



Recent Applications in LC–MS: Food and Flavours

Luigi Mondello¹, Giovanni Dugo¹ and Paola Dugo²,

¹Pharmaceutical-chemical Department, University of Messina, Italy,

²Department of Organic Chemistry and Biochemistry, University of Messina, Italy.

Introduction

Food products are very complex mixtures that contain many nutrients of organic (lipids, carbohydrates, proteins, vitamins) and inorganic (water, minerals, oxygen) nature.

In addition to natural constituents, food products may contain xenobiotic substances that can come from technological processes, agrochemical treatments or packaging materials (residues of pesticides, drugs, toxins, mutagenic compounds, migrants from packaging, metals and inorganic compounds of toxicological concern).

The analysis of food products may be directed to the assessment of food quality and authenticity, the control of technological processes, the determination of nutritional value and the detection of molecules with a possible beneficial effect on human health.

One of the most important research areas concerning food proteins and peptides is to establish a relationship between the structure and functionality of these compounds.

Analytical methods allow the qualitative and quantitative determination of the main components of food products but can also be selective and sensitive enough to permit the detection of minor components, often very important for the assessment of quality and detection of adulteration. Moreover, for the analysis of xenobiotic substances, strict regulation of maximum residue limits (MRLs) has been set for most food products, and analytical methods must allow an accurate determination of analytes at trace level.

In the last decades, chromatography (gas chromatography (GC), high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC)), and capillary electrophoresis (CE) have been largely used in the analysis of food products, ranging from naturally occurring components to xenobiotics. Because of the complexity of food matrices, a sample preparation step is often required, which is sometimes replaced by on-line coupled techniques.

Detectors usually used in combination with chromatographic techniques (GC and HPLC) may be more or less selective and

sensitive, but lack information about the identity of compounds. The coupling of chromatographic techniques and mass spectrometry (MS) overcomes this drawback. MS is a sensitive and selective detector and sometimes preparation steps can be avoided.

GC–MS is a robust technique, used routinely in many laboratories for food analysis; for example for the determination of aroma compounds, pesticide analysis, and more generally, for the analysis of non-polar and semi-polar, volatile and semi-volatile components of food.

More recently, LC–MS, thanks to the development of atmospheric pressure ionization (API) techniques, on quadrupole, magnetic sector or time-of-flight (TOF) instruments, has also had a great expansion into the field of food analysis. Among API-based interface systems, electrospray (ESI) and heated nebulizer atmospheric pressure chemical ionization (HN-APCI) complement one another well with regard to polarity, molecular mass of analytes and chromatographic conditions. Both techniques produce soft ionization, but additional fragmentation can be achieved by performing in-source collision induced dissociation (CID), or by using a tandem MS (MS–MS) or ion trap (IT) instrument.

ESI is useful for polar and ionic solutes ranging in molecular weight from 100 to 150×10^3 dalton. APCI is applicable to non-polar and medium polarity molecules with a molecular weight from 100 to 2000 dalton.

Although the choice of the right interface, as well as the detection polarity are based mostly on the compounds polarity and the HPLC operating conditions, many classes of compounds can give good response with both ionization techniques, sometimes both in positive and negative mode, while for others the choice of one operation mode is obligatory.¹

The number of applications of HPLC–API–MS to food analysis has rapidly increased in recent years. ESI is much more widespread than APCI, but for both techniques the trend is towards an increase in the number of applications.

In the framework of this guide, it is impossible to describe all the recent applications developed in the field of food analysis. For a more extensive description of LC–MS applications to food-related analysis, the reader may refer to many review papers, some of them dedicated to specific areas, such as pesticides² or protein analysis,^{3,4} and others on applications of MS-based techniques for the analysis of compounds of food concern in general.^{5,6}



Successful use of LC–MS in food-related analysis can be found in every area. In this section, a brief overview of the most recent advances in the analysis of the main constituents of food as well as of xenobiotics will be given, with examples of applications developed in the laboratories of the authors.

Natural Substances in Food

Peptides and proteins: Proteins are very important constituents of food, for both their nutritional and functional values. It is also believed that bioactive peptides in the primary sequences make proteins potential health-promoting ingredients.

One of the most important research areas concerning food proteins and peptides is to establish a relationship between the structure and functionality of these compounds. For this purpose, many studies have been directed towards a better structural characterization of food proteins. In food proteins analysis, LC is the most widely used technique for analytical and preparative separations, commonly coupled with UV and fluorescence detectors. On-line LC–MS analysis of food proteins is mainly performed using an ESI interface and a quadrupole mass spectrometer. In fact, the electrospray process produces multiply charged molecular species from proteins because of the presence of many charged amino acid residues.

The potential of HPLC–ESI–MS can be extended by using ‘CID–in source’ (on a single quadrupole mass spectrometer), or using triple quadrupole or ion trap instruments. With these techniques, in addition to determining the mass of food proteins, structural information, post-translational or chemically induced modifications can be determined.

For protein analysis, a new ionization technique, called matrix-assisted laser desorption ionization (MALDI), used in combination with a TOF mass spectrometer, greatly supports research in this area. The MALDI technique was introduced in 1987 by Karos et al.,⁷ and until now has not been routinely coupled on-line with HPLC.

Numerous review articles have demonstrated the capabilities of ESI–MS (both on-line and off-line coupled with HPLC) and MALDI–TOF–MS for the structural characterization of proteins with particular regard to their advantages in terms of sensitivity, mass accuracy and short analysis time.^{3,4}

HPLC–ESI–MS and HPLC–ESI–MS–MS have recently been used for the characterization of low-molecular-mass peptides, respectively for the identification of oligopeptides in Parma ham involved in the development of bitter taste,⁸ and for the separation and identification of low-molecular-mass peptides

released during milk sterilization.⁹ Other studies concern the characterization of major ewe milk proteins by HPLC–ESI–MS and ESI–MS under flow-injection conditions after purification by fast protein LC.¹⁰

Higher resolution can be obtained using a TOF analyser as proved by the determination of modified whey protein by reversed-phase (RP)–LC–ESI–TOF–MS.¹¹ The accuracy of the value calculated from the sequence was better than 0.01%, demonstrating superior performance of TOF to any other mass analysers for proteins.

As an example of the power of MALDI–TOF–MS in protein analysis, the determination of milk authenticity is very rapid and accurate. MALDI–TOF–MS allowed the detection of cow milk in raw ewe and buffalo milk samples and the addition of powdered milk to fresh raw milk samples.¹² Mass accuracy was very high (0.5–1%), time of analysis was very short (100 analyses in one hour) and sensitivity was very good (small amounts of cow milk added to ewe milk and to buffalo milk were detected by evaluating the protein patterns coming from the most abundant whey proteins, α -lactalbumins and β -lactoglobulins).

Lipids

Lipid analysis by HPLC–MS techniques involves the characterization of triglycerides (TAGs), sterols, carotenoids and phospholipids. These molecules are not volatile and therefore not amenable to direct GC analysis. In the analysis of TAGs, once the fatty acid composition of a determined fat or oil is clear (mainly determined by GC analysis of fatty acid methyl esters (FAMES) obtained after trans-methylation), the knowledge of how these fatty acids are distributed within the glycerol molecule is of major interest, with regard to nutritional function, quality control, technological characteristics and authenticity establishment. HPLC–MS with an APCI interface can give very useful information, not only on which fatty acids are present in a TAG molecule, but also on the specific distribution of fatty acids in a TAG, traditionally determined by enzymatic hydrolysis procedures followed by chromatographic analysis.

Figure 1 shows the HPLC–APCI–MS chromatogram obtained for a peanut oil using a C18 column under isocratic conditions (aceton:acetonitrile, 7:3). Under these conditions, the TAGs are separated on the basis of their partition number, PN (PN = CN – 2 DB, where CN is the number of carbons and DB the number of double bonds). Peak identification can be performed by combining PN values with the information from the MS spectrum. Table 1 reports peak identification for the oil analysed. APCI in positive mode produces a $[M+H]^+$ signal and fragments related to the fatty acids linked to the glycerol backbone $[M-(R-COO)]^+$ ($[DG]^+$). The intensity of the $[M+H]^+$ is related to the degree of unsaturation of the molecule: the higher is the degree of unsaturation, the more intense is the pseudomolecular ion. Regarding the $[DG]^+$ ions, their intensity changes mainly in relation to the position occupied by the fatty acid that is removed. The less abundant $[DG]^+$ ion corresponds to the loss of the fatty acid from the *sn*-2 position because this is energetically less favourable than losing a fatty acid from the *sn*-1 or *sn*-3 position. In difunctional TAGs, positional isomers can be distinguished measuring the relative abundance of $[DG]^+$ ions formed. For example, for $[OO]^+/[OP]^+$ in the case of OPO is 4.5 while in the case of POO is 1.6. Mixtures of OPO and OOP at different ratios

show a linear behaviour of the relative abundances of the ions corresponding to the two diglycerides, as can be seen in Figure 2.

With real samples, positional isomers are coeluted in HPLC on C18 columns, and the commercial standards are rarely available. It is well known that non-random distribution of fatty acids in the three positions of glycerol is evident for fats and oils of vegetable and animal origin. Moreover, the distribution is strongly species specific. For example, seed oils tend to have polyunsaturated fatty acids in the *sn*-2 position, in beef tallow about 50% of the fatty acids in the *sn*-2 position are oleic acid, while lard is an animal depot fat with a strong predominance of saturated fatty acids in the *sn*-2 position.

As an example, HPLC-APCI-MS can be used to detect the presence of tallow in lard, as shown in Figure 3 and Table 2.¹³

Figure 1: RP-HPLC-APCI-MS analysis of triglycerides in peanut oil.

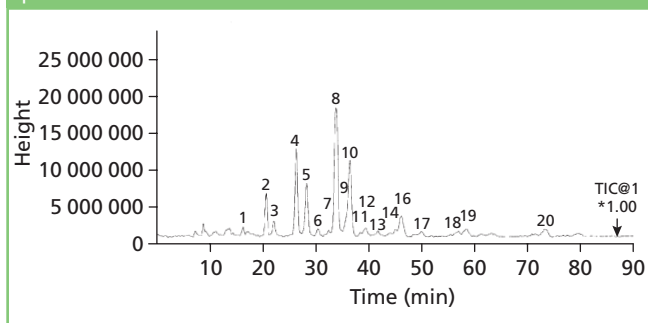


Table 1: Triglycerides identified in peanut oil by RP-HPLC-APCI-MS.

Peak	TAG	DB	CN	NP	[M+H] ⁺	[DG] ⁺	[DG] ⁺	[DG] ⁺
1	LLL	6	54	42	880	LL 600		
2	LLO	5	54	44	882	LL 600	LO 602	
3	LLP	4	52	44	856	LL 600	LP 576	
4	OLO	4	54	46	884	OO 604	OL 602	
5	PLO	3	52	46	858	LP 576	LO 602	PO 578
6	PLP	2	50	46	832	PP 552	LP 576	
7	GOL	4	56	48	912	GO 632	LO 602	GL 630
8	OOO	3	54	48	886	OO 604		
9	SOL	3	54	48	886	LO 602	SL 604	SO 606
10	POO	2	52	48	860	PO 578	OO 604	
11	PSL	2	52	48	860	LP 576	SL 604	SP 580
12	POP	1	50	48	834	PO 578	PP 552	
13	OOG	3	56	50	914	OO 604	OG 632	
14	BLL	4	58	50	940	BL 660	LL 600	
15	OLA	3	56	50	914	LO 602	LA 632	OA 634
16	SOO	2	54	50	888	SO 606	OO 604	
17	POS	1	52	50	862	PO 578	SO 606	PS 580
18	BLO	3	58	52	942	BL 660	LO 602	BO 662
19	AOO	2	56	52	860	AO 634	OO 604	
20	BOO	2	58	54	944	BO 662	OO 604	

TAG = triglyceride, DB = number of double bounds, CN = number of carbon atoms, NP = partition number, P = palmitic acid (C16:0), S = stearic acid (C18:0), A = arachidic acid (C20:0), B = behenic acid (C22:0), O = oleic acid (C18:1, Δ⁹), L = linoleic acid (C18:2, Δ^{9,12}), G = gadoleic acid (C20:1, Δ⁹).

Biologically Active Molecules

It has been demonstrated that some health benefits of food and beverages may depend on the presence of antioxidants, as in olives and olive oil, red wine, berries, fruits and vegetables in general. Compounds with antioxidant activity include phenolic and polyphenolic compounds. The most commonly occurring phenolic compounds in food are simple phenols, phenolic acids and flavonoids.

Because of increased interest in the biologically active compounds in food, many research studies have dealt with detection and quantification of antioxidants. LC-MS is of great help in these instances, because of the complexity of the samples to be analysed and the different distribution of antioxidants in food. Before the widespread use of LC-MS, LC-UV was the technique of choice for polyphenols and flavonoids. Sometimes a photodiode array detector (PDA) was used, but the similarity of the UV spectra and the lack of commercial standards often rendered accurate identification difficult.

RP-HPLC-ESI-MS has been used for more polar phenolic compounds, such as oleuropein and phenolic acids, in olives and olive oil. Although both positive and negative ion analysis are complementary, the latter showed better sensitivity and selectivity for acidic and phenolic compounds.

HPLC-ESI-MS has also been used for many classes of flavonoids, such as flavan-3-ols (catechins), flavanones and anthocyanins.

Because of the limited amount of solvent that can be tolerated for a good efficiency of the ionization process in ESI,

For real complex samples the need for efficient sample purification should not be underestimated.

a splitter is commonly incorporated when conventional HPLC columns are used. The development of small-bore and capillary LC columns allows one to decrease and even suppress the splitter, and increase the sensitivity because of reduced chromatographic dilution.

Figure 4 shows a micro-HPLC–ESI-MS chromatogram of the anthocyanin fraction of a blood orange juice, obtained using a C18 column (150 × 1 mm i.d., 3.5 μm d_p). Elution was achieved using a binary high-pressure gradient at a flow-rate of 40 μL/min. Solvent A was H₂O/HCOOH (9:1) and solvent B was H₂O/HCOOH/CH₃CN (4:1:5). The percentage of solvent B was increased linearly, after an initial hold of 1 min, from 12 to 30% in 25 min; then to 100% in an additional 9 min. ESI was performed in positive mode. For all components ESI ionization allows detection of the molecular ion. Using a single quadrupole, structural information can be obtained by CID-in source, which permits fragmentation between the sugar moiety and the aglycon. From the combination of m/z values obtained for the two analyses, identification of unknown components is possible, as can be seen from the example reported in Table 3. Additional help is available from the analysis of the anthocyanin fraction of several matrices, under the same experimental conditions, and the construction of a homemade library of MS spectra. Although only few anthocyanin standards are available, isomers with the same molecular weight can be distinguished using the information from the MS spectrum in combination with the knowledge of their elution order on C18 columns.¹⁴

Because of the absence or low level of fragmentation, ESI is sometimes used for rapid screening of molecular species present in a complex matrix without chromatographic separation.¹⁵ In particular, MS–MS is considered to be a powerful tool for fast identification, without pre-separation or with low separation efficiency, because of its high selectivity. However, for real complex samples the need for efficient sample purification should not be underestimated. LC pre-separation before MS for

the analysis of complex natural mixtures, such as food samples is very important, as shown in the example reported in Figure 5, in which the ESI-MS analyses of the anthocyanin fraction of a bilberry extract with¹⁶ and without¹⁵ LC pre-separation are compared.

Recently the use of MALDI TOF-MS for the analysis of anthocyanins in wine and fruit juice samples after extraction using a Sep-pak cartridge from a diluted, and in the case of wine de-alcoholized, sample, has been demonstrated.¹⁷

Figure 2: Ratio between [OO]⁺ and [OP]⁺ for mixtures of OPO and OOP at different percentages.

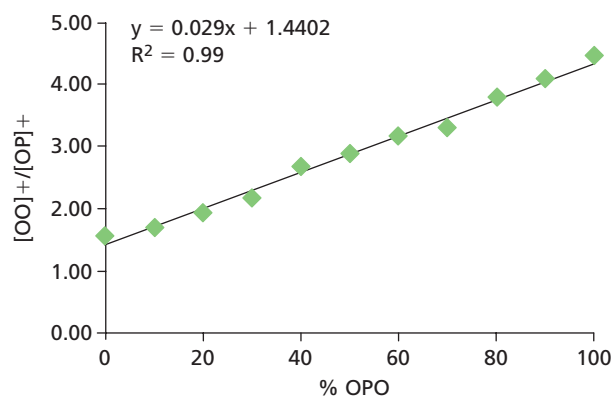


Figure 3: Spectra of POO obtained by RP-HPLC–APCI-MS analysis of (a) lard and (b) tallow.

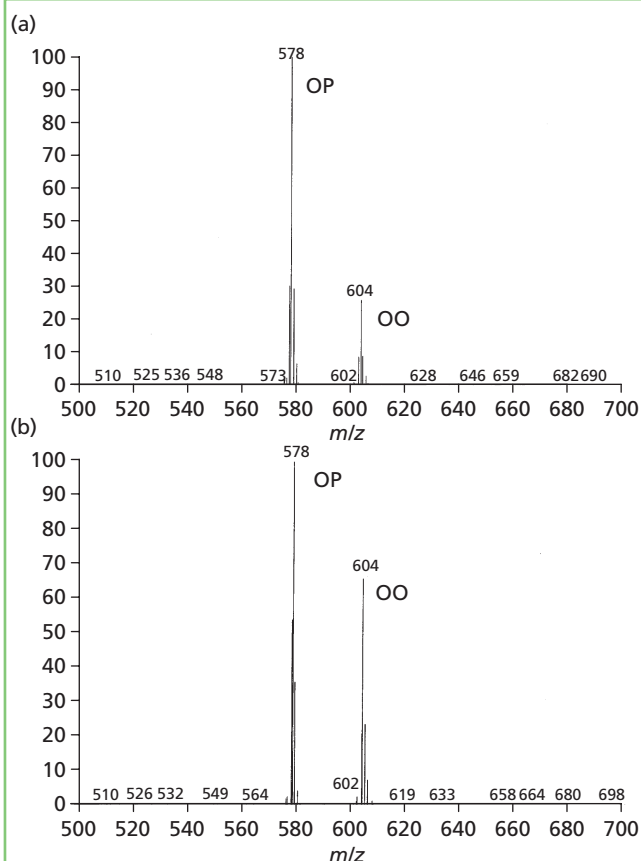


Table 2: Ratio between the positional isomers of OOP in lard, tallow and mixtures of these two animal fats.

	OOP + OPO	OOP : OPO calculated*	OOP:OPO found**
Lard	31%	-	20:80
Tallow	32%	-	100:0
Lard:Tallow 90:10		28:72	30:70
Lard:Tallow 50:50		61:39	60:40
Lard:Tallow 10:90		92:8	91:9

* POO = 20 × (%lard × 0.31) + 100 × (%tallow × 0.32)

OPO = 80 × (%lard × 0.31)

%POO = [POO/(POO + OPO)] × 100

%OPO = [OPO/(POO + OPO)] × 100

**from equation of Figure 2.

Polymethoxylated flavones are present in the peel of some citrus fruits including oranges and mandarins. Their analysis can be important for the detection of quality parameters for citrus peel oils. They can be analysed both in normal- and reversed-phase HPLC, and ionized with both ESI and APCI interfaces in positive mode, giving a better response when APCI is used.

For example, the six polymethoxylated flavones present in orange peel oil, the structures of which are shown in Figure 6, differ only in the number and position of methoxy-substituents. Although the structures are very similar, they give

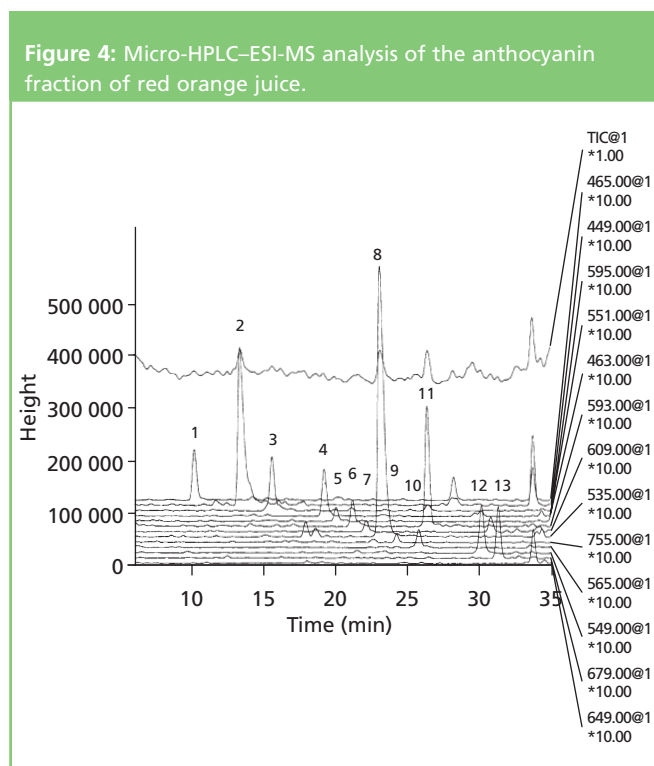


Table 3: Anthocyanins identified in red orange juice analysed by micro-HPLC–ESI–MS.

Peak	Name	RRT	[M] ⁺ (40V)	[A] ⁺ (55V)
1	Dp-3-glucoside	0.76	465	303
2	Cy-3-glucoside	1.00	449	287
3	Cy-3-rutinoside	1.13	595	287
4	Dp-3-(6"-malonyl)-glucoside	1.44	551	303
5	Pn-3-glu	1.49	463	301
6	Cy-?	1.59	593	287
7	Dp- ?	1.66	609	303
8	Cy-3-(6"-malonyl)-glucoside	1.73	535	287
9	Cy- ?	1.80	755	287
10	Pt-3-(6"-malonyl)-glucoside	1.93	565	317
11	Cy- ?	1.98	593	287
12	Pn-3-(6"-malonyl)-glucoside	2.25	549	301
13	Cy-?	2.34	679	287

Dp = delphinidin, Cy = cyanidin, Pn = peonidin, Pt = petunidin.

a different response in APCI depending on whether position 3 is methoxylated or not. In particular, the response is lower for those flavones that have a methoxy group at position 3, as can be observed from the chromatogram in Figure 6 and quantitative data obtained. The same behaviour is observed when UV or PDA detection is used.¹⁸

This is again a problem when quantitative analysis must be performed because of a lack of standards in the field of natural food components.

Other classes of natural components widespread in the vegetal kingdom are coumarins (2-H-1-benzopyran-2-ones) and psoralens (furanocoumarins). These compounds, present in the non-volatile fraction of citrus peel oils, are very important for quality and authenticity assessment. They are odour fixative, and recently interest in their pharmacological and toxicological activities has increased. Coumarins and psoralens are quite small and low-polarity molecules. They can be substituted with hydroxyl, methoxyl, isopentenyl or isopentenylloxyl, geranyl or geranyloxyl groups, usually at positions 5, 7 or 8. For the HPLC–MS analysis of these components, all different modes have been evaluated and ionization does not occur for most of them. A few are ionized using APCI in positive mode, with little sensitivity. It has been observed that the MS response varies in accordance with the position and the nature of the substituent, becoming lower with increasing size of the substituent and when it is located at position 8.¹⁸ In this instance, atmospheric pressure photoionization (APPI) technique can be applied successfully to coumarins and psoralens, giving a good answer for all analytes.¹⁹

Xenobiotic Substances

In the field of food analysis, LC–MS is becoming a very important technique for the determination of xenobiotics mainly because these analytes are present in very small amounts in matrices that are very complex. Moreover, because these xenobiotic substances are often dangerous to human health, ever-lower detection limits are required by regulatory bodies.

Xenobiotic substances may be of different origin, including

- pesticides and veterinary drugs
- toxins
- migrants from packaging and other substances derived from technological processes.

The analysis of these substances requires efficient sample preparation, and trace-level detection and identification.

Pesticides: Although GC in combination with sensitive and selective detection systems such as MSD, atomic emission detection (AED), electron capture detection (ECD), nitrogen-phosphorous detection (NPD) etc. is the technique most commonly used for analysing pesticides in agricultural food products, the use of LC is increasing. In fact, many pesticides are not amenable to GC analysis because of their thermal instability and polarity. Most LC-based methods use conventional detection techniques, such as UV, DAD, fluorescence or electrochemical detection, occasionally combined with postcolumn treatments. These detectors are often not sufficiently selective and sensitive, and cannot be used for multiresidue screening.

LC–MS, and especially LC–MS–MS, can overcome the problems of lack of sensitivity and selectivity presented by the other LC detectors.

Thermally labile pesticides, such as phenylurea herbicides, carbamates, organophosphorous pesticides and ionic pesticides,

have all been determined by LC–MS, mainly using API.² API interfacing techniques have been demonstrated to be invaluable tools for identification of low levels of chemically distinct pesticides in fruit and vegetables. For quantitative analysis, many validated procedures have shown that limits of detection (LOD) lower than the lowest MRL set by the European Union (EU) or the Food and Drug Administration (FDA), can be obtained.^{2,6}

Veterinary drugs: Veterinary drugs, such as antibiotics, are not suitable for direct GC and GC–MS analysis, and need extensive clean-up and time-consuming procedures prior to capillary GC. Numerous commercial test kits have been developed to check the compliance of residue levels of antibiotics in food products at legislative levels based on microbiological assay. These methods are time consuming, complicated and non-specific. As for pesticide residue analysis, HPLC with conventional detection systems, such as UV or fluorescence, presents some drawbacks. As an example, HPLC methods for the determination of the sulphonamide antibacterials require pre- or postcolumn derivatization using fluorescence reagents, and complicated procedures. For some matrices, such as animal liver and kidney, HPLC–UV cannot be used because of severe matrix influences.

LC–MS-based techniques have recently been used for the analysis of many classes of antibiotics used as veterinary

medicines (tetracyclines, sulphonamides, β -lactam antibiotics, such as cephalosporins and penicillins), as well as for the analysis of other drug residues such as growth promoters (β -antagonists, zeranol and trombolone acetate).

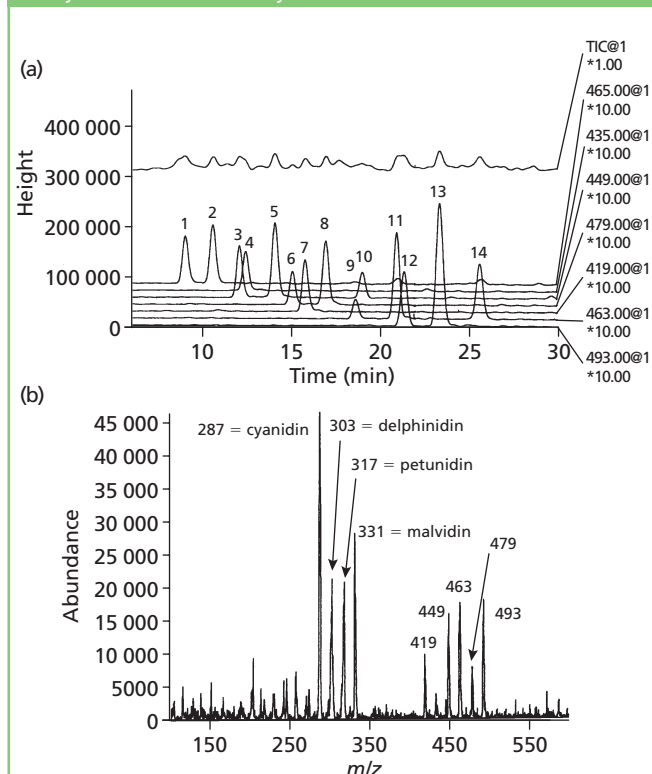
As is the situation for pesticide residues, the most common LC–MS approach for veterinary drugs is based on API, with both APCI and ESI interfaces being used. LC–MS and LC–MS–MS are preceded by sample pretreatment and the HPLC conditions must be modified for LC–MS analysis because of the presence of non-volatile salts in the mobile phase. Sometimes this limit can be overcome; for example, reversed-phase chromatography using a C8 column and a mobile phase containing oxalic acid has been proved to give the best separation of tetracyclines and co-eluting substances. By operating the APCI interface at 475 °C, clogging was not observed because oxalic acid decomposes to CO₂ and H₂O at high temperature.²⁰

Future Trends

LC–MS, mainly using API interfaces, is a powerful tool in the field of food analysis. It provides the advantages of chromatography as a separation technique and those of mass spectrometry for the unambiguous identification of non-volatile and ionic substances, and quantification of compounds in complex matrices.

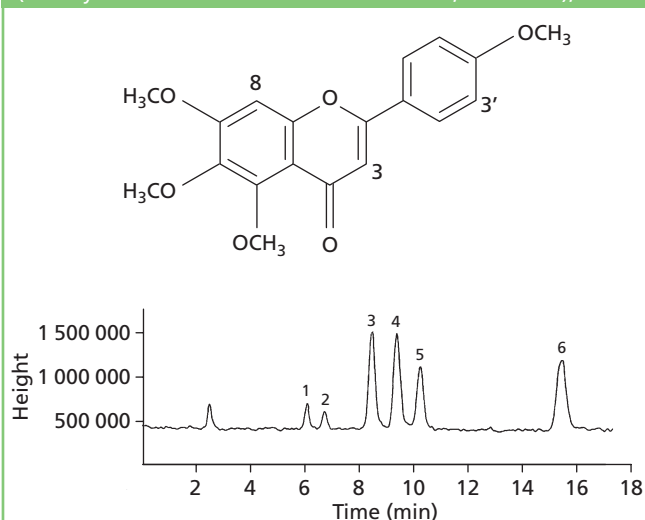
Although a mass spectrometer is a more expensive and complex option than other LC detectors, its use can overcome many other drawbacks: LC–MS and particularly LC–MS–MS

Figure 5: (a) Micro-HPLC–ESI–MS and (b) flow-injection ESI–MS analysis of a black bilberry extract.



Peaks: 1 = Dp-3-gal (MW 465), 2 = Dp-3-glu (MW 465), 3 = Cy-3-gal (MW 449), 4 = Dp-3-ara (MW 435), 5 = Cy-3-glu (MW 449), 6 = Pt-3-gal (MW 479), 7 = Cy-3-ara (MW 419), 8 = Pt-3-glu (MW 479), 9 = Pn-3-gal (MW 463), 10 = Pt-3-ara (MW 449), 11 = Pn-3-glu (MW 463), 12 = Mv-3-gal (MW 493), 13 = Mv-3-glu (MW 493), 14 = Mv-3-ara (MW 463).

Figure 6: RP–HPLC–APCI–MS analysis of the polymethoxylated flavones from a sweet orange essential oil. Column = C18, 250 × 4.6 mm i.d., d_p 5 μm, isocratic elution at 1 mL/min (tetrahydrofuran:acetonitrile:methanol:water, 15/5/22/58), APCI⁺.



Peak	Area % non-corrected	Area % corrected
1 = Sinensetin (3',4',5,6,7-pentaMF)	3.9	3.5
2 = 3,3',4',5,6,7-HexaMF	3.3	4.5
3 = Nobiletin (3',4',5,6,7,8-hexaMF)	25.5	20.2
4 = 3,3',4',5,6,7,8-HeptaMF	25.6	31.9
5 = Tetra-O-methylscutellarein (4',5,6,7-tetraMF)	15.6	14.63
6 = Tangeretin (4',5,6,7,8-pentaMF)	26.1	23.3

greatly simplify sample pretreatment procedures before the LC run, reducing not only the total analysis time but also the method development time.

LC-MS applications in food analysis are rapidly increasing. One of the most important areas of use is represented by the analysis of xenobiotics in food, in which very low detection limits are required. In this field, the development of analytical validated LC-MS and LC-MS-MS methods is increasing.

The future development of LC-MS in food analysis is mainly linked to the increasing use of automatic on-line sample pretreatment and on the development of new sample clean-up procedures. It is also linked to the development of LC in terms of miniaturization (column i.d., particle diameters) and research in the field of new stationary phases. Very important will also be the use of multidimensional (LC-LC) or orthogonal (LC×LC) chromatographic techniques, that will greatly improve method efficiency.

Even though the two ion sources APCI and ESI cover almost entirely the area of non-volatile compounds, development of the APPI interface will prove very useful for very non-polar analytes and for some classes of compounds not ionizable by APCI and ESI techniques.

The development of new MS interfaces will both influence and expand the number of LC-MS applications in food analysis. As an example, MALDI TOF-MS presents some advantages as it can measure the mass of almost any molecules (the limitation regards the TOF analyser), is fast once the sample has been adequately prepared and uses a very small amount of sample solution. MALDI is tolerant of impurities making the direct analysis of simple food extracts possible and permits quantitative analysis using an internal standard.²¹ Even though, at the moment, it is mainly used for large biomolecules such as proteins, the applications of MALDI TOF-MS to other fields of food analysis are starting to appear.

Another important area is represented by the analysis of metals in food and beverages, taking into account their essentiality and toxicity. In fact, the toxicity of a metal can be influenced by numerous factors: age of the subject, type of diet, chemical form of the element, its solubility in fats, and also the contemporary exposure to many metals.

ICP (inductively coupled plasma)-MS is considered the method of choice for trace metal analysis.²² Hyphenation of HPLC with ICP-MS allows elemental speciation analysis with high selectivity and sensitivity.

The general opinion is that LC-MS will complement GC-MS in analytical laboratories in only a few years. The wide applicability of API techniques and the great versatility of ESI and APCI interfaces in the ionizations of analytes for giving molecular weight information, and possible additional fragmentation has been widely demonstrated. The limitation of LC-MS, compared to GC-MS with electron impact ionization is that the spectrum obtained is dependant strictly on the ionization mechanism used. In this way commercial libraries of mass spectra for LC-MS instruments are still not available. However, one can build a customized library in the laboratory, dedicated to relevant compounds of interest (pesticides, veterinary drugs, etc.). This is particularly difficult in the situation of natural compounds for which commercial standards are rarely available. This means that if LC-MS information is not sufficient for structure elucidation, long procedures for the isolation of the analyte from the matrix can

be necessary. In these instances, the use of on-line coupled micro-HPLC-NMR-MS systems will be of great value.

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Luigi Mondello is professor at the Pharmaceutical-chemical Department of the University of Messina, Italy. He received a degree in chemistry from the University of Messina in 1991 and teaches the analytical chemistry and food chemistry courses at the School of Pharmacy, University of Messina and the food chemistry course at the Campus Biomedico in Rome. He is the author of approximately 90 scientific papers, 11 book chapters, 2 reviews, co-editor of a book on multidimensional chromatography (Wiley), and he has been chairman and invited lecturer in national and international congresses and meetings. His research interests include chromatography techniques (HRGC, HPLC, HRGC/MS, HPLC/MS, OPLC) and the development of coupled techniques such as LC-GC-MS, GC-GC, orthogonal GC, LC-LC and their applications in the study of natural complex matrices.

Giovanni Dugo, a graduate in chemistry, has a PhD in the chemistry of natural organic molecules and is now a full professor of food chemistry at the University of Messina, Italy. He is the author of around 200 scientific publications and his main research interests cover the fields of food fats, citrus products and the development of innovative chromatographic techniques and methods for the study of complex matrices.

Paola Dugo has been an associate professor of food chemistry at the University of Messina, Italy since 2000. She received a degree in chemistry from the University of Messina in 1991 and a PhD in Pharmacognosy from the University of Messina in 1996. Her research interests include the study of the composition of citrus essential oils and the study of components with possible biological activity in natural matrices by chromatographic techniques. She is the author of approximately 70 scientific papers and book chapters, and has been a lecturer at national and international congresses and symposiums.