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Determination of abamectin in citrus fruits by liquid chromatography–electrospray ionization mass spectrometry

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Abstract

Liquid chromatography coupled to electrospray mass spectrometry (LC–ES-MS) with positive ion detection was used to determine abamectin in oranges. MS conditions were optimized to achieve maximum sensitivity. The main ion for abamectin was $[M+Na]^+$ at a fragmentor voltage of 180 V. Abundant structural information can be obtained at different fragmentor voltages. The detection limit for the standard solution was 12 pg injected, and good linearity and reproducibility were observed. Abamectin residues were extracted using matrix solid-phase dispersion. Orange samples were homogenized with C_{18} bonded silica placed onto a glass column and eluted with dichloromethane. Recoveries of the abamectin from oranges fortified with approximately 0.01–10 mg/kg ranged from 94 to 99% with an overall average recovery of 96%. The quantification limit is 0.0025 mg/kg, which means detection limit for this analyte could be set at a few hundred picograms per gram of fruit. The presence in the electrosprayed solution of numerous citrus constituents did not interfere significantly with the ionization process of abamectin. The assay procedure provides a simple, rapid, and sensitive method for monitoring residues in oranges. The method was applied to field treatment orange samples.

Keywords: Fruits; Matrix solid-phase dispersion; Extraction methods; Abamectin; Avermectins; Lactones; Pesticides

1. Introduction

Avermectins, which are produced by the actinomycete *Streptomyces avermitilis,* are a family of macrocyclic lactone compounds. The insecticide avermectin B_1 (AVM) has been commercialized under the common name abamectin. Abamectin has been found to be toxic to different species of insects and mites, and is therefore widely used for *Phyllocnistic citrella* control in citrus crops in Valencia. Abamectin is a mixture of two components avermectin B_{1a} and $B_{1b;}$ the former is the major component (≈80%), while avermectin B_{1b} is the minor one (20%). Avermectin residues degrade rapidly, thus forming a variety of products. The only residues of toxicological significance are avermectin and 8,9-Z avermectin [1]. These compounds are neurotoxins and have low LD_{50} values. Since the presence of these residues in fruits can affect consumer health, the regulatory authorities have established maximum residue limits (MRLs) of 0.01 mg/kg for this pesticide.

Several liquid chromatographic methods have been reported for the determination of abamectin in various crops. However, because of the low concentration of residues expected, extensive clean-up of the crop extract prior to analysis is required. HPLC methods using UV detection [2,3] are not sensitive enough to ensure compliance with legisla-

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tion (the MRL set is 0.01 mg/kg) [1]. The use of fluorescence detection provides lower detection limits but requires derivatization with trifluoroacetic anhydride [4–7]. Extensive clean-up procedures of the crop extract and purification of avermectin fluorescence derivatives prior to analysis by HPLC are therefore needed.

The described methods for the determination require complicated homogenization extraction schemes [2–7] or the use of supercritical fluid extraction [8]. A matrix solid-phase dispersion (MSPD) method that is very simple and rapid has been proposed for isolating the pesticide from the citrus samples [9]. Considerable reduction of solvent consumption can be achieved by miniaturizing the scale of sample extraction [10,11]. This makes it possible to screen many samples and apply the method to routine analysis [12].

Mass spectrometry (MS) is rapidly becoming a routine detector for trace analysis of pesticide residues in the regulatory area [13,14]. This detector simplifies clean-up procedures by reducing the time requirements. The literature, however, contains few reports on the determination of abamectin using MS [15–17].

The specific objective of this work was to develop a rapid, specific and sensitive method for the determination of abamectin in citrus fruit. It is based on MSPD microextraction and quantification by liquid chromatography–electrospray ionization-mass spectrometry (LC–ES-MS).

2. Experimental

2.1. Chemicals

Acetonitrile, methanol and dichloromethane, all HPLC grade, were supplied by Merck (Darmstadt, Germany). Ultra pure water was prepared by ultrafiltration of distilled water with a Milli-Q system (Millipore, Bedford, MA, USA).

The solid phase used for MSPD was C_{18} bonded silica (40–60 μm) from Análisis Vínicos (Tomelloso, Spain).

The standard abamectin (92.5%) was supplied by Promochem (Wesel, Germany). Stock solution (978 μg/ml) and working solutions of abamectin were prepared in methanol and stored at 4°C. All the solutions were passed through a 0.45-μm nylon filter before injection into the LC system.

2.2. Instrument and conditions

A Hewlett-Packard (Palo Alto, CA USA) HP-1100 Series LC–MSD system equipped with a binary solvent pump, an autosampler, a photodiode-array detection (DAD) system and a mass selective detector (MSD) coupled with an analytical work station was used. The MSD consisted of a standard atmospheric pressure ionisation (API) source configured as ES. A Kromasil C_{18} (150×4.6 mm I.D., 5 μm) stainless steel column, adapted to a Kromasil C_{18} (3 cm×4.6 mm I.D., 5 μm) both from Análisis Vínicos. The mobile phase used was water 10% in methanol (v/v), isocratic at a flow-rate of 0.5 ml/min. Separations were carried out at room temperature and 5 μl was injected (as standard volume) into the LC–MS system.

The ES–MS interface in positive mode operated under the conditions of 350°C gas temperature, 13.0 l/min drying gas flow, 30 p.s.i. (1 p.s.i.=6894.76 Pa) nebulizer gas pressure and 4000 V of capillary voltage. Full-scan LC–MS chromatograms were obtained by scanning from m/z 50 to 800. Selected-ion monitoring (SIM) of the most abundant ion was used for quantification.

2.3. Sample analysis

Citrus samples (200 g of whole fruit) were prepared using a food processor and mixed thoroughly. An aliquot (0.5 g) of the samples was placed into a mortar (50 ml of capacity) and 0.5 g of the C_{18} sorbent were added and gently blended for 5 min using a pestle, to obtain an homogeneous mixture. This mixture was introduced into a 100×9 mm I.D. glass chromatographic column with a coarse frit (No. 2) and covered with a plug of silanized glass wood at the top. Abamectin residues were eluted with 15 ml of dichloromethane. The eluate was evaporated to dryness with air at 50°C. A 0.5-ml volume of acetonitrile was added and thoroughly mixed in ultrasonic bath for 5 min.

Recovery studies were carried out spiking fresh samples (0.5 g) of orange with the abamectin

fortification solution at different levels: 0.01–10 mg/kg.

3. Results and discussion

3.1. Optimization of HPLC–MS parameters

The abacmectine in the acetonitrile mobile phase gave only a weak electrospray response in the positive ion mode with or without acetic acid. In contrast, the compound in methanolic mobile phase, gave a significantly higher response, and for this reason a methanol–water (90:10, v/v) mobile phase was chosen for the determination.

To optimize the ES-MS conditions, different parameters influencing mass spectra were investigated: gas temperature, drying gas flow, nebulizer gas pressure, and capillary voltage were varied in flow injection analysis (FIA) experiments. The optimum working conditions thus established were used throughout the rest of the experiment (Section 2.2).

In order to identify the main ions for the analyte, FIA was used to introduce the abamectin standard solution (5 μl of 10 mg/l) and then the mass spectra were obtained in full scan mode. The fragmentor voltage was then varied from 40 to 300 V to find the maximum response using the optimum LC–MS conditions. The main ions obtained at each voltage are given in Table 1. At low fragmetor voltages (down to 180 V), abamectin showed molecular ion peak at m/z 895.6 $[M+Na]^+$ with a characteristic fragment at m/z 391.9. A possible fragmentation part way of abamectin is illustrated in Fig. 1.

Minor fragmentation was observed when higher fragmentors were employed (180–240 V). The $[M+Na]^+$ ion was the base peak in the MS spectra, whereas several fragments with relatively low abundance were also detected depending on the extraction voltage; some of these low-abundance ions may be characteristic of abamectin. Since a higher response was obtained at 180 V, this value was chosen for identification and quantification purposes. Additional structural information can be obtained by varying the fragmentor voltage, as can be seen in Fig. 2.

Fragmentor voltages up to 280 V increased fragmentation and under these conditions many fragment ions appeared but none of them is predominant.

Table 1
Mass spectra fragments and relative abundances of ions

Fragmentor voltage (V)	m/z	Relative abundance (%)
80	895.60	19
	391.80	100 (36 000)[a]
	361.60	22
	287.10	12
120	895.60	30
	411.95	25
	391.90	100 (62 000)[1]
	361.60	12
	282.20	11
	84.80	11
140	897.65	9
	896.60	28
	895.60	54
	701.80	11
	392.85	24
	391.90	100 (25 000)[1]
	282.20	19
	84.75	9
180	897.60	19
	896.60	58
	895.60	100 (66 000)[1]
	701.80	19
	310.80	15
	68.75	12
220	897.60	17
	896.60	52
	895.60	100 (60 000)[a]
260	897.60	17
	896.60	48
	895.60	100 (45 000)[a]
	752.00	19
	702.80	14
	701.80	38
	448.20	19
	325.30	11
	180.35	10

[a] Absolute intensity of the base peak.

The curious behaviour of the abamectin which seems to be more fragmented at low voltages can be explained if it is taken into account that the fragmentor voltage is also crucial for an efficient transmission of ions to the quadrupole mass spectrometer. Probably at these very low voltages, the transmission of high m/z ions is not performed adequately while at higher fragmentor voltages (180–240 V) the ion

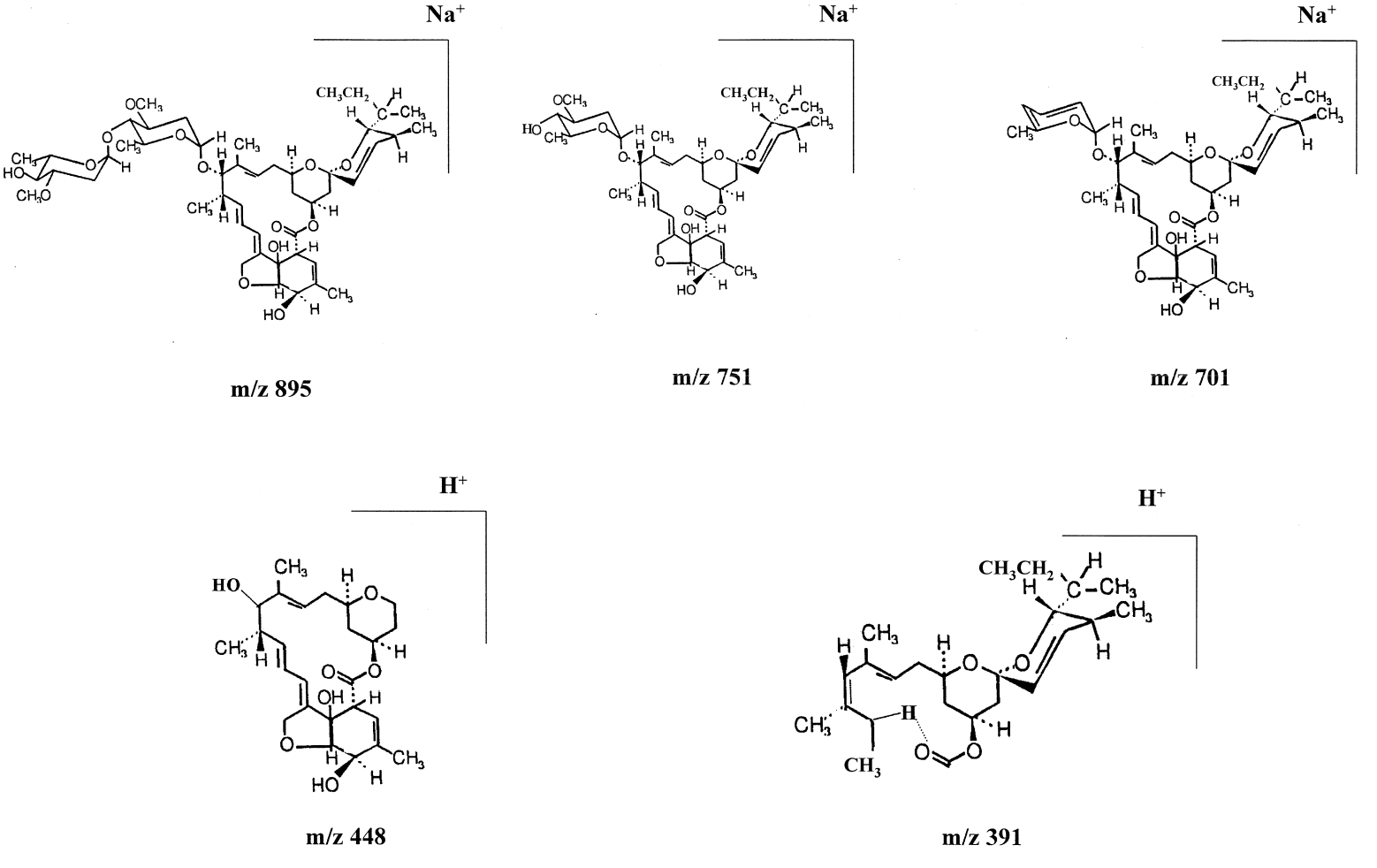

Fig. 1. Tentative identification of abamectin fragmentation patterns.

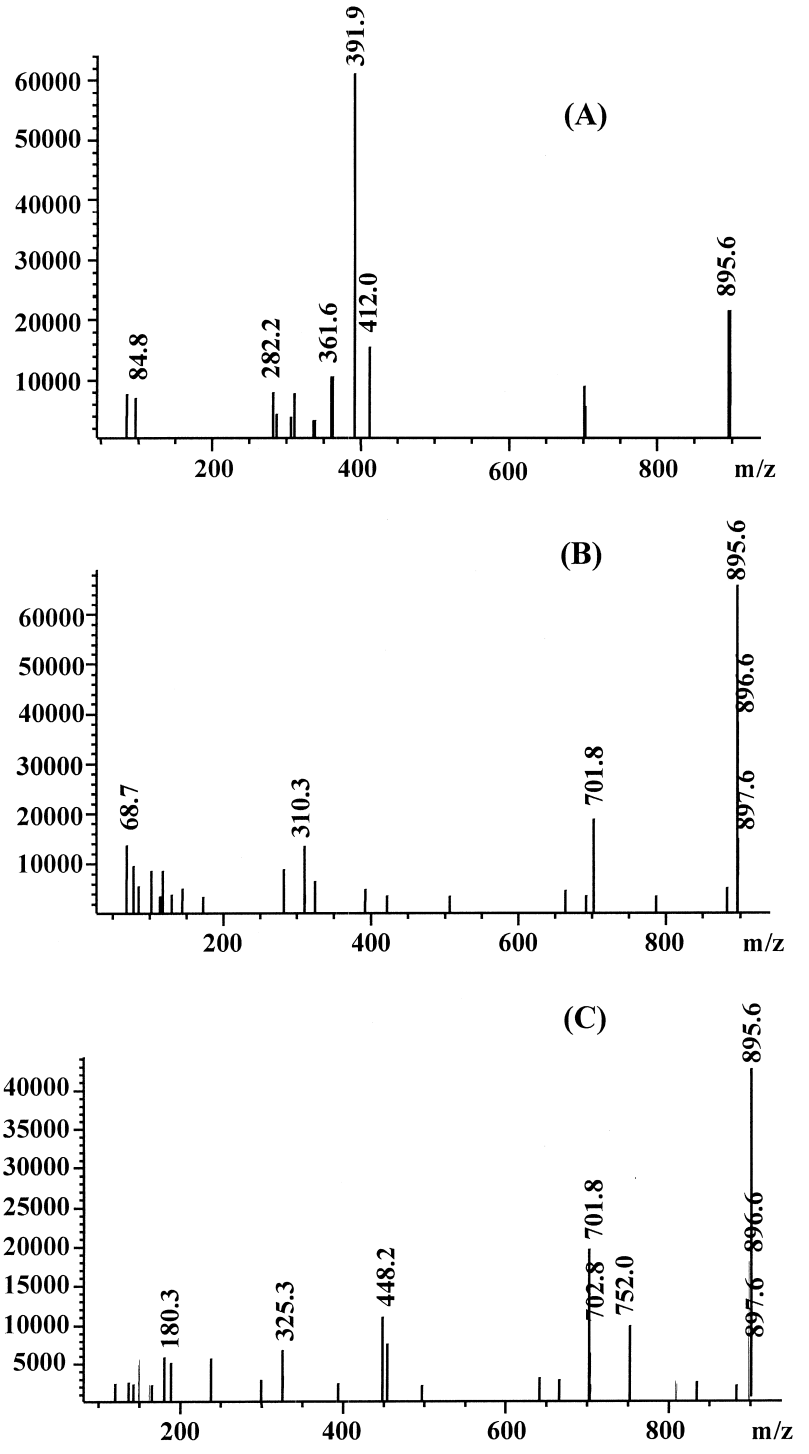

Fig. 2. Variation of the absolute abundance for some fragment ions vs. a fragmentor voltage of 120 (A), 180 (B), 260 (C).

transmission is appropriate and the abundance of molecular ion is more adequate.

3.2. Quality parameters

The calibration graph was plotted (ten points) for standard solutions between 50 pg and 50 ng. A wide range of linearity and good correlation coefficient ($r=0.9973$) were observed. For the study of the reproducibility of the LC–MS method, five replicate determinations on the same day of a standard solution 1 mg/l were carried out under the optimal LC–MS conditions (precision run-to-run). Moreover, five injections of this solution on 3 different days allowed day-to-day precision to be established. The relative standard deviations (RSDs) were 0.5%. for run-to-run and 4.3% for the day-to-day precision; the method, therefore, has a good reproducibility.

The limit of detection (LOD) calculated using the base peak (SIM mode, 180 V, signal-to noise ratio, 3:1) was 12 pg. The LOD for oranges was estimated using oranges free of abamectin spiked at low mg/kg and was 15 pg. By comparing the LODs (based on pg injected for standard solutions and orange samples), it can be deduced that matrix effects, even in complex samples with no clean-up, were not relevant when the described LC–ES-MS technique was used.

3.3. Extraction procedure

The proposed MSPD extraction method is a general method for extraction of pesticides from fruit and vegetables. By the use of this method, abamectin can be extracted in the frame of a multiresidue extraction method.

The accuracy and reproducibility for abamectin in oranges spiked at five levels were evaluated and are shown in Table 2. The mean recovery values at all concentration levels were found to range from 94 to 99% with relative standard deviation (RSD) values $\leq$5%. All values in this table are within the accepted range for residue determination [18].

Typical LC–ES-MS SIM chromatograms of spiked and non-spiked orange samples are shown in Fig. 3. The chromatograms of the orange extract were satisfactory, without interferences at the abamectin elution time area (see blank chromatogram in Fig. 3A). The retention time for abamectin was about 12.5 min. As can be observed in Fig. 3C, the sensitivity was sufficient to confirm the residues at the 0.01 mg/kg level.

Table 2
Abamectin recoveries (%) and relative standard deviation (RSD) from orange samples[a]

Spiked level (mg/kg)	Recovery (%)	RSD (%)
0.01	99	4
0.05	99	5
0.10	94	4
1.0	95	3
10	94	4

[a] $n=3$.

The limit of quantification (LOQ) evaluated as the signal-to noise (S/N) ratio equal to 10 in the selected LC–ES-MS conditions was found to be 2.5 ng/g. If lower concentrations need to be determined this may be possible by either increasing the sample size or injecting a larger volume (up to 5 μl).

The LOQ value is comparable to those published for abamectin analysis in several matrices [3–7] and four times lower than the MRL established by the Spanish Government. The advantages of the proposed MSPD procedure are that it is fast, as it does not involve any clean-up step after extraction of abamectin from vegetable materials; it is simple, for it does not require any derivatization step; easy to perform, and it does not present interferences.

The presence of abundant orange constituents in charge liquid droplets could interfere with the ionization process by complex mechanisms. The lack of interference caused by matrix components was assessed. After measuring for the abamectin the resulting ion signal was averaged and compared with those obtained for the standard solution by applying the *t*-test ($n=9$, $P=0.05$) (Table 3). The calculated *t*-values were lower than the critical value (4.30) with means that the constituents of fruit did not significantly affect the ionization process of abamectin.

The feasibility of using the ES-MS as a detection method for routine determination of abamectin in oranges was evaluated during an entire day of heavy use of the instrument. The ion signals did not show any definite tendency to decrease over 1 working day.

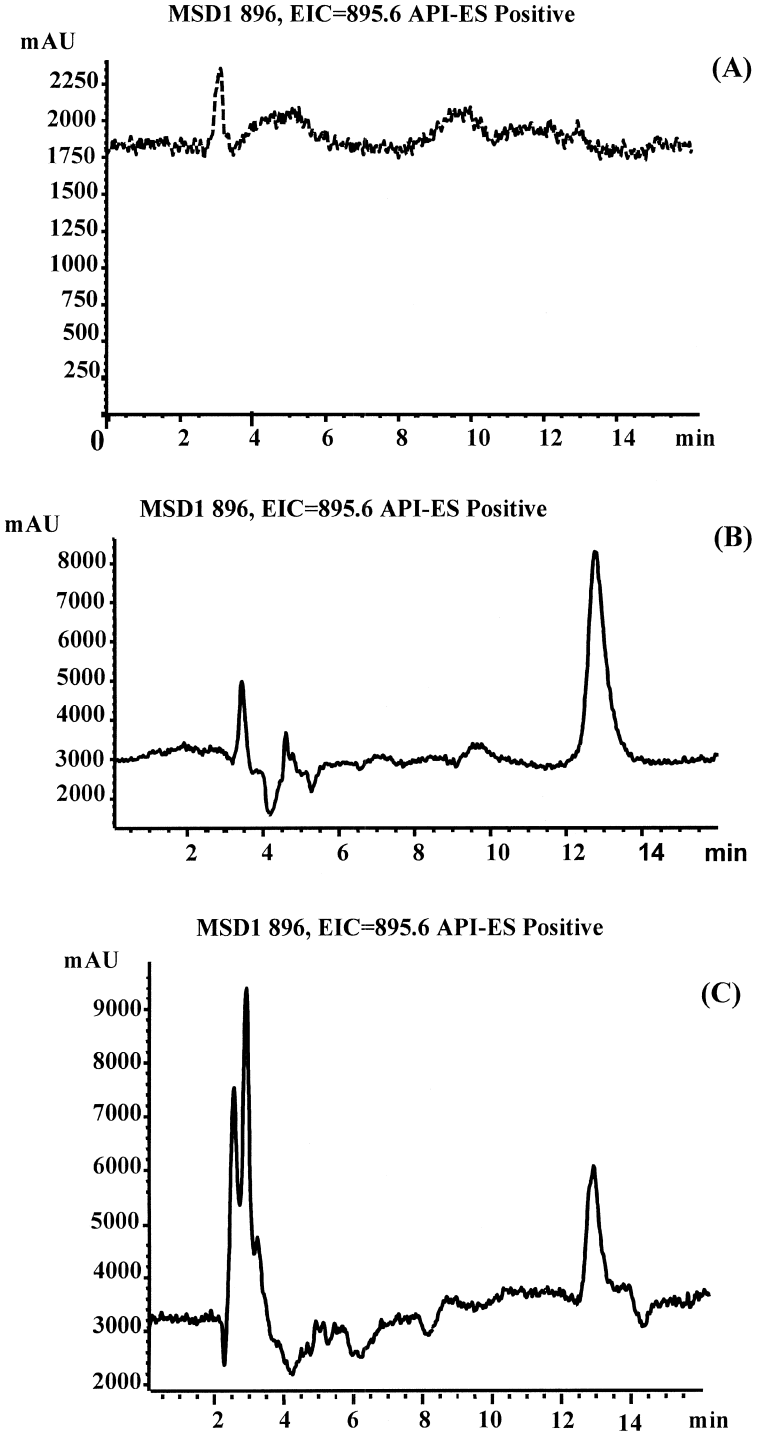

Fig. 3. SIM chromatograms from LC–ES-MS of non fortified orange extract (A), 0.05 mg/kg fortified extract (B), and 0.01 mg/kg fortified extract (C).

Table 3
Matrix effect on the accuracy of the determination of abamectin in citrus by LC–ES-MS

Abamectin (mg/kg)	Analytical data				
	X[a]	SD[b]	RSD[c]	μ[d]	t[e]
0.01	42 341	1561	4	42 616±118	3.93
0.05	121 955	6691	5	123 498±758	2.12
0.1	185 036	7431	4	195 651±4334	1.74
1.0	1 371 148	33 239	2	1 444 953±7288	2.81
10	14 428 020	557 774	4	15 906 069±39 646	3.73

[a] X=mean values of peak areas obtained by analyzing final extracts of three samples of different types spiked with known amounts abamectin.
[b] SD=standard deviation.
[c] RSD=relative standard deviation.
[d] μ±SD=mean values and standard deviations of peak areas calculated by injecting three times known amounts of abamectin.
[e] t=Student's t-test.

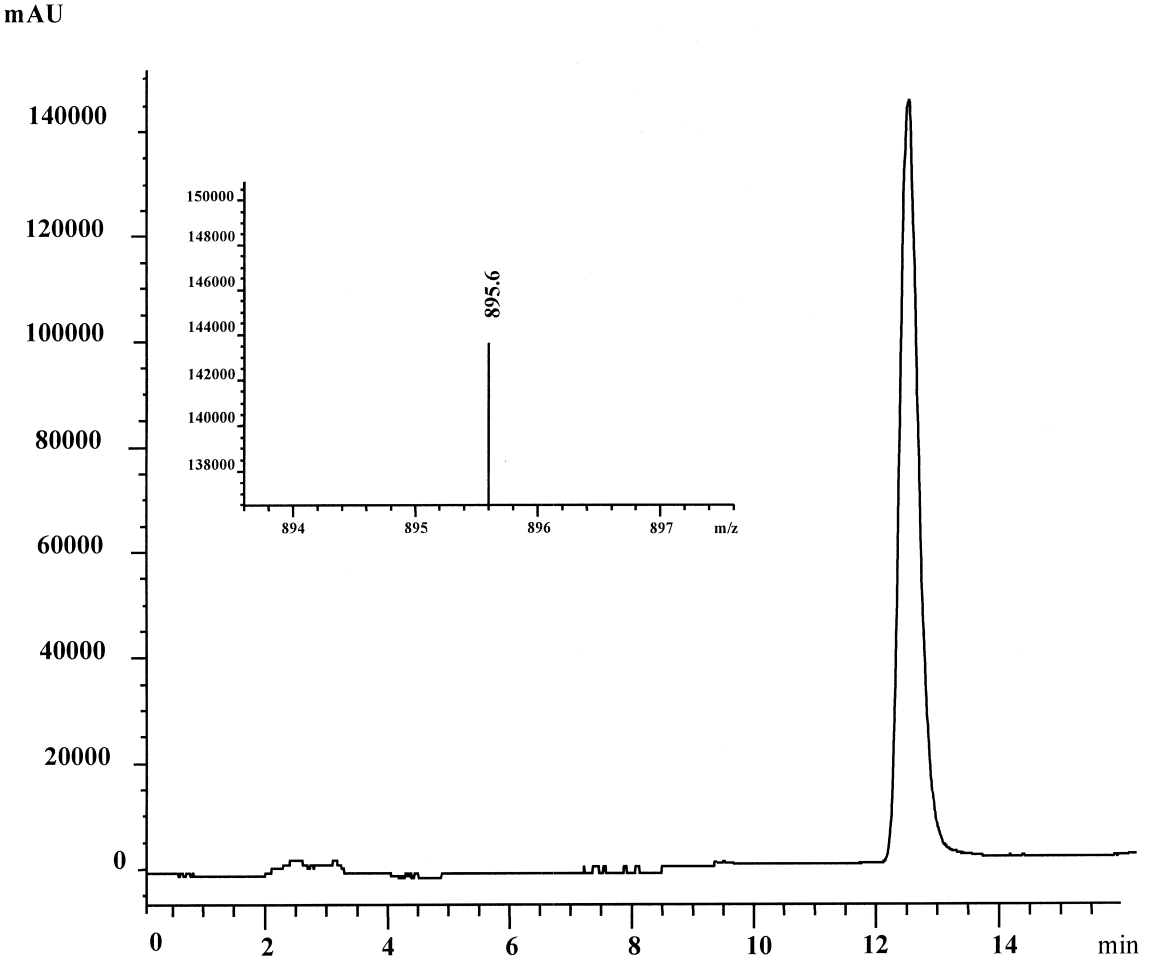

Fig. 4. SIM chromatogram from LC–ES-MS of field treated orange containing 2.4 mg/kg of abamectin.

The residues of abamectin measured in field treated oranges of the Naveline variety, were found to be 0.0025 and 2.4 mg/kg. The determined concentrations are within the studied range and the application of the method proved to be satisfactory.

The abamectin in the samples can be identified/confirmed by comparison of the relative abundance of the most characteristic ions at different extraction voltages. Fig. 4 shows an example of the abamectin identification in a real orange sample.

4. Conclusions

The sensitivity and selectivity of ES-MS detection reduces sample processing time (relative to UV or fluorescence detection) by eliminating time-consuming clean-up steps. Moreover, use of this detection system for pesticide residues in real matrices eliminates the need for additional confirmatory procedures.

The results obtained in this work support the conclusion that LC–ES-MS is an appropriate methodology for pesticide analysis of fruit samples. Moreover, the selectivity and specificity of the determination was enhanced by using relative retention times and ratios of confirmation ions.

The MSPD procedure is very simple, rapid and requires only small sample sizes and solvent volumes. This method makes it feasible to screen many samples and it can be use for monitoring.

Coupling the MSPD and LC–ES-MS procedure allows determining abamectin in orange samples at lower levels than the MRL. This means that the method is suitable for routine analysis, as well as for studying dissipation of this insecticide in field-treated oranges.

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