Review

Applications of liquid chromatography–mass spectrometry interfacing systems in food analysis: pesticide, drug and toxic substance residues

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Abstract

This paper reviews applications of different LC–MS techniques for the determination of xenobiotic substances in foods. Specific examples of contaminants discussed are pesticides, herbicides, insecticides and drugs; concerning toxic substances, mycotoxins, phycotoxins, cyanobacterial toxins, mutagenic and heterocyclic amines and β-carbolines, arsenic, tin and inorganic halogen compounds, packaging materials and various epoxy resins are considered. Advantages and limitations are outlined for the different LC–MS interfacing systems (particle beam, thermospray, atmospheric pressure ionization with electrospray, ionspray and heated pneumatic nebulizer). The impact of developments in instrumental analysis on methodology and the limitations of the various LC–MS methods are discussed. Further, the coupling of LC with element-selective detection systems such as inductively coupled plasma mass spectrometry is discussed, with emphasis on speciation of trace toxic elements in foods.

Keywords: Reviews; Liquid chromatography–mass spectrometry; Food analysis; Interfaces, LC–MS; Pesticides; Drugs; Toxins; Amines; Carbolines; Epoxy resins; Halogen-containing compounds, inorganic; Arsenic; Tin; Mycotoxins

Contents

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1. Introduction

On-line liquid chromatography–mass spectrometry (LC–MS) has been the object of fervent research since the first studies of Tal'roze et al. in 1969 [1] and has developed significantly in the last decade.

GC–MS is extensively applied in both environmental and food analysis. However, many of the analytes, such as pesticides, drugs and toxic substances, are not suitable for GC analysis, as a consequence of their polarity, thermal lability or low volatility. Hence LC has acquired a role of growing importance in food analysis, as is attested by the wide variety of applications reported in recent years. Concerning the LC–MS technique, the advantages lie in a combination of the separation capabilities of LC and the power of MS as an identification and confirmation method. Using conventional LC detectors, detection may be complicated by false-positive results. In such cases, mass spectrometry has proved to be a valuable technique for the unambiguous identification of contaminants in food.

Problems in LC–MS coupling have been addressed by the design of interfacing devices having different strategies. For successful LC–MS applications, new ionization techniques capable of producing ions from highly polar, ionic, heat-sensitive and high-molecular-mass substances were developed. The potential of using the LC mobile phase in interface development and in analyte ionization was recognized by Arpino and Guiochon [2], who reviewed some liquid-based ionization methods such as thermospray (TSP), electrospray (ESP) and fast atom bombardment (FAB); a mechanism common to these interfaces was shown to be the desorption of preformed ions from the liquid phase. An alternative approach was the development of analyte-enrichment interfacing systems, one of the features of these LC–MS interfaces being a free choice of the ionization method independent of LC mobile phase composition. Among the various LC–MS interfaces designed for achieving solute enrichment, moving belt (MB) and particle beam (PB) have been the most successful systems; with both the interfaces conventional EI and CI mass spectra can be obtained, since the mobile phase solvent vapours are removed prior to introduction of the analyte molecules into the ion source. The MB interface nowadays is rarely used, and since no work on xenobiotic substances in food analysis has been reported, MB will not be discussed further.

The particle beam interface derives from the original MAGIC system (monodisperse aerosol generating interface for chromatography) [3], which has been improved by Winkler et al. [4] in redesigning the nebulizer and the momentum separator; with this interface, a concentric pneumatic nebulizer is used and LC flow-rates between 0.1 and 1 ml/min can be applied. Slightly different interfacing systems based on the MAGIC approach have also been created, i.e. the thermabeam interface, in which the nebulization of the column effluent is thermally aided [5], and the universal interface, in which a thermospray nebulizer and a solvent-removing membrane separator between the desolvation chamber and the momentum separator are used [6]. Limitations of the LC–PB–MS system, as compared with other LC–MS interfaces, include lower sensitivity, lower response with high water content mobile phases and difficulty of quantification; in addition, a certain volatility of the analytes is required. One of the effects which can cause a reduction in sensitivity is chromatographic band broadening during transmission through the interface [7]. Non-linearity effects at low analyte concentrations were first described by Bellar et al. [8] and also observed by McLaughlin et al. [9] and Kim et al. [10]. Although this behaviour has been studied by various workers [11–13], it remains not understood. Bellar et al. [8] also reported PB-MS signal enhancement through the addition of a "carrier" to the mobile phase, a phenomenon known as a "carrier effect". Carriers such as ammonium acetate [8], phenoxyacetic acid [14], phenylurea [15], malic acid [10] or the isotopically labelled analogue of the analyte of interest [12] improve transfer efficiency through the PB interface, resulting in lower detection limits and restoration of calibration graphs to linearity. Recently, a micro-aerosol has been designed to interface the liquid effluent originating from a packed LC capillary column and a quadrupole mass spectrometer [16]. This modified version of a PB interface can handle very low eluent flow-rates, of the order of 1 μl/min, offering several advantages in the interface functioning, i.e., insignificant contami-nation by solvent vapours and LC buffers of the vacuum system, the ion source and the analyser. Because of the large reduction in the solvent intake, greater overall sensitivity and better response for mobile phases with high water content have been observed, especially during gradient analysis [17,18]. The PB interfacing system has recently been reviewed by Creaser and Stygall [19], who discussed the interface design and various aspects of quantitative analysis.

Among the different methods, the thermospray (TSP) interfacing system [20] has been one of the most widely applied, but, as a consequence of the introduction of more robust techniques based on atmospheric pressure ionization, the use of this interface is slowly decreasing. TSP is typically used with reversed-phase columns and volatile buffers; ionization may be effected with the use of buffer ions or it may be aided by a filament or a discharge. The buffer ionization mode often yields mass spectra with little structural information; in order to overcome this problem, the other ionization modes or tandem MS have been applied [21]. The processes involved in filament or discharge assisted ionization have a strong resemblance to chemical ionization, and solvent-mediated CI spectra are obtained; these external ionization modes must be used when operating in absence of a buffer, with non-aqueous eluents. With ionic analytes, the mechanism of ion evaporation, as first described by Iribarne and co-workers [22,23], is supposed to be primarily operative; in ion evaporation and also in buffer-assisted ionization, ions are produced spontaneously from the mobile phase. The thermospray approach shows a number of experimental problems, including a wide variation in response to different analytes, the need for a critical control of relevant temperatures during analysis, thermal breakdown of some labile analytes and the lack of structurally informative mass spectra. TSP is typically combined with quadrupole MS instruments, but coupling of TSP to a quadrupole ion trap [24] and to a magnetic sector instrument have also been reported [25,26]. LC–TSP-MS has been

applied in both the qualitative and quantitative analysis of a wide variety of compounds, such as pesticides, surfactants, drugs, steroids, alkaloids and other natural products [21]. LC–TSP-MS has recently been reviewed by Arpino [27,28].

An alternative LC–MS approach involves the use of atmospheric pressure ionization (API) techniques [29–31], which rely upon the formation of ions at atmospheric pressure with some practical benefits for coupling MS with on-line separation systems; in fact, ionization at atmospheric pressure prevents the problem of vaporized solvent entering the vacuum system of the mass spectrometer. A discussion on instrumentation, vacuum system design and ion sources in API systems is given in a recent review of Bruins [32]. API is involved in both electrospray (ESP) and in the closely related ionspray (ISP) techniques. Electrospray ionization [33] has emerged in the last few years and it is a widely applicable soft ionization technique [29,30]. An evaluation of the ESP technique and the instrumentation options has been performed by Voress [34] in a recent product review; he discusses the technology of first-generation ESP interfaces, in which the low-μl/min flow-rate requires capillary columns or postcolumn splitting, and of second-generation ESP interfaces, which allow higher flows by virtue of pneumatically assisted vaporization. The basic second-generation interface is the ionspray, also known as pneumatically assisted electrospray, as originally developed by Bruins et al. [35]. The most recent combination of pneumatically assisted ESP with a coaxial liquid sheath flow allows handling of flow-rates up to 2 ml/min [36,37]. This approach affords improved sensitivity, when using 4.6 mm I.D. LC columns, and the possibility of performing gradient elution [36]. In contrast to the TSP interface, both ESP and ISP interfaces tolerate small amounts of non-volatile buffers and other additives, which can be necessary for the LC separation. For ESP and ISP ionization, the analytes must be ionic or have an ionizable functional group or be able to form an ionic adduct in solution; the analytes are commonly detected as deprotonated species (e.g., $[M - H]^-$, negative ions) or as cation adducts of a proton or an alkali

metal ion (e.g., $[M + H]^+$ or $[M + Na]^+$, positive ions). The ionization of molecular ions, $M^{+\cdot}$, has been observed for some apolar compounds, with polycyclic aromatic hydrocarbons (PAHs) being an example of compounds of interest in foodstuff analysis [38,39]; this capability extends the applicability of ESP-MS to compound types not normally amenable to this technique, e.g., neutral, non-polar substances such as PAHs. Another application area of ESP-MS and ISP-MS is that of organic and inorganic salts [40–43] and organometallic compounds [44,45], even though these papers are limited to fundamental studies or environmental studies.

Pneumatic nebulization in an atmospheric pressure region is also applied in atmospheric pressure chemical ionization (APCI) systems, in which gas-phase ions are formed from a ^{63}Ni foil [46] or most commonly from a corona discharge electrode [47]; LC was first combined with APCI-MS by Horning et al. [48]. In order to combine reversed-phase LC with APCI-MS, Henion's group devised the heated nebulizer (HN) interface [49], which is now commercially available; although this system can handle pure aqueous eluents at flow-rates up to 2 ml/min in addition to involatile buffers, it is not widely used, on account of the limited commercial availability of atmospheric pressure ion sources. Although the HN interface with APCI source is relatively new and at present not widespread, the high eluent flow-rate capability, sensitivity and response linearity make this system a powerful detection tool for conventional LC; it should be noted that the applicability of APCI is restricted to the analysis of compounds with lower polarity and lower molecular mass compared with ESP and ISP. LC–APCI-MS has been demonstrated to be well suited for multi-residue pesticide analysis and for the confirmation and detection of these contaminants in environmental samples [50,51]. The versatility of APCI across chemical classes has also been demonstrated in the biological and pharmaceutical fields [51–56]. Other examples dealing with the application of this technique in food analysis will be discussed later.

As soft ionization techniques such as TSP, ESP and ISP, for which analyte ionization is possible

without a primary ionization source, produce exclusively quasi-molecular ions [27,32,57–59], more sophisticated methods, such as on-line LC–MS–MS, are required to obtain diagnostic fragment ions and, thus, analyte structure elucidation. Identification can often be achieved by using daughter ion MS–MS scans and collisionally induced dissociation (CID), most commonly on a triple-quadrupole instrument. Alternatively, the API methods allow pre-analyser CID experiments, in which fragmentation is induced in the atmospheric pressure source region by applying a voltage to one of the cones which separate the differentially pumped regions of an API source; in this way, dissociation of the quasi-molecular ion occurs and diagnostic structural information can be obtained [60,61]. It is noteworthy that LC–MS–MS methods could facilitate the identification of substances in unresolved LC peaks, since a full-scan CID mass spectrum is characteristic of the structure of the parent compound.

Finally, the coupling of chromatographic separations with element-selective detection systems such as inductively coupled plasma mass spectrometry (ICP-MS) is an attractive technique for trace element speciation. The use of an inductively coupled argon plasma as an ion source for MS was originally proposed by Houk et al. [62]. The recent books and review papers published give evidence that on-line LC–ICP-MS is a technique of growing interest in speciation studies in both the environmental and clinical areas [63–67].

A more detailed description of LC–MS interface technology is given in recent reviews by van der Greef and Niessen [68], Creaser and Stygall [19] and Niessen and Tinke [69]. Continuous flow FAB will not be considered in this review, because xenobiotic substances in foodstuffs have not been analysed using this interfacing system.

In the present overview, applications of the coupled LC–MS with different interfacing systems in food analysis are presented. Topics covered include the LC–MS analysis of pesticide, herbicide and insecticide residues in fruits and vegetables and of drug residues in animal-derived foods. The determination of toxic substances including natural toxins, inorganic ele-

ments and the analysis of packaging residues are also outlined. The survey covers the literature for approximately the last 10 years.

2. Applications

2.1. Pesticide, herbicide and insecticide residues in food

The increasing availability of LC methods for pesticide analysis is essentially the result of their applicability to thermally labile and polar compounds, which would require derivatization before GC analysis. In this respect, triazines, carbamates, phenylureas and their corresponding anilines, phosphorus pesticides, quaternary ammonium compounds and chlorinated phenoxy acids are groups of pesticides to which LC analysis is usually applied. Although UV detectors are the common choice for LC, on-line LC–MS is an interesting approach, which has gained in popularity over the last few years. This technique combines the advantages of LC and MS for the separation and unequivocal identification of pesticides in real matrices, such as foods and environmental samples. However, analyte detectability is often a serious problem in LC–PB-MS and LC–TSP-MS techniques for trace-level monitoring of pesticides. In this respect, off-line and on-line combinations of analyte trace enrichment procedures, such as solid-phase extraction, with LC–MS techniques have proved to be a valuable approach. Recently, much attention has been paid to the development and applications of these off-line and on-line set-ups, but all of these studies have been performed in samples of environmental origin [70–74]. Advantages of these automated approaches are increased sample throughput and enhanced sensitivity; using adequate on-line trace enrichment procedures, detection limits at ng/l levels were achieved for polar pesticides in water samples [71,72]. This aspect has been extensively discussed in recent reviews by Slobodnik et al. [75] and Brouwer et al. [76]. In comparison with the numerous applications of LC–MS methods for the determination of pesticide in various environmental ma-

trices [19,77–80], fewer studies have been carried out on LC–MS determinations of these contaminants in foods. In the determination of pesticide residues in foodstuffs, LC–MS has been applied to fruits, vegetables and cereals.

Fodor-Csorba [81] published a review dealing with methods for the determination of pesticide residues in foods, including supercritical fluid chromatography (SFC), GC and LC. These techniques offer the basis of various assay methods alone or coupled with very sensitive and selective detection systems such as MS.

2.1.1. Particle beam interface

Kim et al. [10] found anion-exchange LC–PB-MS to be an excellent technique for the assay of the herbicide daminozide in apple juice. The solvent system was composed of water, aqueous malic acid (2.0 mM, 35% acetonitrile) and ammonium acetate (0.10 M, pH 6). Malic acid addition (0.4 mM) to the mobile phase was found to be effective for signal enhancement in both full-scan and SIM modes (Fig. 1); with 4 mM malic acid in the mobile phase, the instru-
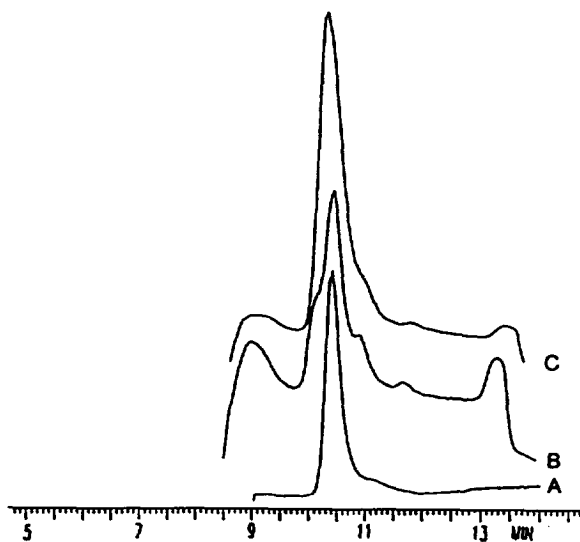

Fig. 1. Anion-exchange chromatography with particle beam mass spectrometric detection (malic acid enhancement, selected ion monitoring) of (A) a daminozide standard, (B) an apple juice sample containing 31 μg/l daminozide and (C) the same apple juice sample spiked with an equivalent amount of daminozide. Reprinted with permission from [10].

ment detection limit was estimated to be 25 μg/l in the SIM mode (Table 1), with a 31-fold enhancement compared with a solvent system without constant addition of malic acid. In addition, with 0.4 mM malic acid in the eluent, the response of daminozide was transformed into a linear relationship in the 0–100-ng range. The increase in the linearity of response and sensitivity deriving from the addition of malic acid to the mobile phase is attributable to the ability of this relatively non-volatile organic acid to function as a carrier for the analyte by forming a constant particle size.

The sensitive detection and confirmation of residues of ethylenethiourea (ETU) in a variety of important food crops have been achieved using an HPLC–PB-MS method [82]. Chromatographic separation of ETU was carried out on a multi-phase column exhibiting both anion-exchange and reversed-phase retention modes, eluting with 5% acetonitrile in water at a flow-rate of 0.25 ml/min. An increase in ETU response and an improved chromatographic peak shape were observed with increasing acetonitrile percentage in the LC eluent, so that postcolumn addition of the organic solvent at 0.2 ml/min gave a final mobile phase composition of 47% acetonitrile in water in the nebulizer. Detection was performed using EI ionization and full-scan acquisition in the mass range 70–300 u. In contrast with the observation of other workers [8], who used a different type of nebulizer, no effect on the PB-MS response for 10 ng of ethylenethiourea was observed when ammonium acetate (10–40 mM) was added to the mobile phase; in addition, it was noted that co-elution of [^{12}C]ETU with the isotopic variant of the analyte [^{13}C]ETU did not influence the molecular ion abundance for injections of 10 ng of ETU. This behaviour indicates that the carrier effect is still not understood and it is not predictable, i.e., apparently depending on the analyte, on the additive and on the interface hardware.

2.1.2. Thermospray interface

An important fundamental study of thermospray mass spectra of several pesticides representative of different classes, including carba-

Table 1
Selected LC–MS applications to pesticide, herbicide and insecticide residues in foods

Compound	Matrix	Stationary phase	Mobile phase	Interface	MS detection mode	Detection limit	Ref.
Daminozide	Apple juice	SGE 250GL	H_2O–2 mM malic acid containing 35% CH_3CN–0.1 M ammonium acetate (3:1:1); flow-rate, 0.25 ml/min	PB	PCI; SIM	0.025 μg/ml	[10]
Ethylenethiourea	Crops	OmniPac PAX 500	5% CH_3CN in H_2O; flow-rate, 0.25 ml/min	PB	EI; full-scan (70–300 u)	0.005 μg/g	[82]
Benomyl	Peaches, apples, tomatoes	Partisil 5 ODS-3	CH_3CN–0.1 M ammonium acetate buffer; flow-rate, 1 ml/min	TSP	PI, filament-on; SIM	0.025 μg/g	[84]
Linuron	Crops	5-μm Spheri-5 C_{18}	Linear gradient elution with CH_3CN–H_2O–0.013 M ammonium acetate	TSP	PI discharge; SIM	0.1–0.25 μg/g	[85]
Folpet, oryzalin			Linear gradient elution with CH_3CN–H_2O; flow-rate, 1 ml/min		NI discharge; SIM	0.025–1.0 μg/g 0.025–0.1 μg/g	
Diflubenzuron	Chillies, plums	Spherisorb ODS2	CH_3OH–H_2O containing 0.05 M ammonium acetate (75:25); flow-rate, 1.0 ml/min	TSP	PI; SIM	0.25 μg/g	[88]
Carbofuran	Potatoes	5-μm Zorbax Rx-C_{18} 5-μm Hypersil ODS	35% CH_3CN in H_2O, containing 5 mM ammonium acetate; flow-rate, 1.0 ml/min 40% CH_3CN in H_2O, containing 5 mM ammonium acetate; flow-rate, 0.4 ml/min	ISP	Full-scan (100–300 u)	0.0025 μg/g	[89]

mates, phenylureas, triazines, anilides, N-heterocyclic and organophosphorus pesticides and quaternary ammonium compounds, was carried out by Volmer et al. [83]. In this work, the dependence of the ion intensities in TSP mass spectra on the vaporizer and gas-phase temperatures under CID conditions was studied. It was demonstrated that by varying the gas-phase and vaporizer temperatures, it is possible to induce controlled chemical reactions during TSP vaporization and ionization, thus overcoming the lack of structural information typical of TSP mass spectra. In addition, techniques for enhancing the sensitivity of TSP detection were discussed for the pesticides, including the variation of the volatile salt concentration. For this purpose, 0.6 ml/min of a 150 mM buffer solution was added postcolumn to the chromatographic eluent, such that a final flow-rate of 1.2 ml/min entered the TSP interface. It was found that operating with a constant value of added salt, the detection limits are virtually independent of the gradient composition. It was observed that as the salt concentration was increased, a significant sensitivity enhancement was achieved at the cost of an increase in fragmentation.

Other examples concerning the study of the behaviour of pesticide standards or applications of these pollutants not strictly related to food analysis are exhaustively covered in recent reviews [75,79].

Determination of methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) in peaches, apples and tomatoes by reversed-phase HPLC–TSP-MS was described by Liu et al. [84]. Because of the instability of benomyl in organic solvents and in water, residues of benomyl in crops were determined by acid hydrolysis of the carbamate to the stable methyl 2-benzimidazolecarbamate (carbendazim). The mass spectrometer was operated in positive-ion, filament-on mode and with SIM of the ions at m/z 192 (carbendazim) and 134 (2-aminobenzimidazole, internal standard). The specificity of MS as a detection system allowed fast chromatography, because none of the crop coextracts provided $[M + H]^+$ ions at m/z 192 or 134. Twenty-five samples each of peaches, apples and tomatoes were assayed; benomyl was detected in numerous cases in peaches (18 positives out of 25 samples) at levels ranging from 0.25 to 3.48 μg/g; four apple samples contained benomyl at levels of 0.15–0.59 μg/g. The minimum detectable concentration in crops was estimated as 0.025 μg/g (Table 1); at the 0.01 μg/g level, recoveries of benomyl were in the range 85–110% in the three food samples analysed; it should be noted that in real samples the actual analyte, carbendazim, could derive from sources other than benomyl.

GC–MS and LC–MS analyses were performed by Mattern et al. [85] for the determination of twenty pesticides extracted from a variety of crops. LC–MS with a thermospray interface was applied for three pesticides which were too polar or heat sensitive to be assayed by GC–MS (folpet, linuron, oryzalin). After extraction, following Luke's procedure [86], the compounds were separated in the reversed-phase mode and detected by MS in the positive-ion discharge mode for linuron or the negative-ion discharge mode for folpet and oryzalin (Table 1). Detection limits were estimated to be in the 0.025–0.5 μg/g range (1.25–25 ng) in all crops analysed, except for spinach, with a calculated detection limit of 1.0 μg/g (50 ng) for folpet. Recoveries at the 0.5 μg/g level were 70–123% with an average R.S.D. of 13%.

An LC–TSP-MS approach was taken by the same research group for the multi-residue assay of non-volatile and thermally labile pesticides in fruits and vegetables, such as apples, beans, peppers, lettuce, potatoes and tomatoes [87]. The aim of this study was to develop an approach which would combine the analysis of carbamates, phenylureas and other pesticide classes into a single procedure. After a reversed-phase chromatographic separation, the mass spectrometer was operated in the positive-ion discharge mode, except for fenvalerate, folpet, iprodione and oryzalin (negative-ion mode). As is shown in Table 2, the detection limits were between 0.025 and 0.25 μg/g for the pesticides assayed in the negative-ion mode, whereas for the positive-ion mode the detection limits were in the 0.25–1.0 μg/g range; except for chlorbromuron, fenvaler-

Table 2
Limits of detection (μg/g) of pesticides in various crops determined by LC–TSP-MS[a] [87]

Pesticide	Apples	Beans	Lettuce	Peppers	Potatoes	Tomatoes
Aldicarb	1.0	1.0	1.0	1.0	1.0 (1)	1.0
Aldicarb sulfoxide	1.0	1.0	1.0	1.0	1.0	1.0
Bufencarb	0.25	0.25	0.25	0.25	0.25	0.25
Carboxin	0.25	0.25	0.25	0.25	0.25	0.25
Chlorbromuron	0.5	0.25	0.25	0.25	0.5 (0.2)	0.25
Diuron	0.25 (1)	0.25	0.25	0.25	0.25 (1)	0.25
Fenvalerate	0.05 (2)	0.05 (2)	0.05	0.05 (1)	0.025 (0.02)	0.1 (1)
Folpet	0.05 (25)	0.25	0.05 (50)	0.05	0.025	0.1 (25)
Iprodione	0.1	0.25	0.1 (15)	0.25	ND[b]	0.1
Linuron	0.25	0.25	0.25	0.25	0.25 (1)	0.25
Methiocarb	0.25	0.25	0.25	0.25	0.25	0.25
Methomyl	1.0 (1)	1.0	1.0 (5)	1.0	1.0	1.0 (1)
Metobromuron	0.25	0.25	0.25	0.25	0.25 (0.2)	0.25
Monuron	0.25	0.25	0.25	0.25	0.25	0.25
Neburon	0.25	0.25	0.25	0.25	0.25	0.25
Oryzalin	0.05	0.05	0.05	0.05	0.025 (0.05)	0.1
Oxamyl	1.0 (2)	1.0	1.0	1.0 (3)	1.0 (0.1)	1.0 (2)
Propoxur	0.25	0.25	0.25	0.25	0.25	0.25
Thiodicarb	1.0	1.0	1.0	1.0	1.0	1.0

[a] EPA tolerances in parentheses.
[b] Interference by potato constituent.

ate, metobromuron and oxamyl in potatoes, the detection limits were lower than or equal to the tolerances formulated by the US Environmental Protection Agency (EPA). Recoveries were usually in the 69–110% range, except for the N-substituted amide carboxin, for which inadequate recoveries between 33 and 54% were found. The mean R.S.D. was 9.1%.

As part of the UK pesticide residue monitoring programme, positive-ion LC–TSP-MS has been performed by Wilkins [88] for the determination of residues of the insecticide diflubenzuron in chillies and plums. With diode-array detection, problems were encountered with the assay of chilli and plum extracts, because of the presence of interfering peaks. By applying the LC–TSP-MS methodology in the SIM mode (m/z 311 and 313 ions monitored), it was proved that the insecticide was not present in the extracts analysed. In addition, UV-absorbing substances in chilli extract were identified from their TSP mass spectra as the pungent compounds capsaicin and dihydrocapsaicin. The detection limit achieved for diflubenzuron was equivalent

to 0.25 μg/g of analyte in crop (Table 1), with recoveries of 90–95%.

2.1.3. Atmospheric pressure ionization techniques

Atmospheric pressure ionization (API) techniques were investigated for the determination of N-methylcarbamate pesticides by LC–MS [50]. The study was accomplished on three representative carbamates (carbaryl, methomyl and aldicarb), spiked at the 0.1 ppm level in green peppers. A comparison of APCI, ionspray, thermospray and particle beam interfacing systems was also performed in terms of sensitivity and identification capabilities. APCI with a heated nebulizer and also ISP with pre-analyser CID and PB with CI yielded both protonated molecules and an intense fragmentation, in the positive-ion mode. Conversely, positive-ion TSP did not provide mass spectra useful for structural elucidation of the tested compounds. In addition to information-rich spectra, the results indicated that APCI-MS experiments provided the greatest sensitivity with limits of detection ranging from

0.05 to 0.18 μg/g; these values are in most cases ten times lower than those achieved by ISP and TSP. The analyte detectability was poor in the case of the PB system, the sensitivity being typically 3–4 orders of magnitude less than that obtained by APCI.

Recently, a method for on-line immunoaffinity chromatography (IAC) with coupled-column LC–MS has been developed for the determination of carbofuran in water and potato samples [89]. An immunoaffinity column was coupled to conventional RPLC columns, using column-switching techniques, and LC detection was achieved with an ion-trap mass spectrometer equipped with an ionspray interface. Assay of a crude potato extract spiked with carbofuran showed a high degree of sample purification, attainable using IAC. In addition, MS coupled on-line with this technique gave full-scan mass spectra of the target analyte, useful for an un-ambiguous identification with minimal sample preparation. As illustrated in Fig. 2, the mini-mum detectable amount of this carbamate pes-ticide in potato extracts was ca. 2.5 ng/g, i.e., a value far below the current EPA regulatory levels for potatoes (1 μg/g for carbofuran and 1-naphthol, its hydrolysis product [90]).

The potential of LC–MS techniques for the determination of non-volatile and heat-sensitive pesticides in foods has been demonstrated. So far, TSP has been the most widely used interface both in the environmental field [75,78,79] and for foodstuff analysis; quantification in the low-nano-gram range is possible, and detection limits adequate for environmental monitoring and for pesticide analysis in complex food matrices have been reported. Although TSP mass spectra lack structural information, the use of discharge or filament-assisted ionization or of MS–MS tech-niques makes LC–TSP-MS suitable for identifi-cation purposes. More structurally informative spectra are obtained using the LC–PB-MS sys-tem, the EI library-searchable and the solvent-independent CI spectra being valuable for uni-vocal identification of unknowns. Despite the general problems of poor sensitivity and non-linearity typical of this interface, several workers have reported good analyte detectability by the careful choice of LC and MS conditions [14,82], and by the addition of an appropriate carrier [10]. More recently, the applicability of API-related techniques for trace-level analysis of pesticides has been demonstrated. Most of the studies performed in the environmental and food areas have used the more versatile ISP and APCI techniques rather than pure ESP. The advantages
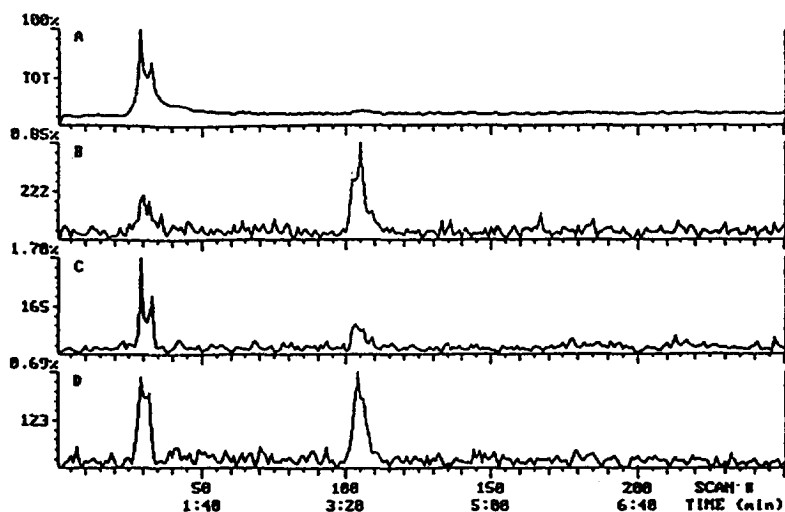

Fig. 2. IAC coupled-column LC–MS analysis of potato extract (2 g equiv. in 20 ml of phosphate-buffered saline). Extract spiked with carbofuran at a level of 2.5 ng/g and pumped through IAC column at 2 ml/min: (A) TIC profile; (B), (C) and (D) extracted ion current profiles for m/z 222, 165 and 123, respectively. Reprinted with permission from [89].

in sensitivity over the PB and TSP techniques suggest that API-based methods are ideal for the determination of a broad range of pesticides.

2.2. Drug residues in food

Antibiotics are a highly important class of substances used in food-producing animals to control bacterial infections and also to preserve health and promote growth. The monitoring of food products for antibiotic residues is a field of increasing importance, because of concern for abuse and for human health. The improper use of these drugs can result in harmful residues in foods, hence the need for improved analytical methods for checking their presence in food. Liquid chromatography has increasingly been used as the method of choice for the analysis of polar, non-volatile and thermally labile antibiotic materials. HPLC is coupled with MS detection when molecular information on the separated antibiotics is required for unequivocal identification.

Recently, review on chromatographic methods for antibiotic residues in different food matrices have been published [91–95]. In these overviews some examples of applications of LC–MS in food and related materials are cited [96–101].

2.2.1. Particle beam interface

LC-PB-MS has been investigated as a potential method for the determination of compounds of pharmaceutical interest found in food products, such as cephapirin in milk, frusemide in kidney tissue and methylene blue in muscle tissue [102]. Investigation on the PB parameters influencing sensitivity indicated that solvent composition, nebulization–desolvation and source temperature played an important role in obtaining optimum sensitivity; in fact, careful optimization of these parameters yielded an order of magnitude better sensitivity. The sensitivity of the PB interface was largest using solvents with low heat capacities (methanol > acetonitrile > 2-propanol > water) and operating at low flow-rates (0.4–0.6 ml/min). By performing detection under full-scan conditions, absolute detection limits of about 100 ng were demonstrated for the

target compounds. When analysing milk or tissue extracts, the mass spectrometer was operated in the SIM mode, allowing the determination of the drugs at ppm levels. A comparison with the qualitative and quantitative results obtained with the TSP interface was also provided. Less structural information was obtained using the TSP source, and the TSP detection limits were more compound dependent than with PB; for some drugs, the sensitivity of detection was lower by one order of magnitude in the case of PB.

HPLC–MS with a PB interface has been chosen as a confirmatory method for some tetracycline antibiotic residues in milk [101]. After a short solid-phase extraction on C_{18} cartridges, drugs were submitted to a reversed-phase LC analysis with MS detection under NCI conditions. The determination in the SIM acquisition mode of the tetracyclines provided a detection limit of ca. 100 ng/ml in spiked and incurred milk samples (Table 3).

The antibiotic chloramphenicol has been successfully determined in calf muscle by LC–PB-NCI-MS, using methane as the reagent gas [103]. The specificity of the method was investigated for three related substances, dehydrochloramphenicol, nitrosochloramphenicol and nitrophenylaminopropanediol. Use of LC–PB-MS allowed the detection of chloramphenicol at levels of 2 μg/kg and the calibration graphs were linear up to 20 μg/kg.

A similar LC–PB-MS approach has been reported also for the determination of ivermectin residues in bovine milk and liver [104]. The specificity necessary for a regulatory confirmation procedure was obtained by monitoring the molecular ion and characteristic fragment ions of the drug under electron-capture NCI conditions. Co-eluting matrix compounds were found to alter the abundance pattern of the analyte and to enhance the total response; this enhancement effect was attributed to improved transmission through the PB interface in a sort of carrier effect, similarly to that reported by other workers. LC–PB-MS with NCI was able to detect low levels of drug residues, the quantification limit being 2 ng/μl in milk and 15 ng/g in liver, i.e., levels not achievable by other MS methods at the

Table 3
Selected LC–MS applications to drug residues in foods

Compound	Matrix	Stationary phase	Mobile phase	Interface	MS detection mode	Detection limit	Ref.
β-Lactams							
Cephapirin Deacetylcephapirin	Bovine milk	Phenyl Spheri-5 (55°C)	30 mM ammonium acetate–2-propanol–CH_3COOH (186:13:1); flow-rate, 1.3 ml/min	TSP	PI; SIM	0.1 μg/g 0.5 μg/g	[110]
Penicillin G	Bovine milk	Phenyl Spheri-5	2-Propanol–CH_3COOH–0.2 M ammonium acetate (25:4:171)	TSP	PI; SIM	0.1 μg/ml	[109]
Penicillins	Meat Milk	Intersil ODS-2	CH_3CN–H_2O–triethylamine (27:77:0.5); flow-rate, 0.5 ml/min	ESP	NI; SIM	0.025–0.1 μg/g 0.002–0.01 μg/g	[117]
Penicillin G, ampicillin, amoxicillin, cloxacillin, cephapirin	Bovine milk	Ultremex 3, C_{18}	40% CH_3CN and 1% CH_3COOH in H_2O (pH 3.0): flow-rate, 0.3 ml/min, post-column split 1:70	ESP	SIM	0.1 μg/g	[116]
cephapirin	Bovine milk ultrafiltrate	Ultremex phenyl (3 μm)	H_2O containing 40% CH_3CN, 1% CH_3COOH (pH 3.0); flow-rate, 0.3 ml/min, post-column split 1:70	ESP	PI; SIM	0.2 μg/ml	[118]
Tetracyclines							
Oxytetracycline, tetracycline, chlortetracycline	Milk	Nova Pak C_{18}	CH_3CN–0.05 M oxalic acid–CH_3OH (5:3:2); flow-rate, 0.5 ml/min	PB	NCI; SIM	0.1 μg/ml	[101]
Chloramphenicol							
Chloramphenicol	Fish, milk	Spherisorb 5 C_{18}	45% CH_3OH in H_2O, containing 0.1% trifluoroacetic acid; flow-rate, 0.4 ml/min	TSP	MS–MS	0.5 μg/g	[106]
Sulfonamides							
Sulfonamides	Meat	TSK gel ODS-80T	0.05 M ammonium acetate (pH 4.5)–CH_3CN (7:3); flow-rate, 0.8 ml/min	TSP	SIM	0.1 ng	[98]
	Salmon flesh	5-μm Vydac 201TP	0.1% trifluoroacetic acid in aqueous CH_3CN (5 to 40% in 20 min); flow-rate, 0.2 ml/min	ISP	PI; SIM	0.025 μg/g for sulfadimethoxine	[99]
	Milk	Nova Pak C_{18}	CH_3CN–25 mM ammonium acetate, pH 6.8 (41:4); flow-rate, 1 ml/min	HN-APCI	SIM	0.0025–0.05 ng/μl	[123]
Other antibacterial drugs							
Moxidectin	Cattle tissues	Partisil 5-C_8	0.1 M ammonium acetate–CH_3COOH (17:83); flow-rate, 1.5 ml/min	TSP	SIM	0.25 μg/g	[111]
Semduramicin	Chicken liver	Hypersil BDS C_{18}	5 mM sodium acetate in CH_3CN–THF (15:3); flow-rate, 0.04 ml/min	ISP	PI; MS–MS SRM	0.03 μg/g	[119]
Erythromycin	Salmon tissue	Zorbax C_8	Linear gradient of H_2O–CH_3CN–formic acid; flow-rate, 1 ml/min	ISP	SIM MS–MS, SRM	0.01 μg/g 0.05 μg/g	[120]
Danofloxacin	Chicken liver, cattle liver	5-μm Hypersil C_{18}	CH_3CN–0.1% trifluoroacetic acid (4:1); flow-rate, 0.05 ml/min	ISP	MS–MS	0.05 μg/g	[122]

time. To compensate for the variations in relative abundances produced by co-eluting material, control milk extracts were spiked with ivermectin standard for the abundance matching requirement of regulatory confirmation.

2.2.2. Thermospray interface

Another technique for the determination of drug residues in foods is LC–TSP-MS. Stout et al. [105] evaluated the techniques of desorption chemical ionization (DCI), LC–TSP-MS and LC–TSP-MS–MS as potential approaches for confirmatory assay of maduramycin α, a polyether ionophore antibiotic, in chicken fat. The former approach was demonstrated to be inadequate, where the latter was judged marginally appropriate. Only LC–TSP-MS–MS adequately resolved the analyte from the tissue co-extractives and provided reproducible mass spectrometric data, useful for identification purposes.

The coupling of the HPLC–TSP system with tandem MS has also been explored by Ramsey et al. [106] to detect residues of chloramphenicol in milk and fish; for this purpose, the CID mass spectrum of the chloramphenicol protonated molecule was recorded using a hybrid tandem instrument. Confirmation of the antibiotic in the food assayed was on the basis of correspondence of the product ion spectra obtained from samples with that of the standard. In both foods, chloramphenicol was identified unequivocally at 0.5 μg/ g. Despite the specificity typical of tandem MS, this method has a higher detection limit than the above-mentioned PB-NCI-MS technique; a significant contribution to the loss in sensitivity seems to be related to the detection of a background of neutral noise, which is attributed to the production of fast neutral species occurring prior to the quadrupole gas cell.

HPLC–TSP-MS was successfully used by Lewis et al. [107] to confirm nicarbazin residues in chicken tissues, after reversed-phase chromatography of the purified extracts. Analysis was performed using negative-ion detection and SIM of three characteristic ions in the TSP mass spectrum of the drug (m/z 164, 272 and 302).

The determination of several sulfonamides in meat by HPLC–MS was performed by Horie et al. [98] using a thermospray interface. The mass spectra obtained from the drugs showed the protonated molecule as the base peak; these ions were monitored in the SIM mode for quantification by means of external standards. For sulfadimidine the detection limit, as determined from SIM experiments, was about 100 pg and similar results were achieved for other sulfonamides.

Voyksner et al. [108] devised a confirmatory HPLC–TSP-MS method for the determination of various penicillin derivatives extracted from bovine milk. For LC–TSP-MS experiments, ammonium acetate buffer replaced the ion-pair reagents used for UV detection. The positive-ion TSP mass spectra of penicillins displayed both $[M + H]^+$ and $[M + Na]^+$ ions, which provided unequivocal proof of the suspected drug residue; many fragment ions were also observed. The sodiated adduct ion was observed because a certain concentration of sodium was present in samples and standards. Applying this methodology, detection limits for the antibiotics studied were estimated to be in the 100–200 ppb range.

Analytical methods utilizing LC–TSP-MS for the assay of penicillin G [109] and of cephapirin and its metabolite in bovine milk have been developed by the same group [110]. The LC–MS procedure devised for penicillin G confirmation in ultrafiltrate milk samples gave a detection limit of 0.1 μg/ml (Table 3). In the case of the antibiotic cephapirin, the principal metabolite was tentatively identified as deacetylcephapirin, both by LC with UV–Vis photodiode-array detection and by LC–TSP-MS. The limits of detection for cephapirin and deacetylcephapirin were 0.1 and 0.5 μg/g, respectively, for the LC–MS technique.

The applicability of the LC–TSP-MS technique to the determination of moxidectin, a potent antiparasitic agent, in cattle tissues and cattle fat was evaluated by Khunachak et al. [111]. The drug was extracted with acetonitrile and the extract was partitioned with hexane. The method proposed eliminated the extensive clean-up and the use of tandem MS, as applied by Tway et al. [112] in the determination of ivermectin, another antiparasitic drug, in animal tissues. In addition to the qualitative confirmat-

ory capabilities of the LC–TSP-MS approach, the quantitative results obtained were within established guidelines for regulatory methods [113] and demonstrated the ability of the LC–MS technique to confirm moxidectin residues in cattle fat at the 250 ppb level (Fig. 3).

More recently, Boison et al. [114] applied the LC–MS technique to screen milk and solid milk products (yoghurt and cottage cheese) for penicillin G residues. After protein precipitation and a solid-phase extraction step on a C_{18} cartridge, penicillin G was eluted and determined under reversed-phase conditions with UV detection; quantification was carried out using penicillin V as an internal standard, yielding a limit of detection of 3 ppb in milk. LC–TSP-MS was chosen as a confirmatory method, allowing the identification of penicillin G at concentrations (5 ppb) close to the detection limit of the LC–UV method. This minimum detectable concentration was significantly lower than that obtained for the same compound by Tyczkowska et al. [109], who used only an ultrafiltration step for milk sample treatment.

2.2.3. Electrospray interface

Nanoscale capillary liquid chromatography has been coupled with quadrupole mass spectrometry through an electrospray interface for
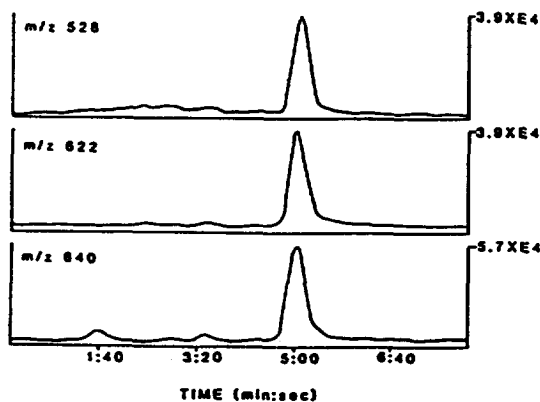

Fig. 3. LC–TSP-MS analysis of moxidectin in cattle fat. SIM traces of m/z 528, 622 and 640 ions from spiked (250 ppb) cattle fat extract. Reprinted with permission from [111].

the determination of a variety of sulfonamides [115]. Detection limits in the full-scan mode were in the low picomole range. In order to confirm the presence of the drugs in a sample, dissociation of the sulfonamides was induced by increasing the skimmer voltage.

β-Lactam antibiotics (penicillin G, ampicillin, amoxicillin, cloxacillin, cephapirin), which are frequently used as antibacterial agents in bovine milk production, have been determined in bovine milk by using LC–ESP-MS [116]. First, by varying the voltage between the capillary and skimmer in the ESP interface, the CID spectra of each drug were investigated; the formation of structurally relevant CID fragment ions was observed for all five antibiotics at high CID voltages (100–200 V). The electrospray response was then studied using different LC eluent combinations and various mobile phase additives; both positive and negative operational modes were applied to obtain characteristic ion signals. The larger the fraction of the organic modifier in the eluent, the higher was the ESP intensity. In addition, it was found that the ESP response, which was poor with a methanol–water (1:1, v/v) as mobile phase, was enhanced by the addition of formic acid or acetic acid, as shown in Table 4. The multi-residue assay of a mixture of five β-lactam antibiotics in milk was carried out using the HPLC–ESP-MS method by performing a postcolumn splitting of the eluent from a 150 × 2 mm I.D. column for successful ESP ionization (flow-rate 4 μl/min). Fig. 4 displays the chromatograms recorded under SIM conditions of a milk ultrafiltrate sample spiked with 143 pg of each drug and of a standard mixture containing 143 pg of each of the five analytes. The detection limit for the simultaneous determination of the drugs examined was 100 ppb.

Different instrumentation was used by Blanchflower et al. [117], who described an LC–ESP-MS confirmatory method for the simultaneous determination of five penicillins in meat and milk. With this system all the LC effluent (0.5 ml/min) could be provided for the ESP-MS analysis; in this way, the limitations of the older ESP instruments, i.e., low effluent flow-rates (generally 5 μl/min) and the use of a postcolumn

Table 4
Comparison between different CID voltages and mobile phase additives[a] [116]

Drug	Mobile phase additive in MeOH–water (1:1)	Relative intensity (%) of $[M+H]^+$ ion at CID $= +80$ V	Relative intensity of [fragment]$^+$ ion at CID $= +160$ V
Penicillin G	None, pH 8.4	30	5
	+ 0.1% (v/v) TFA, pH 2.2	44	21
	+ 0.1% (v/v) FOA, pH 3.1	44	15
	+ 1% (v/v) HOAC, pH 3.4	91	100
	+ 50 mM NH$_4$OAc, pH 7.1	55	61
	+ 50 mM aq. NH$_3$, pH 10.5	100	50
	+ 10 mM HFBA, pH 2.2	68	41
Ampicillin	None, pH 8.4	4	16
	+ 0.1% (v/v) TFA, pH 2.2	5	6
	+ 0.1% (v/v) FOA, pH 3.1	100	100
	+ 1% (v/v) HOAC, pH 3.4	44	90
	+ 50 mM NH$_4$OAc, pH 7.1	9	10
	+ 50 mM aq. NH$_3$, pH 10.5	14	10
	+ 10 mM HFBA, pH 2.2	2	0
Amoxicillin	None, pH 8.4	31	29
	+ 0.1% (v/v) TFA, pH 2.2	3	3
	+ 0.1% (v/v) FOA, pH 3.1	100	96
	+ 1% (v/v) HOAC, pH 3.4	73	100
	+ 50 mM NH$_4$OAc, pH 7.1	3	3
	+ 50 mM aq. NH$_3$, pH 10.5	16	11
	+ 10 mM HFBA, pH 2.2	6	43
Cloxacillin	None, pH 8.4	3	0
	+ 0.1% (v/v) TFA, pH 2.2	63	67
	+ 0.1% (v/v) FOA, pH 3.1	31	100
	+ 1% (v/v) HOAC, pH 3.4	31	100
	+ 50 mM NH$_4$OAc, pH 7.1	56	93
	+ 50 mM aq. NH$_3$, pH 10.5	100	93
	+ 10 mM HFBA, pH 2.2	34	63
Cephapirin	None, pH 8.4	2	4
	+ 0.1% (v/v) TFA, pH 2.2	9	5
	+ 0.1% (v/v) FOA, pH 3.1	100	100
	+ 1% (v/v) HOAC, pH 3.4	89	43
	+ 50 mM NH$_4$OAc, pH 7.1	0.4	2
	+ 50 mM aq. NH$_3$, pH 10.5	24	0.2
	+ 10 mM HFBA, pH 2.2	14	12

[a] CID voltage, voltage measured at the end of the capillary; relative intensity, relative signal intensity of the $[M+H]^+$ ion or [fragment]$^+$ ion; penicillin G, m/z 335 and 160; ampicillin, m/z 350 and 106; amoxicillin, m/z 366 and 349; cloxacillin, m/z 436 and 277; cephapirin, m/z 424 and 111.

flow splitter, which limits the practicality of the technique, were overcome. Acquisition of penicillin signals was performed under time-scheduled SIM conditions, by operating in the NI mode. As reported in Table 3, limits of detection were between 0.025 and 0.1 μg/g in meat and between 0.002 and 0.01 μg/g in milk, hence

below the specified EU maximum residue limits for each of the penicillins.

Recently, LC–ESP-MS has been used by Tyczkowska et al. [118] for confirmation of five β-lactam antibiotics, amoxicillin, ampicillin, cephapirin, cloxacillin and penicillin G, in bovine milk ultrafiltrate; quantification was carried out
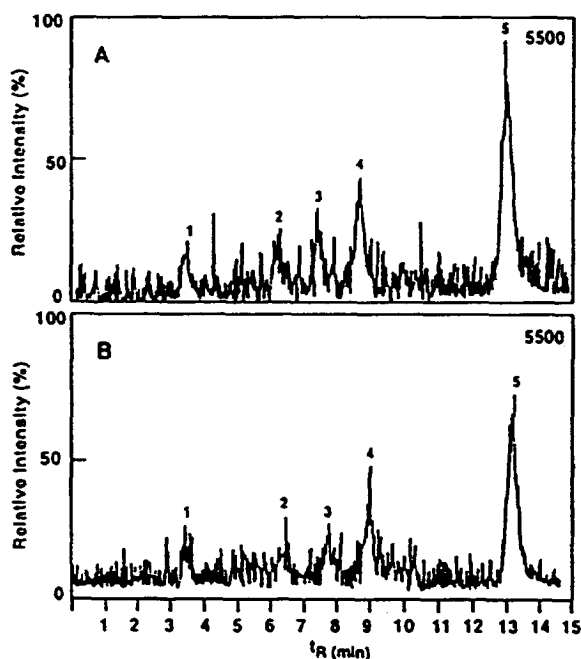
Fig. 4. HPLC–ESP-MS reconstructed total ion current profiles of (A) 143 pg of each drug in milk ultrafiltrate and (B) synthetic mixture of 143 pg per component corresponding to an on-column injection of 10 ng of each drug; SIM data acquisition mode. Numbers at top right indicates ion counts. Peaks: 1 = penicillin G; 2 = cloxacillin; 3 = cephapirin; 4 = amoxicillin; 5 = ampicillin. Reprinted with permission from [116].

by LC with UV photodiode-array detection. The drugs studied were specifically detected in milk sample by operating in the positive-ion mode under SIM conditions; in this way, it was possible to confirm the presence of β-lactams down to 100 pg per component in the milk ultrafiltrate (0.5-μl injections).

As indicated above, LC–ESP-MS can be successfully used to simultaneously determine and confirm β-lactam antibiotics in animal-derived foods. Voyksner and co-workers [116,118] demonstrated the detection of penicillins at the 100–200 ppb level directly in milk ultrafiltrate; they found the positive-ion mode to be more sensitive than the negative-ion mode for this class of compounds. However, the low ppb level of regulatory interest for penicillins was not attained with their method, and they claimed that the use of capillary columns and on-column

concentration of the antibiotics would improve detection limits to the 1–10 ppb range. Detection limits below the EU maximum residue limits for penicillins were achieved by Blanchflower et al. [117], who performed extraction and clean-up steps before LC–ESP-MS analysis. In contrast to the sensitivity observed by Voyksner and co-workers, Blanchflower et al. found that the sensitivity towards penicillins was significantly lower under PI conditions and less than that required to obtain detection limits below the levels of regulatory interest. This different behaviour could be attributed to differences in the type of instrumentation used.

2.2.4. Ionspray interface

On-line HPLC–pneumatically assisted ESP-MS proved to be an attractive approach for the determination of semduramicin and other polyether antibiotics in chicken liver [119]. Trace level confirmation and structure elucidation of these ionophores were achieved as a consequence of the excellent sensitivity of ionspray MS combined with HPLC analysis. Tandem MS CID of the molecular ions enhanced specificity for trace analysis of the antibiotics studied, providing structural information and selective detection of semduramicin in poultry liver down
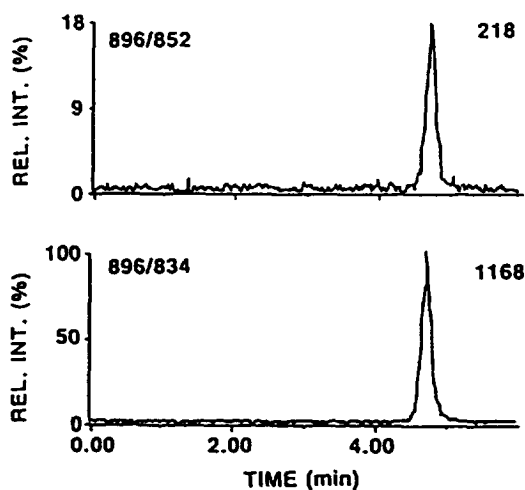

Fig. 5. HPLC–MS–MS SRM of a chicken liver sample spiked with 30 ppb of semduramicin. Reprinted with permission from [119].

to 0.03 μg/g; the specificity of HPLC–MS–MS under selected-reaction monitoring (SRM) conditions is shown in Fig. 5 for a chicken liver sample spiked with semduramicin at this trace level.

Ionspray tandem mass spectrometry was also successfully applied by Pleasance et al. [99] for the determination of 21 antibacterial sulfonamides in cultured salmon flesh. All the analytes displayed simple positive-ion mass spectra, with an intense protonated molecule and no fragment ions of relevant abundance. The use of MS–MS overcame the lack of structural information presented by the ISP mass spectra. A reversed-phase LC separation with gradient elution was developed using UV diode-array detection, to allow the separation of 16 of the sulfonamides. The same mixture was analysed using MS detection by operating in the full-scan acquisition and SIM modes and with tandem MS detection using SRM for increased sensitivity. In this configuration, the LC–MS–MS detection limit for sulfadimethoxine, a representative sulfonamide used in the aquaculture industry, was estimated to be 0.025 μg/g in salmon flesh.

LC–ISP-MS was used by the same group for the determination of erythromycin A and its metabolites in salmon tissue [120]. The reversed-phase chromatographic effluent was fed to a triple-quadrupole mass spectrometer via an ionspray interface. Tandem MS, using CID, provided additional structural information. The LC–MS and LC–MS–MS techniques allowed the detection of the drug at <0.01 and 0.05 μg/g levels, respectively. The combination of these methods permitted the identification of a number of degradation products and metabolites of erythromycin, including anhydroerythromycin and N-demethylerythromycin.

A preliminary study was carried out by McLaughlin and Henion [121] to find a suitable confirmatory method for aminoglycoside antibiotic residues in bovine tissues. A reversed-phase ion-pair separation of spectinomycin, hygromycin B, streptomycin and dihydrostreptomycin was carried out with both pulsed amperometry and ionspray MS detection. Various column phases, mobile phase compositions and

ion-pair reagents were investigated for optimum LC–MS sensitivity. Detection limits were in the low-ng range with both amperometric and ISP-MS detection in the SIM mode.

Chicken and cattle liver were checked for danofloxacin residues by Schneider et al. [122] using LC–pneumatically assisted ESP-MS–MS. After liquid–liquid extraction with dichloromethane, the extracts were analysed by HPLC–MS–MS on a microbore C_{18} column, eluting with acetonitrile–0.1% trifluoroacetic acid mixture (Table 3). Tandem mass spectrometric CID experiments on the protonated molecule of danofloxacin provided two significant daughter ions. Under optimum electrospray and MS–MS operating conditions, the specific monitoring of danofloxacin and the confirmation of its residues in extracts of edible tissues of chicken and cattle were attainable down to 0.05 μg/g.

2.2.5. APCI interface

Six sulfonamides have been separated and determined in milk samples using HPLC with APCI-MS and a heated nebulizer interface [123]. At a sampling cone voltage of 30 V and operating in the SIM mode, rectilinear calibration graphs were obtained for each sulfonamide; e.g., for sulfamethazine, the linear calibration range was 1.25–20 ppb. A simple solid-phase extraction procedure was applied prior to LC–MS analysis of milk; injections of 10–20-μl aliquots allowed the detection of 0.05–0.5 ng of the six drugs.

Although this represents the only application of APCI in drug residue analysis in foodstuffs so far, in the near future this technique is expected to be more often applied because of its versatility and high sensitivity. HN-APCI for LC–MS applications is presumably destined to take over from TSP; on the other hand, the latter has been the technique of choice for the LC–MS assay of drug residues in foods for several years, as is attested by the numerous studies reported in the literature [98,105–111,114]. The applicability of LC–TSP-MS and LC–TSP-MS–MS techniques has been evaluated for confirmatory analyses of many antibiotic systems and, in recent work, detection limits at low-ppb levels have been reported for penicillin G [114]. In addition, LC–

ISP-MS–MS on a triple-quadrupole instrument has also been used for improved specificity for trace assay of the drug studied, providing structural information.

2.3. Toxic substances

2.3.1. Mycotoxins, phycotoxins, cyanobacterial toxins

The study of mycotoxins, which are secondary metabolites of various fungal species, has become of great importance, since the consumption of foods contaminated by the toxins can seriously affect human health. Mycotoxins of particular interest to the problem of food preservation are aflatoxins, ergot alkaloids, patulin, ochratoxin A, zearalenone and fusariotoxin T-2 [124]. Among these, aflatoxins are the most common and highly toxic, aflatoxin B_1 being a power carcinogen. Consumption of rye and other cereal grain contaminated with alkaloids of ergot seems to be associated with a disease called ergotism. Patulin is known to have carcinogenic activity and ochratoxin A is known to be responsible for liver and kidney damage. Other mycotoxins include sphinganine and analogue mycotoxins (SAMs), the name of which derives from their structural similarity to the backbone structure of sphingolipids; their toxicity seems to be associated with esophageal cancer [125] and with the inhibition of ceramide synthase [126]. Hence there is a need to develop methods to detect, identify and quantify these substances for safety evaluation.

In addition, it has been known since early times that human consumption of some fish and shellfish can cause death; the chemistry and biochemistry of these intoxications have been clarified during the last 50 years, and it is now clear that certain microscopic algae produce very potent toxins, which constitute a serious health risk. These algal toxins or phycotoxins, which occur in seafood, are another class of toxins relevant to food analysis; they are divided into paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) toxins. Luckas [127] compiled an overview of the toxicological and chromatographic aspects of phycotoxins in seafood; emphasis was put on the advantages of HPLC methods over bioassays for the analysis of marine toxins. Mass spectrometry was described as a powerful technique for the assay of marine toxins, being valuable for confirmation of toxin identity and characterization of new toxins.

Many blue-green algae, which are found in drinking water reservoirs and in eutrophic lakes, produce cyanobacterial toxins, such as anatoxin-a and saxitoxin, peptide hepatotoxins including microcystin variants and nodularin [128]. These toxins are recognized to contaminate drinking water, with a serious impact on human health, hence analytical methods for monitoring and quantifying these substances are needed.

2.3.1.1. Thermospray interface. Problems associated with HPLC analysis are the sensitivity and selectivity of conventional detectors; the combination of the HPLC system with MS detection provides a sensitive and specific method for the determination of a wide range of mycotoxins in foodstuffs. Rajakylä et al. [129] demonstrated the capabilities of LC–TSP-MS for qualitative and quantitative analysis of various mycotoxins in grain; the compounds examined were deoxynivalenol, patulin, diacetoxyscirpenol, HT-2-toxin, T-2-toxin, zearalenone and ochratoxin A. TSP mass spectra were obtained at two different concentrations of ammonium acetate in the mobile phase (0.001 and 0.05 M); it was observed that at low concentrations of the buffer salt, filament-assisted buffer ionization provided specific fragmentation patterns for most of the analytes. At higher electrolyte concentrations an increase in sensitivity was observed for all mycotoxins by a factor of 5 and the mass spectra displayed a dominant $[M + NH_4]^+$ ion signal and no fragmentation. Evidence of this fact is given in Fig. 6, which compares the TSP positive-ion mass spectra of T-2-toxin recorded using both the filament-on and the filament-off modes. In addition, the TSP method allowed the determination of mycotoxins at concentrations ranging from 0.003 to 0.04 μg/g (Table 5).

The use of HPLC–TSP-MS for PSP saxitoxin assay was described by Wils and Hulst [130]. The analysis was performed in the RP mode with a mobile phase of 0.01 M pentafluoropropionic
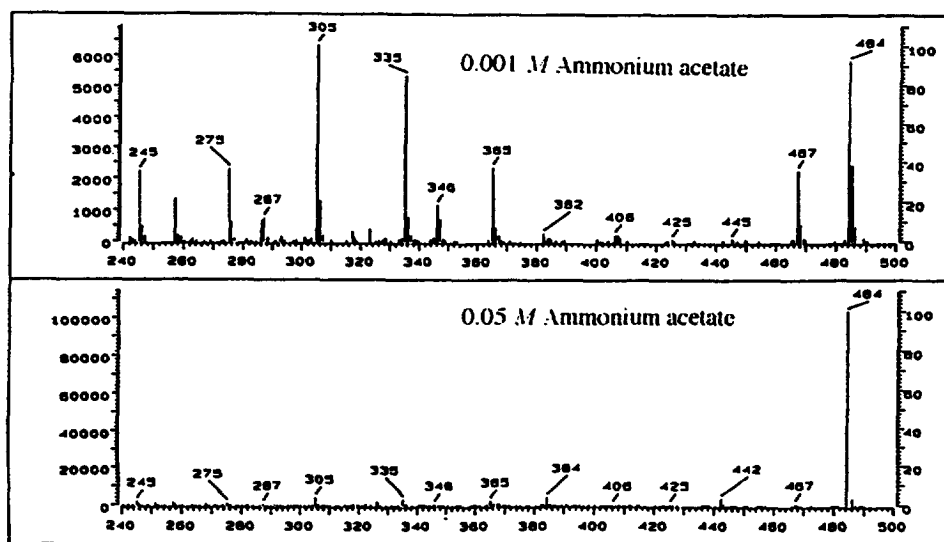
Fig. 6. Effect of ammonium acetate concentration on the LC–TSP-MS positive-ion spectra of T-2-toxin. Reprinted with permission from [129].

acid–acetonitrile (19:1); the TSP mass spectrum of saxitoxin included the protonated molecule at m/z 300 and other characteristic fragments at m/z 150, 162 and 282. Single-ion monitoring at m/z 282 gave a detection limit of 30 ng/ml, when the relatively large volume of 1 ml was injected.

2.3.1.2. Electrospray interface. Cyanobacterial toxins were determined by LC–ESP-MS by Poon et al. [131]. The study demonstrated the applicability of ESP-MS for the determination of low-molecular-mass substances with good sensitivity. Peptide toxins were identified in extracts from toxic bloom material and cell cultures by comparing spectral and chromatographic data with those of authentic toxin standards.

In recent work, negative-ion ESP-MS proved useful in the flow-injection analysis of sphinganine analogue mycotoxins (SAMs) in food matrices, such as corn and vegetable juice [132]. In contrast to positive-ion ESP analysis, ionization in the negative operational mode is less complicated by alkali metal cationization and a simple fragmentation is obtained, making negative-ion ESP assay recommendable for identification of SAMs. CID analyses in both the source region and the collision cell were also indicated as

complementary tools for the determination of these mycotoxins. In positive-mode ESP the AAL toxins TA and TD were detected in spiked extracts of vegetable juice and fumonisin FB_1 was determined in corn spiked extracts with limits of detection ranging from 4 to 10 μg/ml. Positive-mode ESP analysis of a standard solution showed that the detection limit of the AAL toxin TA was 0.4 μg/ml, whereas in extracts of vegetable juice TA had a limit of detection ten times higher (4 μg/ml). Using negative-ion ESP, the detection limits of TA in a standard solution and in the vegetable juice extract were 2 and 4 μg/ml, respectively. These results indicated that the clean-up treatment applied to vegetable juice was more effective in removing the matrix suppression in negative-ion mode than in the positive-ion mode for these substances.

2.3.1.3. Ionspray interface. Pleasance and co-workers [133–135] used ionspray in the determination of PSP and DSP toxins by HPLC–MS. Okadaic acid was determined in plankton and mussel tissue [133,135]; LC–MS analysis of the toxin in plankton gave a detection limit of 2 μg/ml with SIM at m/z 805. An HPLC separation with subsequent ISP-MS detection of PSP

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Table 5
Selected LC–MS applications to toxic substance residues in foods

Compound	Matrix	Stationary phase	Mobile phase	Interface	MS detection mode	Detection limit	Ref.
Mycotoxins and phycotoxins Deoxynivalenol, diacetoxyscirpenol, patulin, HT-2-toxin, T-2-toxin, zearalenone, ochratoxin A	Grain	Vydac 201 HSB 5 Nucleosil 5 C_{18} Spherisorb S 5 ODS-2	A = water (pH 3, CH_3COOH), B = CH_3CN; gradient from A to B in 7 min; flow-rate, 1 ml/min	TSP	Filament-assisted buffer ionization (0.05 M ammonium acetate); full-scan	0.003–0.04 μg/g	[129]
DSP toxins	Plankton	Spheri 5 RP-18	10% CH_3CN in H_2O; flow-rate, 0.2 ml/min	ISP	SIM	2 μg/ml for okadaic acid	[133]
Okadaic acid, dinophysistoxin-1, dinophysistoxin-2	Mussel tissue	Spheri 5 RP-18	A = CH_3CN–0.1% trifluoroacetic acid (60:40), B = 0.1% trifluoroacetic acid; gradient from A to B in 20 min; B held for 5 min; flow-rate, 0.050 ml/min	ISP		0.04 μg/g	[135]
Mutagenic heterocyclic amines and β-carbolines 2-Amino-3-methylimidazo[4,5f]quinoline (IQ) 2-amino-3,4-dimethyl-imidazo[4,5f]-quinoline (MeIQ)	Cooked foods (salmon, sardine, beef)	Ultrasphere ODS	CH_3CN–CH_3OH containing 0.1 M ammonium formate, 0.01 M diethylamine, pH 4.5	TSP	Isotope dilution quantification	0.3 ng/g for IQ	[138]
β-Carboline, 1-methyl-β-carboline	Various foods, alcoholic beverages	Cosmosil C_{18}-P	CH_3OH–0.1 M ammonium formate, pH 3.4 (23:77); flow-rate, 1 ml/min	TSP	PI; full-scan (150–400 u)	N.R.[a]	[142]
Arsenic, tin and inorganic halogen compounds As species	Wine	Wescan Anion/R-IC	A = 2% 2-propanol, B = 50 mM carbonate buffer (pH 7.5); gradient: 0–3 min A–B (70:30); step to 100% B; flow-rate, 1 ml/min for 6.5 min, 2 ml/min to end	ICP	SIM (m/z 75)	He–Ar ICP-MS: As^{III} 0.63, As^{V} 0.37 μg/l, DMA 0.32, MMA 0.80 μg/l	[145]
As species	Club soda Seafood samples	Ionosphere-C or ION 120	20 mM pyridinium (adjusted to pH 2.65 with formic acid) 0.1 M NH_4HCO_3 (adjusted to pH 10.3 with aq. NH_3); flow-rate, 1 ml/min	ICP	SIM (m/z 75)	As^{III} 0.72, As^{V} 0.20 μg/l, DMA 0.34, MMA 0.44 μg/l, 0.01–0.05 μg/g dry mass, 0.36 μg/g for arsenobetaine	[146]
Roxarsone (4-hydroxy-3-nitrophenylarsonic acid)	Chicken tissue	PEP RPC HR 5/10	0.01 M orthophosphoric acid–CH_3OH (95:5); flow-rate, 1 ml/min	ICP	SIM (m/z 75)	0.0018 μg/g of As, 0.0065 μg/g of roxarsone	[148]
Inorganic halogen species	Drinking water, miso soup	GS-220M	50 mM malonic acid–37.5 mM tetramethylammonium hydroxide (adjusted to pH 6.8 with aq. NH_3); flow-rate, 1 ml/min	ICP	SIM (m/z 35, 79, 127)	36 ng Cl, 0.8 ng Br, 25 pg I	[152]
Bromate, bromide	Bakery products	IonPac AS10	150–180 mM sodium hydroxide; flow-rate, 1.0 ml/min	ICP	SIM (m/z 79)	0.006 μg/g for bromate, 0.010 μg/g for bromide	[153]
Tributyltin, triphenyltin	Fish tissue, canned tuna	PRP-1	CH_3OH–H_2O–acetate buffer (94:5:1), pH 6 + 0.004 M sodium pentanesulfonate; flow-rate, 1 ml/min	ICP	SIM (m/z 120)	N.R.	[150]

[a] N.R. = not reported.

toxins and tetrodotoxin was carried out by the same group; isomeric PSP toxins were distinguished both by chromatography and by MS [134].

HPLC–ISP-MS proved to be an excellent technique for the characterization of the oxidation products of paralytic shellfish poisoning toxins by Quilliam et al. [136]. Chromatographic separation of the fluorescent products generated by periodate oxidation of these toxins was carried out under reversed-phase ion-pair conditions with fluorimetric detection. For MS detection, the column eluate was directed at a flow-rate of 20–40 μl/min to a triple-quadrupole mass spectrometer equipped with an atmospheric pressure ionization source and ionspray interface. The mass spectra obtained were useful for characterizing the oxidation products of PSP toxins.

2.3.2. Mutagenic and carcinogenic heterocyclic amines and β-carbolines

Heterocyclic amines are natural mutagenic and carcinogenic substances produced during cooking of proteinaceous foods, having as precursors some amino acids and sugars. The determination of these mutagens in foods involves their identification and quantification, taking into account that they are present in foods at low ng/g levels. Although UV absorption, fluorescence and electrochemical detection systems are all applicable to these compounds, mass spectrometry can selectively detect and confirm their presence in complex food extracts. The chromatographic methods for the assay of these mutagens carcinogens, including GC–MS and LC–MS techniques, were surveyed by Knize et al. [137].

2.3.2.1. Thermospray interface. Heterocyclic amines have been determined in salmon, sardine [138] and beef [138,139] using LC–TSP-MS. In both cases, this LC–MS method allowed the detection of concentrations as low as 0.3 ng/g of 2-amino-3-methylimidazo[4,5-f]quinoline in broiled salmon flesh [138] and in fried beef [139]. Accurate measurements of the mutagenic com-

pounds were possible by using isotopically labeled internal standards.

An HPLC–TSP-MS approach was investigated by Milon et al. [140] for the determination of mutagenic substances present in tryptophan pyrolysates. The formation of two active heterocyclic amines, 3-amino-1-methyl-5H-pyrido[4,3-b]indole and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, at different pyrolysis temperatures and for different periods of times was monitored. The high selectivity and sensitivity of the LC–MS method allowed the detection of these toxic compounds at the low ppb level starting from small amounts of samples.

Among the toxic substances with co-mutagenic activity, β-carboline compounds are worth mentioning; in fact, carcinogens such as aniline and o-toluidine become mutagenic in the presence of β-carbolines [141]. Because of their involatility, these substances are not amenable to direct assay by GC–MS. Adachi et al. [142] determined for the first time β-carboline (norharman) and 1-methyl-β-carboline (harman) in foods and alcoholic beverages by HPLC–MS in the thermospray positive-ion mode. LC–MS analysis proved useful in providing the structural identification of norharman and harman in foodstuffs, as is shown in Fig. 7 for the TSP mass spectra of β-carbolines found in an alcoholic beverage; the chromato-
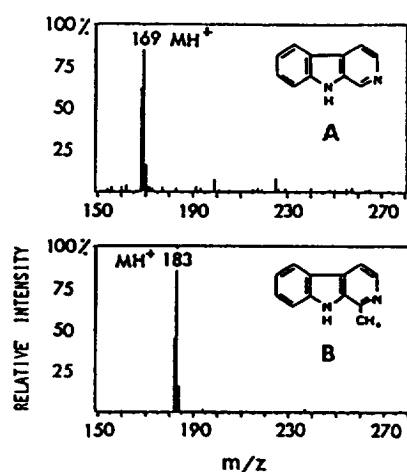

Fig. 7. Thermospray ionization mass spectra of β-carbolines from sake. (A) Norharman, 0.27 μg injected; (B) harman, 2.3 μg injected. Reprinted with permission from [142].
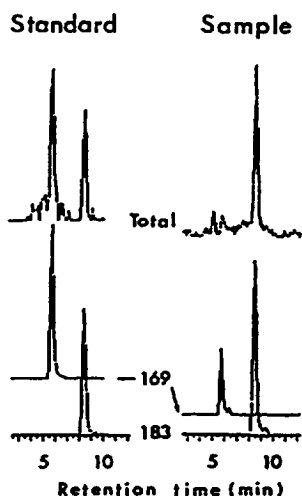
Standard   Sample

Fig. 8. Total ion chromatogram and mass chromatograms of β-carbolines from a standard mixture and sake sample obtained using LC–TSP-MS. The MH^+ ions of norharman (M_r 168) and harman (M_r 182) were obtained at m/z 169 and 183, respectively. In the sake sample the amounts injected were 0.27 μg of norharman and 2.3 μg of harman. Reprinted with permission from [142].

grams of β-carbolines obtained in the assay of a standard mixture and an extract of sake are displayed in Fig. 8. High concentrations of these compounds (67 and 590 ng/ml of norharman and harman, respectively) were found in alcoholic beverages, such as a sake brewed in a rural district and in Kurisake liqueur; in addition, a wheat-derived vinegar and soy sauces were found to contain the highest concentrations of these substances with respect to miso, soybean protein and flour, rye flour and corn starch.

Sen et al. [143] used LC–TSP-MS to determine nitroso-β-carbolines, such as 1-methyl-2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, in seventeen foods. These carcinogenic acids were detected in most of the items assayed, with the highest concentrations found in spiced radish (2500 ppb) and in fermented mustard stems (2730 ppb).

2.3.3. Arsenic, tin and inorganic halogen speciation in food

The analytical approach in most speciation studies involves the combination of the separat-

ory capability of LC with the selectivity and sensitivity of atomic spectrometry. The application of HPLC–ICP-MS to the determination of arsenic compounds in foods has been reported by several workers [144–148]. Since the toxicity of As is known to depend on its chemical form, chemical speciation is needed to determine the class and the amount of arsenic compounds existing in foods and beverages. In fact, of the more environmentally important species, inorganic As^{III} and As^V are the most toxic, methylarsonic acid (MMA) and dimethylarsinic acid (DMA) are fairly toxic and arsenobetaine and arsenocholine are relatively non-toxic.

Concerning the importance of tin speciation in food, the toxicity of tin compounds depends on the number and the nature of organic groups [149], whereas inorganic tin may be considered valuable as an important trace nutrient.

Trace assay of inorganic halogen-containing compounds is likewise important for the comprehension of their nutritional toxicological significance. Inorganic halogen species can be selectively and sensitively determined by ICP-MS coupled with LC. Some examples in food analysis have been reported [152,153].

2.3.3.1. Inductively coupled plasma mass spectrometry.

The identification and the quantification of arsenobetaine and other arsenic species in a dogfish muscle reference material (DORM-1) was carried out by Beauchemin et al. [144] by using HPLC–ICP-MS. In addition, EI-MS and TLC were used for identification purposes, whereas quantification was carried out both by HPLC–ICP-MS and graphite furnace atomic absorption spectrometry (GFAAS). The results indicated arsenobetaine to be the most abundant species (84% of total arsenic) in DORM-1, whereas As^{III}, As^V, MMA, DMA and arsenocholine make up only 4%. In the SIM mode (m/z 75), detection limits of 0.038 μg/g (300 pg) and 0.0088 μg/g (70 pg) of arsenic were reported for the ICP-MS method with and without an LC column, respectively (Table 6).

Sheppard et al. [145] used ion chromatography coupled with plasma MS to separate and detect four As species, As^{III}, As^V, DMA and MMA, in

Table 6
Detection limits of arsenic species [144]

Method	Species	Detection limit (pg As)
HPLC–ICP-MS	Arsenobetaine	300
FIA[a]–ICP-MS	Arsenobetaine	30
FIA[a]–ICP-MS	As(III)	30
GFAAS		20

[a] FIA = flow-injection analysis (HPLC column removed).

wine and club soda, which were chosen as possible vehicles for poisons. Limits of detection of 1.6 μg/l of As^{III}, 2.6 μg/l of As^V, 0.73 μg/l of DMA and 1.8 μg/l of MMA in wine were achieved; in club soda the detection limits were 2.5 μg/l of As^{III}, 3.3 μg/l of As^V, 5.1 μg/l of DMA and 8.1 μg/l of MMA. In addition, they demonstrated that better sensitivity could be obtained using a mixed He–Ar gas ICP as an alternative ionization source; the improved detection limits obtained for the beverages analysed under these conditions are displayed in Table 5. The increase in sensitivity for As with the more energetic He–Ar plasma is shown in Fig. 9 for the separation of the four As species and chloride in wine.

Arsenic speciation by HPLC–ICP-MS has also been reported in seafood samples such as shrimp, crab, dogfish, lobster, mussel, plaice and tuna, with emphasis on minor constituents [146]. After a cation-exchange LC separation, detection was achieved by ICP-MS tuned to the mass of monoisotopic arsenic (m/z 75). In the seafoods investigated, various organoarsenic and inorganic arsenic compounds were identified and quantified. The contents of each organic species (as elemental arsenic) relative to total arsenic were arsenobetaine 19–98%, arsenocholine and trimethylarsine oxide 0–0.6%, tetramethylarsonium ion 0–2.2% and dimethylarsinate 8.2–29%; these species were characterized in all samples, whereas monomethylarsonate was detected only in oyster. The presence of the inorganic compounds arsenite and arsenate was also evidenced with a content ranging from 0 to 1.4% of the total extracted arsenic. Detection limits were in
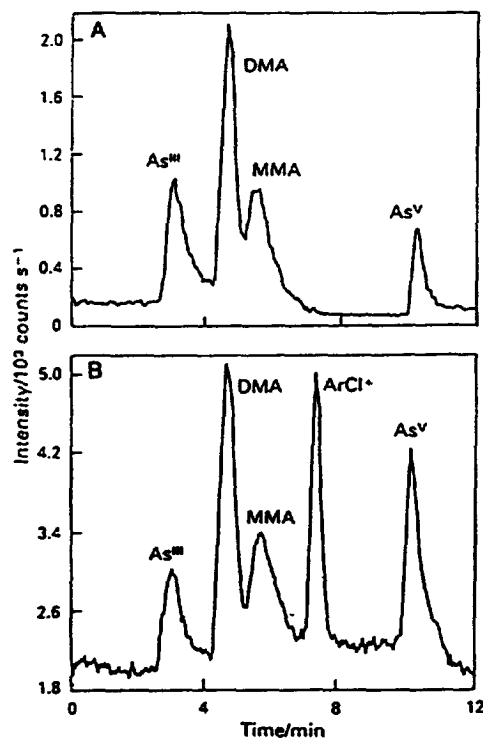

Fig. 9. Separations of the four As species and chloride in wine. Each As species is present at 50 μg/l As. (A) Ar ICP-MS detection and (B) He–Ar ICP-MS detection, monitoring m/z 75. Reprinted with permission from [145].

the range 0.01–0.05 μg/g (dry mass), except for arsenobetaine (0.360 μg/g).

Another study of arsenic speciation by HPLC–ICP-MS was performed recently by Branch et al. [147]. Non-toxic arsenobetaine was the predominant As species present in cod, dab, haddock, mackerel, plaice and whiting. No monomethylarsonic acid was detected in any of the fish samples. In mackerel, dimethylarsinic acid was identified at toxicologically insignificant levels together with a lipid-bound arsenic species. For total As determinations, the technique of nitrogen addition ICP-MS was demonstrated to overcome the potential interference from ^{40}Ar^{35}Cl$^+$ on arsenic measurements.

An RP-HPLC–ICP-MS method has recently been developed for the determination of the growth promoter 4-hydroxy-3-nitrophenylarsonic acid (roxarsone) in tissues from chicken fed on a diet fortified with this compound [148]. Detec-

tion of this organoarsenic compound was by ICP-MS in the SIM mode at m/z 75. A detection limit of 0.0018 μg As/g chicken tissue, corresponding to 0.0065 μg roxarsone per g chicken, was achieved.

Organotin compounds represent other analytes of interest from a toxicological point of view. As a consequence of the use of tributyltin and triphenyltin derivatives, such as tributyltin and triphenyltin chlorides, as additives to antifouling paints for fish nets and ship hulls, the release of considerable amounts of these compounds into the aquatic environment occurs. In addition, triphenyltin tends to accumulate more than tributyltin in fat tissues of fish. In a recent study carried out by Kumar et al. [150], HPLC–ICP-MS was used for the speciation of tributyltin and triphenyltin compounds from a fish tissue certified reference material and canned tuna fish. After supercritical fluid extraction of the organotin compounds from fish samples, the extracts were assayed by ion-pair RPLC with ICP-MS detection by monitoring the tin major isotope at m/z 120. In particular, the tuna fish extract was characterized by a large amount of inorganic tin, probably derived from the soldered can used to store this fish; tributyltin and triphenyl derivatives were also detected in the extract, as shown in Fig. 10. No detection limits were reported.

The same group studied the speciation of inorganic tin and organotin compounds in fish
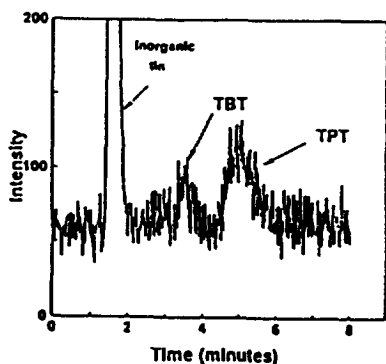

Fig. 10. HPLC–ICP-MS of tin compounds from the tuna extract (monitoring m/z 120). Peaks: TBT = tributyltin; TPT = triphenyltin. Reprinted with permission from [150].

reference material and spiked tuna fish by HPLC–ICP-MS [151]. Determinations were performed using two RP columns with ICP-MS detection in the SIM mode at m/z 120. For the PRP-1 column, linearity covered 2.5–3 orders of magnitude with absolute detection limits of 1.6, 1.5 and 2.3 pg for trimethyl-, tributyl- and triphenyltinchloride, respectively. Higher detection limits were obtained for the same compounds using the ODS-2 column, i.e., 0.4 and 0.7 ng for trimethyl- and tributyltin chloride, respectively.

Trace assay of inorganic halogen-containing compounds is also of interest for the understanding of their toxicological significance. The combination of gel-permeation chromatography (GPC) with element-specific ICP-MS detection has been used in the determination of inorganic halogen species, such as chloride, chlorate, bromide, bromate, iodide and iodate, in drinking water and miso soup [152]. Low concentrations (3.2–5.2 ppb) of iodate but not of iodide were detected in drinking water. Conversely, iodide but not iodate was found in a soup prepared from the drinking water; this is probably due to reduction of the iodate upon prolonged heating in the presence of organic substances. As indicated in Table 5, absolute detection limits ($S/N = 2$) for Cl, Br and I species were 36, 0.8 ng and 25 pg, respectively.

In more recent work, ion chromatography (IC)–ICP-MS was used in the determination of inorganic bromide and bromate in bakery products [153]. Potassium bromate, which is used in the baking industry as a dough conditioner, is partially reduced to bromide during the baking process, in a similar way to that reported for the above-mentioned reduction of iodate to iodide; owing to the health risks related to the use of potassium bromate in baked goods, it is necessary to devise methods capable of detecting bromate at the low-ng/g level. Seven samples of buns and rolls, ten samples of sliced bread and one sample of "brown and serve" Italian roll were submitted to IC–ICP-MS analysis. The peak-height response was linear in the range 1–1000 ng/ml for bromate and 2–500 ng/ml for bromate. For bromate the solution detection limit was 0.6 ng/ml, which corresponds to about

6 ng/g in bread products; 1.0 ng/ml was the solution limit of detection for bromide. The R.S.D. was estimated to be 2.1% for 10 ng/ml of bromate ($n = 10$).

2.4. Packaging residues

As food safety is of primary importance for consumer protection, there is a need for legislative controls to ensure the use of materials in which the migration of substances from packaging into the contained food is reduced. The most efficacious method of control is to approve migration limits for materials intended to come into contact with foods. The EC directive 90/128/EEC specifies an overall migration limit of 10 mg/dm for any residual oligomers used in the production of plastic packagings [154]. Transfer from plastic into food is commonly evaluated by an overall gravimetric migration test, which, however, gives no information about the possibly toxic, migrating compounds. Coupled techniques such as GC–MS and LC–MS have great potential in identifying the hazardous substances and thus in establishing a specific migration criterion. Few examples of the application of LC–MS to the determination and identification of packaging residues have been described; they

are reported in Table 7 in terms of chromatographic and mass spectrometric conditions.

LC–MS has proved to be an advantageous technique for the separation and the characterization of various packaging materials designed to come into contact with foods [155–157,159].

2.4.1. Thermospray interface

A thermospray interface operating in the plasmaspray (PS) mode, i.e., solute ionization accomplished by a discharge electrode with no salt in the mobile phase, has been used in LC–MS experiments aimed at detecting and identifying poly(ethylene terephthalate) cyclic oligomers [155]. The need to operate at unusually high temperatures for LC–TSP–MS (probe tip temperature 370°C) was determined by the difficulty of vaporizing and ionizing these molecules under conventional TSP conditions. Negative-ion spectra of two main peaks were assigned to the cyclic oligomers $(GT)_3$ and $(GT)_4$ from the abundant molecular ions at m/z 576 and 768, respectively.

The characterization by RP-HPLC–TSP–MS of the epoxy resins bisphenol A and bisphenol F and their diglycidyl ethers (BADGE, BFDGE) was the subject of two studies by Gandara and co-workers [156,157]. Epoxy-based solution coatings are used in various applications, including lacquer coatings on food cans and food storage

Table 7
Selected LC–MS applications to packaging residues in foods

| Compound	Matrix	Stationary phase	Mobile phase	Interface	MS detection mode	Ref.
Cyclic oligomers	Poly(ethylene terephthalate)	Nova-Pak C_{18}	H_2O–CH_3CN (2:1) (adjusted to pH 3.5 with formic acid); flow-rate, 2 ml/min	TSP	NI; discharge ionization; full-scan (100–800 u)	[155]
Bisphenol A BADGE	Aqueous-based food simulants	5-μm Spherisorb C_{18}	CH_3CN–0.1 M ammonium acetate (50:50); flow-rate, 1.0 ml/min	TSP	NI; PI, discharge ionization; full-scan (160–600 u)	[156]
Bisphenol F BFDGE	Aqueous-based food simulants	5-μm Spherisorb C_{18}	CH_3CN–0.1 M ammonium acetate (50:50); flow-rate, 1.0 ml/min	TSP	NI; PI, discharge ionization; full-scan (160–600 u)	[157]
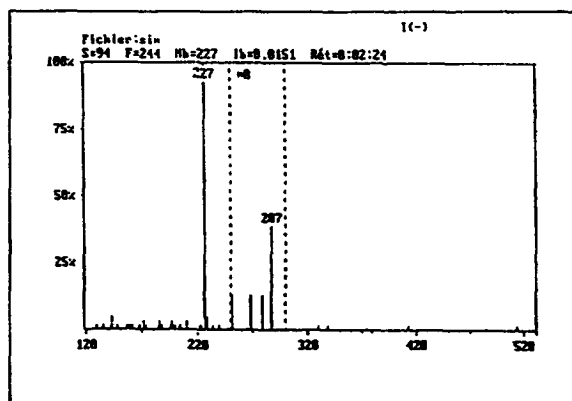
Fig. 11. Thermospray mass spectrum of bisphenol A (negative-ion mode). Reprinted with permission from [156].

vessels. Because of the toxicity associated with unreacted epoxy groups [158], it is necessary to determine the migration of toxic unreacted compounds into food. Three water-based simulated food products [3% (w/v) acetic acid, distilled water and 15% (v/v) ethanol] were subjected to analysis. Bisphenol A was most amenable to negative-ion detection, whereas BADGE could only be detected in the positive-ion mode; in both cases the TSP interface was operated in the discharge ionization mode; the corresponding mass spectra are shown in Figs. 11 and 12. Similar results were obtained for bisphenol F and its diglycidyl ether. BADGE and BFDGE hy-
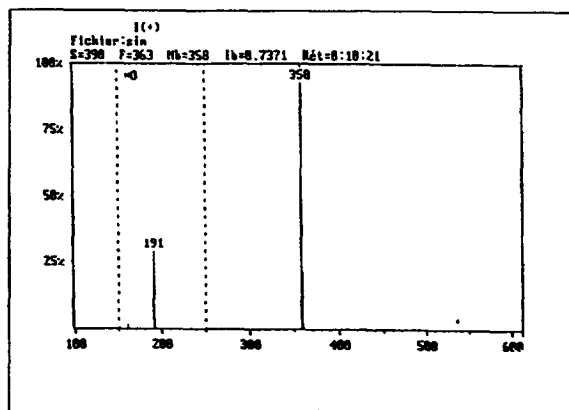

Fig. 12. Thermospray mass spectrum of BADGE (positive-ion mode). Reprinted with permission from [156].

drolysis products were detected and identified in the aqueous-based food simulants.

The same group used LC–TSP-MS for the identification of m-xylylenediamine derivatized with fluorescamine [159]. m-Xylylenediamine is an epoxy resin hardener used in epoxy resin paints destined for food containers; because of its toxicity, determination of this compound at very low levels is required. LC–TSP-MS was used as a confirmatory method for the presence of m-xylylenediamine, since in the chromatogram of the m-xylylenediamine–fluorescamine derivatization reaction mixture, obtained using HPLC–fluorimetric detection, two peaks were observed [160]. By using discharge ionization in the PI mode it was possible to identify the monofluorescamine and the difluorescamine derivatives of m-xylylenediamine from their TSP mass spectra.

2.4.2. Atmospheric pressure ionization mass spectrometry

In a pioneering study, Yamashita and Fenn [161] investigated the applicability of ESP-MS analysis to organic non-polar compounds, such as di-n-butylphthalate. A $1.3 \cdot 10^{-5}$ M di-n-butyl phthalate solution in methanol–water containing a trace of tetramethylammonium iodide was analysed. ESP mass spectra were recorded both at low (+4 kV) and high capillary voltage (+7 kV). It was demonstrated that this molecule desorbs as an ion from dilute methanol solution; at lower voltage, the formation of the ion adduct with the quaternary ammonium ion was observed, whereas in the high-voltage mode, the $[M + H]^+$ ion was formed. Although this fundamental study gave only preliminary results, it indicates the potential applicability of electrospray ionization to this class of foodstuff-related compounds.

ESP-MS and APCI-MS have recently been evaluated for the fast screening of migrating compounds from food contact plastics [162]. The aim of the work was to identify individual components by MS using soft ionization techniques which produce molecular ions, without recourse to HPLC separation. The materials considered in this study were antioxidants, such as substituted phenols (Irganox series) and phosphite esters

(Irgafos series), commonly added to polymers. The Irganox series of antioxidants were best analysed using ESP in the negative-ion mode, whereas the Irgafos compounds required either ESP or APCI techniques in the positive-ion mode. Migration experiments were performed using commercial plastic materials, which were exposed to boiling water for 1 h, and the resulting solutions were compared with calibration standards prepared from the antioxidants. The typical analysis time was 2 min, i.e., about ten times less than that required for HPLC separation of the antioxidants; in addition, qualitative results were in agreement with those of conventional methods.

3. Conclusions

The monitoring of foodstuffs for xenobiotic substances is an area of increasing concern and importance, because of the potential impact on human health; in the last decade, many LC–MS methods have been developed for the determination of pesticide, drug, toxic and mutagenic substance residues in foods of vegetable and animal origin. Many of these methods are compound specific and able to analyse at tolerance levels. By comparing the different LC–MS techniques on the basis of the LC–MS applications to the analysis of xenobiotics in food and related areas, it can be inferred that a universal system does not exist and the choice of an interface is related to the class of compounds considered and to the analytical problem. The introduction of the thermospray interface, which works well with substances of medium polarity, and more recently the development of the API techniques applicable to highly polar compounds, have opened up important application areas of LC to LC–MS. In most cases time-consuming clean-up and derivatization procedures can be avoided. As for TSP, there is a declining trend in the use of this interfacing system in favour of the API techniques, as indicated by the relative diminution of TSP-related papers with respect to those published using ESP/ISP. However, TSP for many years has been the most widely used interface

because of the ruggedness and the possibility of using different ionization modes. Drawbacks of LC–TSP-MS are the requirements for volatile inorganic modifiers and the control of experimental temperatures; the latter parameter is critical to optimum performance, particularly with thermolabile substances. Lack of structural information from LC–TSP-MS experiments can be overcome by the use of LC–TSP-MS–MS; however, the structure-informative fragmentation is obtained at the cost of a more complex method and of more expensive equipment required for MS–MS experiments.

In contrast to TSP, which has confirmatory capabilities and thus is primarily used for analysing non-target compounds, LC–PB-MS appears to have high potential as an identification method for residues of pesticides and some antibiotics in food materials; in fact, PB generates library-searchable EI spectra and solvent-independent CI spectra. However, in general, the non-linear response and poor sensitivity make this technique inadequate for the quantification of contaminants at trace levels in foods; in addition, this technique suffers a limited application range in terms of analyte polarity and molecular mass. Some workers have found that the addition of an "analyte carrier" can significantly improve the detectability to the low-ppb level, even though this effect is dependent on the analyte, the carrier and the interface hardware and it is not predictable. At present, the use of the microflow-rate PB interface is not well developed; the examples reported have demonstrated that this approach could be an effective solution to achieving higher sensitivity of detection with eluents of high water content and to improving chromatographic performance during gradient analysis.

As for the API techniques, ESP and ISP appear to be the most versatile, being suitable for substances ranging from polar to ionic and from low to high molecular mass; the appearance of high-flow-rate ISP has led to a revolution in this area, as this system is compatible with the flow-rates used with conventional HPLC columns. API soft ionization can be complemented by using pre-analyser CID or by recourse to

LC–MS–MS, as realized with the use of a triple-quadrupole system. It must be pointed out that the number of papers published, in the food analysis area, on these interfacing systems is rapidly increasing. The applicability of LC–ESP-MS and LC–ISP-MS to the characterization of inorganic and organometallic compounds is also worth mentioning; as stated above, the usefulness of the API techniques in comparison with ICP-MS in this field is based on their capability to distinguish the various species by providing structural information on analytes, not only to give the total elemental content. Complementary to electrospray and ionspray with respect to the analyte polarity is another API-related technique, APCI with a heated nebulizer interface; APCI is a valuable technique for both structural confirmation and quantitative analysis. Despite the potential of this technique, the number of APCI studies is still too limited to draw conclusions on its performance, but the published results are undoubtedly promising.

As has been discussed, the combination of HPLC with element-selective detection systems such as ICP-MS is of growing interest for trace element speciation in food analysis, from a toxicological point of view. By selecting the appropriate separation mode, excellent detection limits can be achieved by the HPLC–ICP-MS technique. In some instances, enhanced sensitivity of plasma MS has been demonstrated by using alternative ionization sources, such as He–Ar mixed gas ICP; this is particularly true for elements with high ionization energies, such as halogens, As and Se.

As for comparison of the results of quantitative analysis of organic xenobiotic substance residues, ESP and ISP appear to be valuable in terms of analyte detectability, as can be deduced from the tables herein. Detection limits in the low-ng range, i.e., compatible with the concentration levels required for the determination of xenobiotics in food, are also obtained with the TSP interface under optimum conditions. In general, it can be stated that sample preconcentration and clean-up are helpful in achieving the low limits of detection required for trace level analyses for residues in food. The use of the

TSP–MS–MS approach provides enhanced selectivity, generally at the cost of a loss of sensitivity as a consequence of a decreased ion transmission. By performing ESP-MS–MS or ISP-MS–MS, the sensitivity of the more selective LC–MS–MS method can be improved by using SRM.

Although LC–MS has become a mature technique, it cannot be considered routine in food analysis; in fact, LC–PB-MS, which is the simplest and easiest to use system, suffers from inherent limitations; on the other hand, the API techniques, which nowadays appear to be the most effective systems from several points of view, require higher cost instrumentation, particularly when MS–MS equipment is needed to obtain structural information. However, it cannot be excluded that the developments of the interface/ionization technologies could make LC–MS a widespread analytical tool. Finally, a contribution to the routine use of LC–MS in food analysis could derive from the automation of the whole LC–MS system, including on-line sampling treatment.

Abbreviations

APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
BADGE	Bisphenol A diglycidyl ether
BFDGE	Bisphenol F diglycidyl ether
CF-FAB	Continuous flow fast atom bombardment
CI	Chemical ionization
CID	Collision-induced dissociation
DCI	Desorption chemical ionization
DMA	Dimethylarsinic acid
DSP	Diarrhetic shellfish poisoning toxins
EI	Electron impact ionization
ESP	Electrospray
ETU	Ethylenethiourea
FIA	Flow-injection analysis
GFAAS	Graphite furnace atomic absorption spectrometry
GPC	Gel permeation chromatography
$(GT)_n$	Glycol terephthalate oligomers
HN	Heated nebulizer

IAC   Immunoaffinity chromatography
IC   Ion chromatography
ICP   Inductively coupled plasma
ISP   Ionspray
MAGIC   Monodisperse aerosol generating interface for chromatography
MMA   Methylarsonic acid
NI   Negative-ion
PB   Particle beam
PI   Positive-ion
PSP   Plasmaspray
PSP   Paralytic shellfish poisoning toxins
SAM   Sphingosine analogue mycotoxins
SFC   Supercritical fluid chromatography
SIM   Selected-ion monitoring
SRM   Selected-reaction monitoring
TBT   Tributyltin
TPT   Triphenyltin
TSP   Thermospray

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