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Journal of Chromatography A, 882 (2000) 153–173

JOURNAL OF CHROMATOGRAPHY A

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Review

Pesticide residue determination in fruit and vegetables by liquid chromatography–mass spectrometry

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Abstract

An overview is given of pesticide residue determination in fruit and vegetables by liquid chromatography–mass spectrometry (LC–MS). Emphasis is placed on the thermospray, particle beam and atmospheric pressure ionization interfaces including advantages and drawbacks and typical detection limits. The capacity of each interface to provide useful data for identification/confirmation of analytes and the possibility of obtaining structural information for the identification of target and non-target compounds is discussed. Finally, sample preparation techniques are dealt with in relation to their influence on further LC–MS determination. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Fruits; Vegetables; Food analysis; Reviews; Liquid chromatography–mass spectrometry; Interfaces, LC–MS; Pesticides

Contents

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PII: S0021-9673(00)00294-6

1. Introduction

Analysis of fruit and vegetables for pesticide residues is important because of the widespread human exposure to these compounds. Analytical methods are needed to screen, confirm and quantify as many residues as possible in samples whose pesticide treatment history is usually unknown [1]. Multi-residue methods provide the basic tools to the analyst for determining these residues. In this way, gas chromatography (GC) is clearly the technique of choice because of its ability to resolve a single member of a chemical class and individual analytes in suitable prepared extracts containing potential interferences. Unfortunately, universal methods are not available and many pesticides are not amenable to analysis by GC as a result of their thermal instability and polarity. Development of alternative and selective analytical techniques are required [2].

High-performance liquid chromatography (HPLC) or simply liquid chromatography (LC) is very effective in separating non-volatile and thermally labile compounds. Recently developed pesticides together with their degradation products are representative candidates for LC separations because of their medium to high polarity and their thermolability and/or low volatility. Most LC-based methods use common UV, diode array (DAD) fluorescence or electrochemical detection, which are occasionally combined with post-column treatment, e.g. derivatization. They are insufficiently selective and sensitive because of the variety and complexity of food and the small amounts of residues present [3,4].

The lack of sensitive and selective LC detectors has been overcome by combining LC with mass spectrometry (LC–MS). Although the first coupling of LC to MS was reported over 25 years ago and several LC–MS interfaces have been described in the course of time, to combine an HPLC instrument with a mass spectrometer could be considered an 'unnatural marriage' because LC–MS combines an instrument that operates in the condensed phase with other one that operates under vacuum. These technical difficulties have prevented the widespread use of LC–MS in methods developed until ten years ago, when the design of interfacing devices having different strategies repairs these obstacles. At present, the techniques that are used routinely for introduction and analysis of liquid samples by mass spectrometry can be classified in two groups: those that only introduce the sample into the ionic source of the spectrometer, like the particle beam (PB), and those that also allow 'soft ionization' of the sample, such as thermospray (TSP) or interfaces of atmospheric pressure ionization (API). Thanks to them, in a few years LC–MS has become widely accepted as the preferred technique for the identification and quantification of pesticides and other polar and thermally labile compounds [5–7].

Recent developments in the use of LC–MS interfaces to determine pesticides and their degradation products in the environment, in particular polar pesticides, have been covered by several book chapters and reviews [8–10]. These reports focus mainly on water, and procedures for more complex matrices are not extensively dealt with. In 1996, a review examined the applications of these techniques to pesticides and other xenobiotics in food [11]. More recently a book chapter covering the subject of mass spectrometry in pesticide residue analysis has been published [12] but it centers more on the description of the devices that can be used than on incorporating their applications.

However, in recent years the applications of the LC–MS in this matter have burgeoned and the number of related articles published has tripled. For this reason, the present review outlines recent developments in the application of LC–MS in pesticide residue determination from fruit and vegetables for three interface techniques, PB, TSP and API. The identification and confirmation capacity of each interface, and the alternatives to enhance their identification potential is discussed. Attention is devoted to sample preparation techniques, for when LC–MS is combined with appropriate sample treatment procedures, it still ensures the best analytical results and makes it possible to obtain detection limits adapted to determining these residues at trace levels.

The purpose of this review is not to provide an exhaustive description of the interfaces or to cover their principles of operation, but to present some of the advantages and inconveniences associated with them. A discussion of instrumentation, vacuum system design and ion sources in TSP, PB and API systems are given extensively in recent reviews [13–18].

2. LC–MS interfaces

LC–MS technology is still in a state of flux, and the ideal interface has yet to be devised. However, of the different commercial LC–MS interfaces, PB, TSP and API have been applied to pesticide residue analysis. The three methods are aerosol-based techniques. API includes a group of interfaces, commonly called electrospray (ES), ionspray (IS) and atmospheric pressure chemical ionization (APCI). The applications in fruit and vegetables are summarized in Table 1, which also shows the pesticides analyzed by each one.

Carbamates are the family of pesticides most frequent analyzed by LC–MS, probably because they are a well-established group of modern pesticides, their presence has been reported in many foods, and analytical procedures needed for their identification/confirmation and screening. Carbamates and their degradation products are polar, non-volatile and thermally labile. They have been widely determined by the three LC–MS interfaces [44,45]. Of the different classes of pesticides presently in use besides the carbamates, phenylurea herbicides, triazines, organophosphorus pesticides, quaternary ammonium salts and benzoimidazolic fungicides have all been determined by LC–MS. Most of these classes of compounds can not be easily analyzed by

Table 1
Summary of different interface applications to pesticide residue determination in fruit and vegetables

Interface	Flow-rate (ml min^{-1})	Chromatographic conditions	Compounds	Ref.
PB	0.8	Ion-exchange reversed-phase (low water-content eluents)	Daminozide Ethylenethiourea (fungicide metabolite) Carbamates and metabolites	[19–21]
TSP	2	Reversed-phase	Carbamates, benzoylureas Ureas Fenvalerate, folpet, iprodione and oryzalin	[22–25]
ES	0.01–0.2	Reversed-phase	Carbamates Phenylureas Triazines *N*-heterocyclic compounds	[26]
IS	0.2–2	Reversed-phase	Carbamates Thiabendazole Organophosphates Phenylureas Imidazolinone herbicides Ammonium quaternary salts Abamectin	[27–34]
APCI	2	Reversed-phase	Benzoylureas Carbamates Triazines Thiabendazol Clofentezine Fenbutatin oxide Rotenone, cevadine and veratridine Pyrethrin I and II Ryanodine, dehydroryanodine Piperonyl butoxide	[35–43]

GC, and all of them have been analyzed by LC with a variety of detectors [8,10].

Methods using the PB interface have been successfully applied to the analysis of daminozide, ethylenethiourea and several carbamates [19]. The arrangement LC–PB-MS offers the possibility of confirming and determining these compounds.

The TSP configuration allows molecular mass characterization of many carbamates, benzoylureas or imidazolic fungicides, but its generally reported good sensitivity often refers to the monitoring of only a single ion. A solution to solve this problem is to put a TSP on a tandem mass spectrometer. However, TSP has been used without MS–MS for multiresidue determination of pesticides in fruit and vegetables [22,24]

API sources, especially IS and APCI, are by far the most widely used of these three interfaces, by virtue of their compatibility with highly polar water soluble pesticides, reversed-phase LC, and normal LC flow-rates. The API interfaces are rapidly gaining popularity as is demonstrated by the large number of studies using these interfaces for determination of organophosphate pesticides, carbamates, phenylurea and triazine herbicides, benzoimidazolic fungicides, natural pyrethrines, ammonium quaternary salts, abamectin, etc.

2.1. Particle beam

The introduction of the PB interface, originally known as the 'mono-disperse aerosol-generating interface for chromatography' (LC–MAGIC-MS) enables the coupling of a wide range of LC separations to a conventional ion source. Ionization can be by electron impact (EI), chemical ionization (CI), or electron capture negative ionization (ECNI).

The PB technique allows the introduction, in the ionic source of the MS, of a sheaf of neutral molecules of the sample, which can be thermolabile and non-volatile, once, the solvent is eliminated entirely by means of a system based on selective diffusion.

This is practically the only LC–MS coupling technique able to obtain EI spectra compatible with the automatic programs of search and identification of spectra for comparison with the spectra of a spectrum reference library. Unfortunately, PB yields a poor sensitivity and gives a non-linear response. These problems can be partially overcome by the addition of mobile phase additives such as ammonium acetate or ammonium oxalate. Reports on LC–PB-MS for the detection of pesticides in fruit and vegetables are outlined in Table 2.

Kim et al. use anion-exchange chromatography

Table 2
Applications of the LC–PB-MS

Compound	Matrix	Extraction	Stationary phase	Mobile phase	MS mode	Detection limit	Ref.
Daminozide	Apple juice10 ml	Evaporation and treatment with methanol and acetone to precipitate sugars and inorganic salts; final extract: 0.5 ml	Anion-exchange SGE Model 250 GL250×2 mm	Water–aqueous malic acid (20 m*M*, 35% acetonitrile) ammonium acetate (10 m*M*, pH 6.0); flow-rate: 0.25 ml min^{-1}; injected volume: 10–20 μl	CI, PI SIM mode	25 ppb	[20]
Ethylenethiourea	Lettuce Apple sauce Banana Papaya 10 g	Homogenization with aqueous methanol, evaporation and elution through an alumina column with dichloromethane; final extract: 4 ml	Reversed-phase OmmiPac PAX 500 50×4 mm	5% CH_3CN in water; flow-rate: 0.25 ml min^{-1}; injected volume: 100 μl	EI full-scan (70–300 u)	5 ppb	[21]
Methiocarb Baygon Methiocarb sulfoxide Carbaryl	Lettuce 30 g Apple 10 g	Homogenization with dichloromethane; final extract: 1 ml	Reversed-phase 5-μm Hypersil ODS100×2.1 mm	Methanol–sodium acetate 0.05 *M* (70:30) (pH 5); flow-rate: 0.7 ml min^{-1} injected volume: 20 μl	EI full-scan (80–300 u) and SIM	5–20 ng	[19]

PB-MS, with positive-ion chemical ionization using isobutane as the reagent gas to determine daminozide. A method detection limit of 25 ppb is achieved in part by a signal enhancement resulting from the constant addition of malic acid to the mobile phase. Malic acid also modifies the nature of the mass spectrometer response to daminozide, changing it from a nearly cubic to a linear relationship and also changing the relative ion intensities between the base ion $M+1-H_2O$ without and $M+1$ with malic acid [20]. Positive-ion chemical ionization (PCI) is generally used to obtain molecular mass information on labile compounds, which produce weak molecular ions or none at all under EI conditions.

Sensitive detection and confirmation of ethylenethiourea (ETU) residues in a variety of crops have been achieved using PB [21]. Chromatographic separation of ETU was carried out on a multi-phase column exhibiting both anion-exchange and reversed-phase retention modes. The rise in the percentage of acetonitrile in the mobile phase provides an increase in the ETU response and an improved chromatographic peak shape. The LC–PB-MS detection limits for ETU in crops (5 ppb, 1.25 ng) are comparable to those obtained by LC with electrochemical detection.

A simple liquid–liquid extraction procedure has been applied prior to LC–PB-MS to determine carbamates and the degradation product Methiocarb sulfoxide at concentrations lower than those admitted by the European Union (EU) [19]. A mixture of water and methanol was chosen as the mobile phase because the presence of methanol enhances the signal response with the PB interface, mainly when it is used at high concentrations. Ammonium acetate was added as eluent because this compound acts as a carrier and, in general, extends the PB-MS linear range and improves sensitivity. The detection limits for analyte single-ion monitoring mode were between 5 and 30 ng.

In summary, the relatively poor sensitivity of the particle beam interface has been a drawback to its application to food analysis. Addition to the mobile phase of a carrier such as malic acid [20] or sodium acetate [19] and the use of isotopically labeled analogues of the compound [21] can improve the detection limits. The operation of the mass spectrometer in the SIM mode [19,20] and the use of CI [20] can also help to improve pesticide detectability.

2.2. Thermospray

Thermospray was the first truly compatible LC–MS interface and has been more widely applied than LC–PB-MS. The LC effluent enters through a heated vaporizer with an inner diameter of ca. 10 μm where it is partially vaporized and enters into the ion source as an aerosol of vapor and small droplets. These microdroplets contain solvated analyte molecules. As a drop advances through the nebulizer chamber it undergoes desolvation, as a result of the action of a slight heating and a gradual decrease in pressure. This causes the free analyte molecules to appear.

During this desolvation process, the analyte molecules can collide with ions by means of ion–molecule reactions, to ionize by charge-transfer. Ionization of the analytes takes place within the liquid droplets or in the gas phase, although the latter is considered to be the predominant process.

The ions used for collision with molecules can come from different sources. They usually come from adding an electrolyte to the solvent used as mobile phase, such as ammonium acetate or formiate, without the aid of an external source. When the electrolyte can not be added, and/or the solvent is not aqueous, alternative or complementary techniques can be used to achieve the ionization, such as ionization assisted by electrons (filament-on mode) or electric discharges (discharge-on mode). In the case of the techniques attended by electrons, processes of positive-ion and negative-ion CI, as well as ECNI can take place.

The main advantages of TSP are good sensitivity — often refers to the monitoring of only a single ion — and compatibility with conventional size LC. A major disadvantage of thermospray is that its sensitivity is analyte-dependent. Another serious drawback is that, like CI, it produces primarily molecular ions with little of the structurally informative fragmentation provided by EI. This is a major disadvantage for qualitative analysis, but it can be an advantage for target compound significant detection by selected-ion monitoring (SIM) because the desirable traits for a target ion are high mass (for selectivity) and high abundance (for sensitivity);

however, the statistical requirement for confirmation by SIM insists on selecting molecular ion species plus at least three characteristic ions fragments. In practice, expert consensus admits that the correspondence of retention time and molecular mass could provide sufficient specificity for identification of a target compound [4,12]. Applications of the LC–TSP-MS are reported in Table 3.

The first reported application of TSP to analysis of pesticide residues in fruit and vegetables involved determination of benomyl using reversed-phase HPLC [25]. The method is based on the conversion of benomyl to carbendazim with subsequent determination of the latter. Under SIM conditions, the minimum detectable level in apples, peach and tomatoes was 0.025 ppm.

In an effort to develop methodology that would combine the analysis of phenylureas, carbamates and several other pesticides into a single procedure, LC–TSP-MS was evaluated to determine 19 thermally labile and non-volatile pesticides in fruit and vegetables [22]. Aldicarb, aldicarb sulfoxide, bufencarb, carboxim, chlorbromuron, diuron, linuron, methiocarb, methomyl, methobromuron, monuron, nuburon, oxamyl, propoxur and thiodicarb were analyzed in the positive-ion mode. Fenvalerate, folpet, iprodione,

Table 3
Applications of the LC–TSP-MS

Compound	Matrix	Extraction	Stationary phase	Mobile phase	MS mode	Detection limit	Ref.
Benomyl	Apple Peach Tomato 100 g	Extraction with methanol: acetonitrile, acid hydrolysis of benomyl to carbendazim, and partitioning in ethyl acetate; final extract: 1 ml	Reversed-phase 5-μm Partisil 5 ODS-3 250×6 mm	85% acetonitrile and 15% 0.1 *M* ammonium acetate; flow-rate 1 ml min^{-1}; injected volume: 20 μl	PI, filament-on SIM mode	0.025 ppm	[25]
Aldicarb Aldicarb sulfoxide Bufencarb Carboxim Chlorbromuron Diuron Linuron Methiocarb Methomil Metobromuron Monuron Neburon Oxamyl Propoxur Thiodicarb	Apple Bean Lettuce Pepper Potato Tomato 100 g	Homogenization with acetone and partition with light petroleum–CH_2Cl_2; final extract: 4 ml	Reversed-phase 5-μm Spherisorb 220×4.6 mm	Gradient acetonitrile water and 0.013 *M* ammonium acetate solute; flow-rate: 1 ml min^{-1}; injected volume: 50 μl	PI and NI discharge mode SIM mode	0.025–0.1 ppm	[22]
Folpet Linuron Oryzalin	Apples Peaches Potatoes Peppers Spinach Lettuce Snapbeans Sweetcorn 100 g	Homogenization with acetone and partition with light petroleum–dichloromethane; final extract: 4 ml	Reversed-phase Spherisorb 5 μm 220×4.6 mm	Gradient acetonitrile, water and 0.013 *M* ammonium acetate solute; flow-rate: 1 ml min^{-1}; injected volume: 50 μl	PI and NI discharge mode SIM	0.05–1 ppm	[24]
Diflubenzuron	Foodstuff 30 g	Maceration with ethyl acetate and Na_2SO_4 anhydrous and $NaHCO_3$; final extract: 60 ml	Reversed-phase Spherisorb ODS2 100×4.5 mm	Methanol–water (75:25)+ 0.05 *M* ammonium acetate; flow-rate 1 ml min^{-1}; injected volume: –	PI, full scan and SIM MS–MS using argon collision gas	0.25 ppm	[23]

and oryzalin were analyzed in the negative-ion mode. In this study, the results on aldicarb, aldicarb sulfoxide, methomyl, oxamyl, and thiodicarb were disappointing in terms of the detection limit using TSP with both, filament and discharge off. When the TSP source operates in the filament-ionization mode, good sensitivities were obtained but the response was not stable. Operation in the discharge-ionization mode provides the best compromise. Discharge ionization requires no volatile buffer, but LC separations were improved when ammonium acetate buffer was incorporated into the mobile phase. However, losses of sensitivity in the negative mode restricted the use of ammonium acetate. In general, the detection limits obtained by SIM in the PI mode were found to range from 0.25 ppm for the phenylurea compounds to 1 ppm for the carbamates. For compounds such as fenvalerate and folpet, NI detection resulted in detection limits of 0.025–0.1 ppm. All detection limits were lower than or equal to the tolerances set by the United States Environmental Protection Agency (EPA), except those of chlorbromuron, fenvalerate, metobromuron and oxamyl in certain crops.

The same research group reports a rapid analytical procedure for 20 pesticides for which dietary oncogenic risk has been estimated in a variety of crops based on a single extraction step and the use of mass spectrometry for detection and quantification [24]. Folpet, linuron and oryzalin were determined by LC–TSP-MS, whereas the others were determined by GC–MS. A gradient mobile phase consisting of acetonitrile, water and ammonium acetate was used for the elution of linuron, which was analyzed in SIM in the positive-ion discharge mode. A gradient of acetonitrile–water was used for the elution of folpet and oryzalin, which were analyzed by SIM in the negative-discharge mode. Folpet is usually determined by GC, but LC–MS was found to be more sensitive in some crops to folpet than GC–MS; the LC–MS method reported really is a part of the previous multiresidue procedure.

An LC–MS method using TSP in the positive mode to determine diflubenzuron in foodstuffs was reported by Wilkins [23]. The base peak of the spectrum was the 2,6-diflurobenzamide ion (m/z 175) produced by loss of *p*-chlorophenyl isocyanate, and the protonated molecule (m/z 311) was present with an intensity of 80% of the base peak. Due to the interferences at m/z 175, quantification was based on the protonated molecule, and this resulted in a detection limit equivalent to 0.25 mg/kg of analyte in the crop.

Until recently, TSP was considered a very promising technique and was always described as the one most widely used. Studies using pesticides standards to demonstrate the utility of TSP for pesticide detection are too numerous to list here, as are applications to environmental samples. However, in the last three years, this interface has fallen into disuse as a result of progressive introduction of the API system. There has not been new applications to the pesticide residue determination in fruit and vegetables since 1993.

2.3. Atmospheric pressure ionization

API is a ‘soft’ ionization technique that, acting at atmospheric pressure and not at high vacuum as is habitual in other techniques, obtains a high yield. Its effectiveness is, in this respect, several orders of magnitude higher than that of the conventional techniques.

API includes a group of interfaces, commonly called electrospray (ES), ionspray (IS) and atmospheric pressure chemical ionization (APCI). In ES or IS, ionization is brought about by applying a high voltage over the spray, whereas in APCI, it is brought about by using a combination of a heated capillary and a corona discharge. The disadvantage of the original ES interface was the difficulty in achieving low flow-rates (around 20 μl) for conventional LC. At present, however, ES can perform at higher flow-rates (typically 300–500 $\mu l\,min^{-1}$) by directing a gas flow into the effluent stream (designated ‘pneumatically assisted ES’, ionspray, IS, or simply electrospray, ES). The situation improved with APCI, which can operate at a flow-rate up to 2 $ml\,min^{-1}$. However, ES is not prone to thermal degradation as the sample is ionized directly in the liquid phase at quasi-ambient temperature, thus leaving fragile pesticides intact. The main limitation of APCI is that pesticides can undergo thermal degradation in comparison with ES.

API sources share with TSP the disadvantage of producing primarily $[M+H]^+$ ions. However, the

application of an appropriate voltage difference between two zones of an API source generally induces the fragmentation of the primarily formed ions; this mode of operation is called pre-analyzer or collision-induced dissociation (CID) or cone voltage fragmentation (CVF). Commercial instruments have recently been introduced that coupled IS and APCI with ion trap MS–MS. Applications of the LC–API-MS are outlined in Table 4.

2.3.1. Electrospray

A very limited number of applications of ES for determination of pesticides in fruit and vegetables have been published. In fact, only one report was found, and it referred to the important limitations due to the low flow-rates.

The photochemical behavior of series of *N*-heterocyclic compounds, phenylureas and carbamates in a photolysis reactor coupled on-line with an LC–ES was investigated by using these techniques for identification and determination in lettuce and blueberries [26]. The use of LC–$h\nu$-MS, in combination with tandem mass spectrometry (MS–MS), to identify phototransformation products and to establish possible photolytic pathways of pesticides was also described. To combine adequately the flow-rate required for an appropriate HPLC separation and the flow admitted by the ES–MS, a 1:4.5 split was inserted prior to the MS analysis. Approximately 150 $\mu l\,min^{-1}$ of the eluent was admitted into the ES source. On-line photolysis can also be used to induce photolytic reactions to give structurally diagnostic product ions and thus, in turn, to add a significant degree of selectivity to LC–MS analysis.

2.3.2. Ionspray

Rule et al. explore the feasibility of utilizing automated immunoaffinity chromatography as an on-line method of sample preparation for ion spray mass spectrometric analysis of carbofuran in a raw potato extract [33]. By combining IS-MS with this technology, full-scan mass spectra may be obtained that provide confirmation of target analytes with minimal sample preparation.

Several specific multiresidue methods making use of LC–IS-MS to determine pesticides in fruit and vegetables have been evaluated [27,28,32]. Di Corcia et al. [27] studied the feasibility of using LC–IS-MS for measuring traces of *N*-methylcarbamate insecticides in ten different types of fruit and vegetables. Methanol and acetonitrile were tested as organic solvents for the mobile phase. As compared with methanol, acetonitrile provided a significant decrease in the ion signal for carbamates. Spectra for carbamates displayed major peaks for $[M–H]^+$ and $[M–Na]^+$, the later been generally more abundant than the former ones. Moderate amounts of NaCl or HCOOH were added to the mobile phase to know if an increment of the concentration of either H^+ and Na^+ ions was able to improve the yield of transition of ions from the liquid to the gas phase. The increase of both, Na^+ and H^+ concentration had the effect of lowering the ion-signal strength. Analysis of a tomato extract spiked with carbamates at the individual level of 5 ng g^{-1} of vegetable performed by SIM showed that the detection limit of these analytes could be set at a few hundreds picograms per gram of vegetable or fruit.

Lacassie et al. described a multiresidue method for routine quantitative analysis of pesticides of several classes used to treat apples and pears, down to their respective maximum residue limits (MRLs) [28]. It involves a rapid extraction procedure and LC–IS-MS. All the analytes displayed simple positive-ion mass spectra with an intense protonated molecule and only a maximum of one fragment ion of relevant abundance (except for pirimicarb). In SIM mode, the limits of detection and quantification ranged, respectively, from 0.01 to 0.02 $mg\,kg^{-1}$, with relative standard deviation of less than 19%. An excellent linearity was observed for the quantification limits up to 5 $mg\,kg^{-1}$. Intermediate ('inter-assay') precision and accuracy were satisfactory. The method was applied to many fruit samples intended for commercialization.

Fernandez et al. [32] using matrix solid-phase dispersion (MSPD) and LC–IS-MS have analyzed several carbamates. In this study, ES and APCI interfaces, in positive and negative mode, were compared. In the positive mode, the two interfaces gave similar results in terms of sensitivity and structural information because at 20 V corona voltages the fragmentation is minimal. However, ES ionization is preferable because it is a softer technique than APCI and induced lower fragmentation of carbamates such as oxamyl. The detection limits

Table 4
Applications of the LC–API-MS

Compound	Matrix	Extraction	Stationary phase	Mobile phase	MS mode	Detection limit	Ref.
Carbofuran	Potato –	Boiling in aqueous HCl, adjust pH 7.4 and purify on immunoaffinity column; final extract: –	Reversed-phase 5-μm Zorbax Rx C_{18} 150×4.6 mm 5-μm Hypersil ODS 250×2.1 mm	35–40% acetonitrile in water, 5 m*M* ammonium acetate, pH 4; flow-rate: 0.4–1 ml min^{-1}; injected volume: 100 μl	IS, PI CID 60 V full scan 100–300 u and SIM	2.5 ppb	[33]
Aldicarb Aldicarb sulfone Butocarboxym Butoxycarboxim Carbaryl Carbofuran Ethiofencarb Mercaptodimethur Methomyl Oxamyl Pirimicarb Propoxur	Lettuce Tomato Grape Endive Sinach Orange Potato Apple Peach Sugar beet 5 g	Homogenization with methanol, water addition and pass through a carbograph cartridge, the cartridge was turned upside down and eluted with methanol–CH_2Cl_2; final extract: 100 μl	Reversed-phase 5-μm Alltima C_{18} 250×4.6 mm	Gradient: methanol–acetonitrile and water; flow-rate: 1 ml min^{-1}; split the effluent and 40 μl was diverted to ES source; injected volume: 5 μl	IS, PI CID 30 V full scan 70–250 u and SIM	5 ppb	[27]
Atrazine Atrazine-deisopropyl Simazine Propazine Terbutylazine Prometryn Terbutryn Chloroxuron Difenoxuron Diuron Fenuron Linuron Metobromuron Aminocarb Carbaryl Carbofuran Isoprocarb Promecarb Propoxur	Lettuce Blueberry 100 g	Extraction with acetone–water clean-up on C_{18} and partition with CH_2Cl_2; final extract: –	Reversed-phase 5-μm Ultracarb ODS 30 150×3.2 mm I.D.	Methanol water gradient; flow-rate: 0.8 ml min^{-1} split prior to the MS at 0.15 ml min^{-1}; injected volume: 10 μl	ES, PI *hν*-MS MS–MS full scan	–	[26]
Imazethapyr	Rice	Microwave extraction, ionic exchange and C_{18} clean-up; final extract	Reversed-phase TosoHass 50×4.6 mm	–	IS, PI MS–MS	1 ppb	[29]
Carbendazim Thiabendazole Dimethoate Pyrimicarb Methylthiophanate Phosmet Phenoxycarb	Apple Pear 10 g	Homogenization with ammonium acetate and partition with acetone–CH_2Cl_2–hexane; final extract: 1 ml	Reversed-phase 5-μm Nucleosil C_{18} 150×1 mm	Gradient: acetonitrile and 2 m*M* ammonium formate pH 3; flow-rate: 40 μl min^{-1}; injected volume: 2 μl	IS, PI SIM	0.01–0.02 ppm	[28]

Table 4 (*continued*)

Compound	Matrix	Extraction	Stationary phase	Mobile phase	MS mode	Detection limit	Ref.
Chlormequat	Grain 10 g	Homogenization with methanol, water, acetic acid C_{18} cartridge clean-up and residues eluted with methanol–water–acetic acid; final extract: 1 ml	Reversed-phase Spherisorb S5 ODS1 250×2 mm	Acetonitrile–methanol–water–acetic acid containing 50 m*M* ammonium acetate; flow-rate: 0.25 ml min^{-1} injected volume: 20 μl	IS, PI CID 30 V MS–MS full scan 50–150 u MRM	9 ppb	[31]
Chlormequat Mepiquat	Grain 10 g	Homogenization with methanol, water, acetic acid; C_{18} cartridge clean-up and residues eluted with methanol–water–acetic acid; final extract: 4 ml	Reversed-phase Spherisorb S5 ODS1 250×2 mm	Acetonitrile–methanol–water–acetic acid containing 50 m*M* ammonium acetate; flow-rate: 0.25 ml min^{-1}; Injected volume 20 μl	IS, PI CID 30 V MS–MS full scan 50–150 u MRM	6–10 ppb 2–3 ppb	[30]
Abamectin	Orange 0.5 g	MSPD with 0.5 g C_{18} and elution with 15 ml of CH_2Cl_2; final extract: 0.5 ml	Reversed-phase 5-μm Chromasil C_{18} 150×4.6 mm	Methanol–water (90:10); flow-rate: 0.5 ml min^{-1}; injected volume: 5 μl	ES, PI CID 180 V full scan 50–800 u and SIM	0.0025 ppm	[34]
Simazine Atrazine Ametrine Cromazine	Orange Cabbage	–	–	–	APCI, PI MS–MS MRM	0.01–0.1 ppm	[43]
Aldicarb sulfoxide Aldicarb sulfone Methomy 13-Hydroxycarbofuran	Green pepper	Homogenization with acetone partition with CH_2Cl_2	Reversed-phase 5-μm Zorbax Rx C_8 250×4.6 mm	Gradient methanol–water or methanol–formic acid; flow-rate: 1 ml min^{-1}; injected volume: 20 μl	APCI, IS, PB, TSP	0.1 ppm	[42]
Diflubenzuron	Mushrooms 30 g	Homogenization with acetone, extraction into CH_2Cl_2–cyclohexane and clean-up by size-exclusion chromatography (SEC); final extract: 5 ml	Reversed-phase Hichrom S5 ODS2 250×4.6 mm	Gradient methanol–water; flow-rate: 1 ml min^{-1}; injected volume: 50 μl	APCI, NICID 10 V full scan 60–500 u and SIM	0.02 ppm	[35]
Diflubenzuron Clofentezine	Plums Strawberries Blackcurrant-based drinks 35 g	Homogenization with acetone, extraction into CH_2Cl_2–cyclohexane and clean-up by SEC; final extract: 5 ml	Reversed-phase Hichrom S5 ODS2 250×4.6 mm	Methanol–water (80:20) flow-rate: 1 ml min^{-1}; injected volume: 50 μl	APCI, NICID 10 V full scan 60–400 u and SIM	0.02 ppm	[39]
Fenbutatin oxide	Tomatoes Cucumbers Bananas 30 g	Homogenization with $NaHCO_3$, ethyl acetate and Na_2SO_4 anhydrous; final extract: 5 ml	Reversed-phase Hypercarb 100×4.6 mm	Acetic acid (5%, v/v, glacial acetic acid in water) acetonitrile (10:90); flow-rate: 1 ml min^{-1}; injected volume: 50 μl	APCI, PI CID 10 V full scan 80–1100 and SIM	0.02 ppm	[40]

Table 4 (*continued*)

Compound	Matrix	Extraction	Stationary phase	Mobile phase	MS mode	Detection limit	Ref.
Aldicarb sulfoxide Adicarb sulfone Oxamyl Methomyl 3-Hydroxycarbofuran Methiocarb sulfoxide Methiocarb sulfone Aldicarb 3-Ketocarbofuran Carbofuran Carbaryl	Apple Cauliflower Potato Lettuce Celery 40 g	Extraction with methanol, partitioning acetonitrile–CH_2Cl_2 clean-up on charcoal celite; final extract: 2.5 ml	Reversed-phase 5-μm Zorbax C_8 25×4.6 mm	Gradient acetonitrile–water; flow-rate: 1.5 ml min^{-1}; injected volume: 20 μl	APCI, PI SIM	10–100 ppb	[36]
Pirimicarb Carbofuran 3-Hydroxycarbofuran Aldicarb Aldicarb sulfoxide Aldicarb sulfone Thiabendazole Carbendazim Diflubenzuron Clofentezine	Strawberry Plum 30 g	Homogenization with ethyl acetate and Na_2SO_4 anhydrous; final extract: 5 ml	Reversed-phase Hypersil carbamate 250×3 mm	0.05 *M* aqueous ammonium acetate–acetonitrile; flow-rate: 0.5 ml min^{-1}; injected volume: 10 μl	APCI, IS PI and NI CID 10 V full scan 50–500 u and SIM	0.002–0.025 ppb	[38]
Carbaryl	Banana Carrot Green bean Orange Pear Potato10 g	Homogenization with methanol–acetonitrile; partitioning and clean-up celite charcoal; final extract: 1 ml	Reversed-phase Zorbax SB-C_{18} 150×4.6 mm I.D.	Acetonitrile–water; flow-rate: 1 ml min^{-1}; injected volume	APCI, PI CID 20 V SIM	–	[37]
Rotenone Cevadine Veratridine Pyrethryn I and II Ryanodine Dehydroryanodine Piperonyl butoxide	Lettuce Cabbage Cucumber 50 g	Homogenization with water–acetonitrile and clean-up on Envi-C_{18}-SPE and elution with methanol; final extract: 1 ml	Reversed-phase 5-μm Supelco Rx-C_{18} 250×4.6 mm	Gradient methanol–0.01 *M* ammonium acetate; flow-rate: 0.9 ml min^{-1}; injected volume: 100 μl	APCI, PI CID 25 V full scan 150–700 u and SIM	1–200 ppb	[41]
Carbaryl Carbofuran Diethofencarb Ethiofencarb Fenobucarb Isoprocarb Methiocarb Metholcarb Oxamyl Pirimicarb Propoxur Thiobencarb	Orange Grape Onion Tomatoes 0.5 g	MSPD with 0.5 g C_8 clean-up with silica elution with 10 ml of CH_2Cl_2–acetonitrile; final extract: 0.5 ml	Reversed-phase 3-μm Spherisorb C_8 150×5.6 mm	Gradient methanol–water; flow-rate: IS 0.5 ml min^{-1} APCI 1 ml min^{-1}; injected volume: 5 μl	IS, APCI PI and NI CID 20 V full scan 100–310 u and SIM	0.01–0.005 ppm	[32]

were usually in the 0.001–0.01 mg kg^{-1} range, which means that they were 10–100 times lower than the MRLs established by the European Union. Carbamates were not detected with the ES source in the negative acquisition mode at acceptable levels. When APCI was used, the formations of ions $[M\text{-}CONHCH_3]^-$ was observed. The detection limits were ten times higher than those obtained in the positive mode.

Chlormequat, widely used for growth control in cereals, pears and grapes, has been determined in grain [31]. The residue content was determined with LC–MS–MS. Compared to established methods, this method offers minimal clean-up requirements and a high potential for automatization that make it suitable for routine control of MRL compliance. The method was extended to include mepiquat [30]. Quantification was done by the internal standard method, using mass chromatograms of the most intense product ions of mepiquat (m/z 98), chlormequat (m/z 58), and [^{13}C]chlormequat (m/z 61, internal standard). The limits of detection for chlormequat and mepiquat depend of the matrix investigated and were 6–10 μg kg^{-1} and 2–3 μg kg^{-1}, respectively. The performance of the method was demonstrated by analyzing grain material from an inter-comparison study.

Recently, LC–ES–MS has been used by Valenzuela et al. [34] for determination of abamectin residues in oranges; ES can be successfully used to determine and confirm a pesticide as this, with a high relation m/z. Abamectin was specifically detected in oranges by operating in the positive-ion mode under SIM conditions; in this way, it was possible to confirm the presence of abamectin down to 0.0025 μg g^{-1}.

2.3.3. Atmospheric pressure chemical ionization

APCI is a gas-phase ion-molecule reaction process, which leads to the ionization of the analyte molecules under atmospheric pressure conditions. The process is analogous to chemical ionization but the reactant ions are produced by the effect of a corona discharge on a nebulized aerosol of solvent. Due to the atmospheric pressure conditions, the high frequency of analyte/reactant ion collisions ensures high sample-ionization efficiency. The ionization is soft and results predominantly in protonated molecules $[M+H]^+$ in the positive ion mode or deprotonated molecules $[M\text{-}H]^-$ in the negative ion mode.

The use of APCI–MS without coupling to LC for the detection of triazine herbicides in spiked orange peel and cabbage leaf samples has been described [43]. Triazines were thermally stable under the analytical conditions and yield spectra with intense molecular ions at higher m/z values than the matrix. Direct quantification in unextracted samples was achieved by using product ion analysis or multiple reaction monitoring with detection limits of 0.01 to 0.1 mg kg^{-1}.

Pleasance et al. [42] evaluated APCI and IS for analysis of *N*-methylcarbamate pesticides and compared these techniques with TSP and PB interfaces. Fig. 1 shows the chromatograms of the eight carbamates obtained with the different MS interfaces and with UV detection. It illustrates wonderfully the advantages and drawbacks of each interface in terms of sensitivity that are extensively commented on throughout the text. They concluded that an APCI source provides a clear advantage in terms of sensitivity, linearity and range of compounds to which it is applicable. The suitability of LC–APCI-MS for pesticide residue analysis in fruit and vegetables is demonstrated by analyzing of green pepper extract spiked at the 0.1 ppm level with methomyl, aldicarb and carbaryl.

A method involving LC–APCI-MS was first reported for the determination of diflubenzuron in mushrooms and was subsequently applied to determination of diflubenzuron and clofentezine in plums, strawberries and blackcurrant-based fruit drink [35,39]. In preliminary experiments using full scan acquisition, both positive and negative ionization modes were evaluated. No ions attributable to diflubenzuron were observed in the positive mode while the negative mode gave an intense $[M\text{-}H]^-$. Under positive-ion conditions, clofentezine gave a weak spectrum containing $[M+H]^+$ and some higher mass adducts. A much more intense spectrum was observed under negative conditions, and this was dominated by ions corresponding to $[M]^-$. A reporting limit of 0.02 mg kg^{-1}, ten times lower than the lowest MRL (0.2 mg kg^{-1}) set for any of the products investigated, was achieved.

This method has been extended to allow screening of eight additional pesticides (carbofuran, pirimicarb,

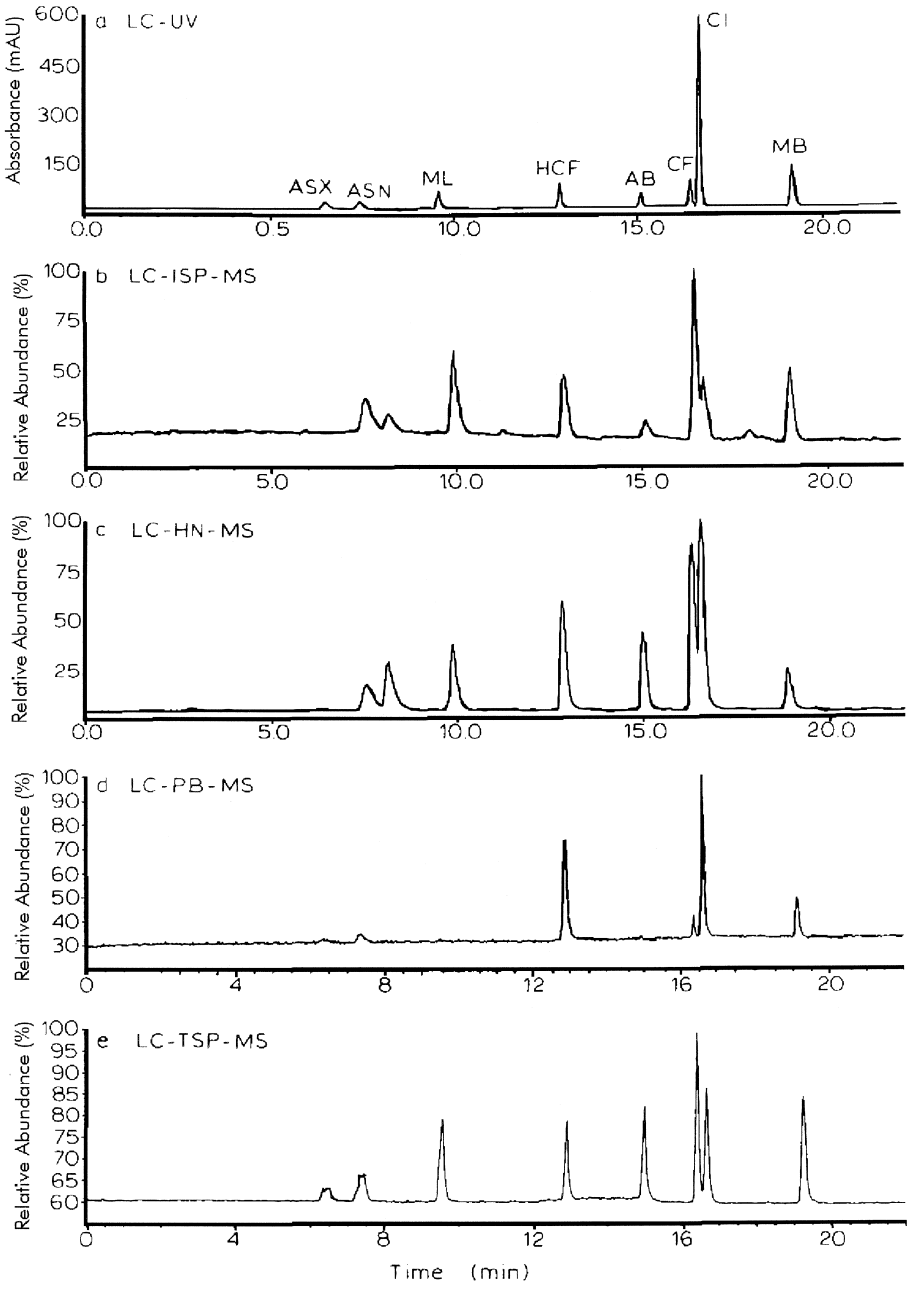

Fig. 1. LC separations of eight *N*-methylcarbamate pesticides with detection by (a) UV (214 nm) and mass spectrometry using (b) ionspray, (c) APCI, (d) PB–EI and (e) TSP interfaces. Conditions: column 250×4.6 mm I.D. Zorbax RX-C_8; mobile phase, aqueous methanol for (a) and (c), modified with formic acid for (b), all at a flow-rate of 1 ml min^{-1}; gradient profile, held at 20% methanol for 4 min followed by a linear gradient to 70% methanol in 11 min and held for 5 min; split ratios (1:20) for (b) and (3:2) for (d), post-column addition of 0.2 ml min^{-1} of 0.5 *M* ammonium acetate for (d); injection volume, 20 μl (20 μg ml^{-1}) for (a) and (d), and 2 μg ml^{-1} for (b), (c) and (e). Peaks: ASX, aldicarb sulfoxide; ASN, aldicarb sulfone; ML, methomyl; HCF, 3-hydroxycarbofuran; AB, aldicarb; CF, carbofuran; CI, carbaryl; MB, methiocarb. From Ref. [42] with permission.

3-hydroxycarbofuran, aldicarb, aldicarb sulfoxide, aldicarb sulfone, thiabendozole, and carbendazim), selected because they or their metabolites are not appropriate for current GC–MS multiresidue techniques [38]. Initial comparison was made between two atmospheric pressure ionization techniques, APCI and IS. The approximate limits of detection were estimated using negative APCI and IS, for diflubenzuron and clofentezine and positive APCI and IS for the remaining eight compounds. These were comparable for most compounds, although APCI was the more sensitive technique for aldicarb and its metabolites and carbofuran. This, and the greater flexibility of the LC flow-rates associated with APCI, led to a choice of APCI as a technique on which to base multiresidue method development.

LC–APCI-MS has proved to be a robust and reliable method for determining fenbutatin oxide in tomato, cucumber and banana extract [40]. After optimization, the system is stable and data have been acquired for up to 120 continuous injections. A decrease in sensitivity is observed over time but this is adequately compensated for by frequent calibration. A routine reporting limit of one-tenth of the lowest MRL and a limit of detection that is 25 times less than the lowest MRL stipulated for these crops has been achieved.

Comparison of APCI with post-column fluorimetry for determination of carbamates indicates than APCI-MS can approach the sensitivity of the fluorescence methods [36]. In general, agreement between the two detection methods was good, although when background was high — as in the case of oxamyl in celery and aldicarb in apple and cauliflower — recoveries determined by MS were considerably lower than those determined by fluorescence detection.

A competitive enzyme-linked immunosorbent assay (ELISA) method for carbaryl quantification in crop extracts was validated by an LC–MS interface with APCI in low concentrate samples [37]. This technique showed levels of sensitivity and selectivity comparable to those obtained by GC–MS, making it possible to analyze the unstable carbaryl and also to verify the degradation of the carbaryl to 1-naphthol in some extracts.

A procedure for determining insecticides used by organic farmers in vegetables has been also reported [41]. In general, detection limits obtained by SIM were found to range from 1 ppb of piperonyl butoxide to 200 for pyrethrin II.

3. Identification and confirmation

The use of a mass spectrometer as detector gives a higher degree of confirmation of molecular identity than methods based on fluorescence or ultraviolet techniques.

3.1. Particle beam

The main advantage of the PB technique is its ability to obtain conventional EI and CI mass spectra in full-scan mode that allows characterization of compounds. In the EI mode, the mass spectra obtained could be compared with those contained in a spectra library, which allows the identification of non-target compounds in real-world matrices. The first study describes the sensitive detection, quantification and confirmation of ETU residues in several food crops using LC–PB-MS. Spectra obtained from crop samples containing as little as 5 ng of ETU were matched with the NBS library reference EI spectrum [21].

The LC–PB-MS system has been successfully used for both identification and quantification of the carbamates methiocarb sulfoxide, baygon, carbaryl and methiocarb. Fig. 2 shows the total ion chromatogram (TIC) corresponding to a sample of lettuce spiked with four carbamates at levels under the maximum allowed by European regulations. Identification of the four carbamates was positive when their EI mass spectra obtained by LC–PB-MS were compared with those contained in the Wiley 138.1. Reference library. As an example, inside the figure, over the chromatogram, the EI mass spectrum obtained and the reference spectrum are shown for Baygon, the least sensitive of the four carbamates. As can be observed, the problem of using the full scan mode is that peaks corresponding to other matrix components coextracted with carbamates appear. Although SIM does not permit identification by comparing with a reference library, for quantification this operating mode is preferred because it is more sensitive and masks interferent signals. The

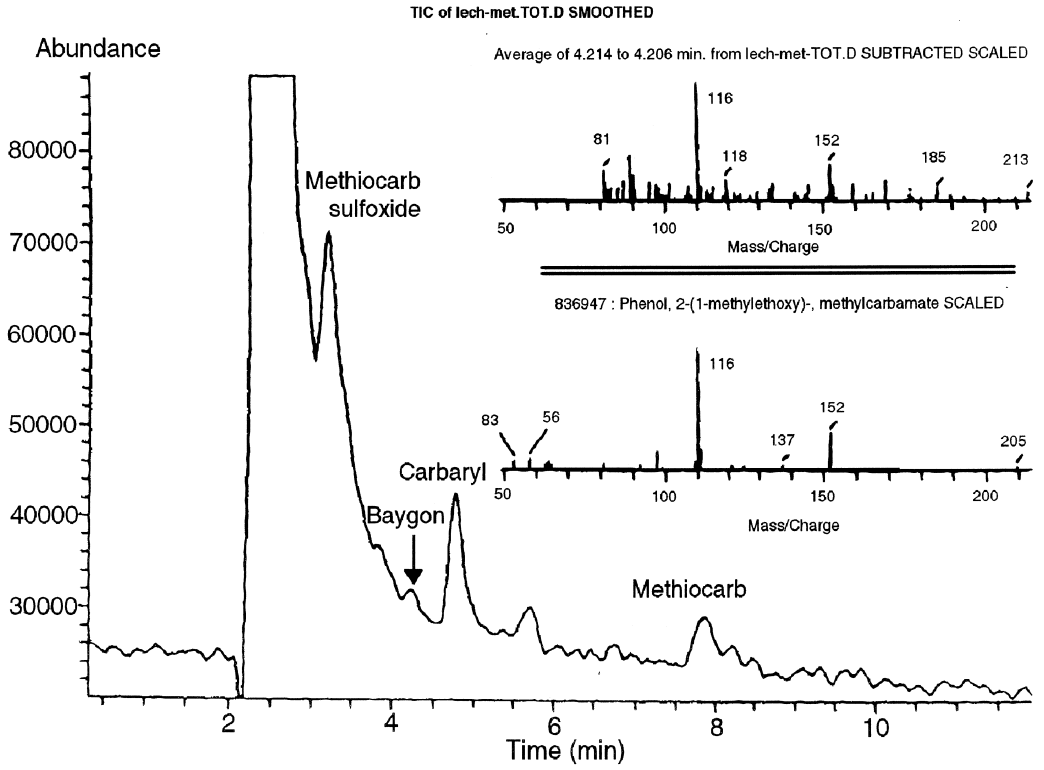

Fig. 2. LC–PB-MS total ion chromatogram of lettuce extract spiked with 0.19 mg kg^{-1} of methiocarb sulfoxide, 2.50 mg kg^{-1} of baygon, 2.28 mg kg^{-1} of carbaryl and 0.16 mg kg^{-1} of methiocarb. From Ref. [19] with permission.

mass analyzer monitor the m/z values characteristics of the selected pesticides instead of scanning low-mass m/z values where the ions will be common to many compounds, and sensitivity will be lost.

Confirmation and quantification of daminozide was carried out by PB mass spectra obtained in SIM in the positive CI mode with isobutane as the reagent gas. Daminozide was confirmed by both the retention time and the presence of characteristic mass ions (m/z ion, relative abundance): 161, M+1, 100%; 162, M+2, 7%; 143, M+1-H_2O, 4%.

3.2. Thermospray and atmospheric pressure ionization

TSP shares with the API techniques the disadvantage of producing primarily $[M+H]^+$ ions, with little of the structurally informative fragmentation provided by EI. This is a major disadvantage for qualitative analysis, but it can be an advantage for target compound detection by SIM provided that, in addition to $[M+H]^+$, some reasonably abundant and structurally significant fragment or adduct ions can be detected. Liu et al. [22] demonstrate that although some analytes coeluted, they could still be analyzed because of the specificity of SIM.

An expensive remedy to the fragmentation problems is to put a TSP ionizer on a tandem mass spectrometer. Under TSP conditions, the major ions observed from diflubenzuron are m/z 175, 311 and 313. It was possible to prove that diflubenzuron was not present by monitoring m/z 311 and 313 because when monitoring m/z 175 there was too much interference [23]. Confirmation of positive results was possible by MS–MS 'multiple reaction monitoring' of the m/z 311 to m/z 158 transition.

The ES/IS and APCI techniques both produce mild ionization which can be complemented by invoking fragmentation-inducing collisions in the interface itself (the so-called pre-analyzer or CID) or

by recourse to LC–MS–MS, as occurs when a triple-quadrupole system is used.

In most multiresidue methods developed by IS and APCI for fruit and vegetables, collision-induced dissociation in the atmospheric pressure source makes it possible to obtain at least one ion of confirmation for each analyte of reasonable intensity [27,28,32,35,38–41]. Fragmentation can thus be induced by varying the orifice voltage; this parameter may be carefully fixed, which is also crucial for an efficient transmission of ions, to obtain the best compromise between sensitivity and fragmentation. Moreover, the selectivity and specificity of the determination were enhanced by using relative retention times and ratios of confirmation. Such 'cone voltage' induced fragmentation provides the potential for generation of alternative confirmatory ions but at the expense of molecular ion sensitivity. As an example Fig. 3a shows the spectrum of diflubenzuron obtained at low extraction and focus voltages where $[M–H]^-$ was the base peak. Higher extraction and focus voltages cause increased fragmentation (Fig. 3b), and under these conditions the deprotonated difluorobenzamide fragment (m/z 156) was the base peak.

Using MS–MS for detection and quantification greatly reduces the risk of false positive findings and eliminates the need for excessive cleanup. This method was used for routine analysis of chlormequat and mepiquat residues in grain [30,31] and imazethapyr and its metabolites in plant matrices [29]. A representative chromatogram obtained from a real sample is shown in Fig. 4.

On-line photolysis can also be used to induce photolytic reactions that give structurally diagnostic product ions and thus, in turn, to add a significant degree of selectivity to LC–MS analyses. Volmer [26] demonstrated this for trace level determination and confirmation of triazine herbicides in blueberry and lettuce extracts. Although the method does not

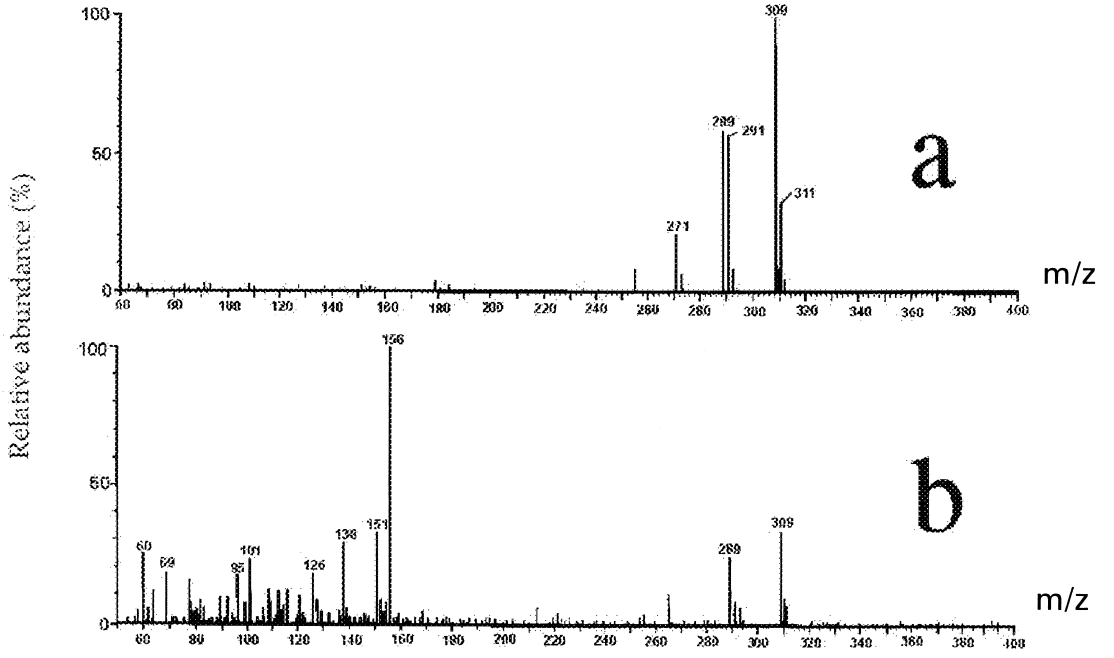

Fig. 3. Spectrum of diflubenzuron obtained at (a) low extraction and focus voltages (5 and 10 V, respectively), the base peak of which is the deprotonated molecule at m/z 309 and (b) higher extraction and focus voltages (30 and 35 V, respectively) the base peak of which is deprotonated difluorobenzamide fragment at m/z 156. From Ref. [35] with permission.

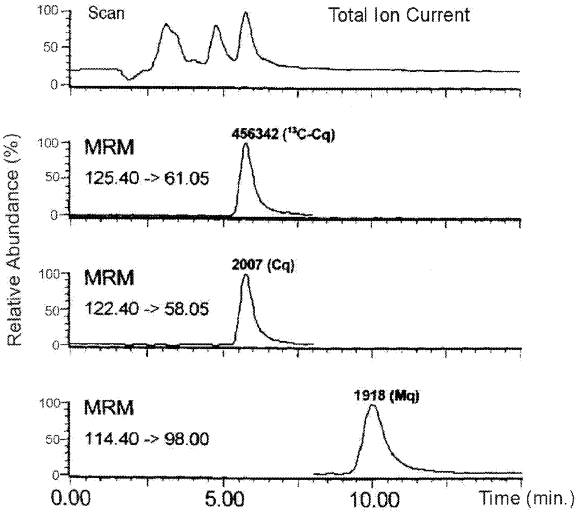

Fig. 4. Chromatograms of a rye sample field incurred with 0.18 mg chlormequat (Cq) kg^{-1} and 0.05 mg mepiquat (Mq) kg^{-1}. The scan chromatogram shows the total ion current of m/z 50–150. The MRM chromatograms show the trace of the product ions m/z 62, 58, and 98 of the quaternary ammonium ions of [^{13}C] Cq, Cq, and Mq, respectively. The height of MRM peaks are given in ion counts. From Ref. [30] with permission.

approach the degree of selectivity provided by MS–MS methods, it can serve as an inexpensive alternative for many applications.

4. Matrix interferences

Liquid–liquid extraction has a long history, and although other techniques have supplanted it in some cases, this technique is still useful. Recently, solid-phase extraction has grown in importance. Most current applications of sample preparation for LC–MS use some variant of these techniques [46].

4.1. Particle beam

Liquid–liquid extraction procedures have been applied prior to LC–PB-MS. Extraction and purification of ETU residues from crop samples were performed using the AOAC method as revised by Krause. The recoveries (mean=85±15%) are typical of those for extraction of ETU residues from a variety of crops. No inferences were detected using full scan from m/z 70–300 [21].

Direct extraction of carbamates from lettuces and apples with dichloromethane has been proposed [19]. One of the major problems observed in LC–PB-MS analysis of the lettuce samples was the broad peak appearing at the beginning of the chromatogram. The peak corresponds to carbohydrates and other polar compounds coextracted with pesticides like carbamates. Since lettuce contains low amounts of carbohydrates, methiocarb sulfoxide was identified and quantified. This was not possible when apple samples were tested, because of their high sugar content [19].

Daminozide is measured in apple juice samples after a sample preparation that involves evaporation and treatment with methanol and acetone to precipitate sugars and inorganic salts [20]. Daminozide was confirmed by both the retention time and the presence of characteristic mass ions, without other matrix peaks.

4.2. Thermospray

Benomyl has proven to be a difficult analytical target because it readily decomposes in many common organic solvents as well as in water. Because of this instability, residues of benomyl in crops are determined by acid hydrolysis of benomyl to the stable compound, carbendazim. The method has been adapted to the determination of benomyl in peach, apples and tomatoes by partitioning with ethyl acetate [25].

A research group has reported a procedure for pesticide determination based on a single extraction step and the use of MS [22,24]. The pesticides were extracted from apples, beans, lettuces, peppers, potatoes, and tomatoes with a slightly modified Luke multiresidue extraction procedure. The Luke procedure was used to extract the pesticides because it is known to be capable of extracting more than 230 pesticides from fruit and vegetables and is thus the most common extraction procedure used by regulatory agencies. The method discussed in these papers has several advantages over current methods. It does not require the derivatization step needed in the

current official methods for determining of carboxim and oryzalin. Different classes of pesticides, such as carbamates and phenylureas are extracted by one procedure. The sensitivity and specificity of the LC–MS in the SIM mode permit the use of the Luke extraction procedure and make it unnecessary to include the clean-up steps in the procedure used. Recoveries were between 69 and 110%, except for carboxim, which had recoveries of 33 to 54%.

During the screening of various fruits and vegetables for diflubenzuron by LC–UV, problems were encountered with the analysis of extracts of fresh chilies and of plums. The extraction procedure involves maceration of the sample with anhydrous sodium sulfate, sodium hydrogen carbonate and ethyl acetate. With the chilies, several interfering peaks were observed, one of which masked the area of potential diflubenzuron response. With the plum extracts, an LC peak was observed in roughly half of the samples. The extracts were analyzed by positive TSP-MS and it was possible to prove that diflubenzuron was not present. Detection in the negative-ion mode was also attempted but was not sufficiently sensitive.

4.3. Ionspray

The residues from samples of apples and pears were extracted using acetone, dichloromethane–hexane. This multi-class/multiresidue extraction method is suitable for both polar and slightly apolar pesticides. Extraction recoveries were between 75 and 98%, except for methyl thiophanate (<20%). No clean-up was necessary and the time required was reduced. Differences between the two matrices were observed. The limits of quantification obtained in apples were slightly higher than in pears [28].

Residues of chlormequat and mepiquat in grain were extracted with methanol–water–acetic acid. Clean-up was achieved using a C_{18} cartridge [30,31]. Most existing methods for determining these compounds possess two main drawbacks in relation to their applicability to routine residue analysis: the need for clean up is excessive and there is a persistent risk of false positive findings. The proposed method addresses both these problems.

Imazethapyr and its metabolites were determined using reduced sample and a microwave-assisted extraction with 100% water. The initial extract could be loaded directly onto a strong cation-exchange cartridge for clean up.

Di Corcia et al. proposed an LC–ES–MS for routinely monitoring low levels of carbamate in fruit and vegetables [27]. Vegetable materials were extracted with methanol. An aliquot of the homogenate equivalent to 5 g of the vegetable material was suitably diluted with water and passed through a Carbograph extraction cartridge. Carbamates were eluted by passing through the cartridge a mixture of dichloromethane and methanol. Recovery of the analytes was better than 80%, regardless of the type of vegetable matrix. The presence in the electrosprayed solution of many vegetable constituents did not interfere significantly with the carbamate ionization process. Under these conditions, well-defined chromatographic profiles were obtained for the 12 carbamates present in vegetables at the individual level of 200 ng g^{-1}, as is shown in Fig. 5.

Thirteen carbamates were analyzed in oranges, grapes, onions and tomatoes by MSPD followed by LC–MS. MSPD was a variation of SPE consisting of blending the sample with a solid-phase to obtain a homogeneous mixture. This mixture, introduced into a glass column was eluted by dichloromethane–acetonitrile. The mean recoveries using C_8 as the solid material varied from 64 to 106%. Matrix constituents did not interfere either [32].

Abamectin residues were also extracted using MSPD [34], homogenizing orange samples with C_{18} and eluting with dichloromethane. Recoveries of abamectin from oranges fortified with approximately 0.01 to 10 $\mu g\ g^{-1}$ ranged from 94 to 99%.

Immunoaffinity chromatography involves the use of antibodies for trace analyte extraction and enrichment directly from a complex matrix. The antibodies are used in columns designed for use with ordinary equipment. The immunoaffinity columns are coupled directly to LC–MS. Direct extraction and detection of carbofuran were demonstrated at low levels from crude potato extract. The purification obtained was superior to that of samples pumped directly onto a reversed-phase trapping column [33].

4.4. Atmospheric pressure chemical ionization

Barnes et al. homogenized the samples with

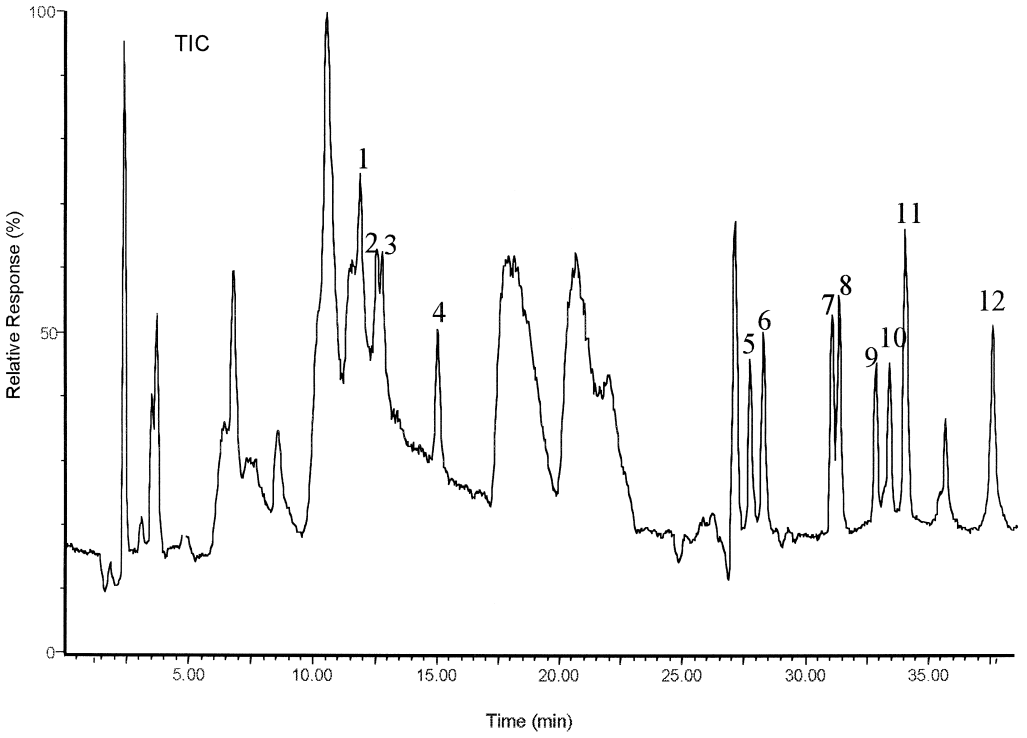

Fig. 5. TIC chromatogram obtained by analyzing a spinach sample amended with 12 carbamates at the individual level of 200 ng g^{-1}: 1, butoxycarboxim; 2, aldicarb sulfone; 3, oxamyl; 4, methomyl; 5, butocarboxim; 6, aldicarb; 7, propoxur; 8, carbofuran; 9, carbaryl; 10, ethiofencarb; 11, pirimicarb; 12, mercaptodimethur. From Ref. [27] with permission.

acetone extracted into dichloromethane–cyclohexane and used clean up by gel permeation to determine diflubenzuron and clofentezine [35,39]. The calibration obtained when using standards prepared in matrix extracts was examined. Differences in slope were observed when compared with calibrations using methanol–water solutions. The differences in slope can be attributed to the presence of co-extractives. These authors reported the same phenomenon when the extraction procedure was based on homogenization with sodium acetate, anhydrous sodium sulfate and ethyl acetate to determine carbamates, benzoylureas and febutatin oxide [38,40]. During investigation into sensitivity and calibration, matrix-matched and solvent-based standard of the same concentration were kept paired but analyzed in a random concentration order. An enhancement or suppression effect due to matrix was observed for most but not all the compounds, and this effect was both compound- and -matrix-dependent.

The instrumental selectivities of the APCI–MS and fluorescent detectors for various carbamates were compared [36]. Five products were spiked with 11 carbamates at levels from 10 to 100 ppb, extracted, and cleaned up using a method based on the AOAC official method. Unspiked products contained substances that produced a response in either the postcolumn fluorimetric detector or the mass spectrometer. Except perhaps in the case of oxamyl in celery, there were no indications of incurred residues because there were no cases in which the response from one detector agreed with that of the other. Carbaryl may have been present in celery because a response occurred with both detectors, but the response in the mass spectrometer was greater than in the fluorescence detector. This result indicates interference in the mass spectrometer. An apparent aldicarb peak showed up in all commodities analyzed by MS but was not observed when the extracts were analyzed with a postcolumn fluorescence detector.

Neither detection method alone ensures definitive identification of residues.

The insecticides used by organic farmers (Rotenone, cevadine, veratridine, pyrethrin I and II, ryanodine and dihydroryanodine) are extracted with acetonitrile–water and are cleaned-up with solid-phase extraction [41]. Results with cucumber and cabbage were essentially the same, with differences in sensitivity due to the mass spectral characteristics of the different interfering compounds found in different vegetables.

In contrast, Nunes et al. determined carbaryl in six vegetable products using a procedure based on the methodology adopted by the FDA for *N*-methylcarbamate determination [37]. The confirmation of carbaryl peaks in samples spiked at low contents was achieved without matrix interferences.

5. Conclusions

LC–MS using PB, TSP and API interfaces is a powerful analytical tool for determining pesticide residues in fruit and vegetables, for it provides the advantages of chromatography as a separation technique and those of mass spectrometry for the unambiguous identification of even non-volatile or ionic substance. Moreover, it offers the possibility of obtaining limits of detection ten times lower than the lowest MRL set by the EU or the FDA for fruit and vegetables. After comparing the different LC–MS techniques on the basis of the applications, it can be inferred that API interfaces are the preferable. In general, with API interfaces a larger number of pesticides have been determined than with PB or TSP.

Although an MS spectrometer is still initially a more expensive and a more complex device that most other LC detectors, once a mass spectrometer is operating, it can be very dependable and reliable, and MS can then eliminate many of the other variables. LC–MS and tandem MS–MS dramatically simplify clean-up procedures, reducing not only sample analysis time but also more important method development time.

LC–MS appears to be a well-established technique. The number of reports in literature on its application to pesticide residues in fruit and vegetables is rapidly increasing. Although it can not yet be considered a routine technique, LC–MS will complement GC–MS in analytical laboratories in only a few years.

Acknowledgements

This work was financially supported by the Generalitat Valenciana, Conselleria de Cultura, Educació i Ciència (GV-AG-03-13).

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