Supercritical fluid chromatography coupled to mass spectrometry: A valuable tool in food analysis

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Abstract

Supercritical fluid chromatography (SFC), although known for several decades, has undergone a growing interest in the last few years fueled by the introduction of modern instruments with improved robustness, and hyphenation to mass spectrometry (MS). This allows the analysis of trace compounds in complex samples with high selectivity, high sensitivity and in a short time, which has contributed to its increased use in the food analysis area. This work reviews the principal applications of SFC-MS in food analysis, highlighting the most important achievements.

Key words: Applications, Food analysis, Food nutrients, Food contaminants Supercritical fluid chromatography, Mass Spectrometry, SFC-MS

1. Introduction

Food analysis is an area of great interest not only from a scientific point of view but also from the social one, because foods and diet play an important role in people's health. Today, food analysis not only focuses on nutrients (i.e, lipids, vitamins, proteins, or carbohydrates) but also food safety is an important issue. Contaminants, adulterants or illicit substances, which are considered to be harmful, need to be analyzed [1]. Aspects related to botanical or geographical origin as well as production systems (i.e. large-scale production, organic production) are also of great interest for the final consumer [2]. Within this context, it can be understood that food analysis is a challenging task. There is a broad variety of sample matrices and the compounds to be analyzed can differ in physical-chemical properties as well as in concentration levels. Moreover, interfering compounds are frequently present and sometimes the target compounds exist at low concentration levels, which makes highly sensitive and selective analytical methods mandatory.

Gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry detectors (MS) are the predominant techniques in food analysis and these are ubiquitous in most laboratories. However, in the last years the increasing demand of fast, selective and sensitive analysis together with environmental friendly concerns, have renewed interest in supercritical fluid chromatography (SFC) [3–5].

Compared with LC, SFC offers several advantages. The high molecular diffusiveness and low viscosity of the mobile phase makes it possible obtain higher rates of efficiency and improved resolutions, and to use higher flow-rates with lower pressure drops; this results in shorter analysis times. Method development is faster, due to the shorter times required for column equilibration, whilst the consumption of organic solvents is lower. Moreover, it offers the possibility of using polar (silica, cyano, ethylpyridine) and nonpolar (C8, C18, C30) stationary phases with a single setup and the same mobile phase components. SFC is considered complementary to LC; the retention behavior of analytes is different and this can facilitate the analysis of complex samples. Compounds that coelute in LC can be resolved in SFC or vice versa. Both techniques can be used in parallel or in multi-dimensional configurations. Moreover, compounds that can degrade in the presence of water can be analyzed in SFC, thus avoiding this

problem. Such is the case of lactones, which can be hydrolyzed when analyzed in reverse phase LC due to the water present in the mobile phase [6]. Additionally, it is complementary to GC; thermally labile compounds or compounds with high molecular weight, which analysis is difficult by GC, can be analyzed using SFC [7].

In modern SFC, the mobile phase is composed of a mixture of CO_2 and a miscible organic solvent. Very often, the separation conditions are not supercritical because the working temperature is below the critical temperature of the mixture. Nevertheless, this is not a problem as the advantages of supercritical fluids are maintained. In the first decade of the present century, SFC was mainly employed for chiral analysis, where it has demonstrated wide applicability [8] and some advantages over normal phase LC. The use of SFC in achiral analysis was less frequent, partially due to the lack of a single type of stationary phase that could provide the widespread applicability of C_{18} in LC, or polysaccharide based columns in chiral SFC. On the other hand, early SFC systems had some drawbacks such as the poor reproducibility of the analyses and the low sensitivity of the UV detectors (usually photodiode array detectors). This lack of sensitivity was due to a high level of baseline noise mainly caused by pressure fluctuations. Pressure control with the older backpressure regulators (BPR) generated pressure variations, which resulted in noticeable changes in the mobile phase refractive index and detector noise. All of this caused a low performance in quantitative analysis, which prevented SFC from being widely accepted for analytical purposes. Nevertheless, the introduction of a new generation of instruments, with improved robustness and performance, has contributed to the renewed interest in this technique. These new systems are based on ultra-high performance liquid chromatography (UHPLC) technology. To exploit the full potential of the new sub-2µm particle size columns and to reach performances similar to UHPLC, the SFC systems required some improvements. New back pressure regulators (BPR) were designed for a better pressure control, reducing the detector noise [9], and also new high pressure pumping systems were developed to deliver accurate flow rates all over a range of higher pressures. Moreover, extra-column band broadening was reduced by using lower injection volumes and shorter tubing with lower inner diameters.

Typically, the silica-based polar stationary phases used in LC (cyano, diol amino…) have been used in SFC, but in some instances it is not the best choice due to certain

limitations such as poor selectivity or poor peak shape, caused by undesirable interactions with residual silanol groups. This is the case of separation of basic or acidic compounds, where the use of additives is necessary to obtain good peak shapes. This fact has contributed to the development of new stationary phases especially designed for SFC such as 2-ethylpyridyne, amino phenyl, poly(butylene terephthalate), which provide good peak symmetries without the use of additives and reinforce the development of achiral applications [10].

Advances in the detection mode have also favored the renaissance of SFC, especially those related with hyphenation to mass spectrometry. The possibility of using MS detectors has expanded the applicability of SFC to other sectors where the information provided by UV detection was not enough to achieve the required levels of accuracy and sensitivity; such is the case of food analysis. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are the most popular ionization sources in the SFC-MS systems. In the early years, APCI was preferred, as it can withstand high flow-rates, and thus considered suitable for SFC-MS. Nowadays, the development of new interfaces has meant that ESI is preferred due to its ability of ionize compounds with a broad range of polarities. Single quadrupole, time-of-flight (TOF), triple quadrupole (QqQ) or quadrupole-time-of-flight (Q-TOF) are the mass analyzers most commonly used with SFC. In fact, there are no limitations regarding mass analyzers that can be coupled to SFC [11]. More recently, the Shimadzu company (Shimadzu, Kyoto, Japan) has commercialized a system in which supercritical fluid extraction (SFE) is online coupled to SFC-MS. With this system, extraction and analysis are simultaneously performed, reducing the total analysis time and the risk of sample contamination or degradation. This could be an interesting approach in food analysis.

SFC has traditionally considered a normal phase chromatography suitable for the analysis of hydrophobic or low polar compounds. However, polar organic modifiers and additives (even small percentages of water) are usually added in order to increase the polarity of the mobile phase and to elute polar compounds with good peak shapes. This broaden the polarity range of compounds that can be analyzed and the scientific areas where SFC can be applied [12]. As far as food analysis is concerned, the number of published papers in this field is increasing in the last few years and in most of them

MS detection is employed. Although a great number are devoted to the analysis of lipids or lipid related compounds [3], analysis of polar compounds such as water-soluble vitamins or amino acids is also described [4,5]. This review summarize the principal applications of SFC-MS in food analysis, during the last ten years.

2. SFC – MS hyphenation

The hyphenation of SFC-MS has greatly improved in the last years fueled by the necessity of increasing sensitivity and selectivity of analysis methods.

In the early years of SFC, this technique was performed using capillary or open tubular columns (capillary supercritical fluid chromatography, cSFC) and pure CO_2 as the mobile phase; therefore, the ionization sources used in cSFC-MS analysis were those employed in GC-MS: electron ionization (EI) or chemical ionization (CI) [13,14]. Nowadays SFC is mainly performed using packed columns (pSFC) and the ionization sources employed in SFC-MS are the LC-type which means, ESI and APCI [15]. . Selection depends mainly on the type of compound to be analyzed and on the mobile phase flow-rate.

In the coupling of SFC to MS, most of the mobile phase (the CO2) is volatilized at the outlet of the backpressure regulator, and only the small amount of the organic solvent, used as modifier, enters the MS interface. Consequently, desolvation efficiency should be better in SFC-MS than in LC-MS. Nevertheless, coupling SFC to MS is a complex task as the decompression process can cause solute precipitation and peak broadening, resulting in a loss of chromatographic performance. Furthermore, some aspects relating to an increase in ionization efficiency and baseline stability should also be considered [16,17]. Therefore, interfacing SFC-MS should deal with all these problems.

In 2005, J.D. Pinkston reviewed the existing SFC-MS interfaces [18], and to date several good reviews concerning this topic have been published [16,17,19], most of them by the research group of D. Guillarme [11,17,20,21].

The interfaces used in SFC-MS coupling can be classified in two main groups:

- Full flow introduction: The full flow from the SFC system enters the MS detector. In this configuration, the BPR is placed before the MS.

- Split flow introduction: The flow from the SFC system is split after the column and before the BPR. A small portion is directed to the MS detector and the biggest part is transferred to the BPR.

For an insight into the different types of interfaces, readers are referred to the above mentioned reviews.

Nowadays, there are two commercially available interfaces, namely (a) “Pre-BPR splitter with sheath pump” and (b) “BPR and sheath pump with no splitter”. In the former, a make-up fluid is added after the column (or the UV detector if used) but before the MS detector, and then the total flow is split before the MS detector. A small portion is directed to the MS detector and most of the flow is transferred to the BPR (Figure 1-A). This interface is commercialized by Waters and Agilent. In the second interface, the total flow is introduced into the MS; the make-up fluid is pumped after the column (or the UV detector if used) and then the total flow is transferred to the BPR and MS detector that are serially connected (Figure 1-B). This interface is commercialized by Shimadzu and Agilent.

In both interfaces, a make-up fluid is introduced before the MS detector, which is beneficial as it prevents solute precipitation when CO2 vaporizes, especially when mobile phases with a small percentage of modifier are used. In addition, the use of a make-up fluid enhances the ionization in ESI, but sample dilution occurs which may negatively affect the sensitivity as it is a concentration dependent ionization source. Nevertheless, the dilution factor remains at suitable values when an active BPR is used and the make-up flow rate is lower than that of the SFC mobile phase. Another problem related to ESI sensitivity appears when there is no splitting. Optimum ESI sensitivity is obtained when the total organic solvent flow rate is low, usually 100 – 300 µl/min; but this flow is higher with interfaces where there is no splitting. This is the reason why the “BPR and sheath pump with no splitter” interface, where the total flow enters the MS detector, is usually considered more suitable for APCI, which is a mass flow dependent ionization source, and the “Pre-BPR splitter with sheath pump” interface is preferred for ESI. However, taking into account that split ratio varies with the mobile phase composition; L Akbal et al. [19] consider that the interface without a splitter can be used with both ESI and APCI in order to have higher precision in certain applications, such

as in the determination of enantiomeric excess, especially when working in gradient mode.

As far as mass analyzers is concerned, the most widely used are single quadrupole (Q), triple quadrupole (QqQ) and quadrupole-time of flight (Q-TOF). Choice depends on the type of compound and the analysis to be performed. Single quadrupole is the cheapest option, but it has the lowest sensitivity and resolution power, despite providing good results in targeted analysis. When higher sensitivity and selectivity are required, the options are QqQ or Q-TOF. QqQ is mainly used for quantitative analysis of trace compounds in complex samples, where a high sensitivity, high scanning speed and wide dynamic range are required. Q-TOF is an alternative to QqQ, and the high resolving power of TOF allows resolution of interfering peaks with the same nominal mass as the analytes, while the signal-to-noise ratio is improved. Q-TOF is generally used for qualitative analysis although it can also be used for quantitative determinations. Orbitrap is the most recently developed mass analyzer. It has high resolution power, but it has scarcely been used in SFC-MS. it is more expensive which could justify its scant use in SFC-MS applications.

Comparing SFC-MS sensitivity with that of LC-MS is a recurrent topic. A priori, the better desolvation efficiency achieved with the SFC mobile phases could favor greater sensitivity, due to the more difficult evaporation of the water rich mobile phases used in reverse phase LC. In fact, sensitivity depends on the type of compound, mobile phase composition, MS interface parameters and equipment characteristics; this makes it difficult to predict better SFC-MS sensitivity as against LC-MS. In addition, in most SFC-MS equipments the MS coupling is performed via a splitter device, while in LC-MS the total flow eluted from the column enters the MS detector; therefore, the sensitivity comparison between the two systems should take into account the split ratio. SFC-MS has generally displayed greater sensitivity when using the older MS instruments, but with the more modern ones the sensitivity achieved is comparable to LC-MS [22]. This could be explained by the fact that in modern MS equipment the desolvation process, using water rich mobile phases, has been improved. The major limitation to SFC-MS sensitivity is the injection volume, which is lower than in LC. Peak distortion is observed when the injection volume is increased and a good selection of sample solvent should be performed [23]. Moreover, in SFC-MS it is possible to use a

make-up solvent for enhancing ionization efficiency and improving sensitivity [24], which is an advantage over LC-MS, where the introduction of a make-up solvent is more difficult to perform. Methanol or ethanol are the most widely used make-up solvents, although the composition and flow rate should be optimized in order to obtain the highest sensitivity. In some instances formic acid, ammonium formate or a small percentage of water have been added to the make-up solvent, thereby improving the results.

A matrix effect has also been observed in SFC-MS [25–32]. This is caused by matrix compounds that coelute with the analytes, and produce an ionization enhancement or suppression. This makes a quantitative analysis more complex. When the same sample has been analyzed using SFC-MS and LC-MS, a different matrix effect has been noticed due to the different elution pattern. The SFC retention mechanism on polar stationary phases is orthogonal to reverse phase LC (RPLC). Polar compounds that elute early in RPLC are the last eluting compounds in SFC; consequently, the elution profile of the analytes and interfering compounds is different, as a result of which the matrix effect observed can also be different [26]. In some cases, the matrix effect observed in SFC-MS was lower than in LC-MS [25], whilst in others it was similar [27]. Occasionally signal suppression was the predominant effect in SFC-MS while signal enhancement was encountered in LC-MS, but this is not a general rule, depending on the sample matrix [25]. To date, there are insufficient findings to determine which technique has a lower matrix effect. As in LC-MS, the matrix effect in SFC-MS is affected by the sample type, the chromatographic column, and the suitability of the sample treatment employed to remove the interfering compounds. When it cannot be completely eliminated, it should be compensated by matrix calibration methods or by using an isotopically labeled internal standard.

3. Analysis of food nutrients

3.1. Lipids

Due to the nonpolar character of CO_2, SFC has traditionally been used for the analysis of lipid compounds. The advantages and limitations have been discussed in several

reviews and book chapters [3,33–35]. FID, UV or light scattering detectors have been used for lipid analysis in the past, but nowadays MS detectors are considered the best option to identify individual molecular species and to determine their structure.

In SFC, using normal phase columns, lipids are separated according to their polarity into lipid classes. In this case, retention time increases with the increased lipid polarity and some kind of separation, based on the fatty acyl composition, can be achieved for individual lipids within one lipid class [36]. On the contrary, using reversed phase columns lipids are separated according to their fatty acyl composition, that means alkyl chain length (acyl carbon number) and degree of unsaturation (number of double bonds). Usually normal phase columns are employed for the separation of lipid classes and reverse phase columns for individual separations of lipids based on their fatty acyl composition [36–39]. When complex mixtures of polar and non-polar lipids are analysed using reverse phase columns, coelution of individual compounds from different lipid classes could happen [37]. Nevertheless, the separation of free fatty acids, fatty acids esters, diacylglycerols (DAGs) and triacylglycerols (TAGs) mixtures has been described using ODS columns [40]. In the case of samples with a high number of lipid compounds very good results were obtained using several core shell ODS columns connected in series [41]. On the other hand, employing polar embebed C18 columns, such as Inertsil ODS-EP, polar lipids were separated based on not only their polarity but also their fatty acyl composition [42].The analysis of TAGs in foods is challenging because of the great number of individual TAGs, the complexity of their structure and the coexistence of many positional isomers. SFC permit high resolution analysis of TAGs, in short analysis times and without sample derivatization.

Initially, TAG determinations were carried out using capillary columns with non-polar (polymethylsiloxane, phenylmethylsiloxane and octyl-methylpolysiloxane) or polar (phenyl-cyanopropylpolysiloxane, cyanopropylphenyl-methylpolysiloxane and polyethyleneglycol) stationary phases. The most important factor controlling separation, especially on the non-polar phases, was the acyl carbon number (ACN). The number of double bonds (DB) influenced the separation of TAGs with the same ACN values, but in some cases, TAGs with the same acyl carbon number and a different DB coeluted. The research group of Manninen et al. developed several cSFC-

MS methods to determine TAGs in different kinds of oils and milk fat [43–45]. They used a LC-APCI interphase.

TAG analysis using pSFC is usually performed by using octadecyl silica (ODS) or silver ion (SI) exchange columns. On ODS columns retention is controlled by the ACN and DB values. Leselier et al. [46,47] found that, on ODS columns and with CO_2/modifier mobile phases, the TAGs retention order is dependent on the equivalent carbon number (ECN) (ECN=ACN-2DB). Retention increased with the increase of ECN and for TAGs with the same ACN retention increased as DB decreased. Moreover, for TAGs having the same ACN or ECN values, a linear relationship between retention and DB was observed. When using SI exchange columns the separation of TAGs is controlled by the degree and distribution of unsaturations.

One of the first applications of SFC-MS with SI exchange columns, was developed by Sandra et al. [48]. They used a commercially available LC-MS interface without any modification and studied the characterization of TAGs in vegetable oils. Two ionization modes were used, APCI and coordination ion spray (CIS-ESI) with silver ions. In both cases, the make-up solvent (methanol in APCI and a methanolic solution of silver nitrate in CIS-ESI) was introduced by means of a T-piece. Analysis time was approximately 80 min. When using APCI the molecular ion $[M-H^+]$ was not detected for fully saturated TAGs; on the contrary, when CIS-ESI was employed the molecular ion $[M-Ag^+]$ was always the most intense, regardless of the degree of unsaturation. APCI made it possible to determine the position where the fatty acids (FAs) are esterified to the glycerol backbone; however, this could not be achieved for highly unsaturated TAGs.

Since this work appeared, the analysis of lipids has considerably improved. Sensitivity and selectivity have increased with the use of QqQ-MS or Q-TOF-MS systems, allowing the identification of regioisomers, which were not previously described, and the analysis of different lipid classes in a single run [36–39]. Moreover, analysis time has been reduced with the use of ultra-high performance supercritical fluid chromatography (UHPSFC) systems and sub 2 μm columns [39–41].

The research group of T. Bamba developed several methods for the simultaneous analysis of different types of lipids, including phospholipids, glycolipids, neutral lipids and sphingolipids, using SFC-MS in APCI or ESI modes [37–39,42,49–51].

Lee et al. [49] developed a SFC-MS method for profiling soybean lipids. Using a cyano column and a gradient of modifier (methanol with 0.1% ammonium formate), TAGs were resolved from the polar lipid phosphatidylcholine (PC). Although some kind of separation was observed for individual lipids, the best results for the TAGs separation were achieved using non polar columns, concretely with three monolithic ODS columns (Chromolith Performance RP-18e, 100 x 4.6 mm) connected in series. In this case, the individual TAGs (including TAGs with high ACN and low DB) were resolved in 8 minutes. Detection was performed using ESI-MS in positive mode, and programmed cone voltage fragmentation was employed for identification. Each TAG ion was found at 35 V, and two types of fragmented ions, diacylglycerols (DAG) and monoacylglycerols (MAG), were confirmed at 50 and 90 V, respectively. The structure of TAGs was determined by the type and peak intensity of DAG fragmented ions, taking into account that FAs at sn-1,3 position are more easily fragmented than at sn-2 position. Some years later, the same research group studied the profiling of several regioisomeric TAGs [50]. In this case, the separation of 16 TAGs was achieved on a C30 column. A gradient of modifier and flow-rate was necessary in order to reduce the retention time of TAGs with high ACN and low DB values. Detection was performed with ESI-QqQ-MS in positive mode and multiple reaction monitoring (MRM). The compounds were eluted in 50 minutes and several isomeric TAG pairs, such as SSO/SOS,SOL/OOO, SLP/OOP, and PPO/POP, were successfully resolved. Applying this method, 70 TAG (C46:0–C60:2) were successfully identified in palm and canola oils, and 20 pairs of TAG isomers were resolved. More specifically, six regioisomeric TAG pairs (PPLn/PLnP, PPL/PLP, PPO/POP, SPLn/SLnP, SPO/SOP and SSO/SOS) were reported by the first time in edible oils.

Zhou et al. [52] used UHPSFC-ESI- Q-TOF-MS for the chemical profiling of TAGs and DAGs in cow milk fat. The best results were obtained using a sub-2 μm ethyl pyridine column. In this case, good peak shapes and resolutions were obtained without adding ammonium formate to the modifier (a mixture of acetonitrile: ethanol 1/1, v/v), 49 individual TAGs and 7 DAGs being separated in 25 minutes in elution gradient mode

(Figure 2). On this column, the TAGs retention was also controlled by the ACN and BD values. TAGs with the lower ACN value eluted the first, but contrary to what was observed on ODS columns, retention of TAGs with the same ACN decreased as the DB value decreased. These results showed a separation behavior more similar to that of cSFC on polar columns such as the cyanopropyl siloxane columns. DAGs displayed greater retention than TAGs and eluted at the end of the chromatogram (within 23.3 and 24.5 minutes). Nevertheless, no conclusion regarding the elution behavior of DAGs could be provided because structure determination was not possible.

Determining free fatty acids (FFAs) is an important task in oil analysis. It can be used to monitor oil degradation, during production or storage processes, as well as to check oil authenticity. SFC offers the possibility of analyzing FFAs at low temperatures and without sample derivatization, simply by dilution in a nonpolar solvent (n-hexane or n-pentane) [53]. This is an advantage over GC methods, where usually a derivatization step is necessary and thermal degradation of polyunsaturated fatty acids (PUFA) may occur at the high temperatures used for separation. In HPLC methods, although derivatization is not required, usually the organic solvent used for lipid extraction is evaporated to dryness and reconstituted in a more compatible injection solvent; while in SFC, the extract can be directly injected into the system. Direct analysis of eight FFAs in edible oils has been described using UHPLC [54], but shorter analysis times and more efficient separations have been obtained with UHPSFC.

At present, most of the SFC studies published employ UHPSFC with sub 2µm columns, with very good results being provided by ODS types. Separation is performed using a gradient of organic modifier (methanol or methanol/acetonitrile 1:1), and the presence of 0.1% of formic acid as an additive is necessary in order to improve the peak shape. The retention mechanism is similar to that observed in RPLC; this means that the elution order of FFAs depends on the ACN and the DB values. The longer and the more saturated the acyl chain length the longer the retention time. Ashraf-Khorassani et al.[55] separated and identified 31 FFA in 7minutes on a HSS C18 column (150 x 3 mm, 1.8µm) using UHPSFC-ESI-QToF-MS in positive and negative modes. Isobaric FFAs were resolved in accordance with the chain position of the double bonds and LODs were similar to those obtained using GC-MS. Meanwhile, Qu

et al. [53] employed a similar column and an ESI-QqQ-MS detector, to determine FFAs in edible oils. Eight FFAs were eluted in 3 minutes and no matrix effect was observed.

3.2. Carotenoids

Carotenoids are natural fat-soluble pigments with important roles in some biological processes. They are classified in two main groups that have different polarities: carotenes without oxygen in its molecular structure, and xanthophylls with oxygen. They are generally analyzed by means of HPLC methods, but the main limitations are the long analysis time and the difficulty of separating cis/trans isomers. C_{18} or C_{30} stationary phases are the most common with C_{30} providing better resolutions but at a cost of higher retentions and longer analysis time.

Several studies have shown the capacity of SFC in the analysis of carotenoids. A reduction in the analysis time and an improvement in resolution between structural isomers, have been the most important achievements. Matsubara et al. [56] published the first work dealing with the use of SFC-MS. They studied the separation of seven carotenoids, more specifically, two carotenes (β-carotene and its structural isomer lycopene) and five xanthophylls (zeaxanthin, neoxanthin, their structural isomers lutein and violaxanthin, and antheraxanthin). The detector was a single quadrupole with ESI ionization. . Using high flow rates (9 ml/min) and a monolithic silica column the compounds were resolved in 4 minutes together with structural isomers. When the method was applied to a direct analysis of algae extracts the use of three monolithic columns connected in series and a flow rate of 3 mL/min was necessary in order to increase resolution and to prevent the coelution of interfering compounds. Some years later, the same research group developed an SFC-MS method for profiling β-cryptoxanthin (βCX) and nine β-cryptoxanthin fatty acid esters (βCXFA) in the peel of citrus fruits [57]. In this case, they used ESI-QqQ-MS and a 5µm column. They achieved greater sensitivity (fentomoles order) although analysis time was 20 min...

Jumaah et al. [58] improved the analysis time by employing UHPSFC-QTOF-MS and a sub-2µm 1-aminoanthracene column. Ten carotenoids (α-carotene, β-carotene, lycopene, canthaxanthin, lutein, zeaxanthin, neoxanthin, β-cryptoxanthin, astaxanthin, and violaxanthin) were determined in rosehip and microalgae extracts in just 6 minutes. On this column carotenes retention is controlled by π-π interactions, thus the greater

the number of double bonds the greater the retention. In addition, hydrogen bonding interactions control xanthophylls retention, and retention increase with the number of hydroxyl groups .ESI and APCI were compared in both positive and negative mode. Neither by using APCI in positive or negative mode nor ESI in negative mode, was any ionization observed for β-carotene or for astaxanthin. However, good signals were obtained with ESI in positive mode.

The research group of L. Mondello is very active in the development of SFC-MS methods for analyzing carotenoids and apocarotenoids in food samples. Apocarotenoids are products of the oxidative and enzymatic cleavage of carotenoids, possessing different bioactive functions in plants and some of them being used as food colorants. Apocarotenoids are generally present at very low concentration, as a result of which the use of a mass spectrometry detector is necessary. Employing a C_{30} fused-core column, Giuffrida et al. [59] developed a pioneer rapid SFC-APCI-QqQ-MS method for analyzing 25 apocarotenoids (including 14 free apocarotenoids and 11 apocarotenoids fatty acids esters) in *Capsicum chinense cv*. red habanero peppers.. Identification was performed in less than 5 minutes and .in order to achieve a high level of such identification both SIM and MRM modes were used simultaneously. The Shimadzu Nexera-UC system, where supercritical fluid extraction is online coupled to SFC-APCI-QqQ-MS, has also been successfully applied to determine carotenoids. Some advantages of this online approach are the lower risk of sample contamination, a lower analysis time and enhanced analytical precision. This equipment was employed by Zocali et al. to determine a large number (over 20) of carotenoids (free carotenoids, carotenoids monoesters, apocarotenoids and carotenoids diesters) in different samples, such as red habanero peppers [60], yellow tamarillo [61], different chilli peppers cultivars [62] and orange peels [63]. In all cases the analysis time was less than 17 minutes, which is really short if compared with conventional LC methods where an off line liquid extraction with organic solvents is necessary. Moreover, a larger number of compounds were identified with the online SFE-SFC procedure and some of them (several ε-apoluteinals and 4-oxo-apo-β-carotenals) were detected for the first time.

3.3 Vitamins

Vitamins are essential nutrients for humans and their intake is necessary for normal and healthy growth. They are classified, according to their solubility, in fat-soluble vitamins and water-soluble vitamins. The two groups have very different polarities, which make it difficult to analyze them simultaneously by means of a single chromatographic method.

The research group of T. Bamba proposed the use of SFC with wide elution gradients of modifier to cover a broad range of polarities, starting with a low percentage of organic modifier (or even pure CO_2) and finishing with 100%. Thus, both polar and nonpolar compounds could be analyzed simultaneously in a single run. They developed an SFC-MS method to analyze simultaneously fat- and water-soluble vitamins [64]. The organic modifier was methanol/water (95/5, v/v) with 0.2% ammonium formate, the percentage ranging from 2% to 100% with gradient conditions. The mobile phase state changed from supercritical to subcritical and liquid, without any discontinuous transitions. The term "unified chromatography" was used to refer to this technique. An important aspect is to avoid the formation of two phases by correctly selecting temperature and pressure. In this case, working at 40ºC and above 10.3 MPa was enough to avoid the two phases over the range of the modifier percentage used. The column selected was a sub 2µm C18SB with C18 chains bonded to non-endcapped silica phase. This stationary phase allows hydrophobic (through C18 ligands) and hydrophilic (through residual silanol groups) interactions, providing good retentions for both fat- and water-soluble vitamins. The presence of water and ammonium formate in the modifier was necessary in order to improve the peak shape of pyridoxine and l-ascorbic acid and decrease the retention of thiamine. Detection was performed using an ESI-QqQ-MS detector, working in MRM mode. Finally, 17 vitamins were separated in a very short time, that is, 4 minutes (Figure 3). The same research group reported the analysis of a water-soluble vitamin, niacin and seven metabolites, in biological fluids also using SFC-ESI-QqQ-MS [65].

Vitamin E is a fat-soluble vitamin composed of a mixture of eight compounds, four tocopherols and four tocotrienols, which is present in vegetable oils. Its analysis has been described using pSFC on conventional diol columns and methyl-terc-butyl ether as the organic modifier [66]; however, in this case δ-tocopherol and γ-tocotrienol were

not completely baseline resolved and the analysis time was long (40 minutes). The separation of tocopherols and tocotrienols was improved by Méjean et al.[67] using a 3μm amino column and ethanol with 0.1 % formic acid as organic modifier. The compounds were determined in soybean oils with an analysis time of 5 minutes. The SFC-QTOF system was equipped with atmospheric pressure photoionization (APPI), ESI and APCI sources; the highest sensitivities and repeatabilities were obtained with APPI in positive mode.

3.3 Other compounds

SFC-MS has also been employed for analyzing free amino acids using as modifier methanol with a small percentage of water, and an additive. Selecting the ionization source (ESI or APCI) depends on the type of amino acid, the ionization mode and the additive employed. D. Wolrab et al. [68] showed that methanol with 50mM ammonium formate and 1% of water are good starting conditions for polar and ionic analytes, whatever the ionization source used in positive mode; however, in these conditions, ESI provided the highest signals in most cases. On the other hand, when working in negative mode, the best results were obtained with methanol containing 50mM ammonium acetate without water.

As far as the stationary phase is concerned, when chiral analysis is not necessary, HILIC phases have been used [68]; but when enantiomeric resolution is required, chiral stationary phase based on polysaccharides [69], teicoplanine [70] or cinchona alkaloids [71] have been selected. Recently, the research group of C. West has developed several methods using SFC-MS and zwitterionic chiral stationary phases (Chiralpak ZWIX (+) and Chiralpak ZWIX (−)) for both chiral [71] and achiral [72] analysis of all free proteinogenic amino acids, which implied a broad range of polarities. These stationary phases contains hydrophilic and hydrophobic groups, which enables the retention of both the most and least polar amino acids. The methods were based on the concept of unified chromatography and a wide gradient of modifier (from 10% to 100%) was employed. When enantiomeric separation was studied, the use of an additive (70 mM ammonium formate) and 7% water was necessary in order to elute all the amino acids, especially the basic ones arginine, histidine and lysine, which were retained in a large extent. Despite some drawbacks (leucine and isoleucine coeluted,

enantiomers of arginine and aspartic acid could not be resolved and cysteine did not elute) 16 out of 19 amino acids were enantiomerically resolved on Chiralpak ZWIX (+). This study shows the capacity of SFC for chiral analysis of free amino acids with a broad range of polarities. When the enantiomeric separation was not required, the method was improved by using 20mM methanesulfonic acid (MSA) and 2% water, and by decreasing the gradient time from 19 min to 7min. The formation of an ion-pair between MSA and the amino acids (through the protonated amino group), reduced the polarity of the analyte decreasing interaction with the stationary phase; this resulted in improved peak shapes and lower retentions. Nevertheless, the use of MSA interfered with the enantiorecognition mechanism and lower enantioresolutions were achieved; this is the reason for it use in achiral analysis. A reversed gradient of flow rate (from 3ml/min to 1 ml/min) and pressure (from 15 to 11 MPA) was employed to avoid reaching the pressure limit. Moreover, the reversed flow rate gradient permitted optimal efficiency for both the early eluting compounds and the last eluting ones. In this case, the 19 amino acids were separated with good peak shapes in 8 minutes (Figure 4). Both methods were applied to the analysis of amino acids in food supplements.

Volatile compounds have also been examined by SFC, but the analysis is usually limited to some compounds from the same family and it is not comprehensively applied to determine compounds belonging to different families. The main limitation is the lack of commercially available columns that can provide efficient separations for different types of volatile compounds. In an attempt to solve this problem, Fujito et al. [73] checked the feasibility of a new polymer-based column, specially developed for SFC, namely, the styrene divinyl- benzene copolymer column (150 x 4.6 mm i.d.;3.5 μm, surface area: 700 m 2 /g). They used a standard mixture of volatile compounds with different functionalities and SFC-APCI-QqQ-MS in polarity switching mode. Good sensitivity rates without the use of make-up solvent were obtained. APCI was employed instead of ESI because terpene hydrocarbons (pinene), two terpene alcohols (menthol, citronellol) and some esters (hexyl acetate, pentyl butylate) could not be ionized in ESI. Twenty-three typical volatile compounds were separated in 8 minutes using a gradient of methanol. The new column provided higher retention and better separation than the conventional silica based ones, such as amino, phenyl or

C18. These preliminary results open up the possibility of using SFC for profiling volatile compounds in food samples.

SFC-MS was applied to determine phenolic acids in olive oil. A good separation was obtained for 12 compounds, yet 4-hydroxybenzoic acid and m-coumaric acid, could not be resolved and gallic and chlorogenic acids showed a severe tailing [74] .

The most relevant applications of SFC-MS in the analysis of food nutrients are summarized in Table 1.

4. Analysis of food contaminants

Food contaminants include pesticides, degradation products originating during food processing, toxins, endocrine disruptors, veterinary drugs or illicit drugs. These compounds can cause different diseases and thus their determination is necessary in order to ensure food safety and fulfill current legislation.

4.1. Pesticides

The analysis of pesticide residues, involves multiple compounds with a broad range of polarities, molecular structures or physical properties. It is usually performed by combining GC-MS and LC-MS methods; this result in a poor throughput and it can also be expensive. Considering that in SFC elution strength can be changed by using a gradient of organic modifier, it is possible to elute a wide group of compounds, from the least to the most polar, with the same equipment. Several papers have demonstrated the usefulness of SFC-MS for a multi-residue analysis of pesticides in short analysis time, with the research group of T. Bamba being very active in this respect.

Ishibashi et al. have developed several high-throughput SFC-MS methods to simultaneously determine a large number of pesticides with different polarities and molecular weights. They used a polar-embedded reversed phase column (Inertsil ODS-EP) as this provided good retention for both polar and nonpolar pesticides. Diquat dibromide (usually analysed by ion chromatography or ion-pair LC) and cypermethrin and tralomethrin (traditionally analysed by GC) were satisfactorily eluted and detected in a single run. Both QqQ-MS [75] and Orbitrap [76] detectors were employed with ESI ionization sources. By means of the latter, 444 pesticides were identified in 20 minutes.

The method was validated using spinach extracts and LODs were below 10 µg/kg for most of the compounds. The number of false positives and negatives was significantly reduced by using monoisotopic as well as isotopic ions along with the high mass accuracy (m/Δm = 7000) and high mass resolution (< 5ppm) provided by the Orbitrap detector. Some years later, Fujito et al. [27] highlighted the importance of optimizing SFC-MS parameters to achieve the highest sensitivity in multi-residue analysis. 441 pesticides, with different polarities and pK_a values, were simultaneously analyzed in 16 minutes by means of a QqQ-MS detector with ESI source in polarity switching mode and MRM acquisition program. In this case, the SFC-MS system (Nexera UC from Shimadzu) was equipped with an interface that transferred all the flow eluted from the SFC column to the MS detector, thus making it possible to compare the sensitivity of the optimized SFC-MS method with that of an LC-MS approach with the same detector. The optimal conditions in SFC-MS were different from those in LC-MS due to the different mobile phase composition. They found that the lower the salt concentration (1mM ammonium formate as additive) in the mobile phase, the higher the sensitivity obtained in both SFC-MS and LC-MS. Moreover, in the case of SFC-MS the appropriate total organic solvent flow rate (modifier and make up solvent) introduced into the ESI source, was between 0.1 and 0.4 mL/min in order to ensure a good sensitivity and repeatability. As far as MS parameters were concerned, capillary voltage in LC-MS slightly influenced signal intensities although better results were obtained at low voltage values. On the other hand, in SFC-MS, the signal was strongly affected by the capillary voltage and the highest signals were obtained at high voltage values (+5/-5 kV). This was explained by the fact that in SFC-MS, the mobile phase usually does not contain water, and consequently the electrical resistance of the mobile phase should be higher compared to that of LC/MS, which usually uses an aqueous solution for the mobile phase. The optimal values of capillary temperature and desolvation line depended on the compound; an intermediate value was the best choice for multi-residue analysis (300ºC and 200ºC respectively) in both SFC-MS and LC-MS. Better results were obtained with SFC-MS, as it displayed greater sensitivity for 386 out of the 441 compounds (i.e., 88%) . The matrix effect was evaluated by analyzing four different vegetables extracts (cucumber, carrot, soybeans and sesame). In this case, although the elution patterns of SFC-MS and LC-MS were different, the

occurrence of a matrix effect in SFC-MS was similar to LC-MS. Nevertheless, the total number of compounds that were not detected and showed poor repeatability was larger in LC-MS than in SFC/MS. This method was adapted by Cutillas et al. [77] for rapid determination of 164 pesticides in tomato, orange and leek samples, with the analysis time reduced to 7 minutes.. A matrix effect was observed, but in a lesser extent than in previous LC-MS methods.

Pérez-Mayán et al. [78] developed an SFC-ESI-QTOF-MS method to determine neonicotinoids in wine samples. Seven neonicotinoids and one transformation product were determined in less than 5 minutes on a sub-2µm ethylene bridged silica column. In this case, a lower capillary voltage was used (3.5 kV) and the presence of water in the make-up solvent (a 75:25 mixture of water/methanol) was necessary in order to improve signals intensities. Comparing the results obtained with SFC and those obtained using reverse phase UHPLC with the same detector and sample treatment, higher separation and ionization efficiencies, as well as a lower matrix effect were obtained with SFC.

Another rapid SFC-ESI-QqQ-MS method was developed by Li et al. [79] for the determination of thiacloprid in greenhouse vegetables and soil. They employed a sub 2µm column and a gradient of modifier (methanol).The analysis time was extremely short, a mere 1.2 minutes.

The enantiomeric analysis of chiral pesticides is of great interest because one pair of enantiomers can display different bioactivity as well as different dissipation rates. Taking into account that SFC is considered one of the most powerful techniques for chiral analysis, several works have described the use of SFC-MS for the chiral analysis of pesticides in different food samples; one of the most active research group in this area is that of F. Dong [28–30,80–83]. In all of these, chiral stationary phases derived from polysaccharides were employed, with the amylose tris(3,5-dimethylphenyl-carbamate) being the one most frequently selected. The detector employed was an ESI-QqQ-MS in positive mode and methanol containing 0.1% formic acid was used as the make-up solvent. Analysis times ranged from 2 to 4 minutes and in most cases, the methods developed were applied to stereoselective dissipation studies of the pesticides.

4.2. Processing contaminants

3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) fatty acid esters are processing contaminants in edible oils and fats. They are generated during the refining process, especially in that of deodorization at high temperatures. These compounds are harmful and it is important that they are evaluated. Their analysis is a challenging task because they occur at very low concentrations in the samples. Using GC-MS methods, a hydrolysis step followed by derivatization with phenylboronic acid is necessary; consequently, MCPD fatty acid esters cannot be distinguished individually. LC methods have limitations in terms of low sensitivity and high consumption of organic solvents. Hori et al. [84], proposed an SFC-ESI-QqQ-MS method for the analysis of fourteen 3-MCPD fatty acid esters in edible oils, using a C_{18} column and a gradient of methanol containing 0.1% ammonium formate. The analysis time was 9 min and sample treatment was not needed, only dilution in the organic modifier. LODs ranged from 0.013 to 0.063 mg/kg, which meant 10 times greater sensitivity compared with the previous HPLC-Q-TOF-MS method. Some years later, Jumaah et al. [85] determined 3-MCPD mono- and diesters, and 2-MCPD mono- and diesters in a single run. They used UHPSFC-ESI-Q-TOF-MS and a sub-2μm 2-picolylamine column. Twenty-six compound were determined in 12 minutes, using pure CO_2 as the mobile phase and without any sample clean-up procedure, simply dilution in heptane. The presence and position of a hydroxyl group in the structure, the number of unsaturated bonds, and the acyl chain length, play a significant role in the separation of MCPD esters. Monoesters were detected as sodium adducts by MS, while diesters were detected by MS/MS in order to distinguish between molecular species with the same molecular mass but with a different position of the fatty acid residues (ions after residue cleavage of fatty acid from the sn-1,3 position are of greater intensity than those after cleavage from sn-2 position).

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic substances that are known to be produced by heating processes, such as roasting and smoking in food manufacturing. PAHs in food are generally determined by GC-MS but the analysis time is 30-40 minutes, and with LC-MS methods sensitivity is low. The research group of T. Bamba developed a novel, rapid and high-sensitive analytical method, for analyzing 15 PAHs in coffee beverages and dark beer. They employed SFC-APCI-MS with a

novel backpressure gradient, in order to achieve low LOQs [86]. In the configuration, the make-up solvent (5% anisole in methanol) was added after the splitter but before the MS detector. Separation was performed on a 3µm ODS-P column with densely bonded octadecyl groups, which is suitable for the separation of planar compounds. When the analytes eluted, the backpressure regulator was practically plugged by raising the backpressure, and the amount of sample introduced into the MS detector was increased. The backpressure was reduced after a compound had eluted, and was increased again when the next compound began to elute. This procedure had no negative effect on reproducibility and repeatability of retention times. The signal to noise ratio (S/N) was increased 2.4-8.0 times and the compounds could be determined in 12 minutes with LOQs below 1 µg/kg (Figure 5). Moreover, this can be reproduced in SFC systems from other manufacturers. If the inner diameter of the line to MS is sufficiently large and less than one third of that of the line to the backpressure regulator, and the length of the line to the MS is less than 0.75 m; the resolution would be unaffected.

The same research group proposed a similar back-pressure gradient to determine acrylamide in beverages, grains, and confectioneries [87]. In this case, a SFC-ESI-QqQ-MS system and a sub-2µm 1-aminoanthracene column were used. Sensitivity was 11-times higher than that obtained in UHPLC- ESI-QqQ–MS, with the same detection system; this permitted evaluation of concentrations lower than 10µg/kg without an SPE clean-up. Moreover, analysis time was under 4 minutes. Acrylamide could be determined in roasted barley tea and coffee at 0.05 µg/kg level, which was not possible when UHPLC was employed.

4.3. Other contaminants

Lipid A is a lipid component of lipopolysaccharides (LPS). LPS are endotoxins located on the cell wall of different Gram-negative bacteria and can cause severe health effects. Thus, lipid A can be used for determining endotoxins from different bacteria. Chen at al. proposed a rapid SFC method with APCI and ion trap mass spectrometry detection to determine lipid A in lettuce and ground beef [88]. Analysis was performed in less than 2 minutes with a cyanopropyl column and 0.2% of diethylamine as additive. Aflatoxins have also been determined using SFC-MS. Lei et al. developed a method

to determine 4 aflatoxins (B1, B2, G1 and G2) in edible oils, using SFC-ESI-QqQ-MS [89]. Separation was achieved in 4 minutes on a sub-2µm 2-ethyipyridine column.

Alkylphenol ethoxylates and tristyrylphenol ethoxylates are used as non-ionic surfactants and their degradation products, alkylphenols and styrenated phenols respectively, show estrogenic activity. These surfactants are still employed in some countries as adjuvants in agrochemical formulations for improving spray efficacy, thus they can be present as contaminants in agrochemical products. Jiang et al. have developed several methods [31,32] to determine these compounds using SFC-ESI-QqQ-MS. In both cases, analysis was performed on a sub-2µm BEH column in less than 5 minutes. The proposed methods were applied to the analysis of cabbage, lettuce, and spinach samples; in all the cases matrix effect was observed.

Last but not least, SFC-MS has also been used for analysing UV-ink photoinitiators (PIs). PIs are catalysts used to initiate a polymerization reaction for the curing of inks and lacquers. They can exist in cardboard or plastic food packaging as residues of the printing process. Without an effective barrier, they can migrate into the food causing food contamination and potential hazards. Zhang et al. [90] used SFC coupled to photodiode array detector and ESI-QqQ-MS, to study the migration behaviour of 13 PIs from a polyethylene (PE) packaging into food simulants. The compounds were separated in 4.5 minutes on an HSS C18 column

The most relevant applications of SFC-MS in the analysis of food contaminants are summarized in Table 2.

5. Conclusions

The number of scientific areas where SFC can be applied are increasing. The introduction of a new generation of instruments with improved robustness and performance, hyphenation to mass spectrometry, the development of new stationary phases and the possibility of using sub 2 µm columns, has expanded the applicability of SFC to other sectors where the required levels of accuracy and sensitivity are critical. In addition, the possibility of modifying mobile phase polarity by adding an organic modifier or low percentages of water, broaden the range of compounds that can be analysed from the nonpolar (lipids) to the most polar (amino acids or water-

soluble vitamins); it is even possible to simultaneously separate a large number of compounds with very different polarities. In food analysis, SFC-MS has provided very good performances for difficult separations of both food nutrients and also food contaminants; moreover, it provides complementary information to LC and it can be applied in targeted and non-targeted analysis.

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Figure Captions

Figure 1. Schematic representations of the commonly used SFC-MS interfaces: A) Pre-BPR splitter with sheath pump, B) BPR and sheath pump with no splitter. *Reprinted from J. Chromatogr. B Anal. Technol. Biomed. Life Sci. Vol.1083, D. Guillarme, V. Desfontaine, S. Heinisch, J.L. Veuthey, What are the current solutions for interfacing supercritical fluid chromatography and mass spectrometry?,pp 160–170 [Ref. 20], Copyright (2018), with permission from Elsevier*

Figure 2. SFC-ESI-QqQ-MS, BPI (base peak intensity) chromatogram of triacyglycerols and diacyglycerols in cow milk fat. Chromatographic conditions: Acquity UPC^2 BEH 2-EP column (150 x 3.0 mm, 1.7 µm), back pressure 1600 psi, flow rate 1.2 mL/min, temperature 50 ºC, gradient of organic modifier (acetonitrile / ethanol 1:1), injection volume 1.0 µL. *Reprinted from Food Chem. Vol.143, Q. Zhou, B. Gao, X. Zhang, Y. Xu, H. Shi, L. Yu, Chemical profiling of triacylglycerols and diacylglycerols in cow milk fat by ultra-performance convergence chromatography combined with a quadrupole time-of-flight mass spectrometry, pp. 199–204 [Ref. 44], Copyright (2014), with permission from Elsevier* .

Figure 3. MRM chromatograms of 17 vitamins. FSV (Fat-soluble vitamins), WSV (water-soluble vitamins). (1) A acetate, (2) A palmitate, (3) D2, (4) α-tocopherol, (5) K2, (6) K1, (7) α-tocopherol acetate, (8) β-carotene, (9) nicotinamide,(10) Nicotinic acid, (11) Pyridoxine, (12) d-pantothenic acid, (13) Biotin, (14) Thiamine, (15) Riboflavin, (16) B12, (17) Ascorbic acid. Method conditions: column C18SB (50 × 3.0 mm i.d., sub 2μm); modifier: methanol/water (95/5, v/v) with 0.2% ammonium formate in gradient conditions, flow rate 1.2 mL/min, column temperature of 40◦C; backpressure: 15.2 MPa (6.0 min),15.2–10.3 MPa (0.2 min), 10.3 MPa (1.6 min), 10.3–15.2 MPa (0.5 min), 15.2 MPa (1.7 min). *Reprinted from J. Chromatogr. A., Vol. 1362, K. Taguchi, E. Fukusaki, T. Bamba, Simultaneous analysis for water- and fat-soluble vitamins by a novel single chromatography technique unifying supercritical fluid chromatography and liquid chromatography, pp. 270–277, [Ref. 59], Copyright (2014), with permission from Elsevier.*

Figure 4. Chromatograms of amino acid standards. Column Chiralpak ZWIX (+) (150 ×3.0 mm, 3 μm). ESI-MS detection with single-ion recording. (a) Hydrophobic side chains (b) Polar side chains and special cases (c) Acidic and basic side chains. *Reprinted from J. Chromatogr. A. Vol. 1616, A. Raimbault, A. Noireau, C. West, Analysis of free amino acids with unified chromatography-mass spectrometry—application to food supplements, pp. 460772, [Ref. 67] , Copyright (2020), with permission from Elsevier.*

Figure 5. Chromatogram of 16 PAHs and backpressure gradient in SFC-MS. (1) BcF, (2) BaA, (3) MCh, (4) CPP, (5) Chr, (6) BjF, (7) BbF, (8) BkF, (9) BaP, (10) DBahA, (11) DBalP, (12) IP, (13) BghiP, (14) DBaeP, (15) DBaiP, (16) DBahP. *Reprinted from J. Biosci. Bioeng. Vol. 126, T. Yoshioka, Y. Nagatomi, K. Harayama, T. Bamba, Development of an analytical method for polycyclic aromatic hydrocarbons in coffee beverages and dark beer using novel high-sensitivity technique of supercritical fluid chromatography/mass spectrometry, pp. 126–130 [Ref. 81], Copyright (2018), with permission from Elsevier.*

Figure 1

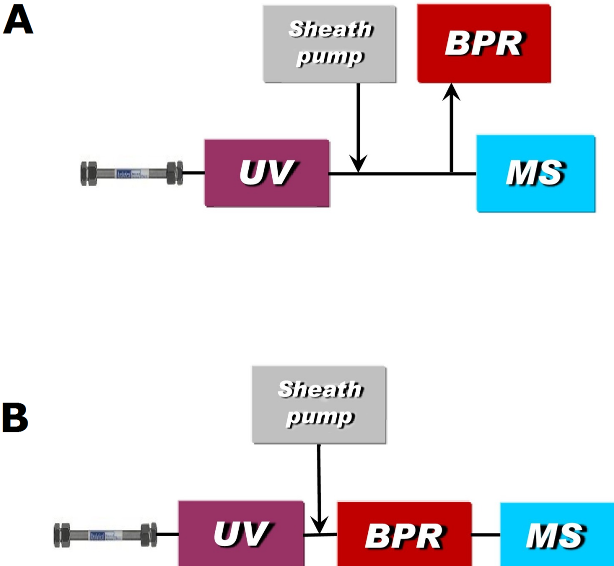


Figure 2

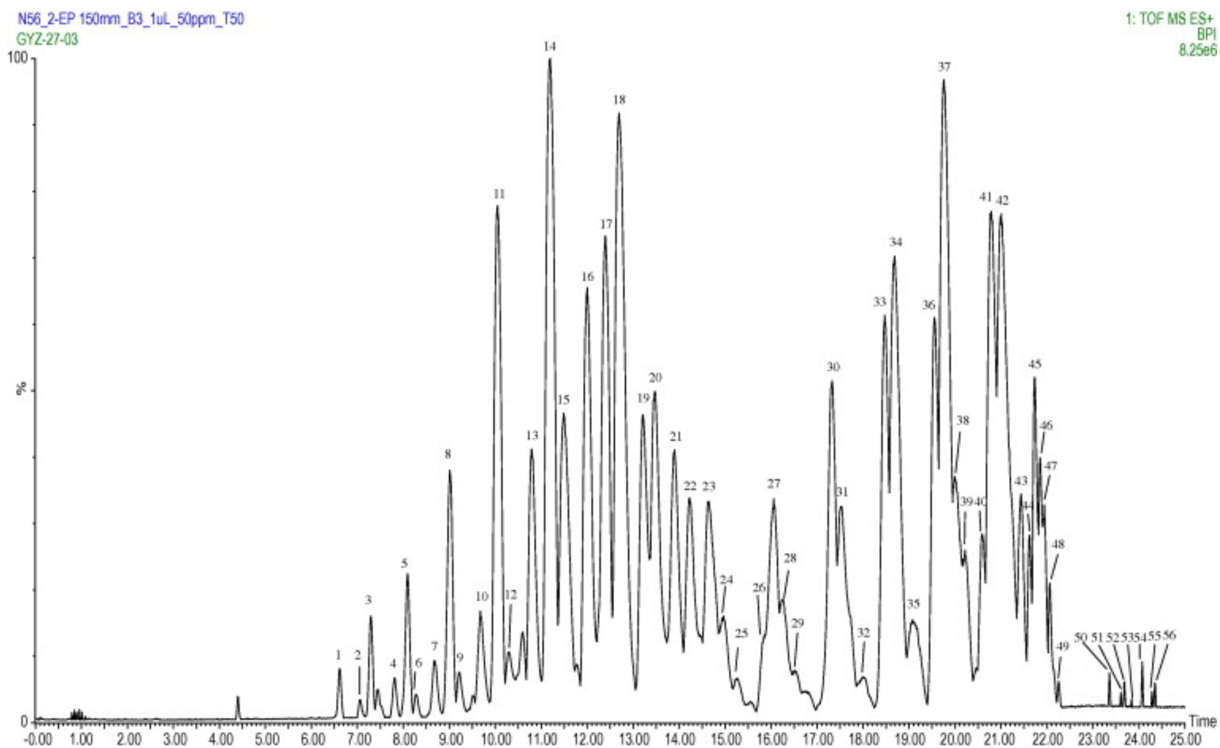


Figure 3

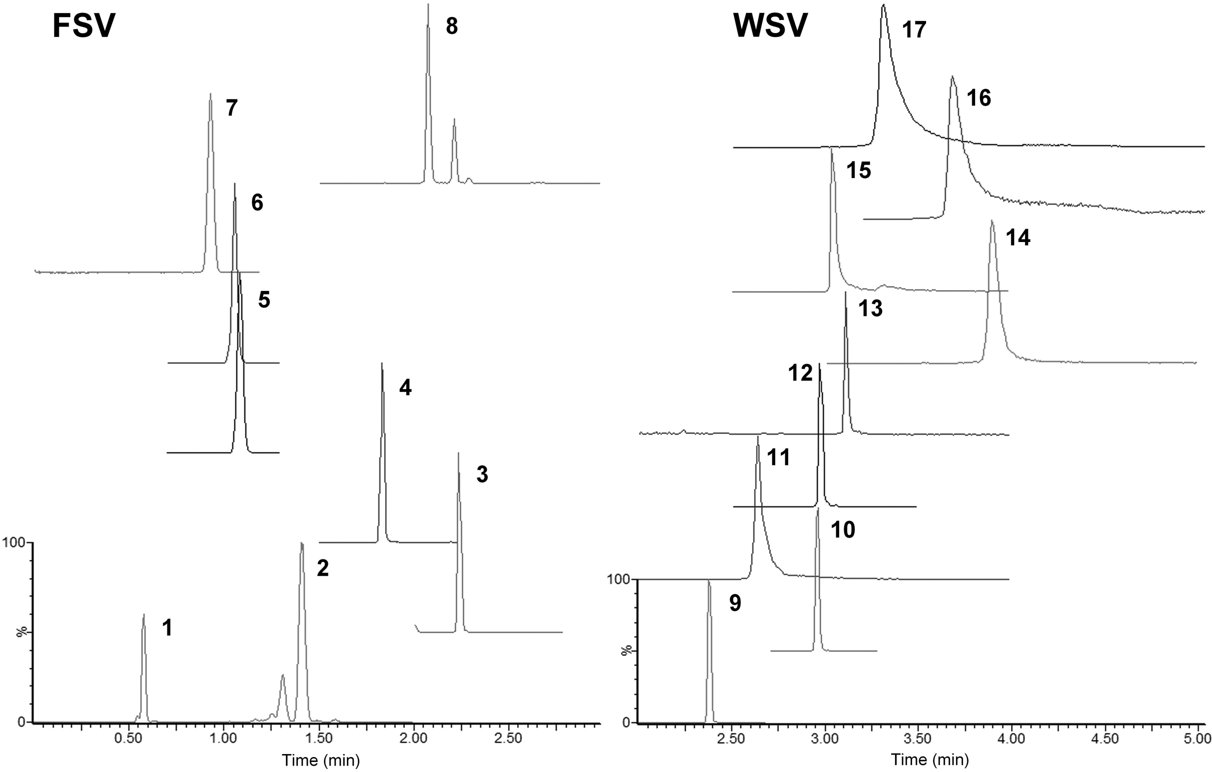
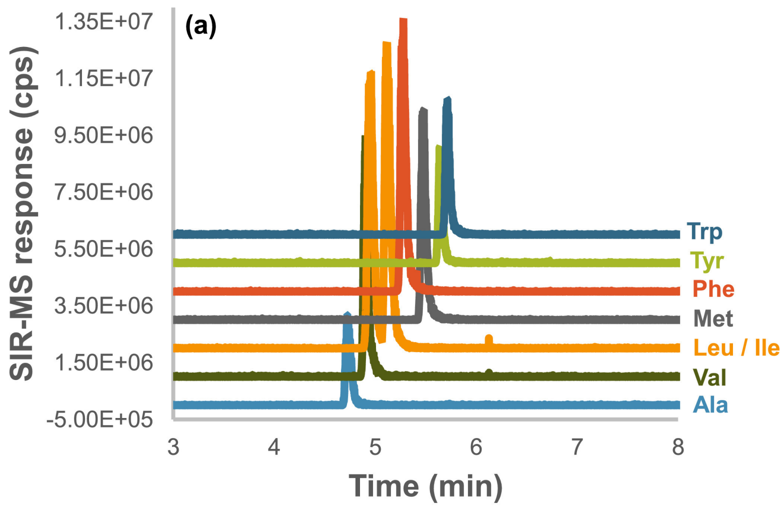
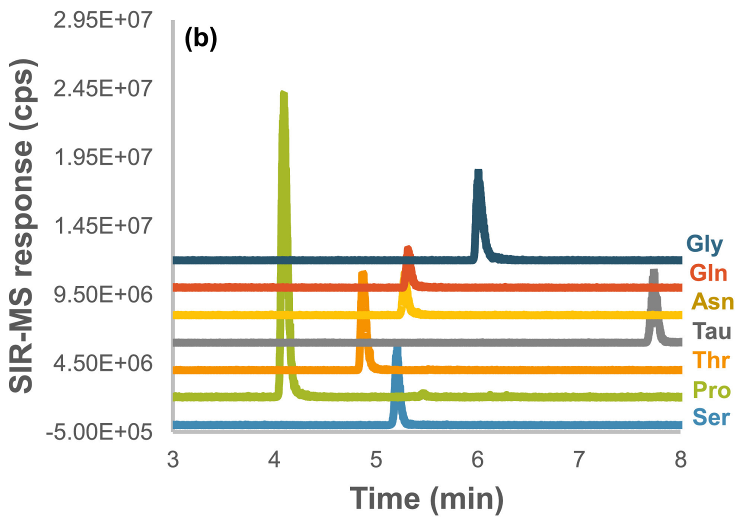


Figure 4





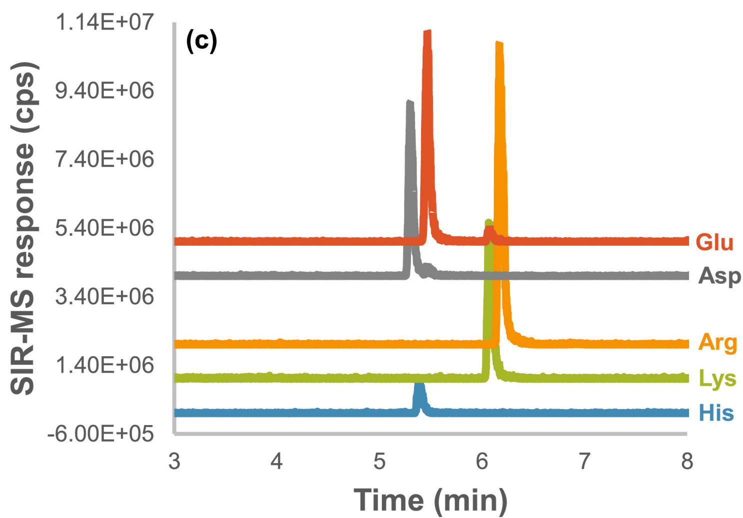


Figure 5

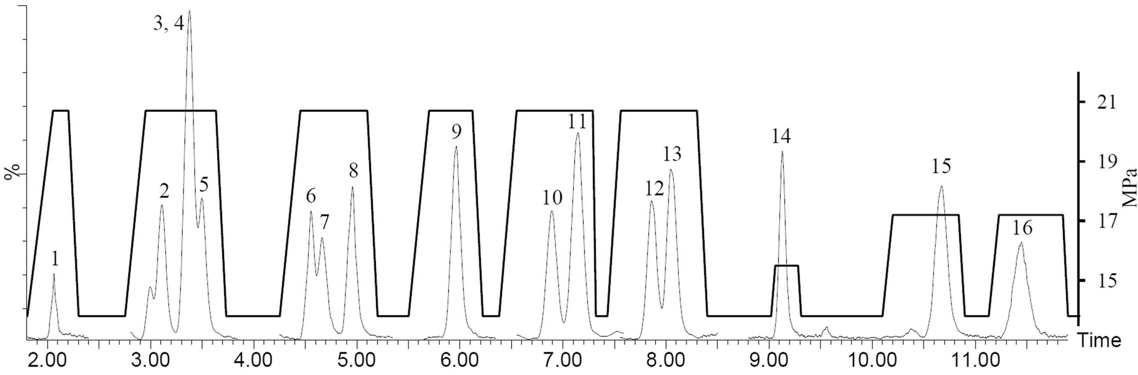


Table 1. SFC-MS applications in the analysis of food nutrients.

Ref	Analyte	Sample	Column	Organic modifier and Chromatographic conditions	Detector
49	TAGs (22 compounds)	soybean oils	Three Chromolith Performance RP-18e columns ((100×4.6 mm ID)	Methanol with 0.1% (w/w) ammonium formate from 20% to 30%, 10 MPa, 3ml/min, 35ºC	ESI-Q-MS positive mode
50	TAGs (C46:0–C60:2) and isomeric TAG pair (70 compounds)	Palm and canola oils	YMC carotenoid column, C30 (250 × 4.6 mm ID; 4 µm)	Methanol with 0.1% (w/w) ammonium formate from 20% to 30%, flow-rate from 2ml/min to 3ml/min, 15 MPa, 35ºC	ESI-QqQ-MS positive mode
52	TAGs and DAGs (56 compounds)	Cow milk fat	Acquity UPC2 BEH 2-EP (150 x 3.0 mm i.d.; 1.7 µm)	Ethanol:acetonitrile (1:1) from 0,2% to 12%, 16 MPa, 1.2 ml/min, 50 ºC	ESI- Q-TOF-MS in positive mode
55	FFA (31 compounds)	Fish oil	Acquity HSS C18 SB (150 x 3.0 mm, 1.8 µm)	Methanol with 0.1% formic acid from 2% to 20%, 1.0 ml/min, 15 MPa, 25ºC	ESI-Q-TOF-MS positive and negative mode
53	FFA (8 compounds)	35 vegetable oil	UPC2 HSS C18 SB column (100 x 3.0 mm, 1.8 µm.)	Methanol/acetonitrile (50:50) with 0.1% formic acid from 3% to 7%, 1.6 ml/min, 15 MPa, 40ºC,	ESI-QqQ-MS negative mode
57	β-cryptoxanthin (βCX) and β-cryptoxanthin fatty acids (9 compounds)	Citrus fruits peel	Inertsil ODS-P (250 x 4.6 mm, 5 µm)	10% Methanol with 0.1% (w/w) ammonium formate, 10 MPa, 35 ◦C, , 3 ml/min	ESI-QqQ-MS positive mode
58	Carotenoids (10 compounds)	Microalgae and rosehip	Waters Acquity UPC2 1-AA (100 mm× 3 mm, 1.7 µm)	Methanol from 5% to 25%, 160 bar ,35 °C, 2 ml/min,	ESI-QTOF-MS in positive mode
59	Apocarotenoids (25 compounds)	Red habanero peppers	Ascentis Express C30 (150 mm x 4.6 mm, 2.7 µm)	Methanol from 0% to 40%, 150 bars, 35ºC, 2 mL/min	APCI-QqQ-MS in positive and negative mode

Table 1. SFC-MS applications in the analysis of food nutrients (continued)

Ref	Analyte	Sample	Column	Organic modifier and Chromatographic conditions	Detector
60	Carotenoids (21 compòunds)	Red habanero peppers	Ascentis Express C30, (150 mm x 4.6 mm, 2.7 µm)	Methanol from 0% to 40%, 150 bar, 35 ºC, 2ml/min	APCI-QqQ-MS in positive and negative mode
61	Carotenoids and apocarotenoids (31 compounds)	Yellow tamarillo	Ascentis Express C30, (150 mm x 4.6 mm, 2.7 µm)	Methanol from 0% to 40%, 150 bar, 35 ºC, 2ml/min	APCI-QqQ-MS in positive and negative mode
62	Free apocarotenoids and apocarotenoids fatty acid esters (27 compounds)	Chilli peppers	Ascentis Express C30, (150 mm x 4.6 mm, 2.7 µm)	Methanol from 0% to 100%, 150 bar, 35 ºC, 2ml/min	APCI-QqQ-MS in positive and negative mode
63	Carotenoids (42 compounds)	Orange peel	Ascentis Express C30, (150 mm x 4.6 mm, 2.7 µm)	Methanol from 0% to 40%, 150 bar, 35 ºC, 2ml/min	APCI-QqQ-MS in positive and negative mode
64	Fat- and water-soluble vitamins (17 compounds)	Standard solution	Acquity UPC2HSS C18 SB, 100 × 3.0 mm i.d; sub 2 µm	Methanol/water (95/5, v/v) with 0.2% ammonium formate from 2% to 100%, 15.2 MPa, 40 ºC, 1.2 ml/min	ESI-QqQ-MS in positive mode
65	Tocopherols and tocotrienols (8 compounds)	Soybean-oil	Amine Luna NH2 column (150 x 2 mm, 3 µm)	Ethanol with 0.1 % formic acid from 3.5% to 8%, 130 bar, 30ºC and 1.5 mL/min	APPI-QTOF-MS in positive mode

Table 1. SFC-MS applications in the analysis of food nutrients (continued)

Ref	Analyte	Sample	Column	Organic modifier and Chromatographic conditions	Detector
71	Free amino acids, chiral separation (16 compounds)	Food supplements	Chiralpak ZWIX (+) and Chiralpak ZWIX (−) (150 ×3.0 mm; 3 μm)	Methanol with 70 mM ammonium formate and 7% water. Gradient from 10% to 100%,150bars, 25ºC and 0.5 ml/min	ESI-Q-MS in positive mode
72	Free amino acids, achiral separation (21 compounds)	Food supplements	Chiralpak ZWIX (−) (150 ×3.0 mm; 3 μm)	Methanol with 2% water and 20 mM methanesulfonic acid Gradient from 10% to 100%, reversed pressure gradient from 15 to 11 MPa, 25ºC, reversed flow rate gradient from 3 to 1 ml/min	ESI-Q-MS in positive mode
73	Typical volatile compounds with a wide variety of chemical properties (23 compounds)	Standard solution	Styrene divinyl- benzene copolymer column (150 ×4.6 mm, 3.5 μm, surface area: 700 m 2 /g)	Methanol from 2 % to 5 %, 15 MPa, 40 ºC, 3 ml/min	APCI-QqQ-MS in polarity switching mode
74	Phenolic acids (12 compounds)	Extra virgin olive oil	Platisil CN (250 mm x 4.6 mm, 5 μm)	Methanol with 7 % water and 0.5 % formic acid. Gradient from 4 % to 50 %, 140 bar, 60 ºC, 3 ml/min.	ESI-Q-MS in negative mode

Table 2. SFC-MS applications in the analysis of food contaminants

Ref	Analyte	Sample	Column	Organic modifier and Chromatographic conditions	Detector
27	Pesticides (441 compounds)	Cucumber, carrot, soybeans and sesame	Shimpack UC-RP (150 x 2 mm, 3 μm)	Methanol with 0.1 mM ammonium formate. Gradiente from 2 % to 80 %; 40ºC, 0.6 ml/min	ESI-QqQ-MS in polarity switching mode
28	propiconazole enantiomers	wheat straw, grape, and soil	Chiralpak AD-3 (amylose tris3,5-dimethylphenyl-carbamate, 150 x 4.6 mm, 3 μm)	7% Ethanol, 2200 psi, 30 ºC , 2 ml/min	ESI-QqQ-MS in positive mode
29	Pydiflumetofen enentiomers	grape and soi	Chiralcel OD-3 (cellulose tris3,5-dimethylphenyl-carbamate 150 x 4.6 mm, 3 μm)	20 % Methanol, 2000 psi, 30 ºC, 1 ml/min	ESI-QqQ-MS in positive mode
30	Prothioconazole and prothioconazole-desthio enantiomers	Tomato, cucumber, and pepper	Chiralcel OD-3 (cellulose tris3,5-dimethylphenyl-carbamate 150 x 4.6 mm, 3 μm)	15 % 2-propanol with 0.2 % acetic acid and 5 mM ammonium acetate, 2000 psi, 25 ºC, 1.5 ml/min	ESI-QqQ-MS in positive mode
31	Nonylphenol ethoxylates and octylphenol ethoxylates (38 compounds)	Cabbage, lettuce, and spinach	Viridis BEH column (100 × 3 mm, 1,7 μm)	Methanol/acetonitrile (3:2). Gradient from 5 % to 30 %; 12 MPa, 30 ºC, 1.65 ml/min	ESI-QqQ-MS in positive mode
32	Tristyrylphenol ethoxylates (30 compounds)	Cucumber, leaves, and soil	UPC^2 Acquity BEH column (100 x 3 mm, 1,7 μm)	Methanol from 5 % to 35 %; 14 MPa, 40 ºC, 1.5 ml/min	ESI-QqQ-MS in positive mode
75	Pesticides (17 compounds)	Brown rice, onion, and spinach	Inertsil ODS-EP (250 × 4.6 mm, 5 μm)	Methanol with 0.1% ammonium formate. Gradient from 5 % to 30 %; 35 ºC, 3 ml/min	ESI-QqQ-MS in positive mode
76	Pesticides (444 compounds)	QuEChERS spinach extracts	Inertsil ODS-EP (250 × 4.6 mm, 5 μm)	Methanol with 0.1% ammonium formate. Gradiente from 5 % to 30 %; 35 ºC, 3 ml/min	ESI-Q- Orbitrap-MS

Table 2. SFC-MS applications in the analysis of food contaminants (continued)

Ref	Analyte	Sample	Column	Organic modifier and Chromatographic conditions	Detector
77	Pesticides (164 compounds)	Tomato, orange and leek	Shimpack UC-XRP (150 x 2 mm, 3 µm)	Methanol with 1 mM ammonium formate. Gradiente from 2 % to 40 %; 150 bar, 40 ºC, 1.5 ml/min,	ESI-QqQ-MS in polarity switching mode
78	Neonicotinoid pesticides and one transformation product (8 compounds)	Wine samples, both red and white	Viridis, ethylene bridged silica BEH (100 x 3 mm, 1.7 µm)	Methanol with 5 mM ammonium acetate. Gradient from 2 % to 30 %; 140 bar, 45 ºC, 1.5 ml/min	ESI-QTOF-MS in positive mode
79	Thiacloprid	Tomato, cucumber and soil	UPC^2 BEH column (100 x 3.0 mm, 1.7 µm)	Methanol from 5 % to 24 %, 1800 psi, 40 ºC, 1.8 ml/min	ESI-QqQ-MS in positive mode
80	Flutriafol enantiomers	tomato, cucumber, apple, grape and soil	Chiralpak IA-3 (amylose tris-3,5-dimethylphenylcarbamate, 150 x 4.6 mm, 3 µm)	12 % Methanol, 2200 psi, 30ºC, 2.2 ml/min	ESI-QqQ-MS in positive mode
81	Isofenphos-methyl enantiomers	Wheat, corn, peanut and soil	Chiralpak IA-3 (amylose tris-3,5-dimethylphenylcarbamate, 150 x 4.6 mm, 3 µm)	10% 2-propanol, 2200 psi, 30 ºC, 2..2 ml/nin	ESI-QqQ-MS in positive mode
82	Fembuconazole enantiomers and chiral metabolites	tomato, cucumber, apple, peache, rice and wheat	ACQUITY UPC^2 Trefoil AMY 1 column (amylose tris-3,5-dimethylphenylcarbamate, (150 x 3.0 mm, 2.5 µm)	Ethanol from 2 % to 40 %, 13.79 MPa, 40 ºC, 1.8 ml/min	ESI-QqQ-MS in positive mode
83	Diniconazole enantiomers	Tea, apple, and grape	Chromega Chiral CCAcolumn (amylose tris3,5-dimethylphenyl-carbamate, 150 x 4.6 mm, 5 µm)	4 % 2-propanol, 2000 psi, 25 ºC, 2 ml/min	ESI-Q-TOF-MS in positive mode
84	3-MCPD fatty acid esters (14 compounds)	Edible oils	Inertsil ODS-4 (250 x 4.6 mm, 5 µm)	Methanol with 0.1 % ammonium formate. Gradient from 5 % to 30 %; 10 MPa, 35 ºC, 3 ml/min	ESI-QqQ- MS in positive mode
85	2-MCPD and 3-MCPD mono- and diesters. (26 compounds)	Corn oil, rapeseed oil, and sunflower oil	Torus 2-picolylamine (100 x 3 mm, 1.7 µm)	Neat CO_2, backpressure gradient from 110 bar to 160 bar, 50 ºC, 1 ml/min	ESI-Q-TOF-MS in positive mode

Table 2. SFC-MS applications in the analysis of food contaminants (continued)

Ref	Analyte	Sample	Column	Organic modifier and Chromatographic conditions	Detector
86	PAHs (15 compounds)	Coffee beverages and dark beer	Inertsil ODS-P column (150 x 3.0 mm, 3 µm)	Acetonitrile with 0.5 % formic acid. Gradient from 0.1 % to 60 %; 40 ºC, pressure and flow rate gradients	APCI-Q-MS in positive mode
87	Acrylamide	Beverages, grains, and confectioneries	ACQUITY UPC^2 Torus 1-aminoanthracene (1-AA) (150 x 3.0 mm, 1.7 µm)	Methanol from 5 % to 47 %; pressure gradient, 40 ºC, 1 ml/min	ESI-QqQ-MS
88	Lipid A	Lettuce or ground beef	Cyanopropyl (30 x 4.6 mm, 5 µm)	25 % Methanol with 0.2 % diethylamine; 150 bar, 40 ºC, 2 ml/min	APCI-ion trap-MS in positive mode
89	Aflatoxins (B1, B2, G1 and G2)	Edible oil	UPC^2 2-EP (100 x 2.1 mm,1.8 µm)	Methanol from 2 % to 20 %, 50 ºC, 1 ml/min	ESI-QqQ-MS in positive mode
90	UV-ink photoinitiators (13 compounds)	Extracts from food simulants	UPC^2 Acquity HSS C18 SB column (100 x 3.0 mm, 1.7 µm)	Methanol/acetonitrile (1:1). Gradient from 0 % to 22 %; 1600 psi, 50 ºC, 1.45 ml/min	ESI-QqQ-MS in positive mode