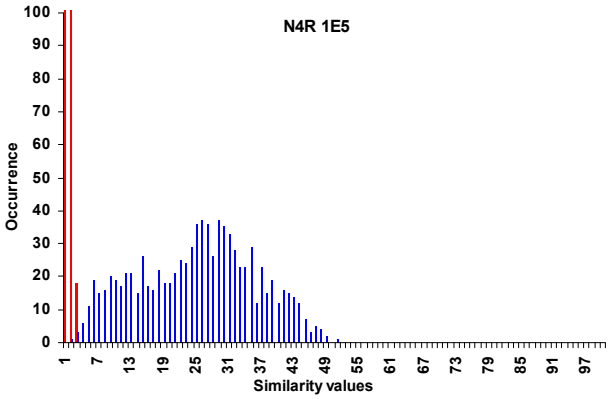


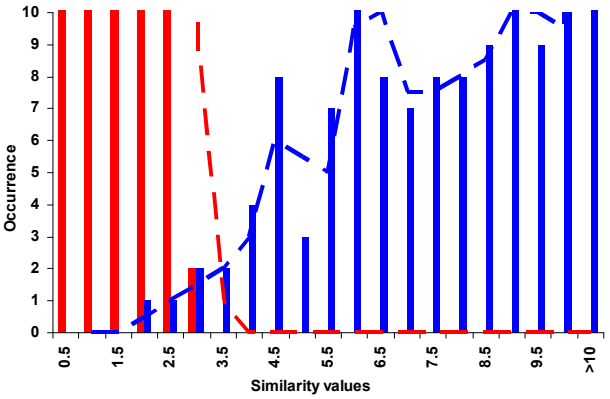


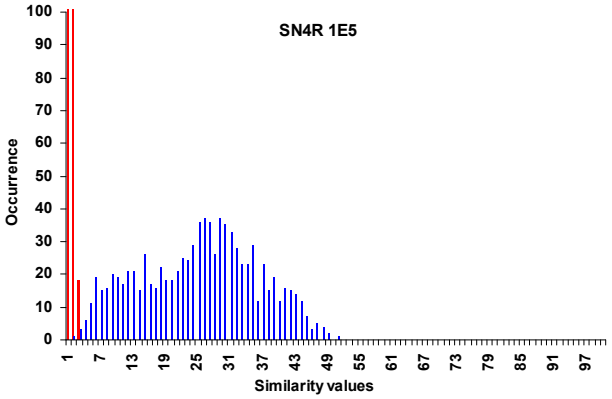
Canberra Index

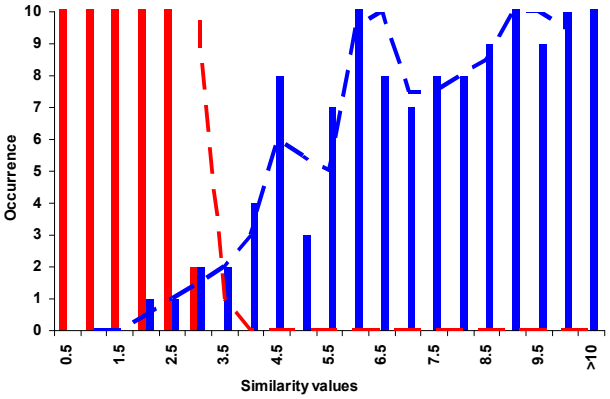






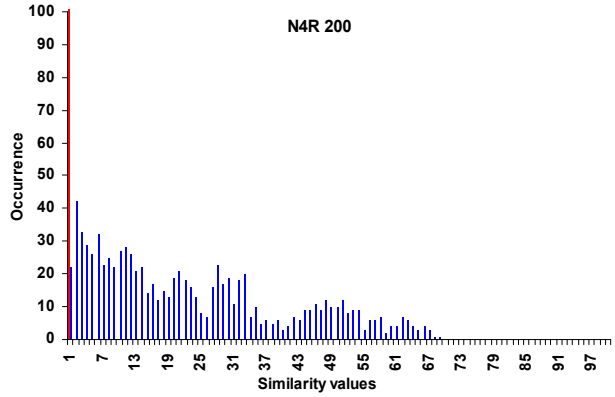


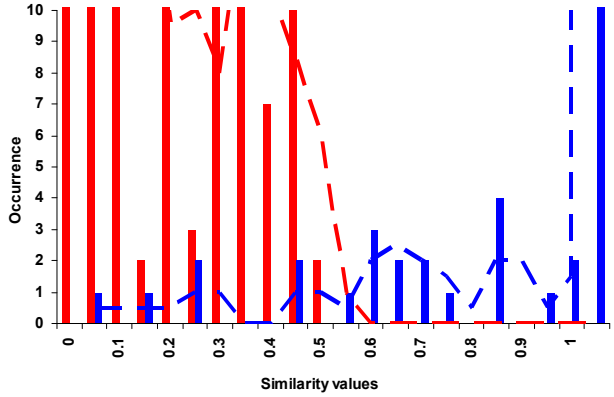


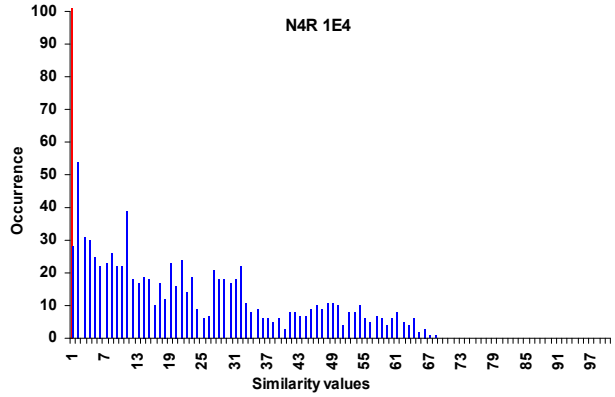


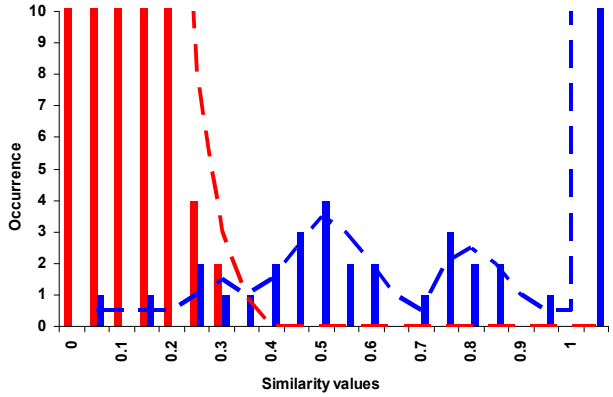
HISTOGRAMS FROM THE FA/SUGAR DATA SET

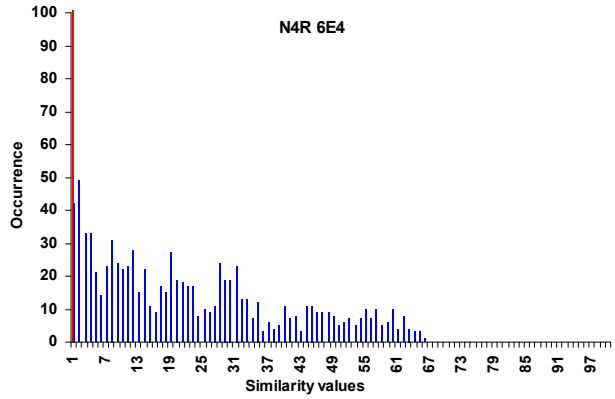
Pearson correlation

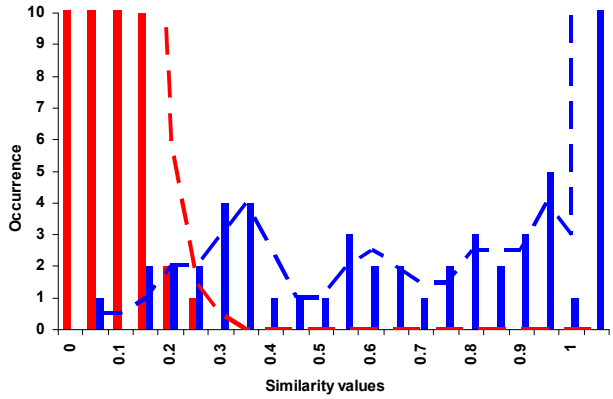


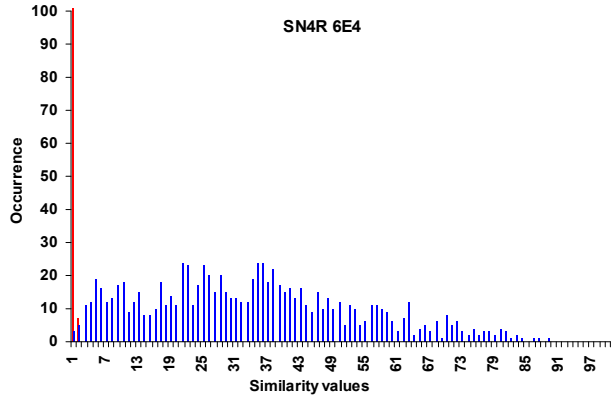


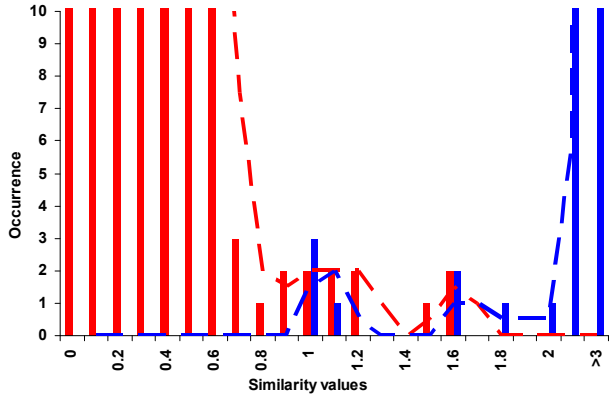




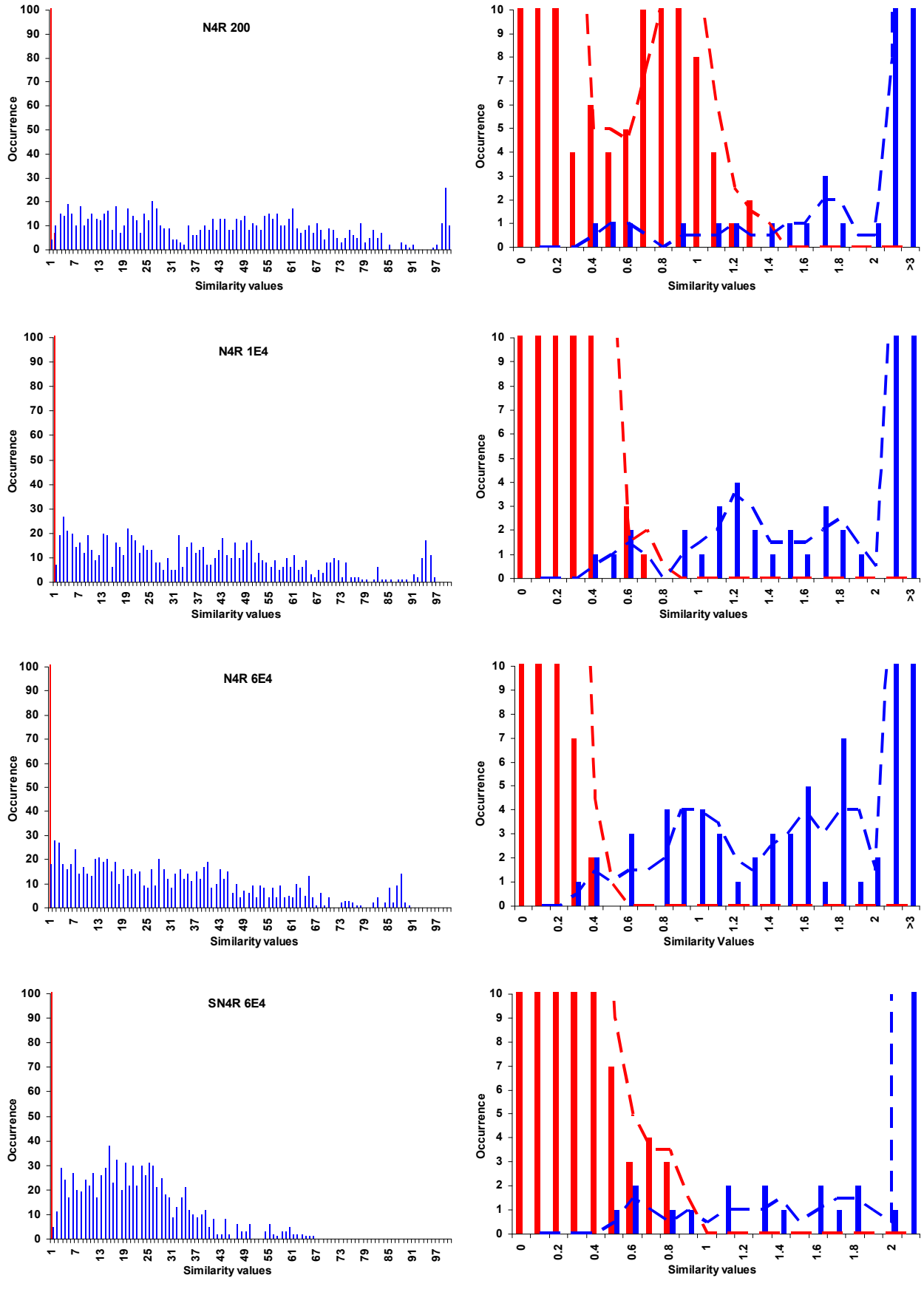








Squared Cosine Function



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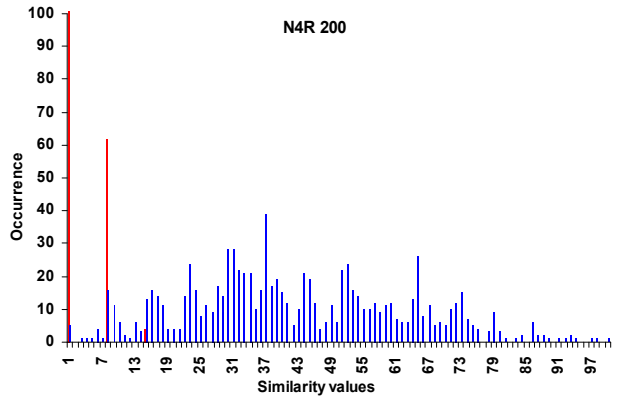
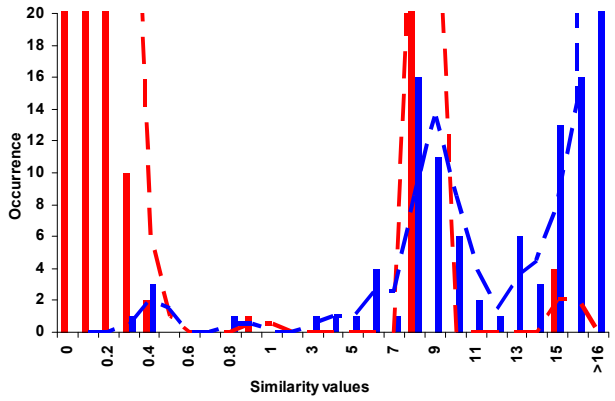
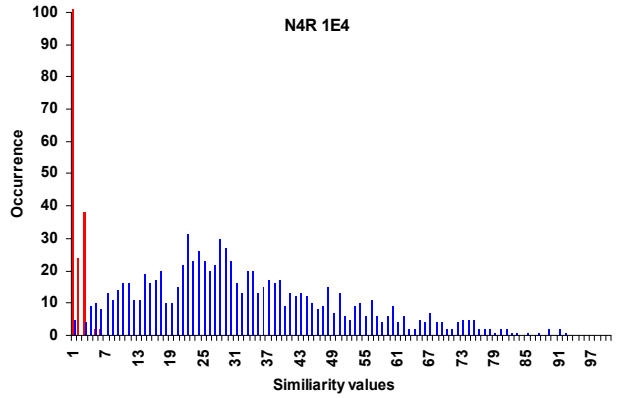
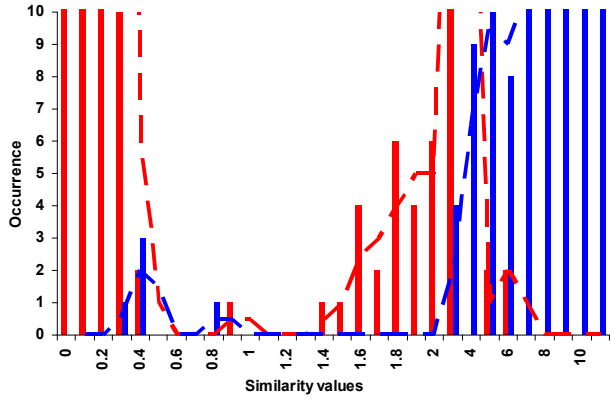
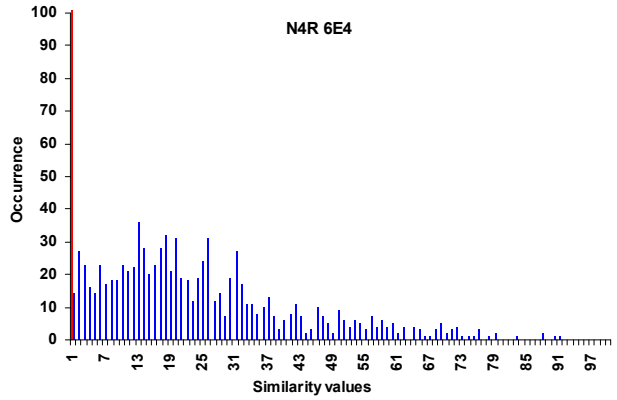
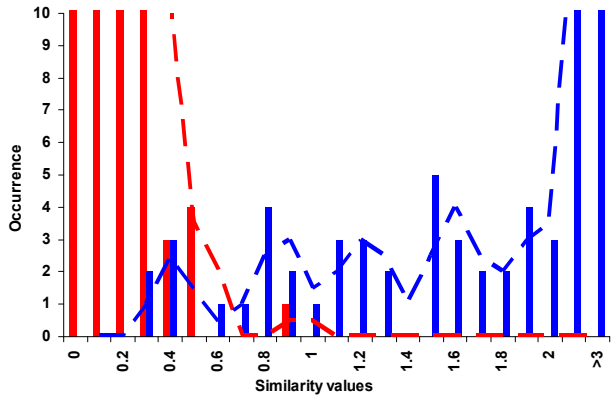


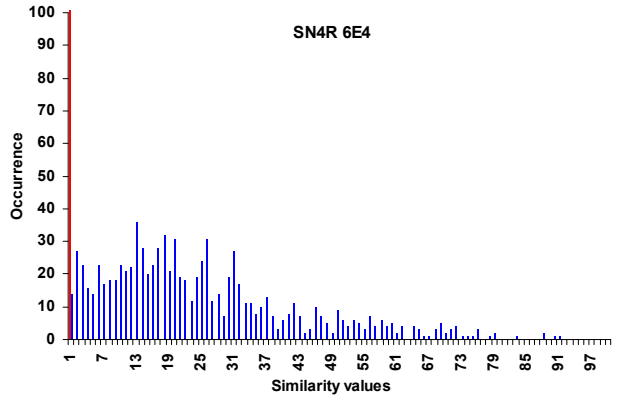


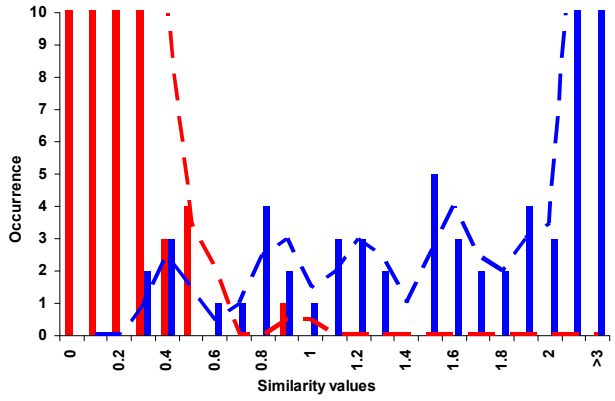




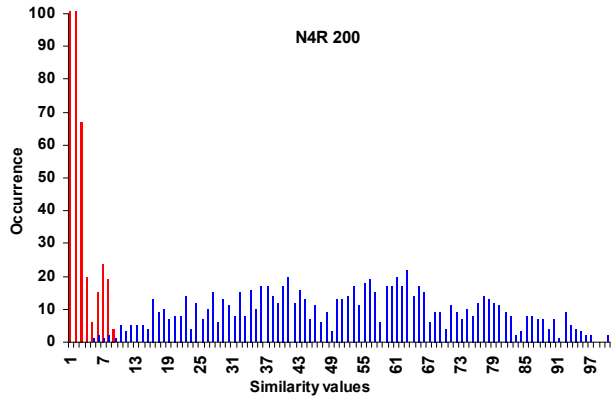


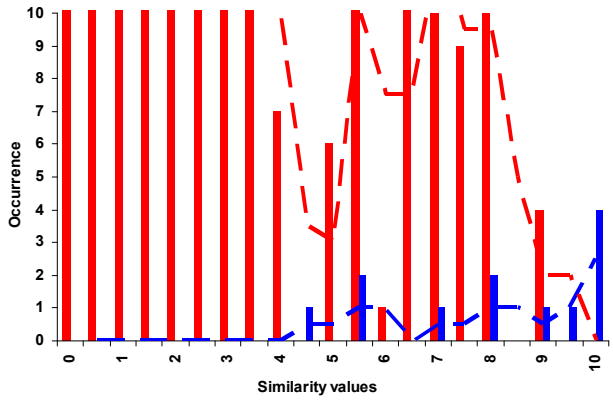


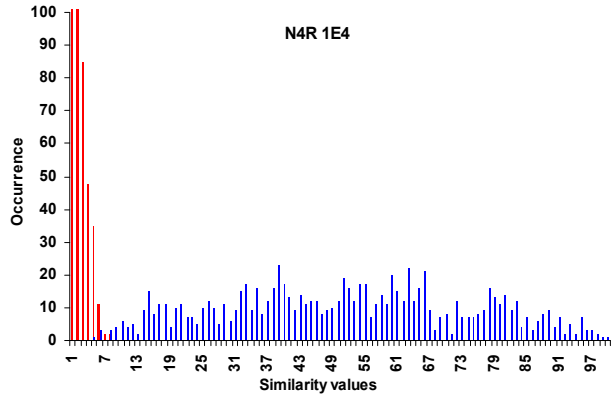


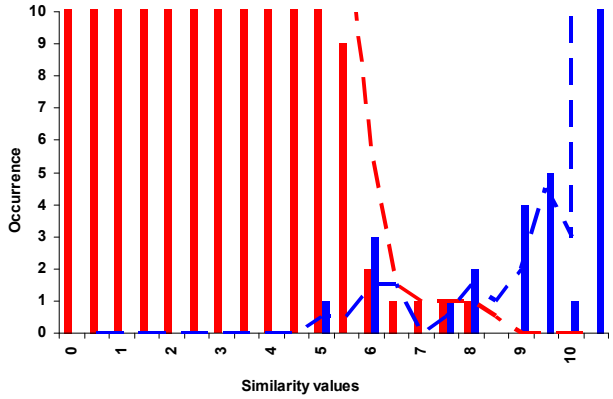


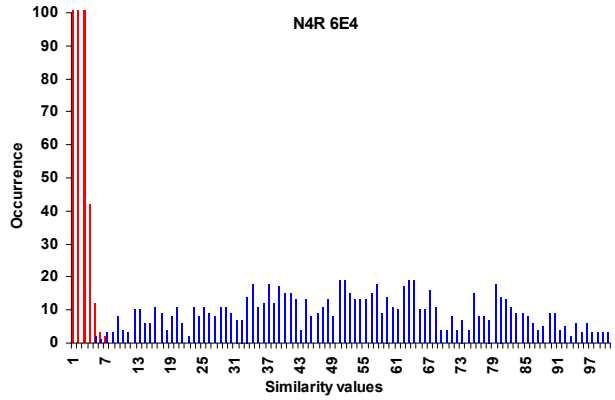
Euclidean Distance

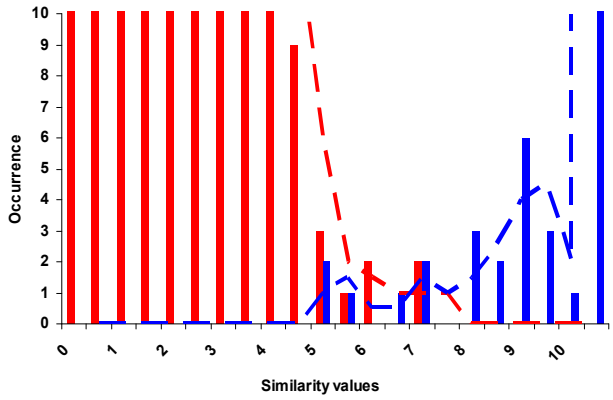


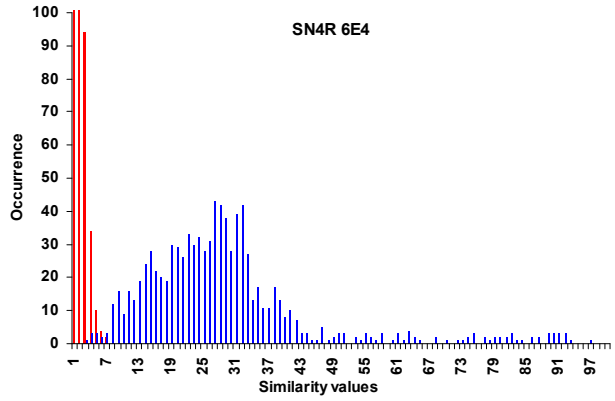


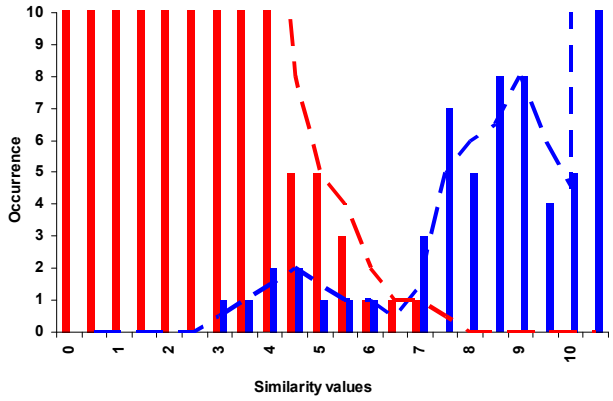




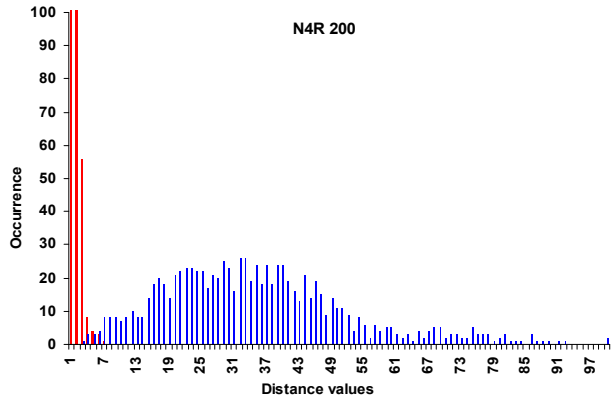


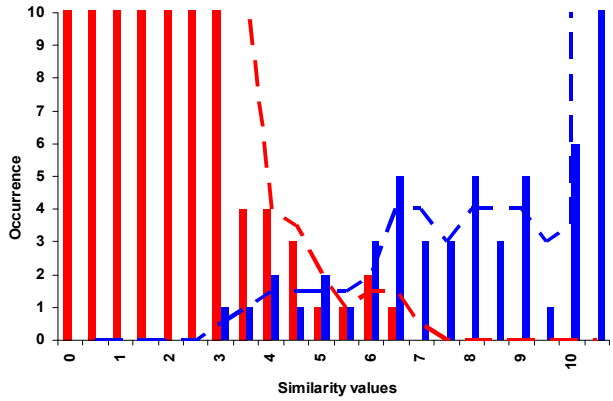


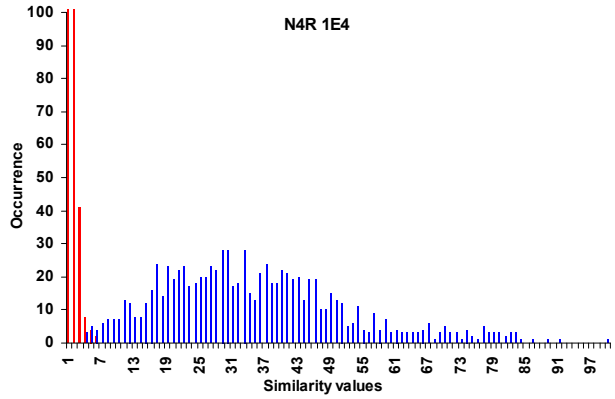


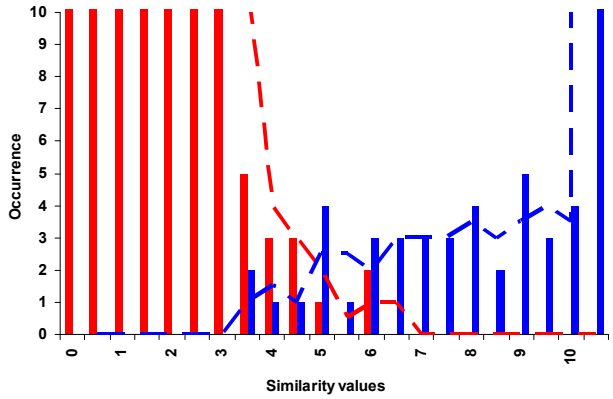


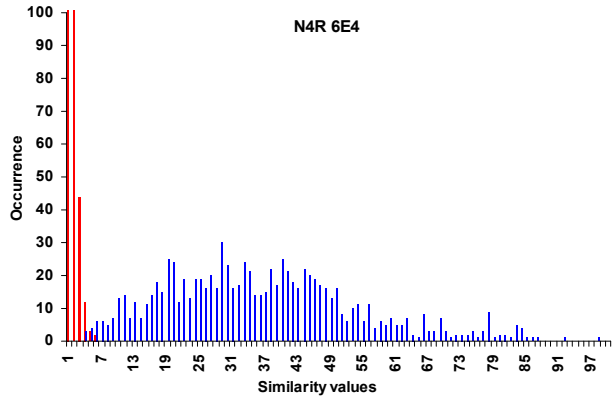
Manhattan Distance

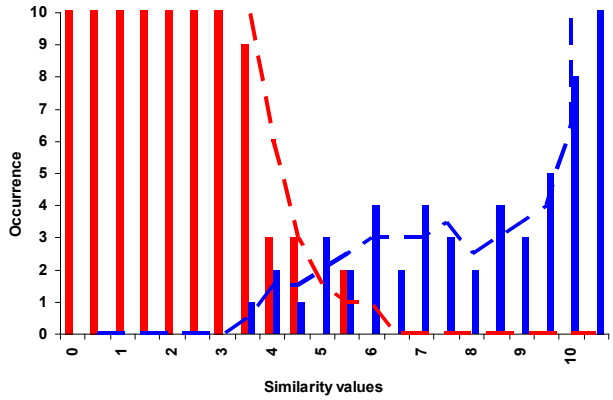


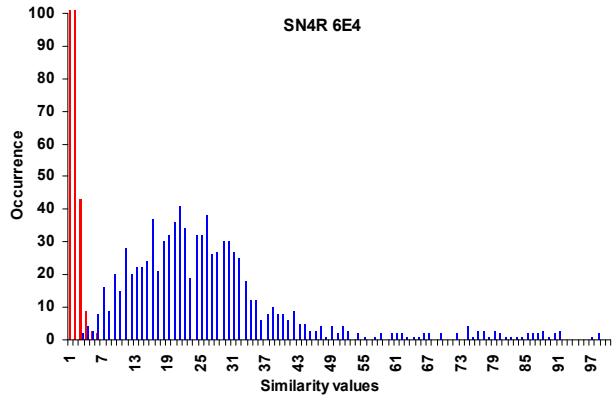


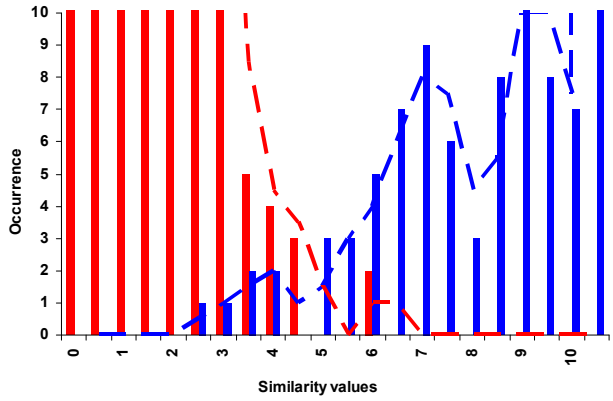












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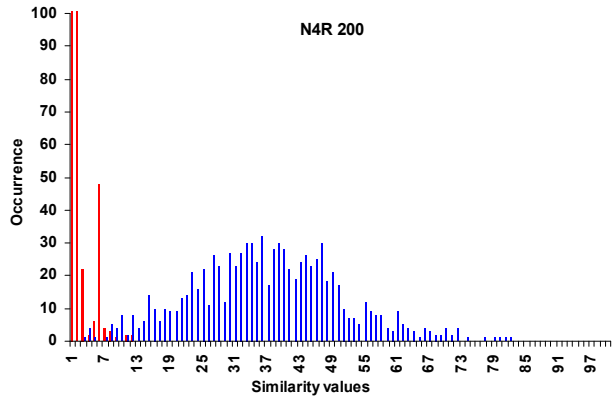

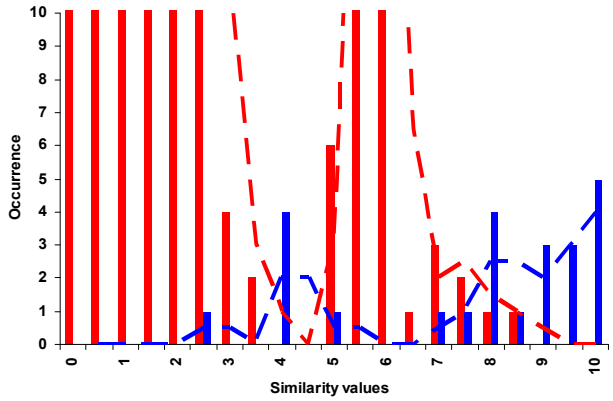

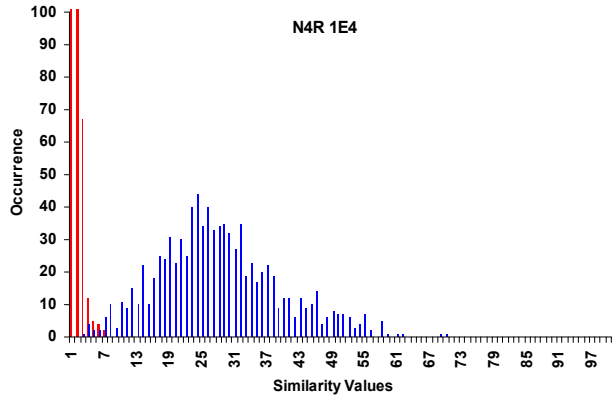

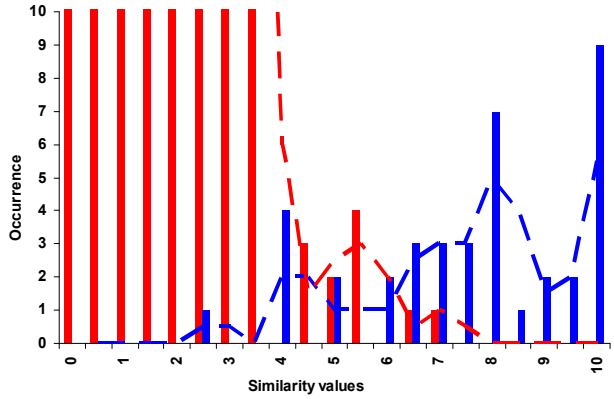

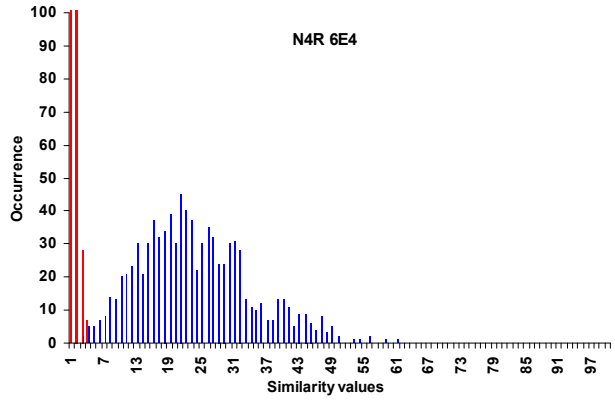

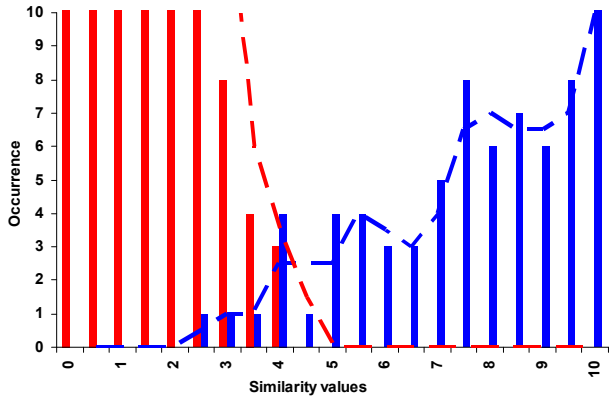

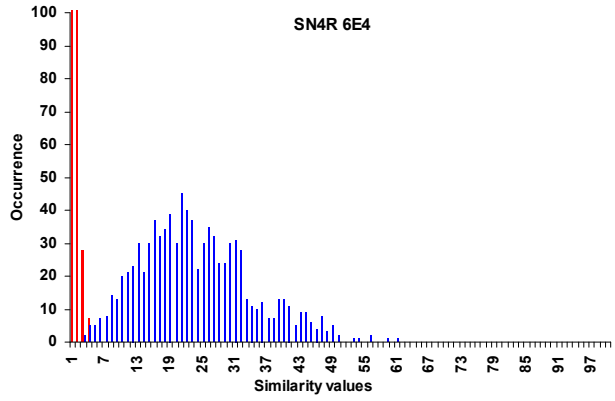

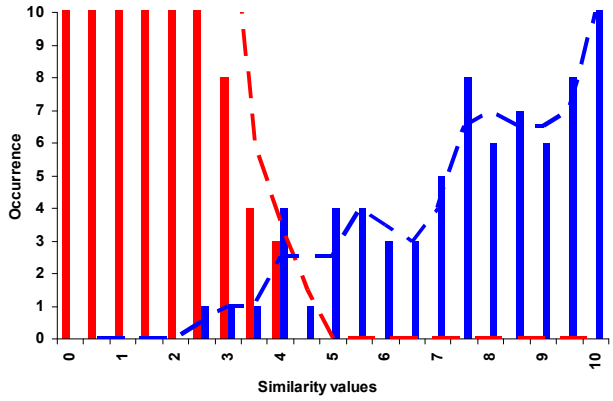

APPENDIX X – SAMPLE GROUPS OBTAINED WITH THE EXCIPIENTS

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
737	02.07.98	11.14	4.92	393.75	Star	rose	Yes	ThioStar	4MTA			Lactose
743	15.04.98	11.15	4.94	399.08	Star	rose	Yes	ThioStar	4MTA			Lactose
1345B	29.06.00	8.01	4.01	249.22	Superman	white	Yes		Amphetamine	48.25	18.60	Lactose (B)
1116A	04.07.00	8.00	4.06	243.02	Superman	white	Yes		Amphetamine	60.55	23.00	Lactose (B)
1265	14.12.00	8.05	4.09	255.97	Superman	white	Yes		Amphetamine	69.18	24.40	Lactose (B)
Z9		9.07	3.54	286.42	Sparrow	white	Yes	Portugal	Amphetamine	19.47	6.80	Lactose
557B		9.09	3.54	287.90	Sparrow	white	Yes	Portugal	Amphetamine			Lactose
557A		9.08	3.63	286.42	Sparrow	white	Yes	Portugal	Amphetamine	15.19	5.30	Lactose
952	22.10.99	8.07	3.49	214.02	Diamond	violet	None	Diamant-B	MDA	58.71	27.50	Lactose
Z197	02.04.04	8.07	2.79	160.60	Diamond	violet	None	Diamant-E	MDA			
1118	19.06.00	8.17	3.30	208.65	Diamond	violet	None	Diamant-C	MDA	55.78	26.10	Lactose
1091	19.06.00	8.18	3.35	213.55	Diamond	violet	None	Diamant-C	MDA	58.45	26.80	Lactose
1204A	10.09.00	8.23	4.15	274.46	Double lightning	violet	None	Diamant-Ecl	MDA	61.10	21.70	Lactose, Mannitol (tr)
1148	13.08.00	8.21	3.54	224.76	Diamond	violet	None	Diamant-D	MDA	52.69	23.10	Lactose, Mannitol (tr)
1209	24.09.00	8.22	3.58	226.53	Diamond	violet	None	Diamant-D	MDA	54.16	23.50	Lactose, Mannitol (tr)
1112F	17.09.00	8.21	3.52	222.63	Diamond	violet	None	Diamant-D	MDA	57.08	24.10	Lactose, Mannitol (tr)
1218A	31.08.00	8.20	4.16	275.14	Double lightning	violet	None	Diamant-Ecl	MDA	70.46	25.30	Lactose, Mannitol (tr)
922	18.09.99	8.30	4.33	243.17	Diamond	violet	None	Diamant-A	MDA	67.79	28.20	Lactose

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1507A	10.02.02	9.04	4.84	298.25	Tasmanian Devil	beige	None		MDEA	78.13	25.22	Sorbitol, Lactose
1444B	26.12.01	6.06	4.23	139.09	Dolphin	blue	None		MDMA	95.91	68.00	Lactose
1445	30.12.01	6.06	4.06	131.09	Dolphin	blue	None		MDMA	91.81	67.60	Lactose
1500	03.02.02	6.05	3.97	128.35	Dolphin	blue	None		MDMA	82.80	63.21	Lactose
1519		6.02	3.96	128.73	Dolphin	blue	None		MDMA	96.66	75.14	Lactose
1112G	17.09.00	7.04	4.37	218.40	"007"	orange	None	JamesBond	MDMA	88.57	38.40	Lactose (B)
1388	30.07.01	7.04	4.98	240.08	"FF"	yellow	None		MDMA	77.72	31.62	Sorbitol
1380A	15.07.01	7.04	4.94	240.81	"FF"	yellow	None		MDMA	86.53	35.41	Sorbitol
1473A	29.06.01	7.07	5.04	242.37	"FF"	yellow	None		MDMA	90.84	35.98	Sorbitol
1207	16.09.00	7.09	3.80	173.88	"Xhi"	dirty white	Yes		MDMA	107.78	58.50	
1498	02.02.02	7.06	4.89	230.79	"Xhi"	rose	Yes strong		MDMA	95.28	40.92	Lactose
1619A	13.02.02	7.05	4.86	227.01	"Xhi"	rose	Yes strong		MDMA	99.14	42.61	Lactose
1635	19.11.02	7.60	4.67	261.15	Peace and love	violet	Yes		MDMA	123.23	44.34	Lactose
1319A	08.01.01	7.97	4.70	259.80	Ferrari	yellow	Yes		MDMA	92.03	34.55	Lactose
1451B	16.01.01	7.96	4.68	258.67	Ferrari	yellow	Yes		MDMA	87.02	31.57	Lactose
1451A	16.01.01	7.99	4.38	255.46	Ferrari	orange	Yes		MDMA	85.70	32.71	Lactose
1668	26.12.03	8.07	4.57	262.49	Heart	rose	Yes		MDMA	61.74	22.97	Lactose
995B	20.12.99	8.06	5.39	361.41	"S"	green	Yes		MDMA	123.65	34.50	Lactose (B)

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
Z60	02.01.99	8.03	4.65	278.66	"STAR DUST"	white	Yes	Weiss	MDMA	78.27	27.00	Lactose
949A	31.01.99	8.01	4.85	294.38	"STAR DUST"	white	Yes	Weiss	MDMA			Lactose
949B	31.01.99	8.03	4.93	296.41	"STAR DUST"	white	Yes	Weiss	MDMA			
1002	02.01.00	8.04	5.47	335.88	"STAR DUST"	dirty white	Yes	EP5	MDMA	90.02	26.11	
1304A	17.05.00	8.07	5.52	336.74	"STAR DUST"	beige	Yes	EP5	MDMA	94.57	27.74	
1061	03.04.00	8.11	5.69	345.95	"STAR DUST"	beige	Yes	EP5	MDMA	92.61	26.60	
Z30	03.10.98	8.06	4.71	291.89	Elephant	beige	Yes		MDMA	90.46	30.00	
985B	14.06.99	8.03	4.93	303.49	Elephant	white	Yes		MDMA	77.72	26.30	
1494D	19.12.01	8.02	5.22	296.95	Elephant	white	Yes strong		MDMA	89.36	29.46	
1416B	01.08.01	8.01	3.24	200.23	Euro	orange	None	Orange	MDMA	120.60	58.40	Lactose
1405	07.09.01	8.02	3.25	201.53	Euro	orange	None	Orange	MDMA	124.60	60.10	Lactose
Z44	30.08.98	8.05	4.85	291.04	Mitsubishi	beige	Yes strong	MitsubishiFIX	MDMA	97.23	34.20	
Z4A	08.08.98	8.06	5.05	317.67	Mitsubishi	beige	Yes	MitsubishiFIX	MDMA	89.49	36.00	
1208	21.10.00	8.06	3.64	237.26	Superman	white	Yes	Superman2-D	MDMA	83.63	34.80	Lactose
1303	17.05.00	8.11	3.44	222.48	Star	beige	None		MDMA	99.27	41.83	
1305	17.05.00	8.09	3.45	220.93	Star	dirty white	None		MDMA	86.39	37.29	
1304C	17.05.00	8.10	3.48	225.19	Star	dirty white	None		MDMA	99.63	40.49	
1306B	17.05.00	8.09	3.41	221.59	Star	dirty white	None		MDMA	91.59	36.28	
1306D	17.05.00	8.10	3.36	219.71	Star	dirty white	None		MDMA	85.83	36.84	

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1306C	17.05.00	8.12	4.74	254.54	Star	dirty white	None		MDMA	87.65	34.08	Lactose
1306A	17.05.00	8.11	4.75	253.18	Star	dirty white	None		MDMA	89.49	34.60	Lactose
1304B	17.05.00	8.10	4.75	255.17	Star	dirty white	None		MDMA	99.05	37.01	Lactose
1460	06.04.01	8.09	3.84	297.35	Star	white	Yes		MDMA	107.84	36.01	Lactose
1528	04.05.02	8.14	4.86	299.65	Marlboro	blue	Yes		MDMA	102.75	33.79	
1524	14.04.02	8.15	4.81	287.90	Marlboro	rose	Yes		MDMA	109.29	37.15	
834	08.08.98	8.13	5.21	299.51	Mitsubishi	beige	Yes	MitsubishiFIT	MDMA	121.69	40.70	Sorbitol
814	23.08.98	8.16	5.19	300.74	Mitsubishi	beige	Yes	MitsubishiFIX	MDMA	98.24	32.00	
1327	17.02.01	8.14	5.06	287.00	Superman	white	Yes		MDMA	80.12	26.81	
1693	17.02.05	8.20	3.69	216.99	Heart	dirty white	Yes		MDMA	19.31	28.15	
1683	10.06.04	8.17	4.07	248.05	Heart	dirty white	Yes		MDMA	76.28	30.60	
1708	08.04.05	8.20	3.69	220.35	Heart	dirty white	Yes		MDMA	53.24	24.20	
Z2A	22.07.98	8.29	4.80	288.73	Elephant	beige	Yes		MDMA	71.94	24.80	Sorbitol
1003	02.01.00	8.65	4.56	320.02	Mitsubishi	dirty white	Yes	uu	MDMA	94.16	29.10	Lactose (tr)
989	21.06.99	8.63	3.40	226.96	Flying bird	beige	Yes	Paix	MDMA	89.39	38.20	Lactose
760	19.07.98	9.03	3.56	250.37	None	rose	Yes	wRoseStrie	MDMA	76.84	31.50	Lactose (B)
788	07.06.98	9.02	3.38	254.31	None	rose	Yes	wRoseStrie	MDMA	85.85	35.80	Lactose (B)
831	26.04.98	9.02	3.58	240.56	None	white	Yes	wBlancStrie	MDMA	78.80	32.00	Lactose (B)
Z14B		9.08	4.86	273.24	None	white	Yes	wBlancStrie	MDMA	93.03	33.60	Lactose (B)

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
985C	14.06.99	9.06	4.61	297.08	Rolling Stones	beige	None		MDMA	87.51	28.90	Sorbitol
985A	14.06.99	9.08	4.63	294.72	Tasmanian Devil	dirty white	None		MDMA	62.43	21.00	Sorbitol
832	29.05.98	9.04	4.76	309.58	Twins	beige	Yes	Jumeaux	MDMA	80.80	26.90	Sorbitol
1380B	15.07.01	9.08	4.40	314.77	Twins	beige	Yes	Jumeaux	MDMA	101.54	31.58	Sorbitol
1473B	29.06.01	9.11	4.41	308.05	Twins	brown	Yes		MDMA	107.45	34.07	Sorbitol
1300	28.03.00	9.04	3.83	297.00	Mitsubishi	white	Yes	MitsubSpots	MDMA	87.77	29.07	Lactose
Z84	14.06.99	9.07	4.65	348.30	Mitsubishi	white	Yes		MDMA	68.95	19.70	Lactose
1111B	17.09.00	9.19	3.35	261.11	Ferrari	beige	Yes	CavFerra	MDMA	92.67	34.10	Lactose
1324	04.02.01	9.14	3.89	294.37	Heart	white	Yes		MDMA	76.67	24.34	Lactose
520	21.01.98	9.13	3.95	285.52	Crown	white	None		MDMA	85.66	30.00	Lactose
523	12.02.98	9.14	4.01	294.90	Crown	white	None		MDMA	85.52	29.00	Lactose (B)
524	12.02.98	9.14	4.04	300.27	Crown	white	None		MDMA	87.08	29.00	Lactose (B)
960	05.12.99	9.09	3.84	295.44	Mitsubishi	white	None		MDMA			Lactose
1309	09.06.00	9.15	3.89	307.71	Ferrari (framed)	white	None		MDMA	100.60	31.86	Lactose
1301	02.05.00	9.18	3.91	308.16	Ferrari (framed)	white	None		MDMA	95.17	30.86	Lactose
942	05.12.99	9.09	3.67	292.18	Ferrari (framed)	white	None	Ferrari-Red	MDMA	72.74	25.00	Lactose
970A	04.02.00	9.09	3.77	292.35	Ferrari (framed)	white	None	Ferrari-Red	MDMA	73.57	25.60	Lactose
970C	04.02.00	9.10	3.72	296.43	Ferrari (framed)	white	None	Ferrari-Red	MDMA	80.91	27.70	Lactose
970B	04.02.00	9.11	3.74	300.29	Ferrari (framed)	white	None	Ferrari-Red	MDMA	87.78	29.20	Lactose

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1223	15.10.00	9.12	4.04	294.13	Mitsubishi	dirty white	Yes		MDMA	80.84	26.30	Lactose
1312	31.07.00	9.14	3.87	302.96	Mitsubishi	beige	Yes		MDMA	118.60	37.41	Lactose
954	12.08.99	9.08	5.53	390.31	Mitsubishi	white	None		MDMA			Lactose
978A	18.02.00	9.09	3.82	296.44	Mitsubishi	white	None		MDMA	102.13	35.40	Lactose
842E	02.06.99	9.12	4.25	317.45	Superman	beige	Yes strong	Superman2-A	MDMA	86.37	27.00	Lactose
894A	16.08.99	9.19	4.35	321.66	Superman	dirty white	Yes strong	Superman2-A	MDMA	73.91	22.80	Lactose
1345A	29.06.00	9.14	3.87	311.16	Heart	beige	Yes	Punchmark	MDMA	63.42	19.86	Lactose
1130	24.07.00	9.18	3.91	315.37	Heart	beige	Yes	Punchmark	MDMA	44.19	14.10	Lactose
1134A	12.08.00	9.16	3.92	315.42	Heart	beige	Yes	Punchmark	MDMA	54.69	16.60	Lactose
1314B	13.01.01	9.21	3.94	305.77	Heart	beige	Yes	Punchmark	MDMA	51.71	15.66	Lactose
1317	05.01.01	9.06	3.90	298.11	Star	white	Yes		MDMA	120.77	39.92	Lactose
Z209	23.02.04	8.01	4.22	239.10	"STAR DUST"	dirty white	Yes	MDEA	MDMA, MDEA			
1310A	19.06.00	8.07	5.01	292.45	Superman	beige	Yes strong	SupermanDef	MDMA, MDEA	86.02	28.74	
1079B	16.04.00	8.04	5.05	296.82	Superman	dirty white	Yes strong	SupermanDef	MDMA, MDEA	86.51	29.00	
836A	06.10.98	9.01	3.19	258.75	Superman	beige	Yes	Superman2-B	MDMA, MDEA	33.13	11.90	Lactose
Z34	04.10.98	9.07	3.22	264.50	Superman	beige	Yes	Superman2-B	MDMA, MDEA	27.41	11.50	Lactose

APPENDIX XI – SAMPLE GROUPS OBTAINED WITH THE ROUTINE METHOD

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
737	02.07.98	11.14	4.92	393.75	Star	rose	Yes	ThioStar	4MTA			Lactose
743	15.04.98	11.15	4.94	399.08	Star	rose	Yes	ThioStar	4MTA			Lactose
1345B	29.06.00	8.01	4.01	249.22	Superman	white	Yes		Amphetamine	48.25	18.60	Lactose (B)
1116A	04.07.00	8.00	4.06	243.02	Superman	white	Yes		Amphetamine	60.55	23.00	Lactose (B)
1265	14.12.00	8.05	4.09	255.97	Superman	white	Yes		Amphetamine	69.18	24.40	Lactose (B)
z9		9.07	3.54	286.42	Sparrow	white	Yes	Portugal	Amphetamine	19.47	6.80	Lactose
557A		9.08	3.63	286.42	Sparrow	white	Yes	Portugal	Amphetamine	15.19	5.30	Lactose
557B		9.09	3.54	287.90	Sparrow	white	Yes	Portugal	Amphetamine			Lactose
z197	02.04.04	8.07	2.79	160.60	Diamond	violet	None	Diamant-E	MDA			
952	22.10.99	8.07	3.49	214.02	Diamond	violet	None	Diamant-B	MDA	58.71	27.50	Lactose
922	18.09.99	8.30	4.33	243.17	Diamond	violet	None	Diamant-A	MDA	67.79	28.20	Lactose
1118	19.06.00	8.17	3.30	208.65	Diamond	violet	None	Diamant-C	MDA	55.78	26.10	Lactose
1091	19.06.00	8.18	3.35	213.55	Diamond	violet	None	Diamant-C	MDA	58.45	26.80	Lactose
1112F	17.09.00	8.21	3.52	222.63	Diamond	violet	None	Diamant-D	MDA	57.08	24.10	Lactose, Mannitol (tr)
1209	24.09.00	8.22	3.58	226.53	Diamond	violet	None	Diamant-D	MDA	54.16	23.50	Lactose, Mannitol (tr)
1148	13.08.00	8.21	3.54	224.76	Diamond	violet	None	Diamant-D	MDA	52.69	23.10	Lactose, Mannitol (tr)
1218A	31.08.00	8.20	4.16	275.14	Double lightning	violet	None	Diamant-Ecl	MDA	70.46	25.30	Lactose, Mannitol (tr)
1204A	10.09.00	8.23	4.15	274.46	Double lightning	violet	None	Diamant-Ecl	MDA	61.10	21.70	Lactose, Mannitol (tr)

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1507A	10.02.02	9.04	4.84	298.25	Tasmanian Devil	beige	None		MDEA	78.13	25.22	Sorbitol, Lactose
1500	03.02.02	6.05	3.97	128.35	Dolphin	blue	None		MDMA	82.80	63.21	Lactose
1519		6.02	3.96	128.73	Dolphin	blue	None		MDMA	96.66	75.14	Lactose
1444B	26.12.01	6.06	4.23	139.09	Dolphin	blue	None		MDMA	95.91	68.00	Lactose
1445	30.12.01	6.06	4.06	131.09	Dolphin	blue	None		MDMA	91.81	67.60	Lactose
1112G	17.09.00	7.04	4.37	218.40	"007"	orange	None	JamesBond	MDMA	88.57	38.40	Lactose (B)
1388	30.07.01	7.04	4.98	240.08	"FF"	yellow	None		MDMA	77.72	31.62	Sorbitol
1380A	15.07.01	7.04	4.94	240.81	"FF"	yellow	None		MDMA	86.53	35.41	Sorbitol
1473A	29.06.01	7.07	5.04	242.37	"FF"	yellow	None		MDMA	90.84	35.98	Sorbitol
1207	16.09.00	7.09	3.80	173.88	"Xhi"	dirty white	Yes		MDMA	107.78	58.50	
1498	02.02.02	7.06	4.89	230.79	"Xhi"	rose	Yes strong		MDMA	95.28	40.92	Lactose
1619A	13.02.02	7.05	4.86	227.01	"Xhi"	rose	Yes strong		MDMA	99.14	42.61	Lactose
1635	19.11.02	7.60	4.67	261.15	Peace and love	violet	Yes		MDMA	123.23	44.34	Lactose
1451A	16.01.01	7.99	4.38	255.46	Ferrari	orange	Yes		MDMA	85.70	32.71	Lactose
1451B	16.01.01	7.96	4.68	258.67	Ferrari	yellow	Yes		MDMA	87.02	31.57	Lactose
1324	04.02.01	9.14	3.89	294.37	Heart	white	Yes		MDMA	76.67	24.34	Lactose
1319A	08.01.01	7.97	4.70	259.80	Ferrari	yellow	Yes		MDMA	92.03	34.55	Lactose
949A	31.01.99	8.01	4.85	294.38	"STAR DUST"	white	Yes	Weiss	MDMA			Lactose
z60	02.01.99	8.03	4.65	278.66	"STAR DUST"	white	Yes	Weiss	MDMA	78.27	27.00	Lactose

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
949B	31.01.99	8.03	4.93	296.41	"STAR DUST"	white	Yes	Weiss	MDMA			
1061	03.04.00	8.11	5.69	345.95	"STAR DUST"	beige	Yes	EP5	MDMA	92.61	26.60	
1304A	17.05.00	8.07	5.52	336.74	"STAR DUST"	beige	Yes	EP5	MDMA	94.57	27.74	
1002	02.01.00	8.04	5.47	335.88	"STAR DUST"	dirty white	Yes	EP5	MDMA	90.02	26.11	
z30	03.10.98	8.06	4.71	291.89	Elephant	beige	Yes		MDMA	90.46	30.00	
z44	30.08.98	8.05	4.85	291.04	Mitsubishi	beige	Yes strong	MitsubishiFIX	MDMA	97.23	34.20	
Z4A	08.08.98	8.06	5.05	317.67	Mitsubishi	beige	Yes	MitsubishiFIX	MDMA	89.49	36.00	
1494D	19.12.01	8.02	5.22	296.95	Elephant	white	Yes strong		MDMA	89.36	29.46	
985b	14.06.99	8.03	4.93	303.49	Elephant	white	Yes		MDMA	77.72	26.30	
1416B	01.08.01	8.01	3.24	200.23	Euro	orange	None	Orange	MDMA	120.60	58.40	Lactose
1405	07.09.01	8.02	3.25	201.53	Euro	orange	None	Orange	MDMA	124.60	60.10	Lactose
1208	21.10.00	8.06	3.64	237.26	Superman	white	Yes	Superman2-D	MDMA	83.63	34.80	Lactose
995b	20.12.99	8.06	5.39	361.41	"S"	green	Yes		MDMA	123.65	34.50	Lactose (B)
1460	06.04.01	8.09	3.84	297.35	Star	white	Yes		MDMA	107.84	36.01	Lactose
1306A	17.05.00	8.11	4.75	253.18	Star	dirty white	None		MDMA	89.49	34.60	Lactose
1304B	17.05.00	8.10	4.75	255.17	Star	dirty white	None		MDMA	99.05	37.01	Lactose
1306C	17.05.00	8.12	4.74	254.54	Star	dirty white	None		MDMA	87.65	34.08	Lactose

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1303	17.05.00	8.11	3.44	222.48	Star	beige	None		MDMA	99.27	41.83	
1305	17.05.00	8.09	3.45	220.93	Star	dirty white	None		MDMA	86.39	37.29	
1304C	17.05.00	8.10	3.48	225.19	Star	dirty white	None		MDMA	99.63	40.49	
1306B	17.05.00	8.09	3.41	221.59	Star	dirty white	None		MDMA	91.59	36.28	
1306D	17.05.00	8.10	3.36	219.71	Star	dirty white	None		MDMA	85.83	36.84	
1668	26.12.03	8.07	4.57	262.49	Heart	rose	Yes		MDMA	61.74	22.97	Lactose
1683	10.06.04	8.17	4.07	248.05	Heart	dirty white	Yes		MDMA	76.28	30.60	
1693	17.02.05	8.20	3.69	216.99	Heart	dirty white	Yes		MDMA	19.31	28.15	
1524	14.04.02	8.15	4.81	287.90	Marlboro	rose	Yes		MDMA	109.29	37.15	
1528	04.05.02	8.14	4.86	299.65	Marlboro	blue	Yes		MDMA	102.75	33.79	
1708	08.04.05	8.20	3.69	220.35	Heart	dirty white	Yes		MDMA	53.24	24.20	
1327	17.02.01	8.14	5.06	287.00	Superman	white	Yes		MDMA	80.12	26.81	
814	23.08.98	8.16	5.19	300.74	Mitsubishi	beige	Yes	MitsubishiFIX	MDMA	98.24	32.00	
834	08.08.98	8.13	5.21	299.51	Mitsubishi	beige	Yes	MitsubishiFIT	MDMA	121.69	40.70	Sorbitol
Z2a	22.07.98	8.29	4.80	288.73	Elephant	beige	Yes		MDMA	71.94	24.80	Sorbitol
1003	02.01.00	8.65	4.56	320.02	Mitsubishi	dirty white	Yes	uu	MDMA	94.16	29.10	Lactose (tr)
989	21.06.99	8.63	3.40	226.96	Flying bird	beige	Yes	Paix	MDMA	89.39	38.20	Lactose
832	29.05.98	9.04	4.76	309.58	Twins	beige	Yes	Jumeaux	MDMA	80.80	26.90	Sorbitol

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1380B	15.07.01	9.08	4.40	314.77	Twins	beige	Yes	Jumeaux	MDMA	101.54	31.58	Sorbitol
1473B	29.06.01	9.11	4.41	308.05	Twins	brown	Yes		MDMA	107.45	34.07	Sorbitol
985c	14.06.99	9.06	4.61	297.08	Rolling Stones	beige	None		MDMA	87.51	28.90	Sorbitol
985A	14.06.99	9.08	4.63	294.72	Tasmanian Devil	dirty white	None		MDMA	62.43	21.00	Sorbitol
1317	05.01.01	9.06	3.90	298.11	Star	white	Yes		MDMA	120.77	39.92	Lactose
1312	31.07.00	9.14	3.87	302.96	Mitsubishi	beige	Yes		MDMA	118.60	37.41	Lactose
520	21.01.98	9.13	3.95	285.52	Crown	white	None		MDMA	85.66	30.00	Lactose
894A	16.08.99	9.19	4.35	321.66	Superman	dirty white	Yes strong	Superman2-A	MDMA	73.91	22.80	Lactose
842E	02.06.99	9.12	4.25	317.45	Superman	beige	Yes strong	Superman2-A	MDMA	86.37	27.00	Lactose
954	12.08.99	9.08	5.53	390.31	Mitsubishi	white	None		MDMA			Lactose
1111B	17.09.00	9.19	3.35	261.11	Ferrari	beige	Yes	CavFerra	MDMA	92.67	34.10	Lactose
1309	09.06.00	9.15	3.89	307.71	Ferrari (framed)	white	None		MDMA	100.60	31.86	Lactose
1301	02.05.00	9.18	3.91	308.16	Ferrari (framed)	white	None		MDMA	95.17	30.86	Lactose
960	05.12.99	9.09	3.84	295.44	Mitsubishi	white	None		MDMA			Lactose
970A	04.02.00	9.09	3.77	292.35	Ferrari (framed)	white	None	Ferrari-Red	MDMA	73.57	25.60	Lactose
942	05.12.99	9.09	3.67	292.18	Ferrari (framed)	white	None	Ferrari-Red	MDMA	72.74	25.00	Lactose
970C	04.02.00	9.10	3.72	296.43	Ferrari (framed)	white	None	Ferrari-Red	MDMA	80.91	27.70	Lactose
970B	04.02.00	9.11	3.74	300.29	Ferrari (framed)	white	None	Ferrari-Red	MDMA	87.78	29.20	Lactose
978A	18.02.00	9.09	3.82	296.44	Mitsubishi	white	None		MDMA	102.13	35.40	Lactose

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Class	Illicit Substance	Qty [mg]	Qty [%]	Diluent
1223	15.10.00	9.12	4.04	294.13	Mitsubishi	dirty white	Yes		MDMA	80.84	26.30	Lactose
1300	28.03.00	9.04	3.83	297.00	Mitsubishi	white	Yes	MitsubSpots	MDMA	87.77	29.07	Lactose
1134A	12.08.00	9.16	3.92	315.42	Heart	beige	Yes	Punchmark	MDMA	54.69	16.60	Lactose
1130	24.07.00	9.18	3.91	315.37	Heart	beige	Yes	Punchmark	MDMA	44.19	14.10	Lactose
1314B	13.01.01	9.21	3.94	305.77	Heart	beige	Yes	Punchmark	MDMA	51.71	15.66	Lactose
1345A	29.06.00	9.14	3.87	311.16	Heart	beige	Yes	Punchmark	MDMA	63.42	19.86	Lactose
831	26.04.98	9.02	3.58	240.56	None	white	Yes	wBlancStrie	MDMA	78.80	32.00	Lactose (B)
Z14B		9.08	4.86	273.24	None	white	Yes	wBlancStrie	MDMA	93.03	33.60	Lactose (B)
Z84	14.06.99	9.07	4.65	348.30	Mitsubishi	white	Yes		MDMA	68.95	19.70	Lactose
788	07.06.98	9.02	3.38	254.31	None	rose	Yes	wRoseStrie	MDMA	85.85	35.80	Lactose (B)
760	19.07.98	9.03	3.56	250.37	None	rose	Yes	wRoseStrie	MDMA	76.84	31.50	Lactose (B)
524	12.02.98	9.14	4.01	294.90	Crown	white	None		MDMA	85.52	29.00	Lactose (B)
523	12.02.98	9.14	4.04	300.27	Crown	white	None		MDMA	87.08	29.00	Lactose (B)
z34	04.10.98	9.07	3.22	264.50	Superman	beige	Yes	Superman2-B	MDMA, MDEA	27.41	11.50	Lactose
836A	06.10.98	9.01	3.19	258.75	Superman	beige	Yes	Superman2-B	MDMA, MDEA	33.13	11.90	Lactose
1310a	19.06.00	8.07	5.01	292.45	Superman	beige	Yes strong	SupermanDef	MDMA, MDEA	86.02	28.74	
1079B	16.04.00	8.04	5.05	296.82	Superman	dirty white	Yes strong	SupermanDef	MDMA, MDEA	86.51	29.00	
z209	23.02.04	8.01	4.22	239.10	"STAR DUST"	dirty white	Yes	MDEA	MDMA, MDEA			

APPENDIX XII – PREPROCESSING METHODS APPLIED TO NIR SPECTRA

Reference	Pre-processing method
Krämer and Ebel (2000)	MSC, 1st Savitsky-Golay derivative (1pt average, 2nd polynomial order)
Chalus et al. (2005)	SNV, MSC, 2nd Savitsky-Golay derivative (5pt average, 2nd polynomial order)
Roggo et al. (2005)	1st Savitsky-Golay derivative (11pt average, 3d polynomial order), mean normalisation
Schneider (2002)	SNV, X + 2, 1/X, 2nd Savitsky-Golay derivative (5pt average, 2nd polynomial order)
Yoon et al. (2004)	SNV, 2nd Savitsky-Golay derivative (11pt average, 2nd polynomial order)
Brigger et al. (2000)	X + 0.2, Kubelka-Munk transformation, MSC
Röseler (2004)	MSC, 2nd Savitsky-Golay derivative (7pt average, 2nd polynomial order)
New	X + 0.2, Log(X), SNV
New2	X + 0.2, Log(1/X), SNV, 2nd Savitsky-Golay derivative (5pt average, 2nd polynomial order)

APPENDIX XIII – SAMPLE GROUPS OBSERVED WITH NIR SPECTROSCOPY

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Sample Class	Illicit Substance	Quantity [%]	Diluent
1112G	17.9.00	7.04	4.37	218.40	"007"	orange	none	JamesBond	MDMA	38.40	Lactose (B)
1473A	29.6.01	7.07	5.04	242.37	"FF"	yellow	none		MDMA	35.98	Sorbitol
1207	16.9.00	7.09	3.80	173.88	"Xhi"	white dirty	yes		MDMA	58.50	
1498	2.2.02	7.06	4.89	230.79	"Xhi"	rose	yes strong		MDMA	40.92	Lactose
1619A	13.2.02	7.05	4.86	227.01	"Xhi"	rose	yes strong		MDMA	42.61	Lactose
Z60	2.1.99	8.03	4.65	278.66	"STAR DUST"	white	yes	Weiss	MDMA	27.00	Lactose
949A	31.1.99	8.01	4.85	294.38	"STAR DUST"	white	yes	Weiss	MDMA		Lactose
1304A	17.5.00	8.07	5.52	336.74	"STAR DUST"	beige	yes	EP5	MDMA	27.74	
1002	2.1.00	8.04	5.47	335.88	"STAR DUST"	white dirty	yes	EP5	MDMA	26.11	
1061	3.4.00	8.11	5.69	345.95	"STAR DUST"	beige	yes	EP5	MDMA	26.60	
949B	31.1.99	8.03	4.93	296.41	"STAR DUST"	white	yes	Weiss	MDMA		
Z209	23.2.04	8.01	4.22	239.10	"STAR DUST"	white dirty	yes	MDEA	MDMA, MDEA		
Z30	3.10.98	8.06	4.71	291.89	Elephant	beige	yes		MDMA	30.00	
985B	14.6.99	8.03	4.93	303.49	Elephant	white	yes		MDMA	26.30	
Z44	30.8.98	8.05	4.85	291.04	Mitsubishi	beige	yes strong	MitsubishiFIX	MDMA	34.20	
Z4A	8.8.98	8.06	5.05	317.67	Mitsubishi	beige	yes	MitsubishiFIX	MDMA	36.00	
1494D	19.12.01	8.02	5.22	296.95	Elephant	white	yes strong		MDMA	29.46	
1310A	19.6.00	8.07	5.01	292.45	Superman	beige	yes strong	SupermanDef	MDMA, MDEA	28.74	
1079B	16.4.00	8.04	5.05	296.82	Superman	white dirty	yes strong	SupermanDef	MDMA, MDEA	29.00	

Sample	Date of seizure	Diam [mm]	Thk [mm]	Weight [mg]	Logo	Colour	Breakline	Sample Class	Illicit Substance	Quantity [%]	Diluent
1327	17.2.01	8.14	5.06	287.00	Superman	white	yes		MDMA	26.81	
1306D	17.5.00	8.10	3.36	219.71	Star	white dirty	none		MDMA	36.84	Maltose (tr)
1306B	17.5.00	8.09	3.41	221.59	Star	white dirty	none		MDMA	36.28	Maltose (tr)
1303	17.5.00	8.11	3.44	222.48	Star	beige	none		MDMA	41.83	Maltose (tr)
1305	17.5.00	8.09	3.45	220.93	Star	white dirty	none		MDMA	37.29	Maltose (tr)
1304C	17.5.00	8.10	3.48	225.19	Star	white dirty	none		MDMA	40.49	Maltose (tr)
1306C	17.5.00	8.12	4.74	254.54	Star	white dirty	none		MDMA	34.08	Lactose
834	8.8.98	8.13	5.21	299.51	Mitsubishi	beige	yes	MitsubishiFIT	MDMA	40.70	Sorbitol
814	23.8.98	8.16	5.19	300.74	Mitsubishi	beige	yes	MitsubishiFIX	MDMA	32.00	
Z197	2.4.04	8.07	2.79	160.60	Diamond	violet	none	Diamant-E	MDA		
1528	4.5.02	8.14	4.86	299.65	Marlboro	blue	yes		MDMA	33.79	
1524	14.4.02	8.15	4.81	287.90	Marlboro	rose	yes		MDMA	37.15	
1693	17.2.05	8.20	3.69	216.99	Heart	white dirty	yes		MDMA, MA	28.15	
1683	10.6.04	8.17	4.07	248.05	Heart	white dirty	yes		MDMA	30.60	
1003	2.1.00	8.65	4.56	320.02	Mitsubishi	white dirty	yes		MDMA	29.10	Lactose (tr)
985C	14.6.99	9.06	4.61	297.08	Rolling Stones	beige	none		MDMA	28.90	Sorbitol
Z14B		9.08	4.86	273.24	None	white	yes	wBlancStrie	MDMA	33.60	Lactose (B)
831	26.4.98	9.02	3.58	240.56	None	white	yes	wBlancStrie	MDMA	32.00	Lactose (B)
788	7.6.98	9.02	3.38	254.31	None	rose	yes	wRoseStrie	MDMA	35.80	Lactose (B)
760	19.7.98	9.03	3.56	250.37	None	rose	yes	wRoseStrie	MDMA	31.50	Lactose (B)
954	12.8.99	9.08	5.53	390.31	Mitsubishi	white	none		MDMA		Lactose