Quantitation and Accurate Mass Analysis of Pesticides in Vegetables by LC/TOF-MS

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A quantitative method consisting of solvent extraction followed by liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) analysis was developed for the identification and quantitation of three neonicotinoid pesticides (imidacloprid, acetamiprid, thiacloprid) commonly used on salad vegetables. Accurate mass measurements within 3 ppm error were obtained for all the pesticides studied in various vegetable matrixes (cucumber, tomato, lettuce, pepper), which allowed an unequivocal identification of the target pesticides. Calibration curves covering 2 orders of magnitude were linear over the concentration range studied, thus showing the quantitation ability of TOF-MS as a monitoring tool for pesticides in vegetables. Matrix effects were also evaluated using matrix-matched standards showing no significant interferences between matrixes and clean extracts. Intraday reproducibility was 2−3% relative standard deviation (RSD) and interday values were 5% RSD. The precision (standard deviation) of the mass measurements was evaluated and it was less than 0.23 mDa between days. Detection limits of the chloronicotinyl insecticides in salad vegetables ranged from 0.002 to 0.01 mg/kg. These concentrations are equal to or better than the EU directives for controlled pesticides in vegetables showing that LC/TOF-MS analysis is a powerful tool for identification of pesticides in vegetables and is a valuable new tool for environmental monitoring of insecticides in food. Robustness and applicability of the method was validated for the analysis of market vegetable samples. Concentrations found in these samples were in the range of 0.02−0.17 mg/kg of vegetable.

Pesticide residues are a major environmental issue in vegetable samples, and the identification and quantitation of insecticides in vegetables is of great importance to individuals and health organizations around the world. 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 United States have increased the standards for human health, workers, and environmental protection. Moreover, the quality standards within the new regulations include the reassessment of the maximum residue limits (MRLs) for vegetables. Therefore, EU directives are setting different MRLs for each pesticide within each food group. Furthermore, the new directive also leads to different MRLs for each EU country, which are still being decided. Within the EU, MRLs have been established for some pesticides in many fruits and vegetables ranging from 0.01 to 3 mg/kg.[1] For fruits and vegetables intended for production of baby food, an MRL of 0.01 mg/kg is applicable for all pesticides.[2] Finally, banned compounds have the lowest MRLs, which is set now at 0.01 mg/kg. This threshold level is also frequently applied for testing compliance with guidelines for organic production, and new methods of analysis should reach these levels.

Use of agrochemicals at various stages of cultivation has, therefore, an important impact in food protection and quality preservation. For this reason, a proper monitoring of pesticide residues is important for the assessment of human exposure to pesticides through foods.[3−15] Traditionally, the screening of pesticides in food has been accomplished by gas chromatography/mass spectrometry (GC/MS) methods.[4] However, many of the new polar and thermally labile pesticides are more readily and easily analyzed by liquid chromatography methods.[3] In this sense, liquid chromatography/mass spectrometry (LC/MS) is becoming a standard tool for pesticide residue analysis in fruits and vegetables.[3,4] For instance, pesticide analysis in food is moving toward LC/MS methods, such as single, triple quadrupole, and LC/MS ion trap mass spectrometry in order to analyze the

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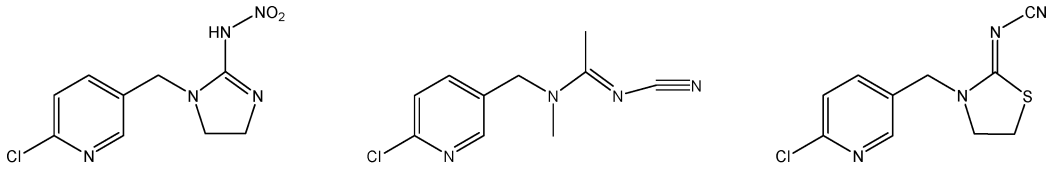

Imidacloprid m/z 256 Acetamiprid m/z 223 Thiacloprid m/z 253

Figure 1. Chemical structures of imidacloprid, acetamiprid, and thiacloprid.

complex matrixes of fruit and vegetable extracts.[16−27] A recent review by Picó et al.[5] on LC/MS analysis of pesticides in food shows that over approximately 100 papers have been published in the past 10 years using LC/MS; however, there are no reports mentioned using time-of-flight mass spectrometry (TOF-MS) for food analysis. Part of the lack of TOF application has been the recent nature of LC/TOF-MS systems being available in the market place as well as the difficulty of performing calibration and quantitation by TOF-MS, which has kept the instrument more of a research tool than a routine tool for environmental monitoring.[28−32] The analytical methodologies employed for monitoring of pesticides in food should be capable of measuring low levels and must provide unambiguous evidence to confirm both the identity and the quantity of any residues detected. In this sense, TOF instruments offer the capability of unequivocal identification (provided by exact mass measurements) of low levels of contaminants, as well as the possibility of quantitation at these low levels.[28] LC/MS determination of pesticides in vegetables has been repeatedly studied, but no attempts have been made to develop a method of analysis based on accurate mass measurements using LC/TOF-MS. Furthermore, the use of TOF-MS allows the capability of nontarget 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 techniques.

The chloronicotinyl (also called neonicotinoid) insecticides were introduced onto the market in the 1990s by Bayer for use against a variety of insects in salad vegetables. The chemical structures of the pesticides are shown in Figure 1. All three compounds shown contain the chloronicotinyl structure; thus, all three compounds have a similar mode of action and target the nicotinic acetylcholine receptor of insects. These compounds were chosen because of their low volatility, which makes them more suitable for LC/MS rather than GC/MS, and because of their occurrence in vegetable samples.[33,34] In this paper, the feasibility of LC/TOF-MS for the detection and quantitation of three chloronicotinyl insecticides (acetamiprid, imidacloprid, thiacloprid) in four salad vegetables (tomato, lettuce, pepper, cucumber) is shown at the MRLs regulated by the EU, the American Food Regulations, and the Japanese Regulations (0.01 mg/kg or ppm).

Although the potential of LC/TOF-MS has been shown for environmental applications, its use in food analysis is still minimal due to some disadvantages and limitations.[28] One of the main limitations is the quantitation due to matrix ion suppression effects in electrospray ionization. Another disadvantage, already noted in some recent papers,[32] is the lack of accuracy at the 1−5 ppm error level, usually needed when analyzing complex matrixes for unequivocal identification of the target analytes. All these considerations have resulted in doubts about the applicability of LC/TOF-MS in routine analysis of pesticides. Therefore, the aim of this study has been (i) to develop a sensitive analytical method to determine three neonicotinoid pesticides commonly detected in vegetables, (ii) to demonstrate the selectivity of the method for the unequivocal accurate identification of such compounds in complex matrixes by performing matrix effect studies, and (iii) to show the linearity obtained by these types of instrumentation in order to carry out quantitation in real samples.

EXPERIMENTAL SECTION

Chemicals. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). A Milli-Q-Plus ultrapure water system from Millipore was used to obtain the HPLC-grade water used during the analyses. Formic acid was obtained from Fluka. Pesticide-grade ethyl acetate and anhydrous sodium sulfate were from Panreac (Barcelona, Spain).

Pesticide analytical standards (purity >96%) were provided by Dr. Ehrenshtofer (Augsburg, Germany). The pesticides selected in the study and their chemical structures are shown in Figure 1. Individual pesticide stock solutions (250−300 μg/mL) were prepared in methanol and stored in the dark at −18°C. Appropriate aliquots of individual stock solutions were diluted in methanol to make a standard working mixture (30 μg/mL for each pesticide).

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From this standard mixture, several solutions at various concentration levels were prepared by dilution with matrix extracts, pure solvents, or both.

Vegetable Extraction. Two-kilogram portions of tomato, lettuce, pepper, and cucumber were obtained from farms that use only pesticide-free agriculture to raise the crops. Prescreening was carried out to be sure of residue-free vegetables by a certified laboratory. Then the samples were homogenized in a low-speed blender, and 15 g of the homogenized vegetable was weighed into a 200-mL PTFE centrifuge tube. A volume of 15 mL of ethyl acetate was added and blended in a Polytron (high-speed blender) for 30 s at 2000 rpm. This step was repeated two more times for a total of 45 mL of ethyl acetate. The combined extracts were then filtered through a thin layer of 20 g of anhydrous Na_2SO_4. 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 ± 5 °C. The dried residue was dissolved by sonication in 15 mL of methanol. The matrix extracts, which contained 1 g of sample/mL, were filtered through 0.2-μm PTFE filters (Millex FG, Millipore) prior to fortification with the analytes or LC/TOF-MS analyses. A previous study showed that no cleanup steps are needed, which results in a convenient and straightforward sample preparation.[6] Samples from market places were extracted using this procedure as well. In a previous study,[6] the extraction recoveries for the three neonicotinoid pesticides were reported to be between the 95 and 103% range.

Validation Studies. All validation studies were performed by using pesticide-free matrix samples previously analyzed. Quantification of sample extracts during validation was done using a calibration curve based on matrix-matched standards (blank extracts fortified with the analytes). The linearity in the response was studied by using pure solvents and matrix blank extract solutions to evaluate possible matrix effects. The blank extracts for each vegetable were initially in methanol, but they were diluted 1:3 with MilliQ water in order to obtain good chromatographic peak shapes for all the analytes. A previous experiment showed that dilution of methanol with water, three times, and increasing the injection volume to 50 μL, restored peak shape for the analytes studied, so this was the general method used in this work. Matrix-matched calibration standards were prepared by dilution of the pesticide stock solution with the individual blank extracts already prepared in MeOH/H_2O (1:3). In this way, the matrix blank residues were fortified with a mixture of acetamiprid, imidacloprid, and thiacloprid at concentrations ranging from 0.005 to 1 mg/kg in order to have a wide range of concentrations. The integrated peak area data of the selected quantification masses (see Table 1) were used to construct the calibration curves. The calibration curves generated were used for quantification purposes. The limits of detection (LODs) were determined as the analyte concentration that gave a signal-to-noise ratio of 3, as calculated by the instrument software, and empirically verified by analyzing pesticide mixtures at these concentration levels in matrix extracts to check the presence of the protonated molecule together with its correct exact mass.

LC/TOF-MS. Liquid chromatography/electrospray/time-of-flight mass spectrometry (LC/ESI/TOF-MS), in positive ionization was used to separate and identify imidacloprid, acetamiprid, and thiacloprid. The analytes were separated using an HPLC (series 1100, Agilent Technologies, Palo Alto, CA) equipped with a reversed-phase C_8 analytical column (Zorbax Eclipse XDB, Agilent Technologies) of 150 mm by 4.6 mm and 5-μm particle diameter. Column temperature was maintained at 25 °C. Mobile phase A was acetonitrile, and mobile phase B consisted of water with 0.1% formic acid. A linear gradient progressed from 15% A (initial conditions) to 100% A in 30 min, after which the mobile-phase composition was maintained at 100% A for 5 min. The flow rate was 0.6 mL/min, and 50 μL of the matrix-matched standards, sample extracts, or both were injected. This HPLC system was connected to a time-of-flight mass spectrometer (MSD-TOF, Agilent Technologies) equipped with an electrospray interface under the following operating parameters: capillary 4000 V, nebulizer 40 psig, drying gas 9 L/min, gas temperature 300 °C, fragmentor 190 V, skimmer 60 V, Oct dc1 37.5 V, Oct rf V 250 V. 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 1 s/spectrum.

Table 1. LC/TOF-MS Characteristic Ions (Protonated Molecule and Fragments) and Relative Abundance (%) of the Neonicotinoid Pesticides, at Two Different Fragmentor Voltages

	190 V		250 V	
compound	m/z	RA	m/z	RA
imidacloprid	256	100	256	10
	210	24	210	20
	209	18	209	60
	175	27	175	100
acetamiprid	223	100	223	22
	126	16	126	100
thiacloprid	253	100	253	28
	126	17	126	100

Mass Measurement Calculations. Elemental composition calculations were performed off-line using the Data Analysis software (Analyst QS, Applied Biosystems, Framingham, MA). This software was used to work with the spectrum generated for every analyte. Potential assignments were calculated using the monoisotopic masses with specifications of a tolerance of 10 ppm deviation and both odd- and even-electron states possible. We can obtain an empirical formula from an accurate experimental mass by imposing the expected number and kind of atoms presents in the molecule. Depending on the tolerance level (ppm or mDa error), the software generates a list of ~10, for instance, from as many as 100 of possible empirical formulas. In this work, the number and types of expected atoms was set as follows: carbons ≤50; hydrogens ≤100; oxygens ≤5; nitrogens ≤5; chlorines ≤1; sulfurs ≤1.

RESULTS AND DISCUSSION

LC/TOF-MS Structural Information. The experiments to select the optimum MS conditions and the appropriate ions were performed by column injection of the standard mix at 1 μg/mL. The optimum working conditions are those reported in the

Table 2. LC/TOF-MS Accurate Mass Measurements for the Neonicotinoid Pesticides and Their Fragments in a Tomato-Matched Matrix

			concentration (0.05 mg/kg)		concentration (0.05 mg/kg)	
compound	elemental composition	theoretical mass	measured mass	error (ppm)	measured mass	error (ppm)
imidacloprid	$C_9H_{11}N_5O_2Cl$	256.0596	256.0596	0.1	256.0597	0.5
	$C_9H_{11}N_4Cl$	210.0667	210.0663	−1.8	210.0664	−1.3
	$C_9H_{10}N_4Cl$	209.0589	209.0587	−0.7	209.0587	−0.7
	$C_9H_{11}N_4$	175.0978	175.0983	2.7	175.0977	−0.7
acetamiprid	$C_{10}H_{12}N_4Cl$	223.0745	223.0746	0.5	223.0749	1.8
	C_6H_5NCl	126.0105	126.0106	0.8	126.0105	0.0
thiacloprid	$C_{10}H_{10}N_4SCl$	253.0309	253.0311	0.7	253.0313	1.5
	C_6H_5NCl	126.0105	126.0107	1.6	126.0103	−1.6

Experimental Section. Drying and nebulizer nitrogen flow rates, vaporizer and drying temperatures, and capillary voltage were investigated, but no important changes on sensitivity were observed when these parameters were varied. The fragmentor voltage was the parameter with more influence on the signal and, especially, on the in-source collision-induced dissociation (CID) fragmentation for each analyte.

Table 1 lists the relative abundance of protonated molecules and fragment ions for the three compounds studied at medium and high fragmentor voltages. The base peak ion observed for all the pesticides was $[M + H]^+$, the protonated molecule. Imidacloprid was the most interesting compound, yielding the fragments at m/z 210, 209, and 175, corresponding to $[M + H - NO_2]^+$, $[M + H - HNO_2]^+$, and $[M + H - NO_2 - Cl]^+$, respectively. The fragment at m/z 210 is an odd-electron ion, and it comes from the loss of NO_2 as a radical fragment from the protonated molecule. The fragment at m/z 209 corresponds to an even-electron ion, and it forms by a loss of HNO_2 (a neutral loss) from the protonated molecule. This ion seems to be more stable than the m/z 210 ion as its relative abundance increases with the fragmentor voltage (see Table 1 for 250 V). Finally, the m/z 175 fragment ion is the result of a chlorine loss from the 210 ion, forming an even-electron ion. Acetamiprid and thiacloprid gave the identical fragment ion at m/z 126 corresponding to $[C_6H_5OCl]^+$, which is one of the characteristic ions for this class of compounds. Imidacloprid does not present this characteristic fragment due to the electron-withdrawing nature of the NO_2 group, which favors the primary CID fragmentation to the m/z 210 and 209 fragments. The fragmentor voltage of 190 V was chosen for further analyses of the three neonicotinoid pesticides.

LC/TOF-MS Accurate Mass Measurements. Table 2 shows the accurate mass measurements obtained from matrix-matched standards (in this case, a tomato matrix) for the protonated molecules and the main fragment ions at two different concentration levels. This table also shows the theoretical exact masses corresponding to these ions as well as the error in accuracy obtained in ppm. Both concentrations gave excellent results for mass accuracy, which were always less than 2 ppm for the protonated ions and less than 3 ppm for the fragment ions. These results show that the use of continuous calibration (a feature of this instrument, see Experimental Section) is effective for accurate mass even across an order of magnitude concentration range in a complex vegetable matrix. It is important to note that the internal reference is being constantly infused at a low flow rate and the software is autocalibrating and storing those results with the raw data, which makes it highly accurate. The agreement (within 2 ppm) demonstrated between the measured and calculated masses serves to verify the proposed elemental compositions and represents a higher order identification of this class of compounds than that based on the nominal mass assignments available from low-resolution LC/MS instruments. The verification of elemental compositions, along with matching retention times and mass spectra, will constitute complementary and unequivocal identification of the analytes in vegetable samples, as shown in this table. Using a system of identification points developed for pharmaceuticals in food,[35] it is possible to score 4 points by achieving accurate mass for the protonated molecule and one fragment ion, which is sufficient for control and identification of banned substances in food.[36] Analytical methodologies must be capable of providing unambiguous evidence to confirm the identity of any target residues, and in this sense, the accuracy of the method developed here shows the ability to achieve this goal.

Figure 2 shows the chromatographic separation of the three chloronicotinyl insecticides, acetamiprid, imidacloprid, and thiacloprid using a C_8 column. The sample contained the pesticides at 0.5 mg/kg in a fortified tomato extract. The extracted ions for each of the three compounds are also shown in the lower half of Figure 2. The window of extraction was clean, which is commonly a feature of accurate mass extraction of ions, where the width of the window of extraction may be narrowed to 0.02 amu or ~10 ppm. In Figure 2, the window of extraction was 0.1 amu, which was sufficient for the identification of the analytes in the matrixes studied.

Lower concentrations were examined to check for accuracy on mass measurements at the low end of the standard curves. Figure 3 shows the extracted ion chromatograms for the three neonicotinoid pesticides spiked in a pepper matrix at a concentration level of 0.05 mg/kg. The corresponding mass spectra are shown in this figure as well. At concentrations as low as 0.05 mg/kg, the extracted ions (m/z 223, 253, and 256) still yielded clean chromatograms, testifying to the importance of accurate mass and its ability to give clean extracted ion chromatograms with a narrow mass window. This accurate mass window is reflected in the

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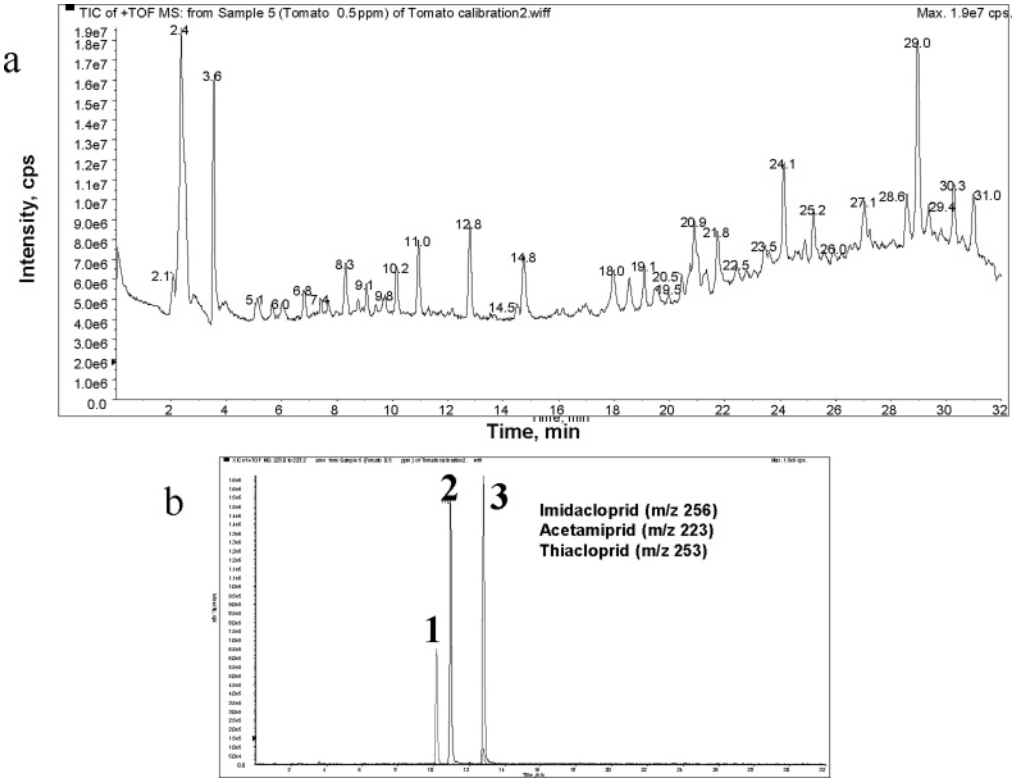

Figure 2. LC/TOF-MS analysis of the three chloronicotinyl insecticides at 0.5 mg/kg in a fortified tomato matrix: (a) chromatogram in full scan and (b) extracted ion chromatograms for the three insecticides. Peak numbers: 1, imidacloprid; 2, acetamiprid; 3, thiacloprid.

determinations of the accurate mass of the protonated molecule of each one of the chloronicotinyl insecticides.

Method Validation. The linearity of the method was calculated on the basis of the limit of linear response to the limit of the quantitative measurements. Calibration graphs of standard solutions and spiked blank extract areas versus concentration were constructed by use of least-squares linear regression analysis. Table 3 summarizes the validation data obtained for the three neonicotinoid pesticides in tomato, pepper, lettuce, and cucumber matrixes including calibration equations, correlation coefficients, and limits of detection. As we can see in this table, the linearity is excellent over the concentration range studied, which is much improved compared with other LC/TOF-MS instruments we have tested previously[29−32] with the same type of detection. The curves were linear in the range studied from 0.005 to 1 mg/kg, and the correlation coefficients were higher than 0.991 for all the pesticides studied. Sometimes it is possible that the calibration curves are linear for the lowest concentration levels only and reach a plateau for the highest concentration levels due to the overloading of the detector. In this work, the concentrations studied are lower and this behavior is not observed due to the higher linear range of the instrument, which is most likely the result of the analog to digital convertor versus the time to digital convertor. Figure 4 shows as an example the calibration curve obtained with a pepper matrix-matched standard.

The LODs were calculated based on a signal-to-noise ratio of 3, and they were empirically verified by analyzing pesticide mixtures at those concentration levels in all matrix extracts. The LODs of the method are reported in Table 3 as well. The LODs were equal or below the EU reporting limits for pesticides in vegetables (which vary from 0.01 to 0.05 mg/kg).

The signal robustness and accuracy precision of the methodology developed were studied by measuring the repeatability and reproducibility of the results obtained. Signal precision was calculated by measuring the areas for a matrix-matched standard (spike level of 0.1 mg/kg). The repeatability (intraday) was assessed by analysis on the same day. The reproducibility (as interday precision) was tested by analysis of samples for five successive days, including several matrices. Both were calculated in terms of relative standard deviation. Table 4 reports these values and shows that for signal precision intraday values averaged 2−3% and reproducibility was 4−5%, showing good performance of the methodology developed in this work.

The variation in the accuracy was evaluated by measurement of the exact masses obtained for each one of the analytes. The same standard was analyzed 5 times on the same day and 10 times for successive days in order to estimate the precision of the method for accurate mass measurements. The results are shown in Table 4. Both intraday and interday values showed a minimal variation of the accurate mass for the three compounds studied (ranging from 0.08 to 0.23 mDa, less than 2 ppm error). In all cases, only the fourth decimal figure in the accurate mass varied, thus showing an excellent precision and accuracy of this methodology provided by the continuous reference calibration and electronic stability of the instrument for the $[M + H]^+$ ion.

Matrix Effects. The occurrence of matrix effects in LC/MS is well known and has an important impact on the quantitation of the pesticides. Matrix effects can both reduce or enhance the response when compared to standards in neat solvents. Matrix effects depend on the instrument and interface used, the analytes, the matrix, and the sample pretreatment procedure.[7] For these reasons, the influence from the matrix can be quite variable. The

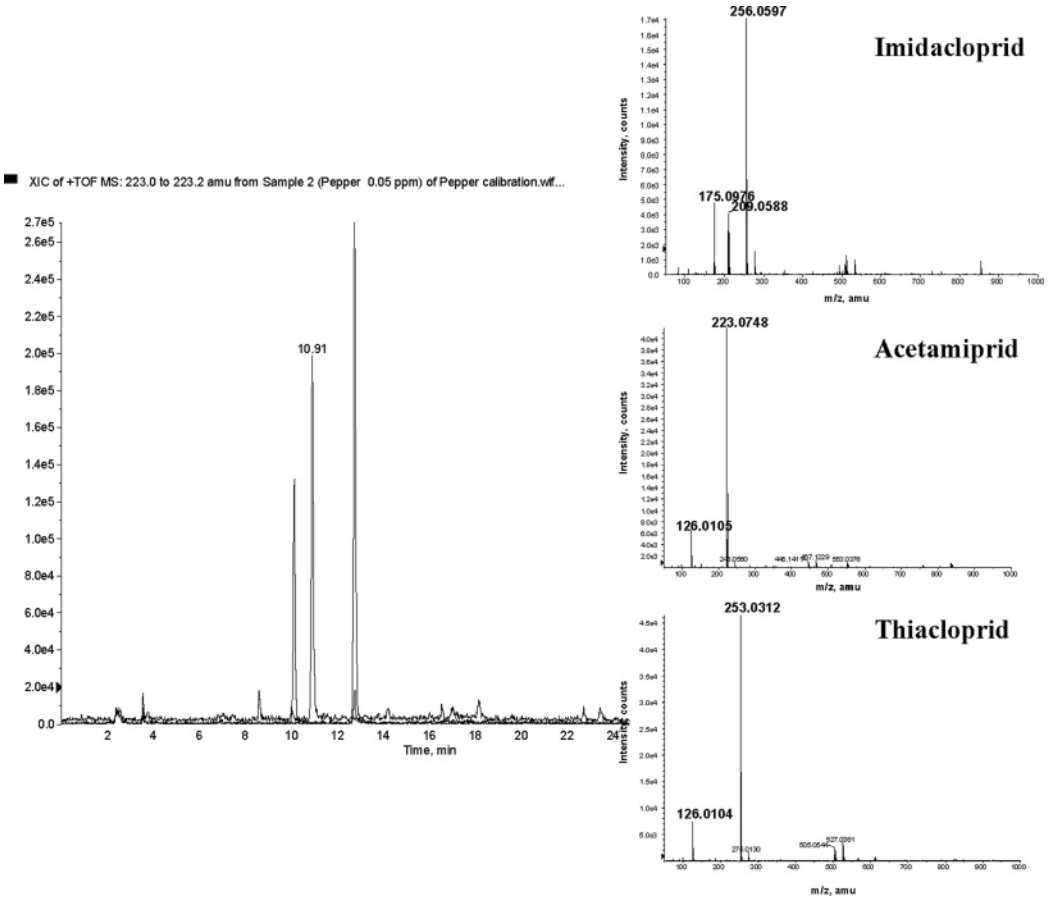

Figure 3. LC/TOF-MS extracted chromatograms of the three chloronicotinyl insecticides at 0.05 mg/kg in a fortified pepper matrix. The corresponding spectra are also shown.

Table 3. LC/TOF-MS Calibration Data for the Neonicotinoid Pesticides (Spiked from 0.005 to 1 mg/kg) Using Matrix-Matched Standards (7 Calibration Data Points at Different Concentrations Were Used)

	matrix	calibration equation	R^2	LOD (mg/kg)
imidacloprid	solvent	$y = 1.0 \times 10^7x + 18867$	0.9993	0.003
	tomato	$y = 1.0 \times 10^7x + 172000$	0.9995	0.01
	pepper	$y = 8.7 \times 10^6x + 453000$	0.9980	0.015
	lettuce	$y = 9.6 \times 10^6x + 143000$	0.9962	0.01
	cucumber	$y = 9.0 \times 10^6x + 19100$	0.9996	0.005
acetamiprid	solvent	$y = 2.0 \times 10^7x + 464616$	0.9974	0.002
	tomato	$y = 2.2 \times 10^7x + 444000$	0.9974	0.003
	pepper	$y = 1.9 \times 10^7x + 678000$	0.9918	0.004
	lettuce	$y = 2.0 \times 10^7x + 503000$	0.9944	0.002
	cucumber	$y = 2.0 \times 10^7x + 433000$	0.9958	0.003
thiacloprid	solvent	$y = 2.0 \times 10^7x + 758528$	0.9953	0.001
	tomato	$y = 2.0 \times 10^7x + 1150000$	0.9936	0.003
	pepper	$y = 1.9 \times 10^7x + 1040000$	0.9922	0.003
	lettuce	$y = 1.9 \times 10^7x + 858000$	0.9904	0.002
	cucumber	$y = 1.8 \times 10^7x + 678000$	0.9930	0.002

effect, expressed as suppression or enhancement, for one specific combination of pesticide and matrix can vary from pesticide to pesticide.

In this work, four commodities were selected for evaluation of matrix effects. The vegetable extracts were analyzed with LC/TOF-MS, and the response was compared to standards in solvents (methanol/water without matrix). An exact determination of matrix effects was done by analyzing standards of different concentrations in pure solvents and in the four matrixes and comparing the slopes of the calibration curves. The variation in the slopes ranged between 87 and 110%, thus showing minimal matrix suppression or enhancement. Alternatively, the matrix effects can be expressed as a ratio of analyte response in matrix-matched standard to its response in solvent standard. The matrix effects measured in this way for the vegetables selected for the method validation at the highest levels of fortification (1 mg/kg) are visually reported in Figure 5.

As can be seen in this histogram, no considerable signal reduction or enhancement in matrix extracts was detected for the three analytes studied (89−103% relative response). The trends

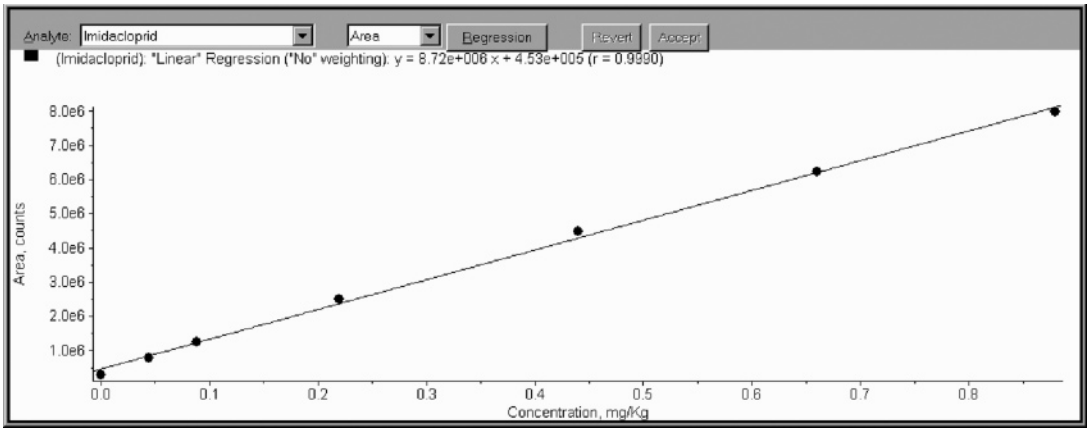

Figure 4. Calibration curve from a pepper-matched standard for imidacloprid obtained by LC/TOF-MS.

Table 4. Quality Parameter Values for the LC/TOF-MS Methodology

	signal precision		precision of accuracy measurement	
compound	intraday repeatability (%)	interday reproducibility (%)	intraday repeatability (σ in mDa)	interday reproducibility (σ in mDa)
imidacloprid (m/z 256.0596)	2	5	±0.11	±0.15
acetamiprid (m/z 223.0747)	3	5	±0.14	±0.23
thiacloprid (m/z 253.0309)	2	4	±0.13	±0.08

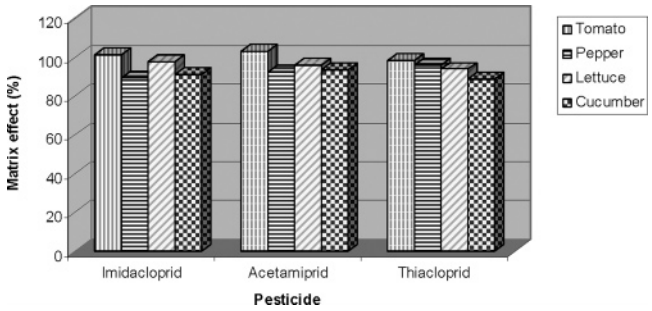

Figure 5. LC/TOF-MS relative response (%) of standard prepared in extracts (1 g of matrix/mL of extract) relative to standard in pure solvent. Matrix effect (%) = peak area of matrix-matched standard/peak area of solvent standard × 100.

Table 5. Concentration of the Three Neonicotinoid Pesticides in Vegetable Samples by LC/TOF-MS

samples	compound	concentration (mg/kg) LC/TOF-MS	concentration (mg/kg) LC/TQ/MS[a]
1 (tomato)	thiacloprid	0.09	0.09
2 (pepper)	imidacloprid	0.17	0.16
3 (cucumber)	imidacloprid	0.06	0.06
	acetamiprid	0.02	0.03
	thiacloprid	detected <LOD	nd

[a] Values obtained from Coexphal Laboratory using approved methods and validated by EU guidelines. nd, not detected. The comparison with another LC/MS (triple quadrupole) method is also reported in the table.

observed for all the matrixes studied and the three pesticides were similar, supporting earlier observations that differences in matrix effects between commodities are usually much smaller than the difference between any matrix and clean standard solutions.[7] An important feature is that the calibration equations are virtually identical when they were constructed from each type of vegetable or from the calibration solutions (curves not shown here).

Quantitative Analyses of Market Samples. The optimized analytical procedure was used to analyze processed vegetable products obtained from local markets. To quantitate the samples, calibration was performed by external matrix-matched standards to eliminate the matrix effect and to obtain a more realistic determination. Table 5 shows the concentrations for the three neonicotinoid pesticides found in three real samples by LC/TOF-MS and the concentration reported by an official established methodology using a triple quadrupole instrument.[6] 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 analysis for the determination of neonicotinoid pesticides at lower levels than the MRLs in all the vegetables studied. LC/TOF-MS is a sensitive technique and provides confirmation of identity, which is an important feature when low MRLs are introduced for certain commodities.

CONCLUSIONS

In this work, it was observed that liquid chromatography in combination with the use of time of flight is capable of discriminating more efficiently than nominal mass detection by LC/MS between the analyte and matrix signal. TOF-MS seems to be a good alternative in terms of both detection levels and structural information with the possibility of identification of unknown

nontarget compounds provided the availability of full-scan spectra data. High precision in the accurate mass measurements (~2 ppm) was achieved, thus validating the performance of the methodology.

The excellent selectivity and sensitivity allows identification and quantification of low levels of neonicotinoid pesticides in vegetable matrixes. The quantification is a feature of LC/TOF-MS that has not been possible in the past.[36] Calibration curves presented excellent linearity over the range studied. Furthermore, these low levels allow application of the developed method at the concentrations required by current regulator laws for baby and organic foods. The sensitivity of the method is sufficient to enable testing of compliance with baby food regulations (i.e., 0.01 mg/kg for all pesticides[2] and maximum residue limits established in the EU (0.01 mg/kg or higher.[1] Moreover, the availability of full-scan data provided by LC/TOF-MS together with the capability of obtaining accurate mass measurements for all the peaks detected allows screening for nontarget (e.g., banned) compounds in vegetable samples. LC/TOF-MS may turn into a highly useful technique to identify and confirm pesticide residues in fruits and vegetables, becoming a valuable addition to existing analytical tools.

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