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What are we determining using gas chromatographic multiresidue methods: tralomethrin or deltamethrin?

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Abstract

The analytical behaviour of the relatively new pyrethroid insecticide tralomethrin has been evaluated by using gas chromatography (GC) with electron-capture and mass spectrometry (MS) detectors, and liquid chromatography (LC)–atmospheric pressure ionization mass spectrometry with electrospray interfacing. Under the GC conditions commonly used in pesticide residue analysis, it was found that tralomethrin is transformed into deltamethrin (in a reproducible way) in the injector port of the GC system. Results obtained in this work indicate that the GC multiresidue methodologies routinely applied in the analysis of pyrethroid pesticides in foods cannot distinguish between these two pesticides, and the chromatographic signal obtained at the retention time of deltamethrin/tralomethrin can be really quantified as either deltamethrin or tralomethrin, including when it is confirmed as deltamethrin by MS. Under the LC–MS conditions assessed in this work, deltamethrin and the two diasteroisomers of tralomethrin were well separated and identified.

Keywords: Pesticides; Tralomethrin; Deltamethrin; Pyrethroids

1. Introduction

Tralomethrin is a relatively new non-systemic pyrethroid insecticide effective for the control of a range of agronomic pests, particularly *Lepidoptera* in cereals, fruits, vegetables and other crops, at application rates as low as 7.5–20 g active ingredient (a.i.)/ha [1]. In Spain, it is commercialised by DuPont under the trade name Traker, and it is already widely used as an effective substitute of some traditional insecticides whose use in Europe are strongly restricted or prohibited. Maximum residue limits (MRLs) for tralomethrin have been established in some European countries, but they are not yet harmonised at the European Union level. Examples of these MRLs are: 0.01 mg/kg for all fruits and vegetables in Spain [2], and 0.50 mg/kg for lemons, grapes, apples or peaches in Italy [3]. Tralomethrin (R–CHBr–CBr_3; molecular mass: 665) is a mixture of two active diasteroisomers, which are partially transformed into deltamethrin (R–CH=CBr_2; molecular mass: 505), by elimination of a molecule of bromine, in both plants and the environment [1,4]. However, the ''residue definition'' until now used to establish MRLs for tralomethrin, and to monitor tralomethrin residues in foods, is just ''tralomethrin'' [2,3,5]. The structures of tralomethrin and deltamethrin are given in Fig. 1.

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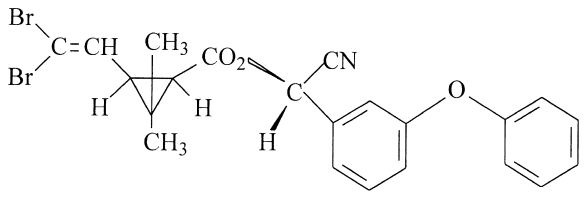
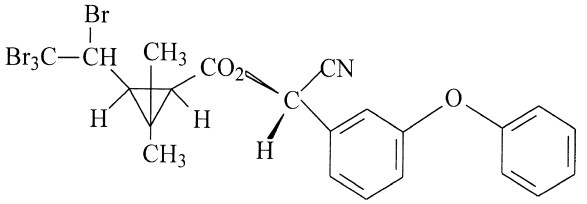


Deltamethrin



Tralomethrin

Fig. 1. Molecular structures of tralomethrin and deltamethrin.

Up to now, few analytical procedures have been reported for the determination of tralomethrin residues, including a high-performance liquid chromatographic (HPLC) method with radiometric detection (RAM), which was applied to the analysis of water, sediment and fish tissue [6], and different gas chromatographic (GC) methods with electron-capture detection (ECD) or mass spectrometry (MS) detection described for the analysis of fruits and vegetables [7], milk [8], or soya oil [9]. Initially, GC–ECD and/or –MS multiresidue methods routinely used, and validated, in many food control laboratories to determine pyrethroids residues in fruits and vegetables [10–12] could be also applied to determine tralomethrin residues in these matrices [13].

The main objectives of this work were: (a) to evaluate the analytical behaviour of tralomethrin by using GC–ECD and GC–MS, and compare the analytical parameters obtained for tralomethrin with those obtained for deltamethrin; and (b) to evaluate an ethyl acetate-based multiresidue extraction method to be applied to the analysis of tralomethrin residues in peppers. Owing to the results obtained in the GC studies, which showed that it is not possible to distinguish between tralomethrin and deltamethrin residues with this technique, additional studies on the analytical behaviour of both pesticides by LC–MS were also performed.

2. Experimental

2.1. Reagents and materials

Acetone, ethyl acetate and cyclohexane were pesticide residue grade. Acetonitrile and water were LC grade. Anhydrous sodium sulfate was pesticide residue grade. Certified standards of tralomethrin (90.0% purity) and deltamethrin (99.0 purity) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standard solutions of tralomethrin (0.35 mg/ml) and deltamethrin (0.69 mg/ml) were prepared in acetone. Standard solutions for GC analysis were prepared by suitable dilution of the stock standards solutions with either ethyl acetate–cyclohexane (1:1) or blank pepper extracts. Standard solutions for LC analysis were prepared from the stock standard solution in acetonitrile–water (4:1). Pure standards and standard solutions were stored in dark at −20°C.

2.2. GC–ECD analysis

GC–ECD measurements were performed with a Model 3800 gas chromatograph from Varian (Walnut Creek, CA, USA) equipped with a Model 1079 injection port, and a Model 8200 Cx autosampler (for split/splitless injections); fitted with either a DB-5MS or a DB-1701 fused-silica capillary GC column (J&W, Folsom, CA, USA) of 30 m×0.25 mm I.D., 0.25 μm film thickness. The DB-5MS column temperature programme was: 60°C for 1 min, 25°C/min to 180°C, 5°C/min to 280°C, and hold for 9 min. The DB-1701 column temperature programme was: 90°C for 1 min, 30°C/min to 180°C, 4°C/min to 280°C, and hold for 13 min. In all cases, a 1 μl volume was injected with the split closed for 0.75 min, and the carrier gas was helium (99.999% purity) with electronic flow control at 1.2 ml/min. Other GC operating conditions were: 250°C injector temperature (or other as indicated in Results and discussion); 300°C detector temperature; and 30 ml/min make-up gas flow (nitrogen). A Varian Star

4.5 Chromatography Workstation was used for chromatographic data processing.

2.3. GC–MS analysis

GC–MS analyses were performed with a Varian 3400 gas chromatograph–Saturn 3 ion trap mass spectrometer equipped with a Model 1077 injection port, and a Model 8200 Cx autosampler (for split/splitless injections); fitted with a DB-5MS fused-silica capillary GC column (30 m×0.25 mm I.D., 0.25 μm film thickness). Operating conditions for GC–MS were: 2 μl injection volume; 9 p.s.i. helium (99.999% purity) column head pressure; 0.75 min splitless time; 250°C injector temperature; 60°C initial oven temperature for 1 min, ramped to 180°C at 25°C/min, then to 280°C at 5°C/min, and held at 280°C for 15 min; 280°C transfer line temperature; and 220°C ion-trap manifold temperature (1 p.s.i.= 6894.76 Pa). MS measurements were performed with electron impact (EI) at 70 eV in the full scan mode (total ion current, TIC) over the mass range of m/z 60 to 650 at 1 scan/s from 6 to 35 min. For GC–MS we utilised Saturn GC–MS Version 5.2 software for data collection.

2.4. LC–MS analysis

LC–MS analyses were performed with a HP Series 1100 liquid chromatograph and a HP 1100 MSD-G1946A atmospheric pressure ionization (API) mass spectrometer equipped with electrospray (ESI) G1948A and atmospheric pressure chemical ionization (APCI) G1947A interfaces (Hewlett-Packard, Palo Alto, CA, USA). The chromatographic separation was carried out with a LiChroCART 125-4 Superspher 100 RP-18 column (Hewlett-Packard), and isocratic elution with acetonitrile–(50 m*M* ammonium formate in water–acetonitrile, 95:5, acidified by adding formic acid, pH 3.5) (80:20) as mobile phase at 1 ml/min flow-rate. Analyses were performed with the ESI interfacing technique in the positive mode of operation. The operating parameters were: 10 ml/min drying gas flow-rate; 50 p.s.i. nebulizer pressure; 3000 V capillary voltage; 325°C drying gas temperature; and 60 V fragmentor voltage. MS measurements were performed in full scan mode over the mass range of m/z 50 to 800.

2.5. Extraction procedure and recovery tests

The extraction procedure assessed to analyse residues of tralomethrin in peppers was a modification of the ethyl acetate–GC multiresidue extraction method developed by the Swedish National Food Administration for fruits and vegetables [10]. A brief description of the assessed extraction procedure is as follows: weigh 37.5 g of thoroughly homogenised sample and blend with 100 ml ethyl acetate and 20 g anhydrous sodium sulfate for 5 min. Filter the solvent phase through a glass fibre filter with a 10 g sodium sulfate layer, and dry the filtrate by shaking with 15 g sodium sulfate. Transfer 25 ml of the ethyl acetate layer to a 100-ml round-bottomed flask and concentrate to approximately 2 ml on a rotary vacuum evaporator at 37°C. Transfer the concentrate quantitatively to a graduated test tube, adjust the volume to 5 ml with ethyl acetate and then to 10 ml with cyclohexane. Filter the extract through a 0.45-μm microfilter by suction with a 10-ml syringe. The obtained extract contains 0.94 g sample per ml and is ready to be analyzed by GC–ECD.

Tralomethrin recovery tests were conducted on pepper samples previously analysed and demonstrated not to contain any residues of tralomethrin or deltramethrin. Pepper samples were spiked with tralomethrin at three different levels, 0.01, 0.11, and 0.15 mg/kg, by adding a suitable volume (80 or 10 μl) of tralomethrin standard solutions (50 or 70 mg/l) to 37.5 g of homogenised blank pepper sample in a blender jar. Five replicates of the 0.11 and 0.01 mg/kg spikes, 10 replicates of the 0.15 mg/kg spikes, and a number of blank pepper samples were analysed. Analyses were performed by GC–ECD using the DB-1701 column (0.11 and 0.01 mg/kg spikes) or the DB-5MS column (0.15 mg/kg spikes). In all cases, recoveries were calculated using analytical standards of tralomethrin prepared in extracts of blank pepper samples.

3. Results and discussion

GC–ECD analysis of tralomethrin standards showed that the retention time values of the only chromatographic peak obtained for this pesticide, in both the DB-5MS and DB-1701 columns, are exactly

the same than those obtained for deltamethrin standards. Under the GC–ECD conditions indicated in the Experimental section, retention times were of 30.6 and 37.7 min in the DB-5MS and DB-1701 columns, respectively. GC(DB-5MS)–ECD chromatograms obtained for a solvent standard of tralomethrin (1.10 mg/l) and a solvent standard of deltamethrin (0.80 mg/l) are compared in Fig. 2. Likewise, Fig. 3 compares the GC(DB-1701)–ECD chromatograms obtained for standards of tralomethrin and deltamethrin prepared in blank pepper extract (matrix standards) with a concentration of 0.04 mg/kg in both cases.

On the other hand, the retention time of the only peak obtained for tralomethrin in the GC–MS system was also exactly the same than that obtained for deltamethrin (32.0 min). In addition, the mass spectra obtained for both tralometrhin and deltamethrin standards were totally equivalent to the mass spectrum of deltamethrin from the mass spectra library. Figs. 4 and 5 show the GC–MS chromatograms and mass spectra obtained for solvent standards of tralomethrin (34 mg/l) and deltamethrin (43 mg/l), respectively, using the GC–MS conditions indicated in the Experimental section.

Once the identity of the standards of tralomethrin and deltamethrin was confirmed by means of LC–MS (see below), the only way to explain the GC results obtained is that the two isomers of tralomethrin are transformed into deltamethrin in the GC injector port, by elimination of a molecule of bromine. The other possible explanation (the two isomers of tralomethrin and deltamethrin having fortuitous equal retentions in the two types of columns utilised, and the tralomethrin isomers undergoing loss of bromine in the MS source) was rejected. Note that the LC–MS chromatograms presented below, and also the LC–UV analysis carried out by Dr. Ehrenstorfer to certify the purity of the tralomethrin standard, show that the two isomers of tralomethrin are easily separated by LC. Therefore, if tralomethrin was not transformed into deltamethrin,

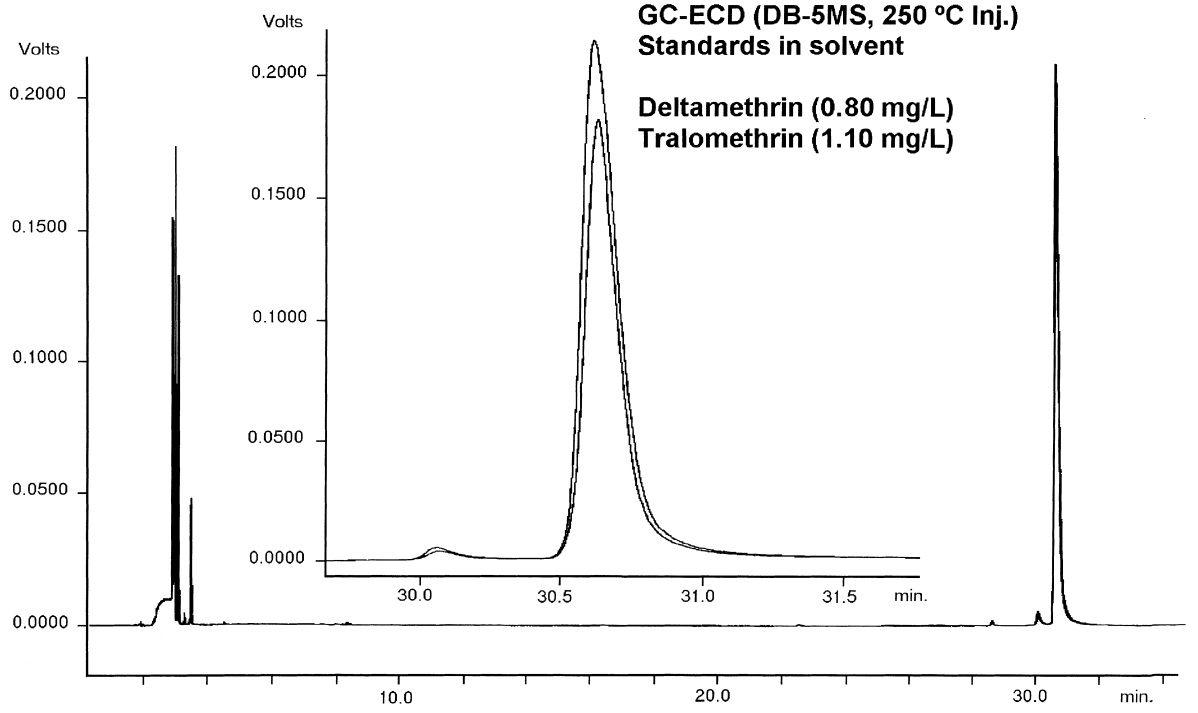


Fig. 2. GC–ECD chromatograms (DB-5MS column) obtained for a solvent standard of tralomethrin (1.10 mg/l) and a solvent standard of deltamethrin (0.80 mg/l), including an amplified detail from 29.5 to 32.0 min.

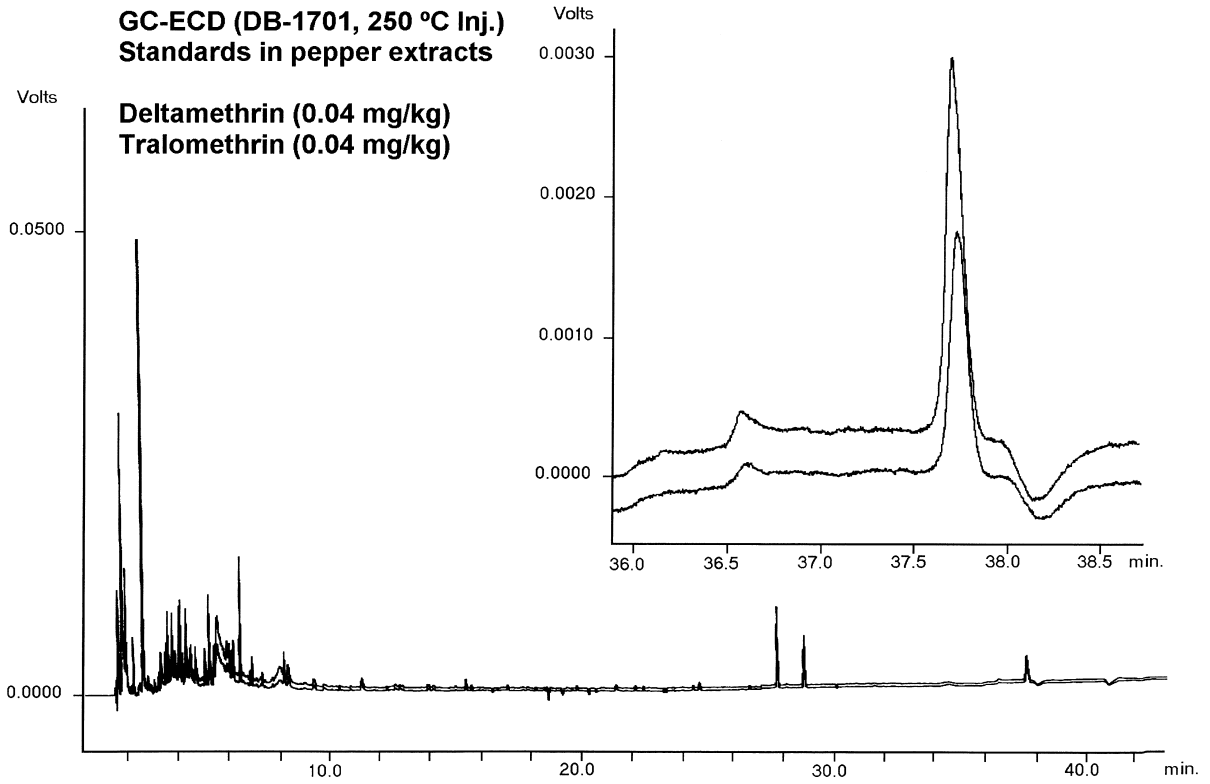


Fig. 3. GC–ECD chromatograms (DB-1701 column) obtained for a matrix standard of tralomethrin (0.04 mg/kg) and a matrix standard of deltamethrin (0.04 mg/kg), including an amplified detail from 36 to 39 min.

it would be expected to obtain two peaks for tralomethrin in GC, at least in one of the two columns of different polarity used in this work.

Tralomethrin/deltramethrin relative response factor (RRF=peak area/mass concentration for tralomethrin divided by peak area/mass concentration for deltamethrin) in the GC(DB-1701)–ECD system was obtained by analysing different matrix standards of tralomethrin and deltamethrin with concentrations ranging from 0.01 to 0.20 mg/kg. As indicated in Table 1, the RRF values determined were close to 0.58 in all cases. Table 2 shows the RRF values obtained in the GC(DB-5MS)–ECD system by analysis of solvent standards of tralomethrin (1.10 mg/l) and deltamethrin (0.80 mg/l) under the chromatographic conditions indicated in the Experimental section, but using different injector temperatures (from 200 to 300°C). Results in Tables 1 and 2 indicate that the tralomethrin/deltamethrin relative response factor in the GC–ECD systems was reproducible and close to 0.6 when injector temperatures of 240–300°C were used. Also, the tralomethrin/deltamethrin RRF obtained in the GC–MS system ranged from 0.55 to 0.60 when the quantification of the standards of both pesticides were made on the ion chromatograms of m/z 181, 253 or 172.

At this point, it is important to note that a RRF value of 0.76 should have been obtained if the transformation of tralomethrin into deltamethrin would have been quantitative (0.76=deltamethrin molecular mass/tralomethrin molecular mass). We are not sure why an experimental RRF value of ~0.60 is obtained, but some possible reasons could be: an incomplete transformation, the effect of different physical processes during the injection, or that the actual purity degree of the standards utilised was different to the certified value. Therefore, further work will have to clarify the physico–chemical processes involved in the GC injection of tralomethrin. Despite this, the results obtained in this paper

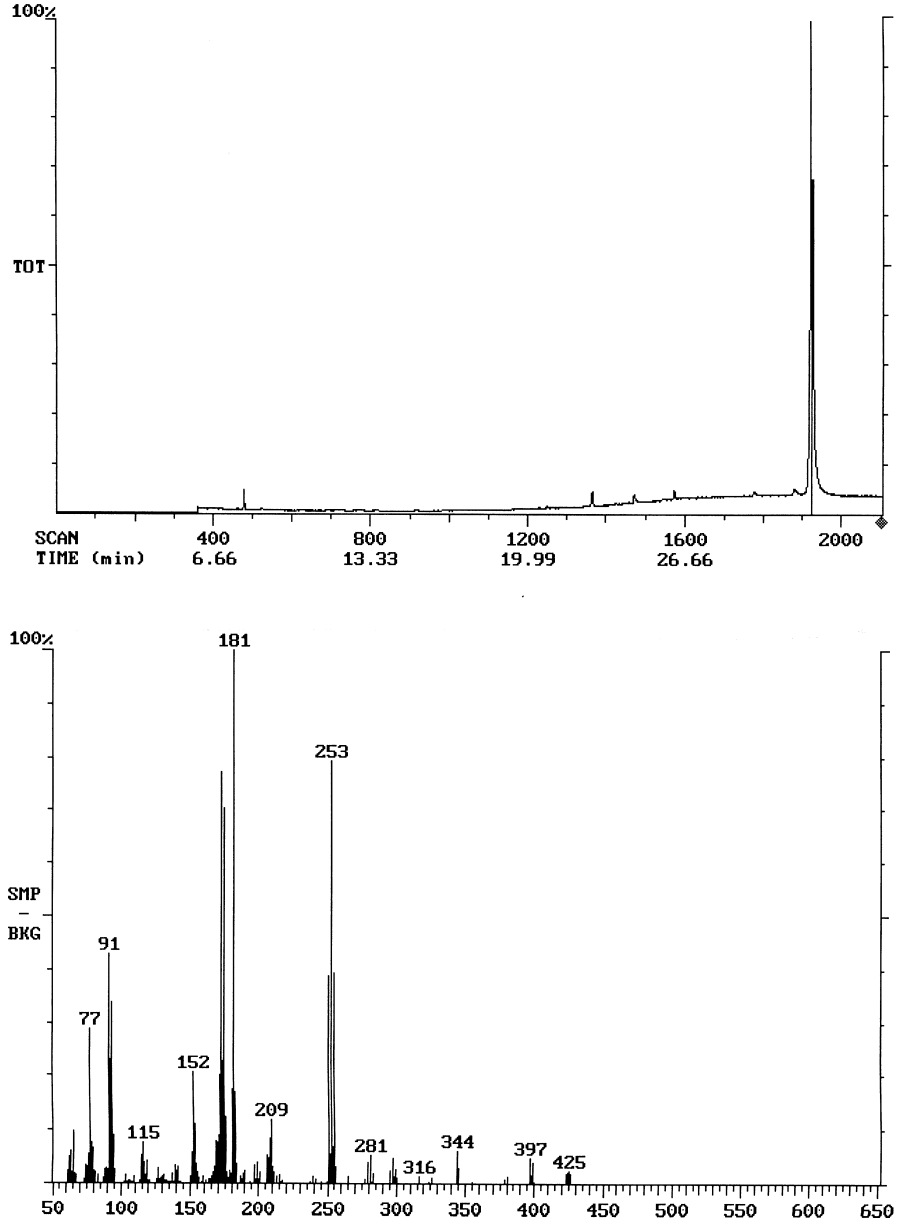


Fig. 4. GC–MS chromatogram and spectrum (t_R=32 min) obtained for a solvent standard of tralomethrin of 34 mg/l.

are enough to affirm that tralomethrin is transformed into deltamethrin in the injector port of the GC system, and demonstrate that by using just conventional GC multiresidue methods it is not possible to distinguish between deltamethrin and tralomethrin, even if using GC–MS.

The results obtained in some additional GC–MS analyses of tralomethrin and deltamethrin standard solutions, which were carried out with a temperature programme in the injector (60°C for 6 s followed by ramping to 280°C at 10°C/min) and keeping all the other GC–MS conditions as described in the Experimental section, were the same as those obtained with conventional hot injections. Hence, tralomethrin

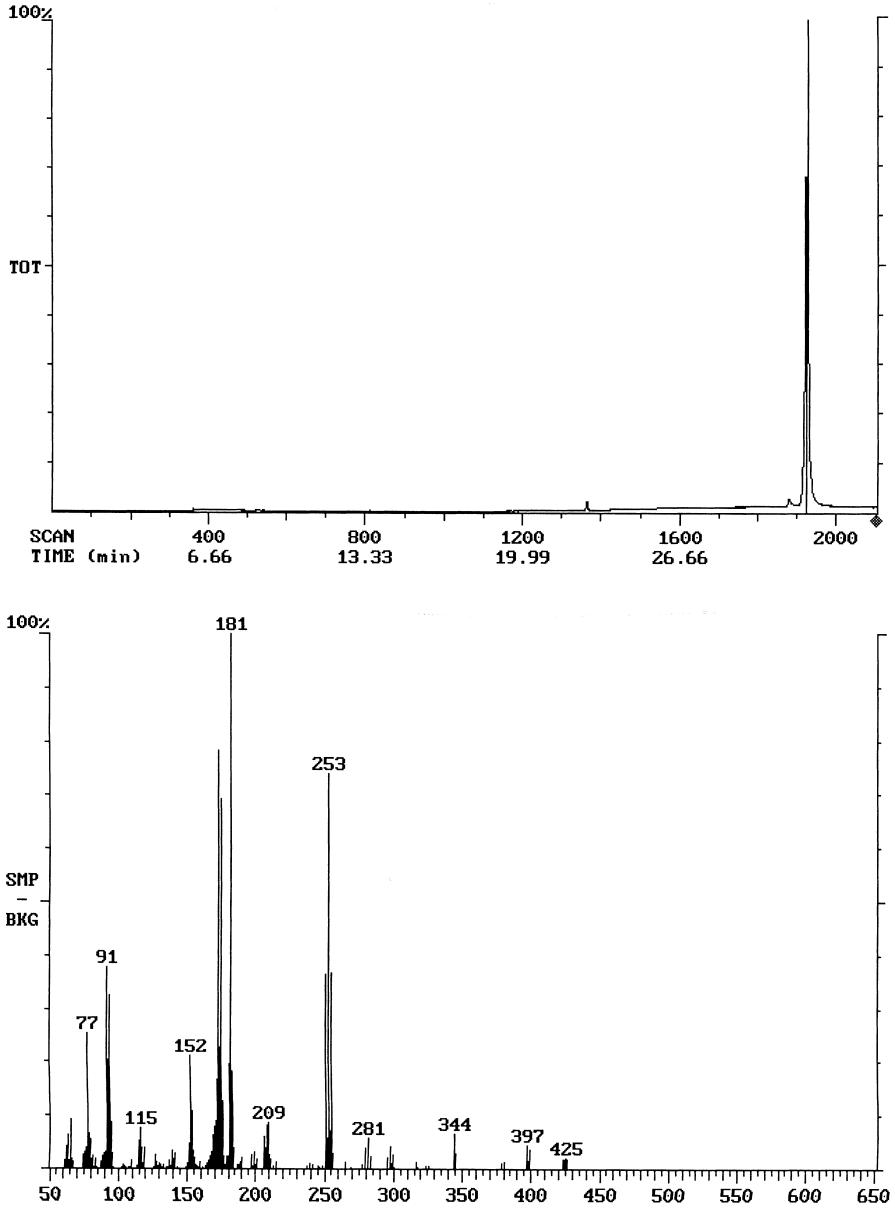


Fig. 5. GC–MS chromatogram and spectrum (t_R=32 min) obtained for a solvent standard of deltamethrin of 43 mg/l.

gave just one chromatographic peak at the same retention time as the peak obtained for deltamethrin, and with exactly the same mass spectrum. Also, the RRF found under these conditions was again close to 0.6. Therefore, no additional information was obtained from these experiments, except that the problem persists when programmed temperature injection is used.

Recovery values obtained for tralomethrin from spiked pepper samples by using the above described multiresidue extraction method and the GC–ECD system are given in Table 3. The overall mean

Table 1
Tralomethrin/deltamethrin relative response factors (RRFs) obtained by GC(DB-1701)–ECD analysis of different matrix standards of tralomethrin and deltamethrin with an injector temperature of 250°C

Concentration (mg/kg)	RRF
0.01	0.57
0.04	0.60
0.10	0.59
0.20	0.57

Table 2
Tralomethrin/deltamethrin relative response factors (RRFs) obtained by GC(DB-5MS)–ECD analysis of solvent standards of tralomethrin (1.10 mg/l) and deltamethrin (0.80 mg/l) using different injector temperatures

Injector temperature (°C)	RRF
200	0.44
220	0.49
240	0.57
250	0.58
260	0.57
280	0.59
300	0.58

recovery and the corresponding relative standard deviation (RSD) ($n=20$) are 108% and 20%, respectively. These values can be considered acceptable according to the within-laboratory method validation criteria proposed by a recent AOAC/FAO/IAEA/IUPAC Expert consultation for multiresidue analysis of pesticides [14]. The assessed multiresidue extraction method has already been demonstrated to be suitable for the analysis of deltamethrin residues in different fruits and vegetables [10], and it could be that, in general, any GC multiresidue method validated for deltramethrin is also valid for tralomethrin.

All these results indicate that by using the analytical methodologies currently applied in almost all the pesticide residue control laboratories around the world to determine residues of deltamethrin in fruits and vegetables (which are GC multiresidue methods), it is not possible to assure if a residue confirmed as deltamethrin in a sample (by using two GC columns of different polarity or by GC–MS) is really a residue of deltamethrin, a residue of tralomethrin, or a residue of both pesticides. In a case that a crop was treated with tralomethrin the analysed sample will probably contain both compounds since tralomethrin is partially transformed into deltamethrin in plants [1]. In all cases, the residue could be quantified by using either standards of deltamethrin or standards of tralomethrin. One easy way to avoid all these difficulties at the time of determining deltamethrin and tralomethrin residues by using GC multiresidue methods would be to make a change in the ''residue definition'' of both pesticides, our proposal being: ''sum of deltamethrin and tralomethrin determined as deltamethrin''.

Another possibility to solve this problem would be to make the routine residue analysis of both pesticides by LC–MS. At present, many laboratories are introducing various LC–MS methods in routine analysis for most of the non-GC-amenable pesticides [15], but this technique is not usually applied to determine pyrethroid residues. In this work, some preliminary tests on the analytical behaviour of tralomethrin and deltamethrin by using LC–MS were performed with the main objective of confirming the identity of the certified standard of tralomethrin. After assessing different LC–MS modes of operation and conditions already described for residue analysis

Table 3
Tralomethrin recoveries obtained from spiked pepper samples by using the ethyl acetate extraction–GC–ECD multiresidue method described in the text

Spiking level (mg/kg)	*n*	Recoveries (%)	Mean recovery (%)	RSD (%)
0.15	10	89/108/102/90/96 95/103/95/90/104	97	7
0.11	5	106/101/103/96/104	105	4
0.01	5	109/136/154/100/172	134	22

of a number of pesticides in fruits and vegetables [16], the LC–ESI-MS technique in the positive ion mode of operation was selected for the analysis of tralomethrin and deltamethrin standards. LC–MS chromatograms and spectra obtained in the scan mode for standard solutions of tralomethrin and deltamethrin of 34 mg/l, under the analytical conditions described in the Experimental section, are presented in Figs. 6 and 7, respectively. It can be seen that deltamethrin and the two diasteroisomers of tralomethrin are efficiently separated using the selected LC conditions. The mass spectrum obtained for deltamethrin under the selected LC–MS conditions is characterised by the m/z ions 506 (M+1)

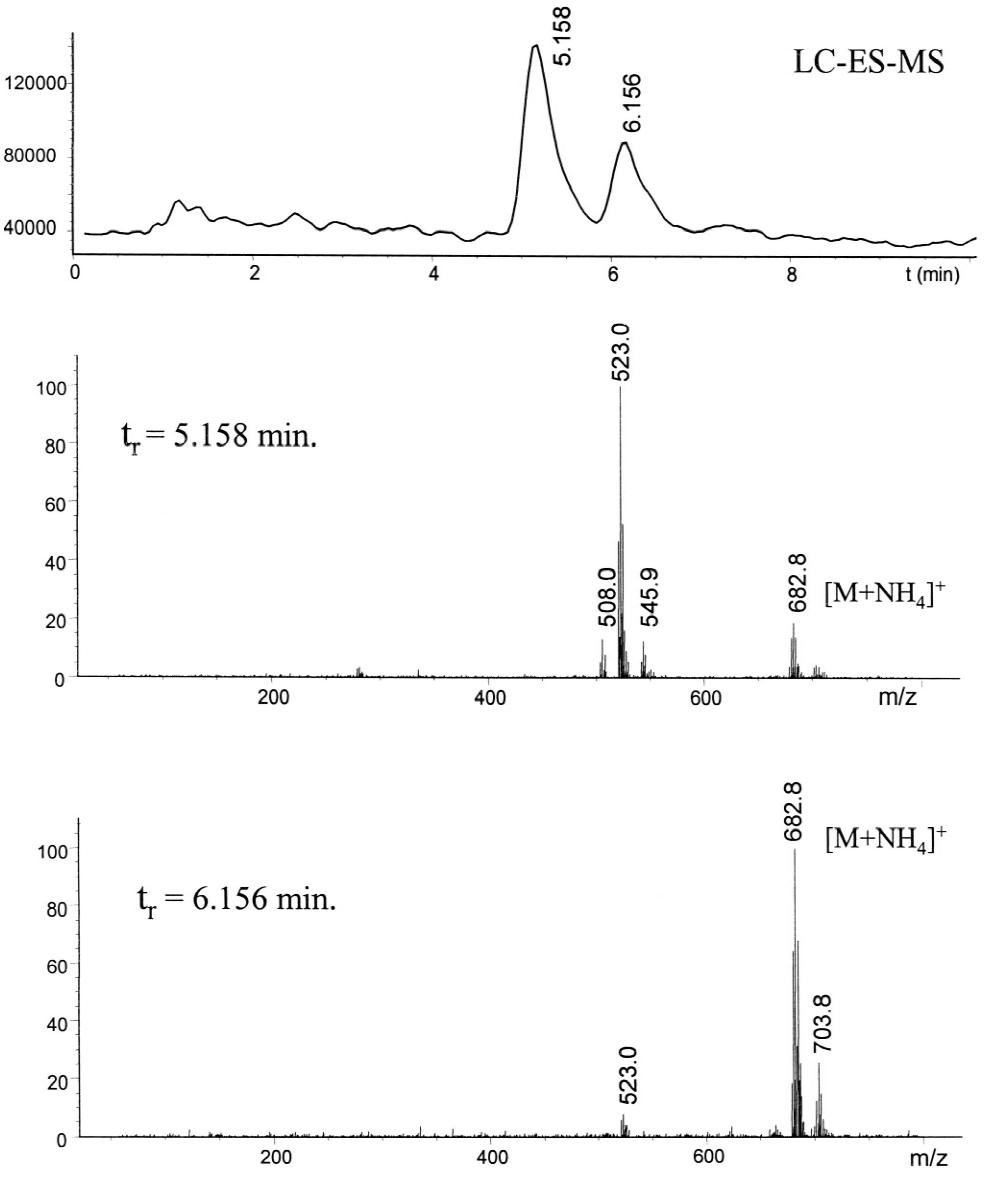


Fig. 6. LC–MS chromatogram and spectra (t_R=5.2 min and t_R=6.2 min) obtained for a solvent standard of tralomethrin of 34 mg/l (LC–MS conditions are indicated in the text).

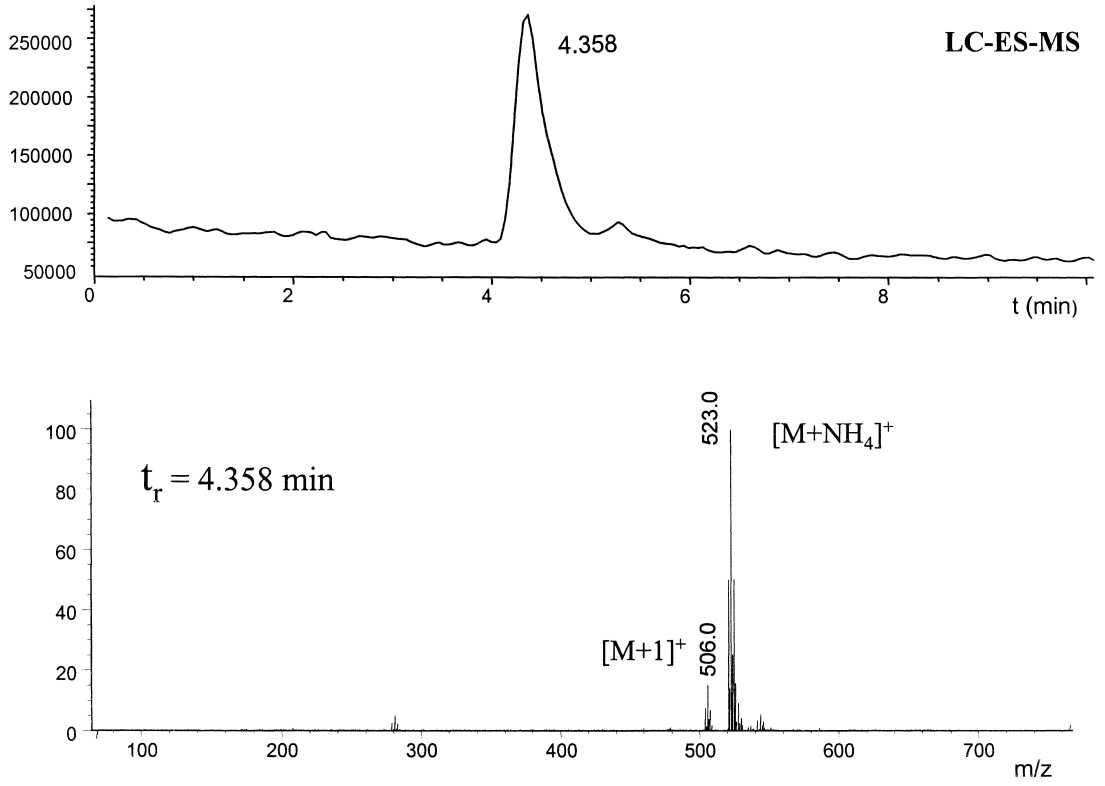


Fig. 7. LC–MS chromatogram and spectrum (t_R=4.4 min) obtained for a solvent standard of deltamethrin of 34 mg/l (LC–MS conditions are indicated in the text).

and 523 ($M+NH_4^+$). Mass spectra obtained for the two diastereoisomers of tralomethrin are characterised by the m/z ions 683 ($M+NH_4^+$) and 523, but the relative abundance of these two ions are very different for each one of the two diasteroisomers. These results indicate that the m/z ions 506, 523 and 683 could be used in future work to develop and validate an LC–MS method in the selected ion monitoring mode to determine tralomethrin and deltamethrin residues in vegetable samples.

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