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Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography–time-of-flight mass spectrometry

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Abstract

In this work, a new multi-residue methodology using liquid chromatography–time-of-flight mass spectrometry (LC–TOF-MS) for the quantitative (routine) analysis of 15 pesticide residues has been developed. The analytical performance of the method was evaluated for different types of fruit and vegetables: pepper, broccoli, tomato, orange, lemon, apple and melon. The accurate mass measurements were compared in different matrices at significantly different concentration levels (from 0.01 to 0.5 mg/kg) obtaining accuracy errors lower than 2 ppm, which is well within the accepted limits for elemental confirmation. Linearity of response over two orders of magnitude was demonstrated ($r > 0.99$). Matrix effects resulting in suppression or enhancement of the response were frequently observed, most notably in broccoli and citrus. Instrumental limits of detection (LOD) were between 0.0005 and 0.03 mg/kg depending on the commodity and pesticide studied, all being within European Union regulations for food monitoring program. Finally, the methodology was applied to the analysis of two samples from an inter-laboratory exercise. The high degree of confirmation for target pesticides by accurate mass measurements demonstrated the applicability of the method in routine analysis. This study is a valuable indicator of the potential of LC–TOF-MS for quantitative multi-residue analysis of pesticides in vegetables and fruits.

Keywords: Pesticides; Time-of-flight; Mass spectrometry; Food analysis; Vegetables

1. Introduction

In recent years, the established regulations regarding the maximum residue levels (MRLs) in commodities have become more and more stringent. The European Union (EU) has set new Directives for pesticides at low levels in vegetables in order to meet these health concerns. For example, new laws such as the European Directive 91/414/EEC, or the Food Quality Protection Act (FQPA) in the USA have increased the standards for human health, workers, and environmental protection. The quality standards include the re-assessment of the maximum residue limits, which are typically lower than the previous ones. For example, in Europe the new Directive also lead to the harmonization of the MRL for each EU country and, in some cases, individual country MRLs are not decided. Therefore, EU directives are setting different MRLs for each pesticide within each food group. Typically, the MRLs range from 0.01 to 3 mg/kg depending on the commodity and pesticide [1]. For fruits and vegetables intended for production of baby food, an MRL of 0.01 mg/kg is applicable for all pesticides [2], and finally, banned compounds have the lowest MRLs at 0.01 mg/kg.

The low MRLs have fostered the development of more powerful sensitive analytical methods to meet the requirements in complex samples, such as food. In this sense, liquid chromatography–tandem mass spectrometry (LC–MS–MS) with triple quadrupole in selected reaction monitoring (SRM)

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mode has become so far, the most widely used technique for the quantitation of (polar) pesticides in food as reported extensively in the literature [3–14]. On the other hand, high-resolving power mass spectrometric techniques such as time-of-flight mass spectrometry (TOF-MS) have been applied in the environmental field mainly for structure elucidation or confirmation purposes [15–19]. LC–TOF-MS has been applied for confirmatory analyses rather than for quantitation mainly because of well-known limitations such as the narrow dynamic range. Another disadvantage has been the lack of accuracy of some instruments to achieve the 2–5 ppm error level, usually needed when analyzing complex matrices for unequivocal identification of the target analytes [19,20]. However, the use of TOF-MS techniques has become necessary in the last few years for the unequivocal identification of contaminants and veterinary drugs in meat [21] and to achieve the EU requirements regarding the number of identification points for a positive finding [22]. In addition, the use of TOF-MS allows the capability of non-target identification, because the full-spectrum is recorded at all times, which is not possible with standard monitoring practices that use single ion monitoring or multiple reaction monitoring (MRM) techniques.

Finally, this paper presents a detailed overview of the accuracy and precision of a multi-residue method for pesticides in complex fruit and vegetable matrices. The present strategies and technical development of such methodology are described in regard to identification and quantification.

2. Experimental

2.1. Chemicals and reagents

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Chemical structures for the pesticides studied in this work are shown in Fig. 1. Individual pesticide stock solution (200–300 μg/ml) were prepared in pure methanol and stored at −18 °C. HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Formic acid was obtained from Fluka. Pesticide-grade ethyl acetate and anhydrous sodium sulphate were from Panreac (Barcelona, Spain).

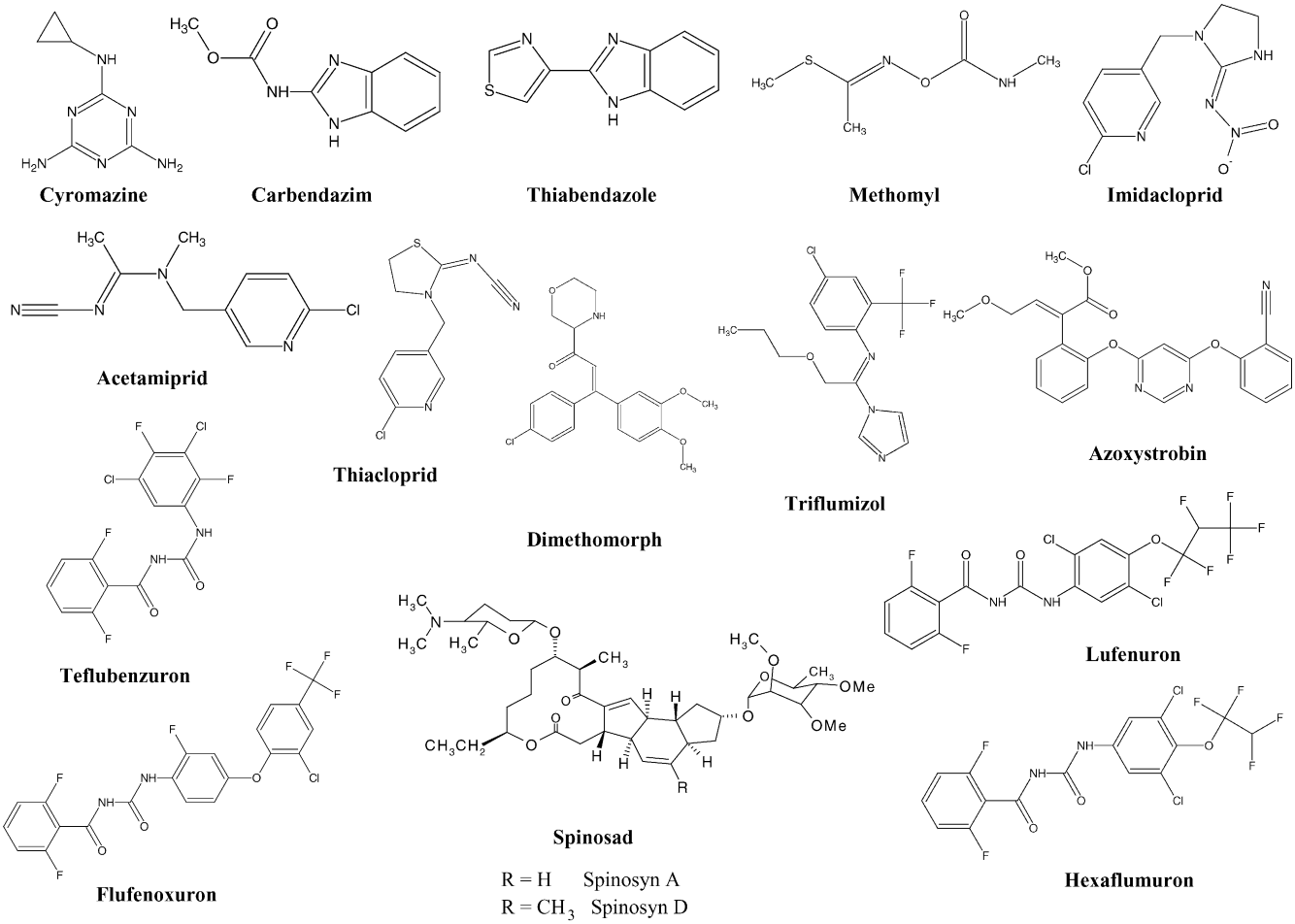

Fig. 1. Chemical structures of the selected multi-class pesticides.

2.2. Sample treatment

Vegetable samples were obtained from the local markets. "Blank" vegetable and fruit extracts were used to prepare the matrix-matched standards for validation purposes. The extraction procedure was as follows: a 15-g portion of sample previously homogenized was weighted in a 200 ml PTFE centrifuge tube. Then, 90 ml of ethyl acetate and 1 ml NaOH (6.5 M) were added and the sample was blended in a Polytron (high-speed blender) for 30 s at 21,000 rpm. The extract was then filtered through a layer of 20 g of anhydrous Na_2SO_4. After that, the solid was washed with 50 ml of ethyl acetate and the combined extracts were evaporated to dryness on a vacuum rotary evaporator using a water bath at $45 \pm 5\,^{\circ}C$. The remaining residue was dissolved by sonication in 15 ml of methanol. The extracts obtained this way, containing 1 g of sample per ml, were filtered through 0.45 μm PTFE filters (Millex FG, Millipore, Milford, MA, USA) before analysis.

Quantitation of sample extracts during validation was done using a calibration curve based on matrix-matched standards (blank extracts fortified with the analytes). The matrix blank residues were fortified with a mixture of the pesticides studied at concentrations ranging from 0.01 to 0.5 mg/kg in order to have a wide range of concentrations. The integrated peak area data of the selected quantification masses were used to construct the calibration curves. The linearity in the response was studied by using standards prepared in pure solvent and by comparing it with matrix-matched extract solutions to evaluate possible matrix effects. The limits of detection (LODs) were determined as the analyte concentration that gave a signal-to-noise of 3, as calculated by the instrument software, and empirically verified by analyzing pesticide mixtures at these concentration levels in matrix extracts.

2.3. LC–TOF-MS

Liquid chromatography–electrospray ionization–time-of-flight mass spectrometry (LC–ESI–TOF-MS), in positive ionization was used to detect the pesticides. The separation of the selected pesticides was carried out using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1100, Agilent Technologies, Palo Alto, CA, USA) equipped with a reversed-phase C_8 analytical column of 150 mm × 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25 °C. The injected sample volume was 50 μl. Mobile phases A and B were acetonitrile and water with 0.1% formic acid, respectively. The optimized chromatographic method held the initial mobile phase composition (10% A) constant for 5 min, followed by a linear gradient to 100% A in 25 min. The flow-rate used was 0.6 ml/min. A 12-min post-run time back to the initial mobile phase composition was used after each analysis. This HPLC system was connected to a time-of-flight mass spectrometer Agilent MSD TOF (Agilent Technologies) with an electrospray interface, using the operational parameters included in Table 1. LC–TOF-MS accurate mass spectra were recorded across the range from 50 to 1000 *m/z*. The data recorded was processed with the Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany) with accurate mass application-specific additions from Agilent MSD TOF software. The mass axis was calibrated using the mixture provided by the manufacturer over the *m/z* 50–3200 range. A second orthogonal sprayer with a reference solution was used as a continuous calibration using the following reference masses: 121.0509 and 922.0098 *m/z* (resolution: 9500 ± 500 at 922.0098 *m/z*). Spectra were acquired over the *m/z* 50–1000 range at a scan rate of one second per spectrum. Optimization of LC–TOF-MS parameters such as capillary voltage, nitrogen flow, drying temperature and fragmentor voltage were carried out by chromatographic separation of the mixture of pesticides under the conditions described above.

Table 1
LC–TOF-MS operational parameters in positive ESI ion mode

Parameter	Value
Capillary voltage	4000 V
Nebulizer pressure	40 psig
Drying gas	9 l/min
Gas temperature	300 °C
Fragmentor voltage	190 V
Skimmer voltage	60 V
Octapole DC 1	37.5 V
Octapole RF	250 V
Mass range (*m/z*)	50–1000
Resolution	9500 ± 500 (922.0098)
Reference masses	121.0509; 922.0098

3. Results and discussion

3.1. LC–TOF-MS parameter optimization

The main instrumental parameters (drying and nitrogen flow rates, vaporizer and drying temperatures and capillary voltage) were optimized to provide the best possible sensitivity (Table 1). However, the effect of all these parameters in the studied ranges did not affect significantly the signal of the analytes, except for the fragmentor voltage, which played an important role in both the sensitivity and fragmentation patterns. This parameter is important, because it provides valuable structural information (characteristic fragmentation for each pesticide), making attainable the accurate mass of each characteristic fragment ion together with its elemental composition [17,18], which can be used with the molecular ion for confident identification criteria.

For this reason, the voltage value was studied in the range from 120 to 250 V under optimized source conditions (see Table 1). Fragmentor voltages of 250 V or higher led to extensive fragmentation even of the reference masses. Voltage values of about 120 V provided minimal fragmentation in most pesticides. Two medium voltage values were explored for proper optimization: 190 V (a medium value

Table 2
Optimization of fragmentor voltages

	m/z	Relative abundance (V)	
		190	230
Cyromazine	167[a]	100	100
	125	<5	22
	85	<5	24
Carbendazim	192[a]	100	17
	160	43	100
Thiabendazole	202[a]	100	100
	175	<5	9
Imidacloprid	278[b]	–	38
	256[a]	100	48
	210	24	20
	209	20	56
	175	24	100
Acetamiprid	245[b]	–	25
	223[a]	100	77
	126	15	100
	99	–	12
Thiacloprid	275[b]	–	24
	253[a]	100	83
	126	16	100
	99	–	11
Hexaflumuron	483[b]	100	100
	461[a]	94	80
Teflubenzuron	403[b]	100	100
	381[a]	64	30
Azoxystrobin	404[a]	100	19
	372	31	100
	344	–	11
Dimethomorph	388[a]	100	100
	301	<5	10
Triflumizol	346[a]	24	5
	278	100	100
Methomyl	185[b]	13	7
	163[a]	6	–
	106	32	14
	88	100	86
	73	20	100
Lufenuron	533[b]	100	100
	511[a]	70	70
Flufenoxuron	511[b]	100	100
	489[a]	92	46
Spinosad A	732	100	100
	544	<5	<5
Spinosad D	746	100	100
	558	<5	<5

[a] Protonated molecule (used for quantitation).
[b] Sodium adduct.

which provides a mild in-source CID fragmentation), and 230 V (for extensive fragmentation). A comparison of the typical fragment ions obtained and their relative abundances is summarized in Table 2. Some compounds presented characteristic fragmentations at a higher fragmentor voltage such as the neonicotinoid pesticides (imidacloprid, acetamiprid, thiacloprid) [23]. Similarly, cyromazine, carbendazim, azoxystrobin and dimethomorph needed a high voltage to fragment as well. On the other hand, methomyl gave abundant fragmentation even at the low fragmentor voltage of 190 V. Finally, thiabendazole, hexaflumuron, teflubenzuron, lufenuron, flufenoxuron and spinosad did not present a clear fragmentation even at a high fragmentor voltage. As a compromise value between sensitivity for quantitation (using the protonated molecule) and "rich" information mass spectra, a value of 190 V, was chosen for further experiments. In some exceptional cases (i.e. triflumizol), a lower voltage yields better sensitivity as well as enhanced fragmentation. It is important to note the presence of the sodium adduct as a base peak for the four benzolylurea pesticides (hexaflumuron, teflubenzuron, lufenuron and flufenoxuron). However, the protonated molecules are also present in the spectrum and they can be used for quantitation.

Using the capabilities of optimized in-source fragmentation, LC–TOF-MS becomes an attractive tool for the unequivocal identification of pesticides. In fact, the proposed approach fulfils the EC criteria for the spectrometric identification and confirmation of organic residues and contaminants, which are based on the use of identification points (IPs). The 2002/657/EC European Commission Decision establishes the need to obtain three IPs to confirm organic residues of drugs in food (four if they are banned substances) [21]. Using the accurate mass of the protonated molecule along with that of an additional characteristic fragment ion, the proposed technique meets these regulations.

3.2. Accurate mass measurements

The accurate mass measurements were carried out with the following procedure. The *m/z* of the analytes (using a mass interval of 0.2 Da), was extracted from the total ion chromatogram (TIC), to obtain an extracted ion chromatogram (XIC). Once the background of the XIC was subtracted, the accurate mass spectrum of the compounds was obtained. The accurate mass of the protonated molecule was used for both confirmation and quantitation purposes in all cases, except for methomyl, which presented a main fragment (*m/z* 88) as a base peak in the spectrum and in this case the sodium adduct $[M + Na]^+$ was the one used for quantitation to avoid background interferences at the low mass range. The accurate mass data of the molecular ions were then processed through the software, which provided a list of possible elemental formula. Once all the possible elements and a minimum and maximum number of each of those were set along with a threshold value for errors (i.e. 5 ppm), a list of empirical formulae ordered by error (ppm) was automatically provided together with the double bond and ring equivalent number (DBE).

The list of tentative empirical formulae can be drastically reduced by defining the minimum and maximum number of atoms for each molecule. This is the case for analytes with

one or more chlorine atoms, number that can be easily deduced from the isotopic profile of the accurate mass spectra. As an example, the accurate mass spectra of triflumizol and hexaflumuron obtained in a matrix-matched standard of pepper sample are shown in Fig. 2. As it can be seen in this figure, both the chlorine isotope pattern and its abundance either in the molecular or in the fragment ion can be easily used as a valuable tool for identification [15]. In addition to this, a major number of the proposed formulae are often "chemically incoherent" because they contain atoms that are not present in most organic compounds. This fact also helps in the unequivocal identification of the targeted species and the assignment of its correct elemental composition.

The accuracies obtained in the mass measurements of the protonated molecules of the selected pesticides on matrix-matched standards are shown in Table 3 (using a tomato extract fortified with 0.05 mg/kg of each pesticide as an example). The errors obtained were less than 2 ppm in most cases. The widely accepted accuracy threshold for confirmation of elemental compositions has been established at 5 ppm. Therefore, the mass measurement accuracy along with the characteristic retention time, usually provides unique elemental composition assignment. In addition, the mass measurement accuracy is also easily achieved for all the characteristic fragment ions, providing, thus, a double-set of information for unequivocal identification.

The good accuracy results obtained can be attributed to the way the instrument process "all the data, all the time" and calculates the accurate mass. The instrument uses a dual-nebulizer ion source and an automated calibrant delivery system, which introduces the internal reference masses (121.0509 and 922.0098) at a very low flow rate, combined with a software package, which is constantly auto-calibrating and recording the results of the internal reference masses along with the raw data. This strategy provides enhanced accuracy compared to many previous TOF instruments, in which the mass calibration was external.

The effect of different concentration levels and matrix complexity on the accurate mass measurements was evaluated in all the matrices tested at different concentration levels across the working range (0.01–0.5 mg/kg). No significant differences were observed in the accuracy obtained in the various matrix-matched standards compared to those prepared with pure solvents, keeping the error far below 5 ppm, with average values of about 2 ppm in all the pesticides (results not shown for all matrices, only for tomato matrix in Table 3).

3.3. Selectivity with accurate mass

The selectivity of LC–TOF-MS relies on the resolving power of the instrument on the *m/z* axis. The higher the resolution provided by the instrument, the better the selectivity for unequivocal identification. Taking into account that the resolving power of a TOF instrument is in the range of 5000–10,000 [19] it can discriminate between "isobaric" interferences within 0.05 Da mass difference (using an ion at 350 *m/z* for example). Therefore, an isobaric interference in LC–TOF-MS analyses would arise only if an interfering species with the same time retention of the target analyte had the same exact mass (differences less than 0.05 Da). This selectivity is significantly higher than that provided by any other LC–MS instruments. In addition, and as an alternative, a characteristic fragment ion accurate mass could be employed for quantitation in order to avoid this potential isobaric interference, using an optimized fragmentor voltage.

On the other hand, as a comparison with triple quadrupole instruments, the accurate mass measurement capabilities of TOF instruments can provide valuable evidence for an unavoidable isobaric interference, which might occur in complex samples. This kind of interferences yield well-known overestimation errors for example in LC–Q–MS and

Table 3
LC–TOF-MS accurate mass measurements in a tomato extract fortified with the pesticide mixture

Compound	Formula	Retention time	Selected ion	*m/z* experimental	*m/z* calculated	Error	
						m Da	ppm
Cyromazine	$C_6H_{10}N_6$	3.2	$[M+H]^+$	167.1040	167.10397	0.029	0.17
Carbendazim	$C_9H_9N_3O_2$	6.1	$[M+H]^+$	192.0767	192.07675	−0.05	0.27
Thiabendazole	$C_{10}H_7N_3S$	7.5	$[M+H]^+$	202.0430	202.04334	−0.34	1.7
Methomyl	$C_5H_{10}N_2O_2S$	12.3	$[M+Na]^+$	185.0355	185.03552	−0.02	0.11
Imidacloprid	$C_9H_{10}N_5O_2Cl$	15.7	$[M+H]^+$	256.0597	256.05957	0.12	0.47
Acetamiprid	$C_{10}H_{11}N_4Cl$	16.6	$[M+H]^+$	223.0742	223.07450	−0.3	1.3
Thiacloprid	$C_{10}H_9N_4ClS$	17.7	$[M+H]^+$	253.0308	253.03092	−0.12	0.48
Spinosyn A	$C_{41}H_{65}NO_{10}$	20.9	$[M+H]^+$	732.4668	732.46812	−1.32	1.81
Spinosyn D	$C_{42}H_{67}NO_{10}$	21.9	$[M+H]^+$	746.4832	746.48377	−0.57	0.77
Dimethomorph	$C_{21}H_{22}NO_4Cl$	22.8	$[M+H]^+$	388.1310	388.13101	−0.01	0.03
Azoxystrobin	$C_{22}H_{17}N_3O_5$	24.3	$[M+H]^+$	404.1243	404.12409	0.20	0.50
Triflumizol	$C_{15}H_{15}N_3OF_3Cl$	25.9	$[M+H]^+$	346.0925	346.09285	−0.35	1.0
Hexaflumuron	$C_{16}H_8N_2O_3F_6Cl_2$	27.2	$[M+H]^+$	460.9885	460.98889	−0.39	0.85
Teflubenzuron	$C_{14}H_6N_2O_2F_4Cl_2$	27.6	$[M+H]^+$	380.9816	380.98152	0.077	0.20
Lufenuron	$C_{17}H_8N_2O_3F_8Cl_2$	28.6	$[M+H]^+$	510.9854	510.98570	−0.30	0.58
Flufenoxuron	$C_{21}H_11N_2O_3F_6Cl$	29.2	$[M+H]^+$	489.0440	489.04351	0.49	1.0

Concentration, 0.05 mg/kg.

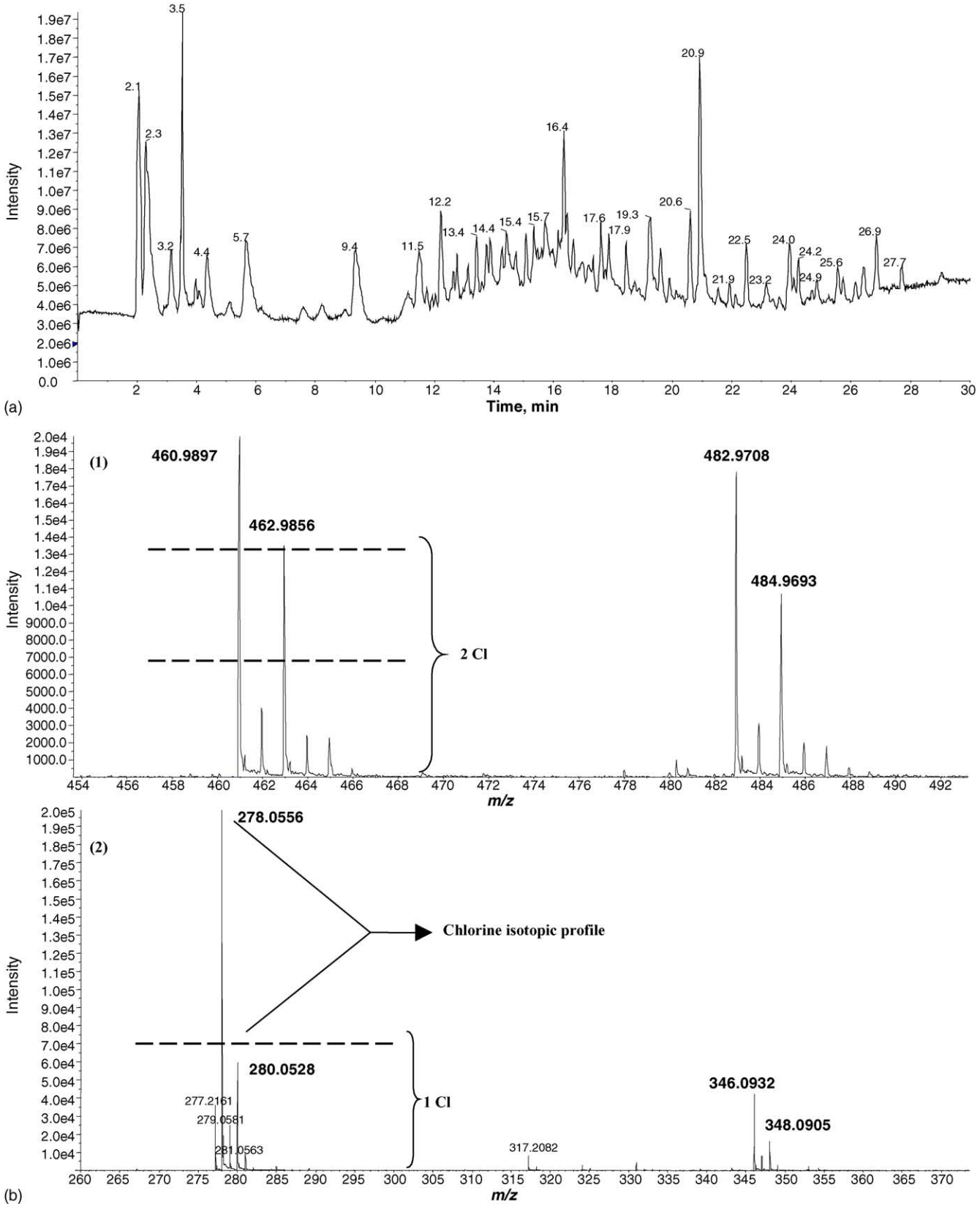

Fig. 2. Evidence of the chlorine isotopic pattern by LC–TOF-MS: (a) total ion chromatogram of a pepper-matched standard solution fortified at 0.05 mg/kg and (b) accurate mass spectrum for hexaflumuron (1) and triflumizol (2).

LC–(TQ)–MS–MS instruments. This is the case of overestimation errors due to the contribution from the signal of the ^{13}C isotope of another compound which is one unit mass lower than the target compound [17]. This contribution cannot be avoided (and even detected) with LC–MS(–MS) instruments in SIM or SRM modes. Taking advantage of the *m*/*z* resolving power of LC–TOF-MS, accurate mass measurements can, at the least, unravel the existence of these inevitably isobaric interferences. In this sense, to circumvent these interferences, other characteristic fragment ions from the accurate mass spectra of the targeted species could be employed "a posteriori" since the full-scan spectra is recorded at all times by LC–TOF-MS.

3.4. Analytical performance: quantitation

To confirm the suitability of the method for analysis of real samples, matrix-matched standards were used in the calibration. Quantitation was carried out under full-scan conditions by using the extracted ion chromatograms (XIC) (usually with a 0.2 Da window) of the protonated molecule for each pesticide, except for methomyl, which gave the sodium adduct. Peak areas of the extracted ions were used for quantitation. Linearity was evaluated by analyzing these standards solutions at six different concentration levels in the range 0.01–0.5 mg/kg. The linear calibration curves of four of the selected pesticides in different matrices are plotted in Fig. 3. As it can be observed in this figure, the linearity of the analytical response across the studied range is excellent, with correlation coefficients higher than 0.992 in most cases. Up to now, the analytical linearity of TOF instruments has not enabled its application for quantitative purposes. These instruments usually suffered from narrow dynamic ranges, requiring mathematical algorithms, such as the "time to digital correction", in order to attain a longer linear dynamic range. This has been a severe limitation on the applicability of TOF-MS for quantitation purposes. An analog to digital convertor (ADC) is used in this work providing thus, an enhanced linear dynamic range and this makes possible its successful applicability to pesticide residue routine analyses.

The reproducibility, repeatability and accuracy of the method were also evaluated on matrix-matched solutions at different concentration levels. The repeatability study was carried out by injection of the same standard solution five consecutive times in the same day. The reproducibility study was carried out for 5 successive days using the same solution. The relative standard deviation (RSD) values obtained from run-to-run and day-to-day precision and accuracy studies are summarized in Table 4 at three different concentration levels. From the results obtained, the developed method was

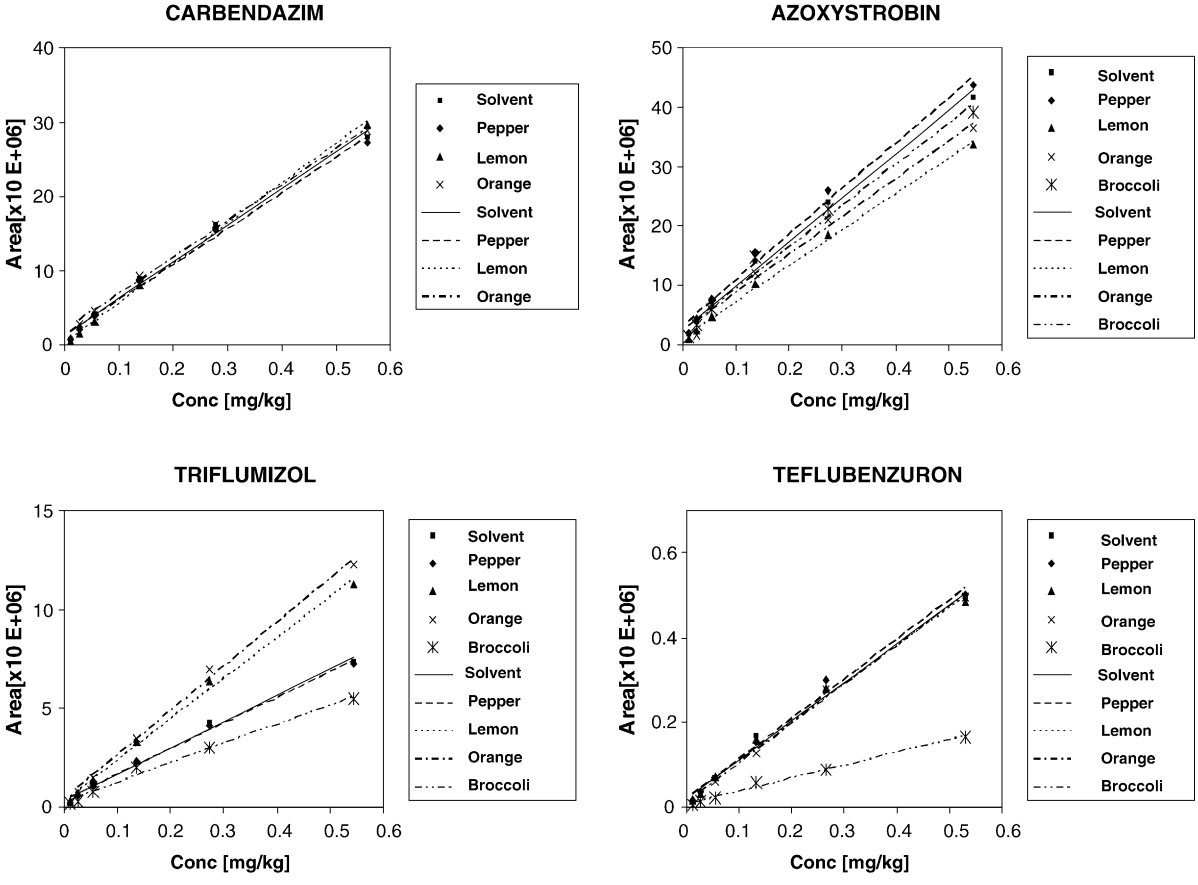

Fig. 3. Matrix-matched calibration plots for carbendazim, azoxystrobin, triflumizol and teflubenzuron in different fruit and vegetable matrices.

found to be precise (with run-to-run instrumental RSD values between 0.8 and 7% and day-to-day RSD values between 2 and 10%). The accuracies and precisions obtained compare favorably against those traditionally affordable with other LC–MS instruments widely accepted for quantitative purposes. As an example, a typical total ion chromatogram from a 0.01 mg/kg orange-matched standard together with the extracted ion chromatograms used for quantification purposes for some of the selected pesticides is shown in Fig. 4.

The LODs were estimated from the injection of matrix-matched standard solutions at concentration levels corresponding to a signal-to-noise ratio of about 3. The results obtained in three different matrices are included in Table 5. Triflumizol, azoxystrobin and spinosad were the most sensitive compounds with the lowest LODs in all three matrices. The matrix of broccoli yielded the higher LODs due to the ionization suppression encountered by electrospray of this type of matrix as it is commented in the next section. In general and in all cases, with the exception of methomyl, the LODs obtained meet the requirements regarding the MRLs imposed by the existing European regulations [2].

3.5. Matrix effects

The occurrence of matrix effects in LC–MS is well known, playing an important role in the quantitation of the pesticides, especially when electrospray ionization is used. Matrix effects can both reduce or enhance the response when compared to "solvent" standards. The signal suppression–enhancement depends strongly on the interface used (ESI in positive mode usually suffers higher signal suppression/enhancement than negative mode), on each individual pesticide, on each matrix tested (i.e. the amount of matrix per ml of extract) and also on the sample treatment procedure (extraction solvent, clean-up

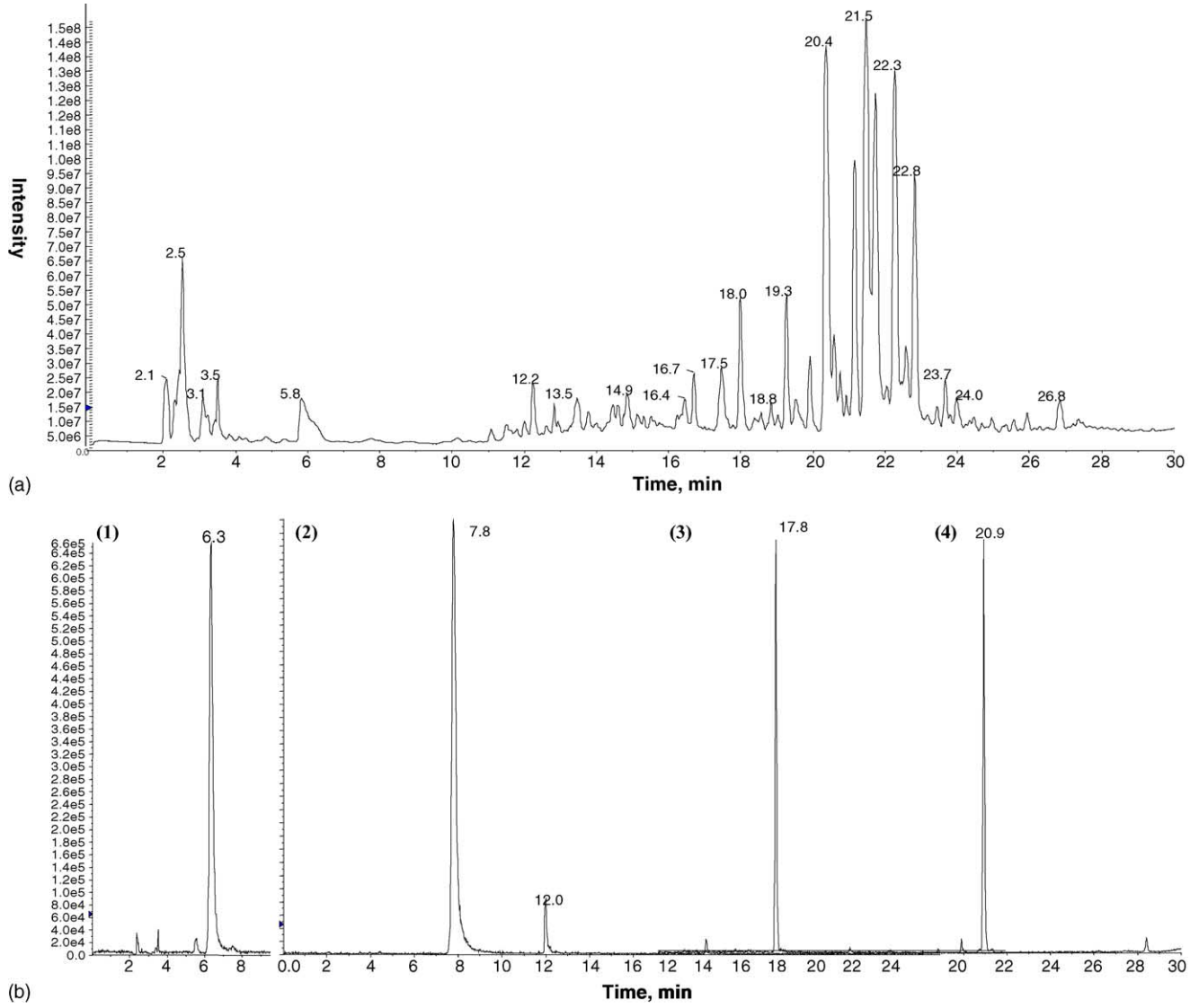

Fig. 4. (a) LC–TOF-MS total ion chromatogram corresponding to an orange-matched standard fortified at 0.01 mg/kg with the pesticide mixture; (b) extracted ion chromatogram (XIC) for quantitation purposes. Peak numbers: (1) carbendazim, (2) thiabendazole, (3) thiacloprid and (4) spinosyn A.

Table 4
Accuracy and RSD values obtained from the intra- and inter-day study for validation ($n = 5$)

Amount added	RSD (%)		
	Intra-day (mg/kg)		Inter-day (mg/kg)
	0.05	0.5	0.25
Cyromazine	7.1	3.8	5.9
Carbendazim	2.7	3.9	6.0
Thiabendazole	2.2	1.5	3.6
Methomyl	6.0	6.6	10
Imidacloprid	3.1	5.9	6.1
Acetamiprid	0.8	0.8	7.7
Thiacloprid	2.1	3.2	10
Spinosad	2.6	1.2	2.6
Dimethomorph	2.9	2.3	10.4
Azoxystrobin	3.1	2.4	5.7
Triflumizol	3.8	2.3	9.4
Hexaflumuron	2.6	2.0	11
Teflubenzuron	5.8	2.8	10
Lufenuron	5.6	4.5	8.0
Flufenoxuron	5.3	6.6	9.2

Intra-day values correspond to a melon-matched standard solution at two different concentrations and inter-day values correspond to a pepper-matched standard solution at 0.25 mg/kg.

Table 5
LODs obtained by LC–TOF-MS in three matrices

Compound	LODs (μg/kg)		
	Orange	Pepper	Broccoli
Cyromazine	5	5	10
Carbendazim	4	5	8
Thiabendazole	5	10	5
Methomyl	10	30	50
Imidacloprid	5	10	12
Acetamiprid	3	5	9
Thiacloprid	1	4	5
Spinosad	0.8	1	2
Dimethomorph	5	2	8
Azoxystrobin	0.8	0.3	0.5
Triflumizol	0.5	0.9	5
Hexaflumuron	8	10	25
Teflubenzuron	7	10	20
Lufenuron	5	10	23
Flufenoxuron	4	10	20

procedures, etc.). To evaluate the signal suppression/matrix effects, the slopes obtained in the calibration with solvent-based standards for each pesticide were measured and the slope ratios (matrix/solvent) in the seven different matrices tested were calculated. Results are shown in Table 6. For example, both carbendazim and thiabendazole presented negligible matrix effects in any type of matrix as observed from the slope ratios. This fact is also confirmed in the matrix-matched calibration plot for carbendazim from Fig. 3. Thus, in this case, solvent-based standards could be used for accurate quantitation of real samples. On the other hand, for example, in the case of triflumizol, the signal obtained strongly depends on the matrix, as it can be noticed from the different coefficients (matrix/solvent slope) included in Table 6. In this case, matrix-matched calibration must be used for quantitation purposes for every type of matrix or sample. The nature of the matrix also plays an important role in the matrix effects for some specific compounds. As it can be noticed in Table 6, in the case of the broccoli extract, the last four eluting pesticides (hexaflumuron, teflubenzuron, lufenuron and flufenoxuron) presented a higher matrix suppression signal, due probably to the hydrophobic complexity of this matrix.

3.6. Pesticide residues in market samples

To evaluate the effectiveness of the proposed methodology, it was applied to the analysis of two samples from an inter-laboratory comparison test for pesticide residue analysis organized by TestQual (http://www.testqual.com/). The results obtained are shown in Table 7. All the target compounds covered by the comparison test were properly identified (carbendazim, methomyl, imidacloprid and hexaflumuron).

Table 6
Evaluation of matrix effects: comparison of the calibration curve slopes

	Matrix: solvent (MeOH/H_2O)		Slope matrix/solvent						
	Equation	r	Pepper	Tomato	Apple	Broccoli	Lemon	Orange	Melon
Cyromazine	$y = 2.0 \times 10^7 x + 1600000$	0.992	1.06	1.05	1.05	0.73	1.22	1.03	1.20
Carbendazim	$y = 4.9 \times 10^7 x + 1220000$	0.997	0.97	0.99	0.89	0.98	1.08	0.99	1.03
Thiabendazole	$y = 7.3 \times 10^7 x + 1300000$	0.998	1.00	0.96	0.90	0.93	1.10	1.14	1.05
Methomyl	$y = 1.6 \times 10^6 x + 33000$	0.994	0.70	0.79	0.93	0.56	1.26	1.51	0.78
Imidacloprid	$y = 9.8 \times 10^6 x + 10000$	0.998	0.72	0.94	0.79	0.48	0.57	0.60	0.93
Acetamiprid	$y = 2.5 \times 10^7 x + 500000$	0.997	0.76	0.92	0.87	0.55	0.68	0.72	1.06
Thiacloprid	$y = 2.2 \times 10^7 x + 740000$	0.996	0.82	1.00	0.83	0.55	0.59	0.63	0.95
Spinosad	$y = 6.5 \times 10^7 x - 700000$	0.997	1.12	1.08	1.04	1.01	1.19	1.12	1.04
Dimethomorph	$y = 9.1 \times 106x + 190000$	0.995	0.97	1.13	1.03	0.69	0.88	1.00	0.97
Azoxystrobin	$y = 7.3 \times 10^7 x + 2620000$	0.996	1.04	0.88	0.94	0.95	0.82	0.87	1.00
Triflumizol	$y = 1.3 \times 10^7 x + 350000$	0.996	0.98	0.98	0.85	0.74	1.55	1.68	1.25
Hexaflumuron	$y = 1.1 \times 10^6 x + 17000$	0.999	0.96	0.78	0.70	0.22	0.95	1.16	1.05
Teflubenzuron	$y = 9.1 \times 105 + 20000$	0.996	1.02	0.77	0.57	0.33	0.98	1.01	0.89
Lufenuron	$y = 8.2 \times 10^5 x + 2000$	0.998	1.04	0.95	0.78	0.23	0.78	1.14	0.98
Flufenoxuron	$y = 1.7 \times 10^6 x + 9000$	0.995	1.00	0.89	0.71	0.28	1.12	1.32	1.04

Table 7
Comparison of LC–TOF-MS and LC–MS results for the analyses of pesticide residues in certified fruit samples

Sample/pesticide	TestQual value[a]	LC–TOF-MS	LC–MS
Apple			
Carbendazim	0.32	0.21	0.17
Methomyl	0.27	0.32	0.24
Strawberry			
Carbendazim	0.30	0.25	0.27
Hexaflumuron	0.22	0.27	0.26
Imidaloprid	0.09	0.10	0.12
Methomyl	0.58	0.53	0.45
Spinosad	–	0.13	0.15
Azoxystrobin	–	0.14	0.17

Concentration units, mg/kg.
[a] Assigned value provided by the inter-calibration exercise.

Moreover, two more non-target pesticides studied in the developed method were also identified (spinosad and azoxystrobin) in one of the samples. The results obtained with the developed LC–TOF-MS method were compared with those obtained with an LC quadrupole MS method in SIM mode. Values reported were significantly very close between the two methods, thus verifying the feasibility of the LC–TOF-MS method for the quantitative analyses of vegetable samples. The applicability of the method is thus demonstrated by data of real samples showing that LC–TOF-MS is suitable for the analysis of pesticides at low concentration levels, together with additional information of accurate mass measurements.

4. Conclusions

A study to evaluate the usefulness of LC–TOF-MS for quantitative analyses of pesticides in fruit and vegetable samples was carried out. The results obtained showed a good analytical performance in terms of sensitivity and selectivity of the method. Good precision and accuracy was achieved in all cases for all matrices studied. Matrix effects were evaluated as well for seven different fruit and vegetable matrices; matrix effects resulting in suppression or enhancement of the response in more than 20% were frequently observed, most notably in broccoli and citrus. The ruggedness of the method was demonstrated by analysis of real samples and a comparison with a single quadrupole instrument was performed showing a good correlation in the values obtained by both methodologies. Furthermore, LC–TOF-MS offers higher quality structural information for unequivocal identification of target compounds provided by elemental composition formula information. The resolving power, accurate mass measurement capability and full spectral sensitivity also makes LC–TOF-MS attractive as a tool for identifying non-target "unknowns" compounds in complex matrices. Future work includes the exploration of the possibilities of the instrument for identification of non-target compounds.

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