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Liquid chromatography–tandem mass spectrometry for the identification of impurities in d-allethrin samples

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Abstract

A previous GC/MS study highlighting the impurity profile of the synthetic pesticide d-allethrin is extended here to validate and confirm the impurities identity through the development of soft ionisation HPLC–MS methods. To accomplish this, we developed a reverse phase LC–MS analysis in gradient elution with two distinct soft ionisation techniques, the atmospheric pressure ionisation with electrospray source (API–ESI) and the chemical ionisation (APCI). A single quadrupole and an ion trap, which allowed the simultaneous determination of the molecular masses and structural information of the impurities by acquisition of collisionally induced (CID) product ions spectrum and in-source fragmentation, were employed as analysers. Single quadrupole and ion trap analysers resulted perfectly matching in the d-allethrin impurity fragmentation patterns. All the main impurities over 0.1% identified by GC/MS were confirmed. Results indicate that the proposed HPLC/MS method was found appropriate to confirm the presence of impurities such as chrysolactone, chloro allethrin derivatives, allethrolone and chrysanthemic acid, excluding their formation under GC/MS strong ionisation condition.

Keywords: d-Allethrin; Impurities identification; HPLC–ESI/APCI–MS; Single quadrupole analyser; Ion trap analyser

1. Introduction

Synthetic pyrethroids account for more than 30% of the world trade in insecticides [1]. The increasing consumption and the broad application of these insecticides require reliable analytical methods for their quality control. In this view, the purity evaluation of bulk chemical is of great importance to safeguard the quality and subsequently the efficacy of the final product.

d-Allethrin (DA) (Fig. 1) represents one of the most widely used pyrethroids. Even if several methods are reported for the analysis of DA in various specimens such as milk, air and food [2–8], however, the impurity profiling of commercial DA was not object of systematic study.

The analytical profile of DA was first approached by us through the development of a GC–flame ionisation detection (FID)–MS method for purity determination and for the structural characterisation of major impurities in raw material (over 0.1% peak area/total peaks' area) [9]. Various sources of commercial batches are in fact marketed with about 90% purity.

An enantioselective HPLC method for the determination of the DA stereoisomeric composition was also published [10]. DA is in fact a mixture of esters derived from (1*R*,3*R*) *trans* and (1*R*,3*S*) *cis* chrysanthemic acid (CA) in 80/20 ratio and (*R*) and (*S*) allethrolone (AL) alcohol and the stereochemical composition is crucial for activity and toxicity.

Object of this work was to develop a suitable LC–MS method for the characterization of the structure of DA impurities under soft ionisation conditions, re-evaluating the impurity profile of DA. In fact, the strong conditions for sample evaporation, chromatographic separation and ionisation source used in GC–MS might induce chemical instability and/or formation of condensation or degradation products, reducing sensitivity. The potential analyte instability may prevent the accurate identification and determination of the real sample impurities.

A further validation for the identification of DA labile contaminants (allethrin *cis–trans* chloro derivatives) and the

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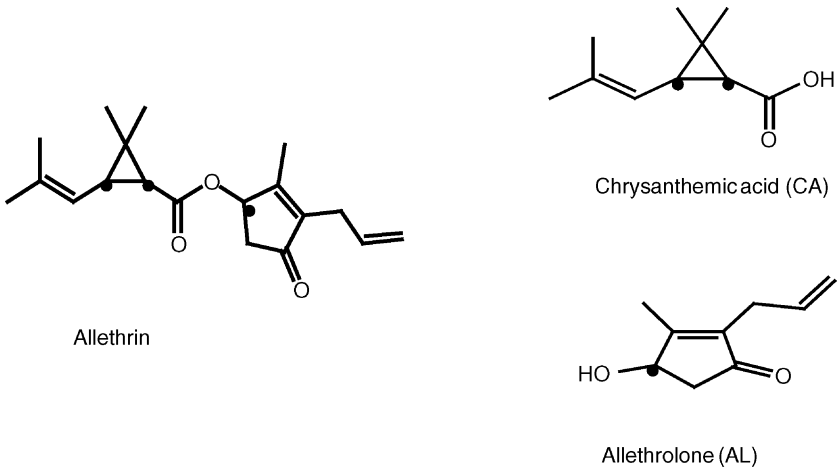


Fig. 1. Structures of: (I) allethrin; (II) chysanthemic acid; and (III) allethrolone. Stereogenic centres are bold.

elimination of the artefacts which might be formed at high temperature were therefore pursued in the present study. To accomplish this, we developed a reverse phase LC–MS analysis by gradient elution with two distinct soft ionisation techniques, the atmospheric pressure ionisation with electrospray source (API–ESI) and the athmospheric pressure chemical ionisation (APCI). Focusing on the DA impurity structure profile, two analysers were then employed: a single quadrupole and an ion trap, which allowed the simultaneous determination of the molecular masses and structural information of the impurities by acquisition of collision induced dissociation (CID) product ions scan and in-source fragmentation. Finally, the results obtained with the various techniques were compared.

2. Experimental

2.1. Chemicals and solutions

Standard d-allethrin (DA) (Fig. 1) [2,2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylic acid, 2-methyl-4-oxo-3-(2-propenyl)-2-cyclopenten-1-yl ester] was purified by flash chromatography, identified by NMR and characterized by HRGC and HPLC.

Chrysanthemic acid (CA) (Fig. 1) (2,2-dimethyl-3-(2-methyl-propenyl)-cyclopropanecarboxylic acid) stereoisomers were obtained by hydrolysis of correspondent methyl ester, commercially available from AgroChemie (Hungary) and purified by distillation. (*R*,*S*) Allethrolone (AL) (Fig. 1) (4-hydroxy-3-methyl-2-(2-propenyl)-2-cyclopenten-1-one) was synthesised as reported in literature [11,12].

Chrysolactone (4,4,7,7-tetramethyl-3-oxabicyclo[4.1.0] heptan-2-one) was prepared as previously reported [13].

Allethrine chloro derivatives, such as 2,2-dimethyl-3-(2-chloro-2-methyl-propyl)-cyclopropanecarboxylic acid, 2-methyl-4-oxo-3-(2-propenyl)-2-cyclopenten-1-yl ester, were synthesized from chloro CA [14] by esterification with the corresponding alcohol.

Sodium hydroxide was from Fluka (Milan, Italy), hydrochloric acid (1 N) was from Riedel-de Haën (Hanover, Germany).

The organic solvents for chromatographic separations were of HPLC grade from Romil Pure Chemistry (Cambridge, UK). Formic acid was from Sigma (Milan, Italy). Purified water from a TKA ROS 300 system (Millipore, Ireland) was used to prepare buffers and standard solutions.

2.1.1. Solutions

Standard solutions of DA (0.3 mg/mL), AL (0.15 mg/ml), chrysolactone (0.01 mg/mL), CA and DA chloroderivatives (0.13 mg/mL) and sample solutions of DA as raw material (0.5 mg/mL) were prepared by dissolving the appropriate weight of the pure reference standards in acidified (0.5% formic acid) mixture methanol/water 1:1.

2.2. Apparatus and chromatographic conditions

LC–MS analysis were performed using a Jasco PU-1585 liquid chromatograph (Jasco Corporation, Tokyo, Japan) interfaced with a Finnigan LCQ-Duo mass detector (ThermoFinnigan, San Jose, CA, USA), equipped with an ESI source and an ion trap analyser. The ESI system employed a 4.5 kV spray voltage (positive polarity), a capillary temperature of 220 °C and a capillary voltage of 3.0 V. The in-source fragmentations were carried out using a normalised collisionally energy of 20–23%.

The analysis was performed also on a HPLC/MSD Agilent 1100 Series system (Agilent Technologies Inc., USA) equipped with ESI and APCI sources, with a single quadrupole analyser. The ESI conditions were as follows: fragmentor 80 V for the collisionally induced dissociation (CID), driving gas 12 mL/min, temperature 300 °C, nebulizer pressure 40 psi, capillary voltage 4.0 kV (positive polarity). The APCI conditions were as follows: fragmentor 60 V, driving gas 6.0 mL/min, temperature 300 °C, vaporizer temperature 350 °C, nebulizer pressure 50 psi, capillary voltage 3.0 kV, corona current 4 μA. Two different types of chromatographic conditions were employed when using the two-ionisation sources: system A (ESI) and system B (APCI).

System A: Reverse phase chromatographic separations were performed on a Waters XTerra^{TM} C_{18} (3 μm, 2.1 mm × 100 mm ID) column, using gradient elution from A (0.5% formic acid

in water)/B (0.5% formic acid in acetonitrile/methanol 80/20) 80/20 (v/v), to A/B 45/55 (v/v) in 10 min, to A/B 30/70 (v/v) in 70 min, at a flow rate of 0.2 ml/min. The injection volume was 5 μl.

System B: Reverse phase chromatographic separations were performed on a HP C_8 (5 μm, 4.6 mm × 250 mm ID) column, using gradient elution from A (0.4% formic acid in water)/B (0.4% formic acid in acetonitrile/methanol 80/20) 80/20 (v/v), to A/B 45/55 in 10 min, to A/B 30/70 in 70 min, at a flow rate of 1.0 ml/min; the injection volume was 20 μl.

The mass chromatograms were recorded in total ion current (TIC), within 80 and 500 *m/z*, in single ion monitoring (SIM) on the $[M+H]^+$ monoisotopic species, in MS/MS and CID mode for the impurities.

3. Results and discussion

3.1. Chromatographic conditions

In the course of the present studies, mixtures of acidic (formic acid) methanol, acetonitrile and water were used as the eluent components for the gradient RP-HPLC separation of *cis–trans* DA stereoisomers and the related impurities. A typical chromatogram of a bulk DA sample is shown in Fig. 2, where the LC–ESI–MS (Fig. 2a) and LC–APCI–MS (Fig. 2b) chromatograms obtained with single quadrupole analyser in TIC modality and positive polarity are reported.

It has to be noted that under these conditions a satisfactory resolution of DA *cis* and *trans* stereoisomers from the main impurities was obtained. The impurities and DA *cis* and *trans* stereoisomers were separated and identified by ESI ion trap and single quadrupole MS and reported in Table 1 with the relative retention times and structures. In this study, by incorporating formic acid into the eluent the soft electrospray (API–ESI) ionisation could be used for the investigation of the thermally labile parent drug and its similarly labile impurities.

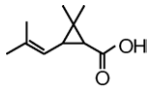
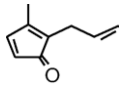
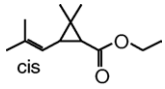
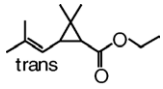
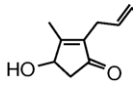
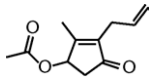
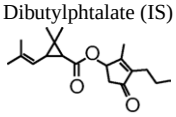
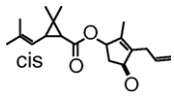
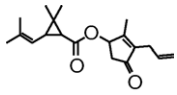
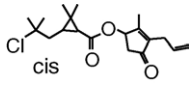
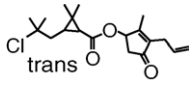
The two *cis* and *trans* allethrin diastereoisomers in 80/20 ratio were well separated using both system A and B. In system A (Fig. 2a) a microbore C18 stationary phase was used for the ESI analysis while in system B (Fig. 2b) a regular C8 stationary phase was adopted for APCI ionisation. System B was chosen in order to test the effect of a stationary phase with reduced lipophilicity on the overall chromatographic selectivity and its suitability to this application. As a result, under system B chromatographic conditions a reduced number of peaks were revealed (Fig. 2b). In particular, the *cis* chloro DA derivative showed the same retention time as *cis* DA. However, in the chromatogram obtained by using extract ion current mode (XIC) at 339 *m/z* in APCI, the *cis* chloro derivative DA was selectively identified (Fig. 3b) and by using XIC at 305 *m/z* the *cis* and *trans* allethrin derivatives with the saturated chain were selectively extracted.

3.2. Impurities determination by LC–MS

In the course of the above-mentioned GC–MS earlier studies [9], the molecular peaks of the chloro derivatives DA impurities were not visible in the mass spectrum obtained by EI and CI ionisation modes. Moreover, the confirmation of some impurities such as the chrysolactone, CA and AL resulted necessary to exclude the potential degradation of DA and/or internal condensation of CA under GC/MS experimental conditions.

Liquid-chromatography coupled to UV detection (LC–UV), because of its general application, ease of use and low level of chemical and electrical background, is still the most commonly used technique for the detection and quantitative determination of impurities. However, its lack of selectivity and the fact that it gives little or no qualitative information on the analytes

Table 1
DA impurities as identified by GC–MS, LC–MS and tandem mass analysis

GC–MS		MW	Structure	LC–MS
Peak no.	Area (%)			Peak no.
1	0.50	92		
2	1.12	168		4
		135		3
3	0.03	196		
4	0.10	196		
5	1.05	168		2
6	0.67	152		1
7	0.11	194		
8	–		Dibutylphtalate (IS)	
9	0.24	304		9
10	0.22	302	Isomer	
11	19.84	302		6
12	72.76	302		7
13	0.12		Not identified	
14	0.23	302	Isomer	
15	0.20	302	Isomer	
16	0.71	338		5
17	0.62	338		8

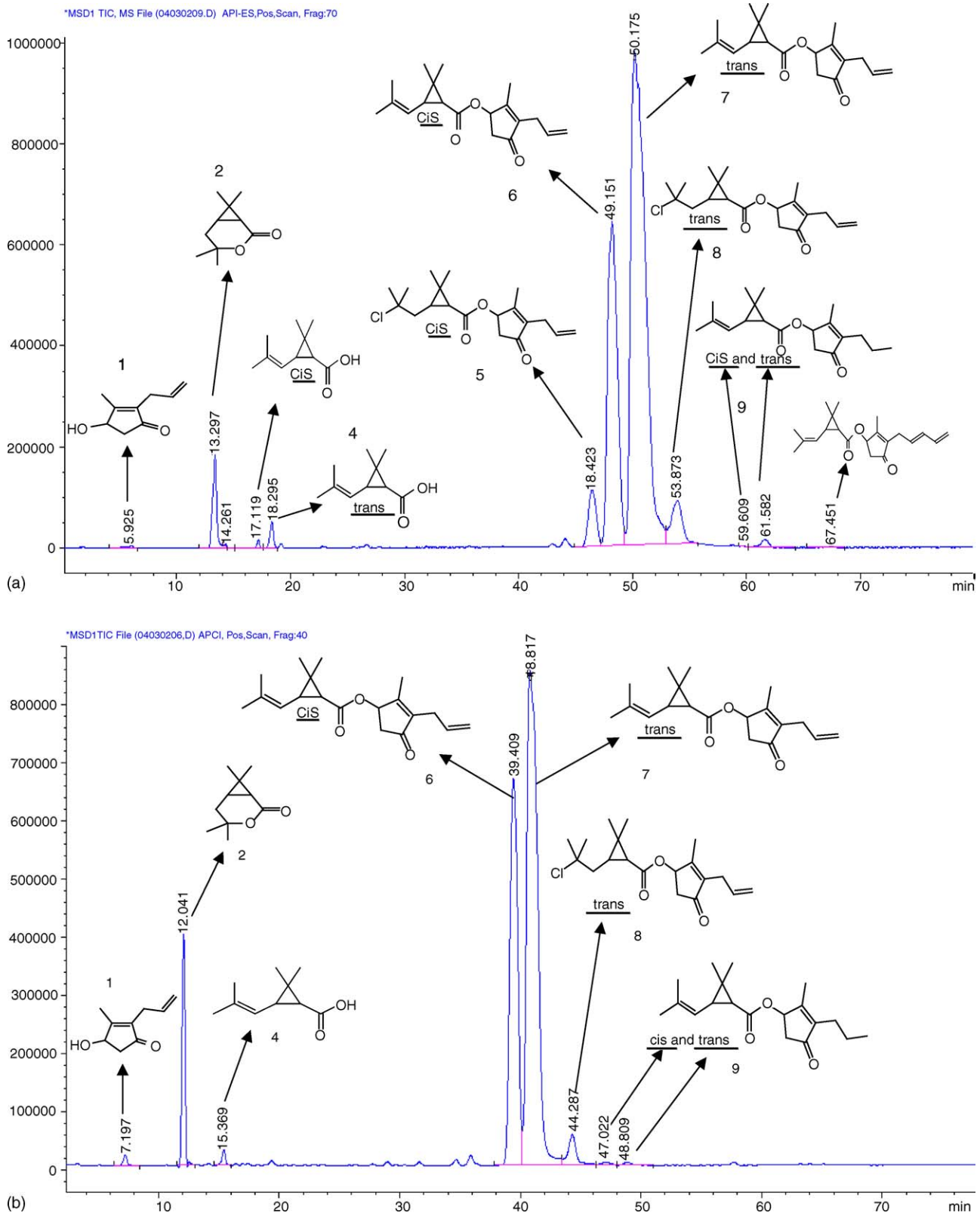


Fig. 2. (a) HPLC–ESI–MS chromatogram of d-allethrin sample acquired in TIC modality. Chromatographic conditions: System A: Waters XTerra^{TM} C_{18} (3 μm, 2.1 mm × 100 mm ID) column, gradient elution from A (0.5% formic acid in water)/B [0.5% formic acid in acetonitrile/methanol (80/20) (v/v)] 80/20 (v/v), to A/B 45/55 (v/v) in 10 min, to A/B 30/70 (v/v) in 70 min, at a flow rate of 0.2 ml/min. The injection volume was 5 μl. Single quadrupole analyser. (b) HPLC–APCI–MS chromatogram of d-allethrin acquired in TIC modality. Chromatographic conditions: System B: HP C_8 (5 μm, 4.6 mm × 250 mm ID) column, gradient elution from A (0.5% formic acid in water)/B [0.5% formic acid in acetonitrile/methanol (80/20) (v/v)] 80/20 (v/v) to A/B 45/55 in 10 min, to A/B 30/70 in 70 min, at a flow rate of 1.0 ml/min; the injection volume was 20 μl. Single quadrupole analyser.

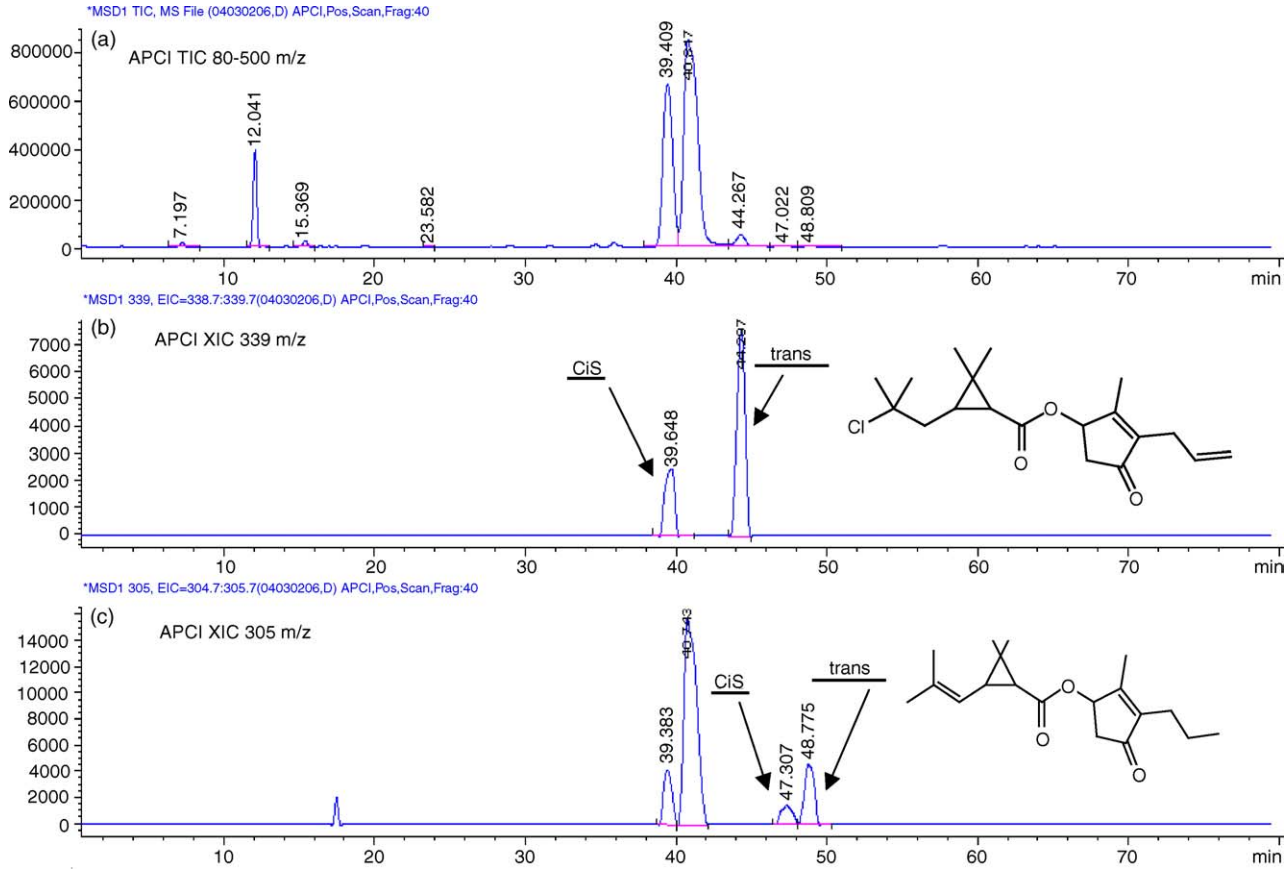


Fig. 3. HPLC–APCI–MS chromatograms of d-allethrin (a) acquired in TIC modality. Chromatographic conditions as in Fig. 2b. Extracted chromatogram at: (b) 339 *m*/*z* (XIC) and (c) 305 *m*/*z* (XIC).

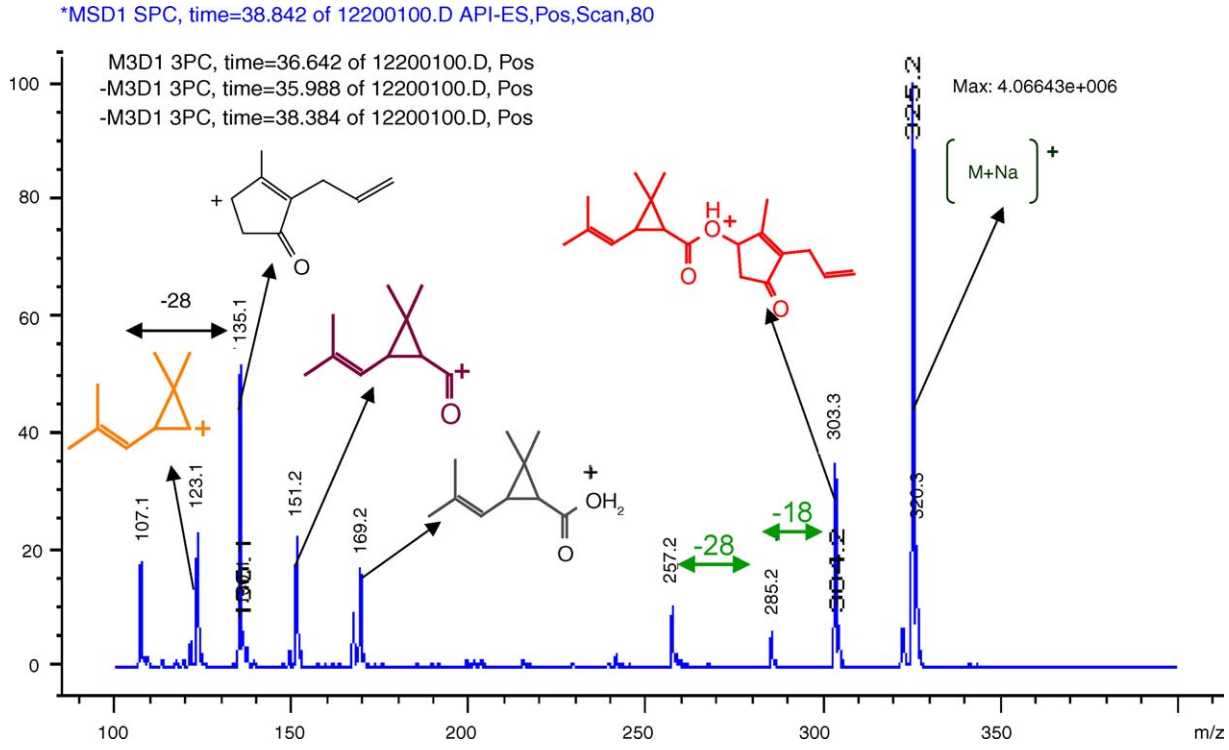


Fig. 4. CID spectrum obtained on peak nos. 6 and 7 (*cis* and *trans* d-allethrin) in chromatogram 2a and 2b.

makes it of little use for identification of unknown impurities or degradants. In addition, impurities are not always detectable by UV.

Conversely, full-scan LC–MS allows for the simultaneous detection and molecular mass determination of the analytes while tandem mass spectrometry (MS/MS) provides fragmentation mass spectra which are very useful for structural characterisation. However, the possible high level of chemical noise in the total ion chromatogram (TIC) when the mass spectrometer is used in the full-scan mode can be detrimental to the detectability of analytes present at very low level.

In order to confirm the presence of the DA impurities under soft conditions, reverse phase LC–MS analysis in gradient elution coupled to a soft (API–ESI and APCI) ionisation technique was therefore employed for the investigation of the thermally labile parent drug and its similarly labile impurities and has the advantage to operate at ambient temperature. LC–MS with ESI and APCI sources was adopted with single quadrupole and ion trap analysers in positive polarity. MS/MS parameters were optimised on the DA standard solution operating in direct infusion mode. The conditions for the measurement of precursor ions were optimised in the single MS scan mode.

Product ion spectra were recorded by scanning Q over the relevant mass range. After the determination of the product ions, the conditions for the nitrogen-collision-induced dissociation were optimised. The fragmentation behaviour of the main components separated in the chromatogram of DA samples was investigated by LC–MS/MS and a fragmentation mechanism was proposed.

Concerning HPLC–API–ESI analysis with single quadrupole analyser (Fig. 2a), in the CID mass spectrum (Fig. 4) of both *cis* and *trans* DA (peak nos. 6 and 7 in chromatogram of Fig. 2a and b), the sodium adduct m/z $[M + Na]^+ = 325$ is well detectable along with the molecular related ion $[M + H]^+ = 303$; the ion at m/z 169 derives from the cleavage of the ester bond between CA and AL and corresponds to the protonated acid, the ion at m/z 135 is the dehydrated alcohol AL $[M + H - H_2O]^+$, which is present also in the CID spectrum (Fig. 5) of allethrolone (peak no. 1; Fig. 2a), revealed as impurity in DA sample. The ions at m/z 151 and 123 derive from the ion 169 by the loss of a water molecule and the loss of the carboxy group (–COOH), respectively.

The identified impurities are reported in Table 1 with their area%, structure and molecular weight. LC–MS analysis (CID spectra are reported in Figs. 5 and 6) confirmed the structures of most impurities previously identified by GC–MS [9]. The fragmentation of the impurities eluted in the LC chromatogram proceeds similarly to that of DA. In particular, for CA (peak no. 4) CID–MS spectrum shows the same ions with m/z 169

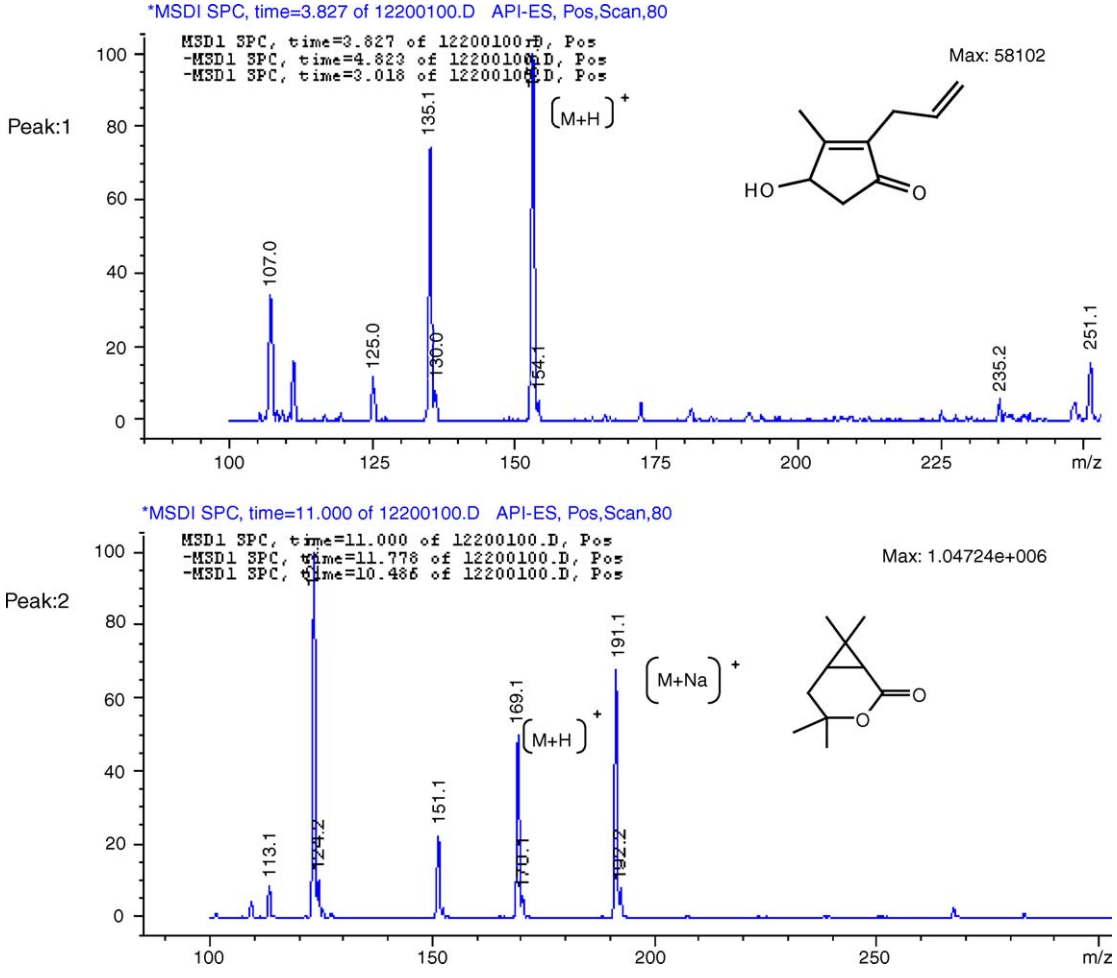


Fig. 5. CID spectra obtained on peak no. 1 (AL) and peak no. 2 (chrysolactone) in chromatogram 2a and 2b.

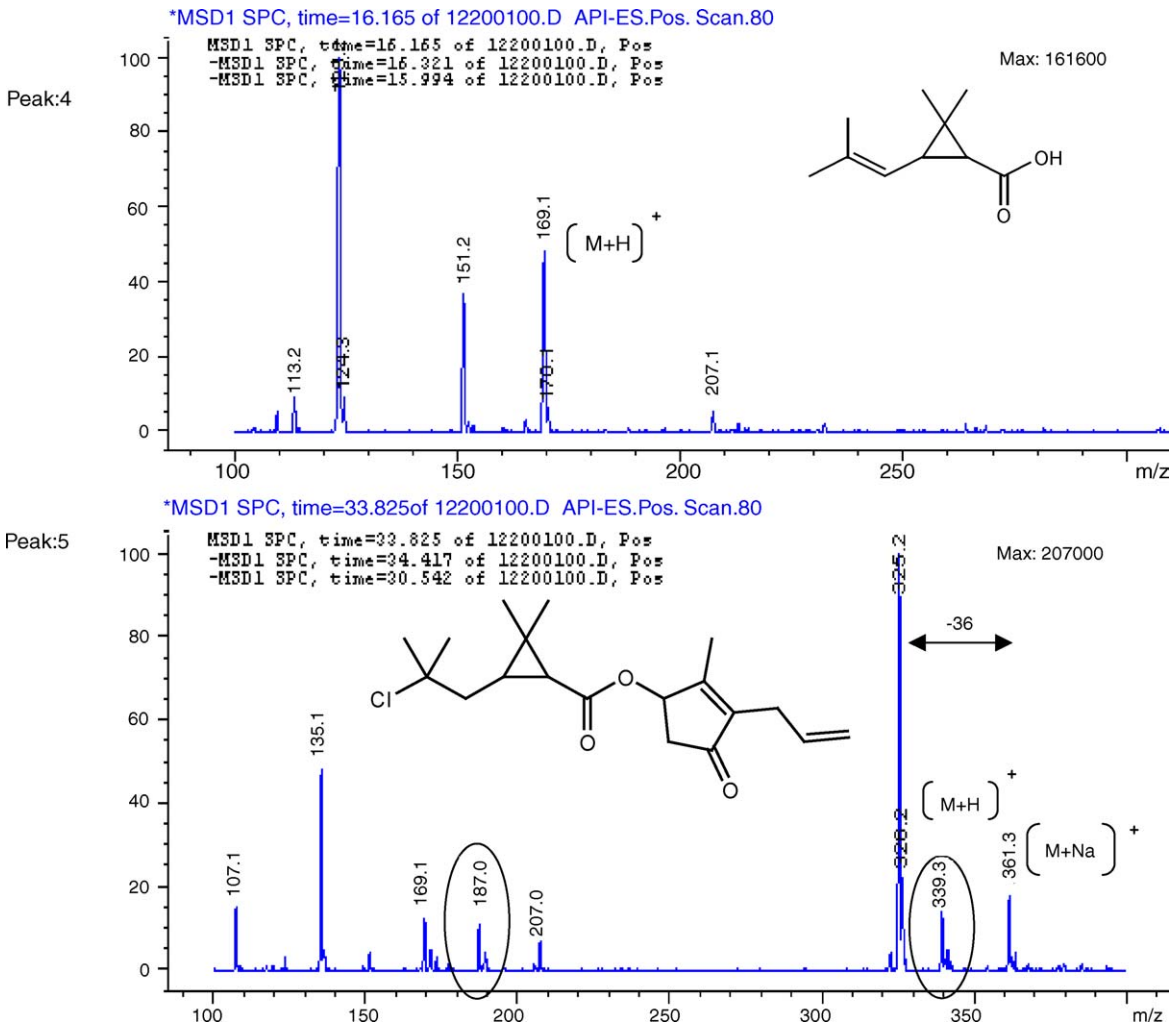


Fig. 6. CID spectra obtained on peak no. 4 (CA) and peak no. 5 (chloro allethrin derivative) in chromatogram 2a and 2b.

(protonated CA) (Fig. 6) and the ions at *m/z* 151 and 123 which derive from the ion 169 by the loss of a water molecule and the carboxylic group, respectively. The *cis* and *trans* CA were separated and identified as resolved peaks (peak no. 4 of Fig. 2a) eluting at 17 and 18 min under the API–ESI conditions by injecting the pure reference standards. In fact, the MS spectra showed the same fragmentation patterns for the two couples of diastereoisomers not distinguishing between *cis* and *trans* CA.

Peak no. 1 in the LC chromatogram (Fig. 2a) was identified as AL by the prominent molecular ion form $[M+H]^+$ at *m/z* 154 and by the ion at *m/z* 135, the dehydrated alcohol AL $[M+H-H_2O]^+$ in the CID spectrum (Fig. 5).

Therefore, allethrolone (peak no. 1) and the free chrysanthemic acid (peak no. 4) were found in the analysed sample under LC–MS reported conditions as well as by GC–MS, excluding the formation of these impurities under the GC–MS analysis conditions for DA degradation.

The chrysolactone found in the GC–MS analysis of DA samples was thought to potentially derive from an internal cyclisation of chrysantemic acid at high temperature. In the LC–APCI–MS analysis it was clearly identified as peak no. 2 by injection of the synthesised standard. Its chromatographic behaviour (Fig. 2a and b) is different from CA as its retention time is shorter (t_R = 12 min) than CA (t_R = 15 min), under the reported reversed phase gradient conditions. Conversely, the prominent ion form $[M+H]^+$ = 169 *m/z* and fragmentation pattern (Fig. 5) is very similar to CA (Fig. 6). In this case, the liquid chromatographic analysis was essential in chrysolactone identification.

The allethrin *cis–trans* chloro derivatives were properly identified only by LC–MS (peak nos. 5 and 8) because, due to their instability, the molecular peaks were not revealed in their mass spectra under GC–MS, EI and CI modes. In Fig. 6, the CID mass spectrum of the *cis–trans* chloro derivatives are reported, while the extracted ion current (XIC at 339 *m/z*) chromatogram is reported in (Fig. 3b). Further identity confirmation was obtained by direct injection of pure synthetized standards by checking the relative retention times and by overlapping mass spectra. Peak no. 5 resulted into *cis* and peak no. 8 *trans* chloro derivatives. The chlorine isotope pattern is clearly observed for $[M+H]^+$ (peak at *m/z* 339), $[M+Na]^+$ = 361 and in the *m/z* 187 (151 + 35) ion derived from fragmentation of the chloroallethrin ion that has resulted from the cleavage of the ester bond (Fig. 6).

The analogue of allethrin with the saturated chain side of the alcohol moiety was identified as peak no. 9 (Figs. 2a,b and 3c), by the prominent molecular ion form $[M+H]^+$ at *m/z* 305 and by the ion at *m*/*z* 137, the dehydrated saturated alcohol AL $[M+H-H_2O]^+$.

Concerning HPLC–API–MS analysis with ion trap analyser, the DA sample related mass chromatograms were acquired in the total ion (TIC) and single ion monitoring mode (SIM) on the $[M+H]^+$ monoisotopic species of the impurities. Impurities were further characterized by MS/MS spectra. The same molecular ion fragmentation patterns as obtained in CID mode with the single quadrupole were observed (data not shown), further confirming the DA purity profiling.

A few impurities which were found at concentration below 0.1% in GC–MS analysis were not detected by using HPLC–MS conditions (Table 1), however such a low sensitivity is not required.

4. Conclusion

DA commercial samples were analysed by reverse phase gradient elution LC/MS with API–ESI source and APCI adopting both single quadrupole and ion trap analysers in positive polarity in order to validate the impurity profiling obtained by GC–MS.

Almost all important peaks in the DA mass chromatogram with area over 0.1% were identified and are mechanistically explainable as they are inherently related to molecular structure. Abundant ions are associated with fragmentation processes that are energetically favored. The important diagnostic indicators are a large portion of ions that resembles the DA breakage into AL and CA and the facile loss of water and carboxylic acid moiety. The ions displaying a chlorine isotope pattern could be readily used to highlight the presence of chloro in chloro DA derivatives, which can be only preliminarily suggested by GC–MS analysis. Furthermore, single quadrupole and ion trap analysers resulted perfectly matching in the DA impurity fragmentation patterns. Hence, each analyser can be independently used for the reliable impurity profiling of DA. Therefore, the HPLC intrinsic selectivity associated to the soft ionisation conditions coupled to the MS structure identification resulted an optimal instrumental combination for an unambiguous DA impurity identification. However, the lower sensitivity achieved by the proposed HPLC–MS methods, under the conditions employed, suggests to adopt the combined GC–MS and HPLC–MS analyses in order to complete the impurity profiling of DA. This combined methodological approach offers the opportunity of an effective quality control of commercial batches of DA from different sources.

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