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Advantages of Atmospheric Pressure Chemical Ionization in Gas Chromatography Tandem Mass Spectrometry: Pyrethroid Insecticides as a Case Study

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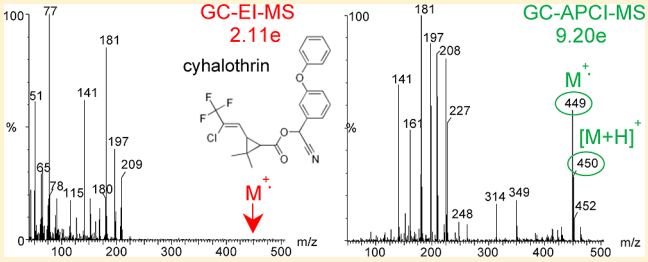
Supporting Information

ABSTRACT: Gas chromatography coupled to mass spectrometry (GC/MS) has been extensively applied for determination of volatile, nonpolar, compounds in many applied fields like food safety, environment, or toxicology. The wide majority of methods reported use electron ionization (EI), which may result in extensive fragmentation of analytes compromising selectivity and sensitivity. This might also complicate the application of tandem MS due to lack of specific/abundant precursor ions. Pyrethroids are examples of compounds with this behavior. In this work, the potential of atmospheric pressure chemical ionization (APCI), a softer form of ionization, combined with GC and a triple quadrupole mass analyzer was investigated, taking pyrethroids as a case study and their determination in fruit and vegetables as example application. Ionization and fragmentation behavior of eight pyrethroids (bifenthrin, cyfluthrin, cypermethrin, permethrin, λ-cyhalothrin, fluvalinate, fenvalerate, and deltamethrin) by APCI were studied. The formation of a highly abundant (quasi) molecular ion was the main goal because of the enhanced selectivity when used as precursor ion in tandem MS. The addition of water as a modifier was tested to promote the generation of protonated molecules, resulting in notable improvement of sensitivity and selectivity for most compounds. The excellent detectability (low detection limits (LODs) <20 fg achieved) when using APCI combined with state-of-the-art tandem MS was demonstrated for real samples. Additionally, matrix effects were evaluated in terms of signal enhancement/suppression. Depending on the matrix, different degrees of suppression were observed, on average reducing the signal in matrix to 55% of that in solvent. The results presented in this paper demonstrate the potential of APCI as new source for GC/MS that could be applied to other analytical problems apart from those illustrated in this work.



Gas chromatography coupled to mass spectrometry (GC/MS) has been widely applied in the field of pesticide residue analysis. Electron ionization (EI) has been the most widely used ionization source. Typically, the molecule is highly fragmented during ionization. For many compounds, characteristic mass spectra are obtained, but in other cases, fragment ions are less specific, or fragmentation is too extensive, compromising sensitivity. Furthermore, the highly diagnostic molecular ion is often absent in EI. An example of compounds with less favorable EI are pyrethroids, which are used all over the world to control a wide range of insects in agricultural fields, in greenhouses, and in postharvesting storage.[1]

Pyrethroids have been detected in environmental, biological, and food samples, where they are generally present at low concentrations.[1−4] Although some references dealing with liquid chromatography (LC) coupled to MS have been reported,[5−7] in general, the method of analysis involves GC with electron capture detection, single quadrupole MS,[1,3,4,8,9] or tandem MS (MS/MS) using triple quadrupole (QqQ) or three-dimensional quadrupole ion trap (QIT) mass analyzers.[10−13] The use of tandem MS greatly increases selectivity and sensitivity, especially when dealing with complex matrixes, thus enabling low detection limits (LODs). The precursor ion in tandem MS preferably needs to be of relatively high mass and abundance in order to obtain a product ion mass spectrum of analytical significance and to achieve good signal-to-noise (S/N) ratio and low detection limits. In the case of pyrethroids, this is challenging due to their extensive fragmentation by EI. Softer ionization techniques in GC/MS would overcome this issue, in those applications where the extensive EI fragmentation and the absence of the molecular ion in the spectrum would be considered a disadvantage. Chemical ionization (CI) can be a more favorable alternative due to the production of protonated molecular ions and reduced fragmentation. Thus, the combination of CI and MS/MS for enhancing chromatographic and mass spectral signals has been reported.[14,15] However, several compounds (cypermethrin, cyfluthrin, and

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esfenvalerate) did not produce significant high mass ions, even under optimized methanol CI conditions and underwent significant fragmentation to give low mass ions.[14] As many pyrethroids possess one or more halogenated atoms, some researchers have used GC/NCI-MS for their determination with methane as reagent gas.[2,16,17] In general, although CI source has been available in GC instruments for a long time, it has been less used than EI. Positive or negative CI can give better selectivity than EI for several pesticides, which results in chromatograms with reduced matrix interference,[18−21] but suffer from higher signal intensity variation compared to EI. CI is rarely used in multiresidue methods, because it is not as universal as EI and requires various injections of the sample to cover a wider range of analytes.[22,23]

Atmospheric pressure chemical ionization (APCI) can be an attractive alternative to EI (less fragmentation) and CI (more universal). Although it has primarily been used to interface a mass spectrometer with LC, this ionization interface can also be applied to GC. The ionization mechanism occurring in the APCI source is of low-energy (soft), which generates spectral data typically rich in molecular or quasi-molecular ion information. In MS/MS, because of the reduced fragmentation when using APCI, the selection of the precursor ion is no longer a compromise between selectivity and sensitivity. Additionally, the use of this interface enables the combination of GC separations with a wide range of advanced mass spectrometers which initially were specifically developed for combination with LC.

Since the first developments of APCI,[24−28] GC/APCI was never fully commercialized, probably because of the high costs of the specialized instrumentation needed at that time. Nowadays, new APCI sources are commercially available that can be interfaced to both GC and LC instruments.[29,30] This adds versatility and extends analytical capabilities giving flexibility to determine volatile and semivolatile compounds of low and intermediate polarity, traditionally analyzed by dedicated vacuum GC/MS instruments. GC/APCI in combination with time-of-flight (TOF) MS has been applied to metabolic profiling.[31] Recently, preliminary results on the use of APCI in combination with QTOF MS were shown[32] to be promising. However, further research is required to have a better understanding of the possibilities of this source in different fields, e.g., environmental analysis or food safety. The aim of this work is to investigate the potential of GC coupled to a state-of-the art QqQ mass analyzer using the APCI source for the sensitive detection of pyrethroids with determination in fruit samples as example application.

EXPERIMENTAL SECTION

Reagents. See Supporting Information.

Instrumentation. *GC/(APCI)(QqQ)MS/MS.* An Agilent 7890A GC system (Palo Alto, USA) equipped with an Agilent 7683 autosampler was coupled to a triple quadrupole mass spectrometer, Xevo TQ-S (Waters Corporation, UK), using an APGC source, operating in APCI mode. The GC separation was performed using a fused silica DB-5MS capillary column, 30 m × 0.25 mm i.d., film thickness 0.25 m (J&W Scientific, USA). The oven temperature was as follows: 100 °C (1 min); 25 °C/min to 150 °C; 10 °C/min to 300 °C (3 min). Splitless injections of 1 μL using a straight empty deactivated liner from Restek were carried out at 280 °C. Helium 99.999% (Carburos Metálicos, Spain) was used as carrier gas at 2.5 mL/min.

The interface temperature was set to 310 °C using N_2 as auxiliary gas at 250 L/h, makeup gas at 320 mL/min, and cone gas at 170 L/h. The APCI corona pin was operated at 1.8 μA, and cone voltages between 5 and 40 V were selected. The ionization process occurred within an enclosed ion chamber. By placing an uncapped vial with water (modifier) in a specially designed holder located in the source door, the relative abundance of molecular ions and protonated molecules could be influenced. "Charge transfer conditions" and "proton transfer conditions" refer to the situation without and with intentional presence of water in the source, respectively. For MS/MS measurement, argon 99.995% (Carburos Metálicos, Spain) was used as collision gas at a pressure of 4.15×10^{-3} mbar in the collision cell.

GC/(EI)(QqQ)MS/MS. See Supporting Information.

RESULTS AND DISCUSSION

GC Analysis of Pyrethroids. Synthetic pyrethroids contain two or three chiral centers, making them a family of chiral pesticides with a large number of stereoisomers. In GC, some of these isomers can be separated but enantiomeric pairs are not. Thus, multiple peaks can be observed in the gas chromatogram for individual pyrethroids, corresponding to the separation of diastereoisomers.[2,33] For the quantification of pyrethroids, often, all peaks corresponding to a certain pyrethroid are integrated and summed.[2,34] This eliminates issues related to lack of information on exact diastereoisomer composition of the pyrethroid reference standards and with isomer conversion occurring during the sample preparation and/or GC analysis. This has also been done in this work for calibration curves and quantification of positive findings. However, it should be noted that in legislation the maximum residue limits may apply to certain specific isomers. Examples include *cis*-deltamethrin, λ-cyhalothrin, RS/SR fenvalerate, RR/SS fenvalerate, and τ-fluvalinate in products of plant origin in the EU.[35]

In GC/EI-MS, the helium gas flow rate used is typically 1 mL/min because this is considered optimum for GC separation but also ensures a stable vacuum. The APCI-MS instrument used in this work can handle higher helium flow rates which may be advantageous because it (i) may reduce analyte degradation or isomer conversion in the inlet (faster transfer of the analytes from the inlet to the analytical column during splitless injection), (ii) facilitates elution of the analytes at lower GC temperatures, and (iii) leads to faster elution of the analytes (shorter GC run time). On the other hand, higher flow rates may affect GC resolution. This was examined through the critical isomer pairs (e.g., τ-fluvalinate, cypermethrin). Between 1 and 5 mL/min, no dramatic differences were observed. At the temperature program applied, the optimum resolution was achieved at 2.5 mL/min and was used for further experiments with GC/APCI-MS/MS.

Background in EI MS/MS Determination of Pyrethroids: Drawbacks. As is widely known, pyrethroids present highly fragmented EI spectra, where the molecular ion is often absent and the main m/z ions correspond to small parts of the molecule, most of them common to other pyrethroids. The use of rather nonspecific fragment ions as precursor can complicate both quantification and identification processes (see Table S-1, Supporting Information).

As an example, the SRM transition commonly used for the quantification of three different pyrethroids (λ-cyhalothrin, $C_{23}H_{19}ClF_3NO_3$ (MW = 449); fenvalerate, $C_{25}H_{22}ClNO_3$

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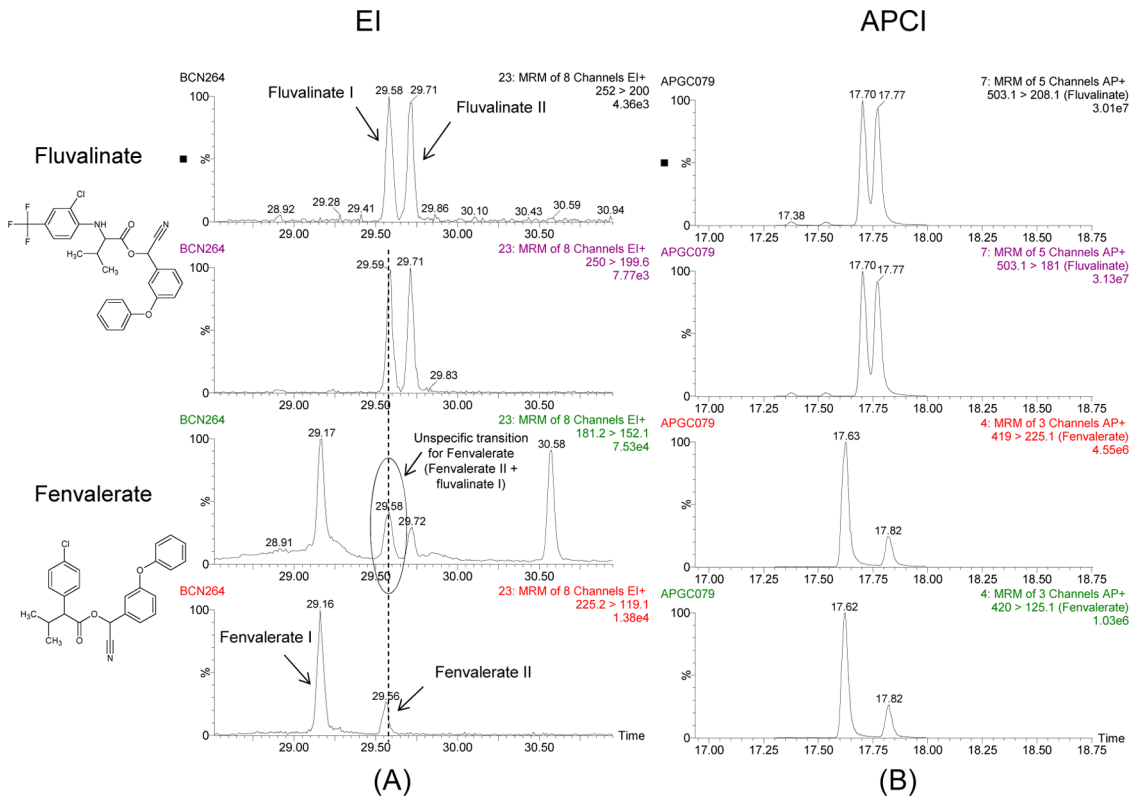

Figure 1. GC/MS/MS chromatograms for τ-fluvalinate (top) and fenvalerate (bottom) in a standard in solvent (250 ng/mL). (A) EI; (B) APCI.

(MW = 419); and deltamethrin, $C_{22}H_{19}Br_2NO_3$ (MW = 503) is exactly the same, 181($C_{13}H_9O$) > 152($C_{11}H_4O$). The selected precursor ion 181 (base peak of the three EI spectra) is a common fragment of the three pyrethroids, corresponding to the two phenyl rings with the ether linkage. This is not a big problem when enough chromatographic separation exists between the target analytes, which is the case for these three compounds. However, in the case of fenvalerate, which coelutes with the first peak of τ-fluvalinate, the above-mentioned transition 181 > 152 used for its quantification is also present in τ-fluvalinate I (Figure 1a). This can be solved by selecting another SRM transition for fenvalerate (for example, 225 > 119), but this one is less sensitive.

A worse situation occurs when analytes have both quantification and confirmation transitions in common. This is the case for a pair of pyrethroids (cyfluthrin, $C_{22}H_{18}Cl_2FNO_3$; and cypermethrin, $C_{22}H_{19}Cl_2NO_3$) where 163($C_7H_9Cl_2$) > 91(C_7H_7) and 163 > 127(C_7H_8Cl) (see Table S-1 and Figure S-1, Supporting Information). Although an alternative transition of similar sensitivity could be selected for cyfluthrin (226 > 206), identification by ion ratios can be complicated when both pyrethroids are present in standards or samples and chromatographically overlap.

Ionization and Fragmentation Behavior of Pyrethroids in GC/APCI-MS(/MS). The "soft" ionization behavior of the new interface was tested using standards in solvent. The pyrethroids bifenthrin, permethrin, and fenvalerate, for which the molecular ion ($M^{+\bullet}$) is absent in the EI spectrum, showed a peak corresponding to $M^{+\bullet}$ under APCI ionization. In the case of fenvalerate, the molecular ion even became the base peak of the mass spectrum. This behavior, when using N_2 as makeup gas, could be explained by the creation of nitrogen plasma by the corona discharge needle, N^{2+} and N^{4+}, which in the case of charge transfer reacts directly with analyte molecules.[32]

The rest of pyrethroids, λ-cyhalothrin, cyfluthrin, cypermethrin, τ-fluvalinate, and deltamethrin, showed a mixture of two quasi-molecular ions corresponding to $M^{+\bullet}$ and $[M + H]^+$. For λ-cyhalothrin, cyfluthrin, cypermethrin, and τ-fluvalinate, the intensity of $M^{+\bullet}$ was higher than $[M + H]^+$, in contrast to deltamethrin, for which the formation of $[M + H]^+$ was more favorable. An explanation for the observed protonation might be the presence of water vapor traces in the source, which readily promote the formation of the protonated molecule instead of the molecular ion.

As an illustrative example, Figure 2 shows the MS spectra for λ-cyhalothrin. As can be observed, $M^{+\bullet}$ is practically absent in the EI spectrum. However, very different results were obtained under APCI where $M^{+\bullet}$ and $[M + H]^+$ became clearly visible. Despite the softer ionization, still a number of fragments were obtained. While some fragments were observed for both EI and APCI (e.g., m/z 141, 181, 197, 209), the overall spectra were clearly different, with higher m/z ions being more abundant in the APCI spectrum.

In Table 1, the major ions obtained with APCI under charge transfer conditions are given. Ions obtained with EI are also included for comparison.

The cone voltage applied affected the ionization/fragmentation. Too low voltages could lead to poor ion extraction, but too high voltages could promote undesirable fragmentation in the source. Values between 5 and 50 V were tested in order to select the optimum value for each compound. No significant differences on in-source fragmentation pattern were observed, although voltages higher than 40 V generally led to a loss of abundance of the molecular ion and/or protonated molecule. For each compound, the optimized cone voltage that gave the

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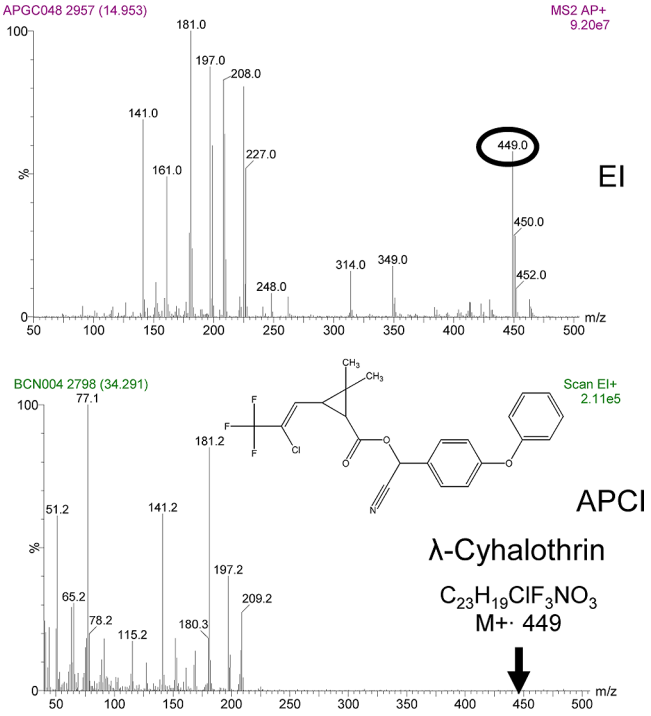

Figure 2. EI (bottom) and APCI (top) spectra for λ-cyhalothrin.

highest intensity for the quasi-molecular ion (typically between 5 and 40 V) was selected for further research (Table 2).

Finally, the fragmentation of the pyrethroids in the collision cell was studied. The quasi-molecular ion was selected as the precursor ion (Table 2), and fragmentation was performed at collision energies in the range of 5−30 eV. In the case of bifenthrin, permethrin, and fluvalinate, also certain fragment ions (see Table 2) were selected to study their fragmentation as they were more abundant than the quasi-molecular ion. Generally, product ion spectra by APCI were much richer in fragment ions than by EI where the choice of precursor ions was limited to less specific lower m/z fragment ions. This favored the selection of several SRM transitions for each pyrethroid which is beneficial for identification purposes.

As an example, Figure 3 shows EI and APCI product ion spectra at 20 eV for bifenthrin and deltamethrin. In the case of EI, the precursor ion was m/z 181 for both analytes, due to their similar EI spectra. However, in APCI, the molecular ion ($M^{+\bullet}$) of each pyrethroid could be selected as precursor ion (m/z 422 for bifenthrin and m/z 503 for deltamethrin). The SRM transitions finally selected for all the studied pyrethroids are included in Table 2. Selection of transitions for the GC/(APCI)MS/MS method was made as a compromise of sensitivity and selectivity (i.e., background noise), at the different collision energies tested.

Ionization and Fragmentation Behavior of Pyrethroids in GC/APCI-MS(/MS) Adding Water as Modifier. As explained above, for several pyrethroids, both $[M + H]^+$ and $M^{+\bullet}$ were observed in the APCI spectra. This fact encouraged us to introduce water as a modifier to promote the formation of the protonated molecule. Water was added on purpose as modifier, and the presence/absence and/or improvement on the signal of the protonated molecule was evaluated. This proton transfer behavior could be explained because the nitrogen plasma reacts with water, or any proton source, and indirectly transfers protons to the analyte.[32]

The modifier was placed in an uncapped vial, which was located within a specially designed holder placed in the source door. For permethrin and fenvalerate, that showed only $M^{+\bullet}$ under charge transfer conditions, the formation of protonated molecule was also favored. In the case of permethrin, $M^{+\bullet}$ even disappeared, concentrating the signal completely in $[M + H]^+$ ion, while for fenvalerate, the signal was undesirably divided into two quasi-molecular ions (molecular ion and protonated molecule). In the cases where a mixture of $M^{+\bullet}$ and $[M + H]^+$ was observed even under charge transfer conditions (λ-cyhalothrin, cyfluthrin, cypermethrin, fluvalinate, and deltamethrin), the use of water as modifier favored the formation of the protonated molecule, and in all cases, the $M^{+\bullet}$ ion

Table 1. Relative Abundance of Ions Obtained during EI and APCI Ionization[a]

						APCI									
		EI spectrum (m/z and relative abundance)				charge transfer conditions					proton transfer conditions				
compound	mass														
bifenthrin	422	181	165	166		181	361	**422**			181	361	362		
		(100)	(60)	(45)		(100)	(50)	(30)			(100)	(70)	(40)		
λ-cyhalothrin	449	181	197	209		181	197	208	**449**	450	227	208	197	450	
		(100)	(50)	(30)		(100)	(90)	(80)	(50)	(20)	(100)	(60)	(70)	(60)	
permethrin	390	183	165	153		183	355	**390**			183	355	373	391	
		(100)	(20)	(15)		(100)	(60)	(25)			(100)	(70)	(40)	(25)	
cyfluthrin	433	163	199	206	226	226	206	**433**	434		191	226	434		
		(100)	(40)	(60)	(20)	(100)	(75)	(50)	(30)		(100)	(85)	(50)		
cypermethrin	415	163	181	209		181	208	**415**	416		191	209	416		
		(100)	(90)	(50)		(100)	(80)	(30)	(30)		(100)	(50)	(55)		
fenvalerate	419	125	181	209	225	181	208	225	**419**		181	208	226	**419**	420
		(100)	(60)	(30)	(10)	(100)	(80)	(90)	(90)		(100)	(75)	(50)	(30)	(30)
τ-fluvalinate	502	250	181	208		250	483	**502**	503		181	208	250	503	
		(100)	(25)	(10)		(100)	(50)	(40)	(30)		(100)	(80)	(50)	(75)	
deltamethrin	503	181	209	253		253	208	279	**503**	504	279	253	504		
		(100)	(40)	(30)		(100)	(70)	(60)	(100)	(40)	(100)	(70)	(100)		

[a]EI = electron ionization (70 eV). APCI = atmospheric pressure chemical ionization. Bold value, molecular ion. Underlined value, protonated molecule.

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Table 2. Experimental Conditions of the Optimized GC/(APCI)MS/MS Method for Pyrethroids under Both, Charge and Proton Transfer Conditions

			charge transfer conditions						proton transfer conditions					
tR (min)	compounds	M	precursor ion (m/z)	cone voltage (V)	product ion (m/z)	Q/q_i ratio	dwell time (s)	collision energy (eV)	precursor ion (m/z)	cone voltage (V)	product ion (m/z)	Q/q_i ratio	dwell time (s)	collision energy (eV)
14.15	bifenthrin	422	180.9	30	115.0	1.1	0.050	30	181.0	30	115.0	1.1	0.080	30
					141.0	1.2		30			165.0	Q		30
					153.0	1.2		20			166.0	14.3		20
					165.0	Q		10						
			361.0	20	181.1	2.2	0.050	20						
					193.1	4.1		20						
			422.0	20	287.0	15.8	0.050	20						
					322.0	33		10						
					361.0	15.7		20						
14.94	λ-cyhalothrin	449	449.0	20	141.0	Q	0.027	30	450.0	40	141.0	14	0.097	20
					181.0	1.1		30			157.0	34		30
					197.1	1.0		20			161.0	211		10
					314.0	7.6		20			197.0	88		10
					414.1	5.7		20			225.1	Q		10
15.91	permethrin	390	355.0	10	113.0	1.0	0.027	10	355.0	10	319.0	Q	0.052	10
					319.0	Q		10						
			390.0	30	255.0	7.8	0.027	20	391.0	10	183.1	6.2	0.052	30
					265.0	5.05		20			243.0	18.4		10
					290.0	8.9		10			319.1	7.1		10
					339.0	9.7		20			337.0	20.3		10
					354.0	5.8		10			355.0	7.0		10
16.43	cyfluthrin	433	433.0	20	199.0	3.1	0.052	20	434.0	40	91.0	3.1	0.046	30
					206.0	Q		30			127.0	2.7		30
					227.0	1.9		10			155.0	20		30
					397.0	1.7		10			162.9	10		20
											191.0	Q		10
16.76	cypermethrin	415	415.0	30	116.0	2.6	0.052	30	416.0	10	91.0	3.3	0.046	30
					181.0	1.7		30			127.0	2.9		30
					209.1	Q		10			155.0	20		30
					379.0	1.8		10			162.9	11		20
					388.0	27		10			191.0	Q		10
17.79	fenvalerate	419	419.0	30	125.0	1.2	0.052	20	419.0	40	125.0	6.4	0.030	10
					139.0	18		20			139.1	15.4		20
					147.0	195		10			152.0	13.3		20
					225.0	Q		10			225.0	Q		10
									420.0	10	125.0	6.1	0.030	10
											139.1	11.4		20
											226.1	3.3		10
17.68	τ-fluvalinate	502	483.0	20	181.1	2.5	0.052	20	503.0	40	181.1	Q	0.030	30
					208.1	7.3		20			208.0	1.1		10
					216.1	22		20			250.0	22		20
					231.1	6.8		20						
			502.0	20	250.0	Q	0.052	5						
18.34	deltamethrin	503	503.0	40	181.0	13	0.042	30	504.0	10	171.1	7.6	0.080	20
					344.1	15		20			199.9	7.5		30
					397.0	10		10			226.0	65		20
					424.0	9.1		10			250.9	11		20
			504.0	5	171.0	3.5	0.042	20			278.8	Q		10
					250.8	5.5		20						
					278.8	Q		20						

disappeared, enhancing $[M + H]^+$ formation (see Table 1, and deltamethrin example in Figure S-2, Supporting Information). In all cases, fragment ions were also observed, and no conclusions regarding the more or less fragmentation behavior could be obtained. Bifenthrin was found to be negatively affected by the addition of water as modifier. The molecular ion disappeared, but no $[M + H]^+$ was formed instead. In this case, m/z 181 was observed as base peak, similar to the APCI spectrum under charge transfer conditions and EI.

Next, the pyrethroid fragmentation in the collision cell was studied, by the selection of the protonated molecule (except for bifenthrin and permethrin for which fragments were also

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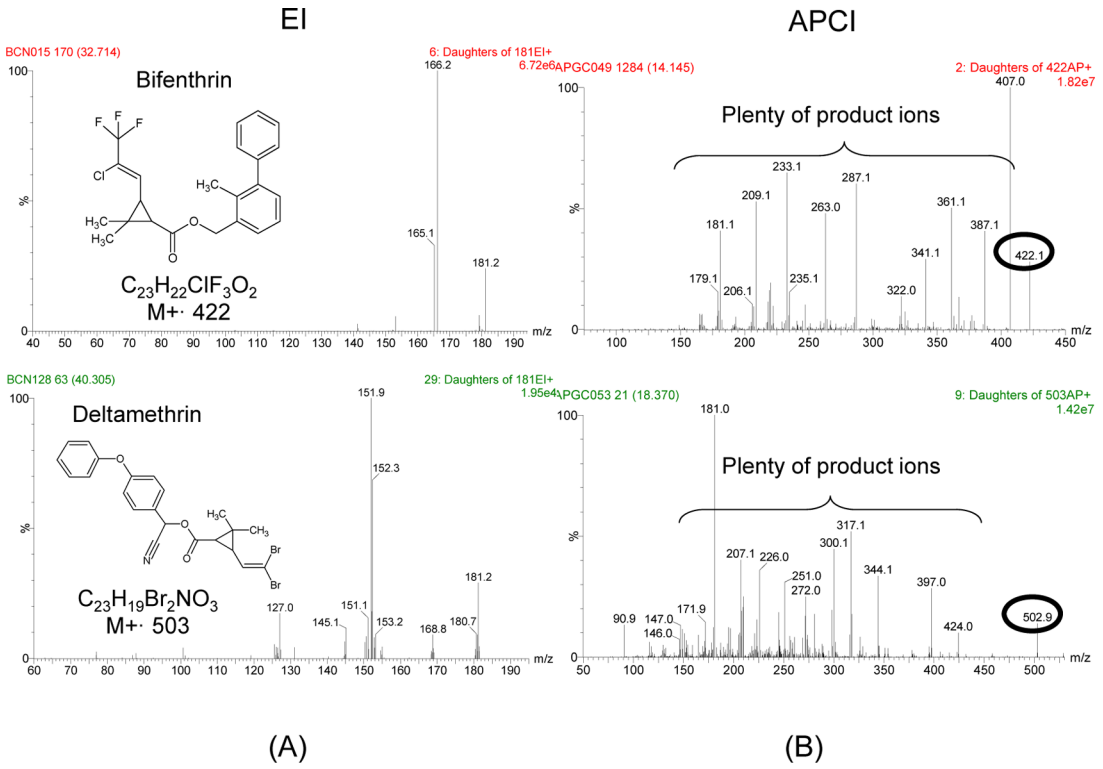

Figure 3. (A) Product ion spectra for precursor ion m/z 181 generated in the EI source for bifenthrin (top) and deltamethrin (bottom). (B) Product ion spectra for APCI precursor ions m/z 422 (bifenthrin) and 503 (deltamethrin).

selected) as the precursor ion (Table 2). Different collision energy values (between 5 and 30 eV) were tested. In general, CID spectra from $M^{+\bullet}$ and $[M + H]^+$ were different (see Figure S-3, Supporting Information). As expected, most product ions coming from the odd electron $M^{+\bullet}$ were also odd ions, and product ions coming from the even electron $[M + H]^+$ were even ions due to neutral losses in both cases.[36] The selected reaction monitoring (SRM) transitions optimized for each compound are given in Table 2.

Selectivity of the Transitions: APCI vs EI. As explained above, we showed a case where the selection of nonspecific transitions could end up with drawbacks in the identification and/or quantification processes of esfenvalerate in the presence of fluvalinate I (Figure 1a). As shown along this work, in the APCI spectra, the molecular ion or protonated molecule was rather abundant in most cases. This allows one to select the molecular ion/protonated molecule as precursor ion for tandem MS experiments. This would result in more selective SRM transitions compared to EI, where the precursor ion used to be a fragment ion, sometimes common to several pyrethroids. The selection of specific SRM transitions for fluvalinate and fenvalerate solves this problem (Figure 1b) due to the ability to select the molecular ion of each compound, improving the detection process.

These benefits are even more evident when dealing with the determination of cyfluthrin and cypermethrin. Under EI-MS/MS conditions, the transitions selected for both pyrethroids are exactly the same, and full GC separation or the use of less sensitive transitions is required for identification. On the contrary, with APCI, alternative and more specific transitions can be used (Figure S-1, Supporting Information).

Linearity, Precision, and LODs. Linearity of absolute response of analytes was established by analyzing standards solutions, in triplicate, in the range of 0.05−500 ng/mL. Under charge transfer conditions, the correlation coefficients (r) were higher than 0.99, with residuals lower than 20% for all the compounds in the following ranges: 0.05−500 ng/mL (fenvalerate), 0.1−500 ng/mL (bifenthrin), 0.5−500 ng/mL (permethrin, λ-cyhalothrin, and fluvalinate), and 5−500 ng/mL (cypermethrin, cyfluthrin, and deltamethrin). Under proton transfer conditions, the response was higher for all pyrethroids except for fenvalerate, so the linearity could be extended down to 0.1 ng/mL for deltamethrin, fluvalinate, λ-cyhalothrin, cypermethrin, cyfluthrin, and permethrin. As commented above, bifenthrin was negatively affected by the addition of water as modifier and the molecular ion disappeared. The use of fragment m/z 181 as precursor ion allowed more sensitive detection down to 0.1 ng/mL (which was the same as obtained under charge transfer conditions). Fenvalerate was the only compound that showed lower sensitivity under proton transfer conditions. Figure 4 shows the excellent detectability that can be reached by GC/APCI-MS/MS (proton transfer conditions). At 0.1 ng/mL (0.1 pg pyrethroid on-column), the S/N was still around 50 for all pyrethroids, except fenvalerate (approximately 10 times lower S/N). The repeatability of the response was tested by running standard solutions at different concentrations (5 and 25 ng/mL for experiments under charge transfer conditions, and 1 and 25 ng/mL under proton transfer conditions). In both cases, RSDs were lower than 15% (see Table S-2, Supporting Information). This shows that, although under charge transfer conditions a mixture of both molecular ion and protonated molecule occurred for some compounds, this formation was stable and reproducible.

Dwell Time vs Sensitivity and Precision. A detailed study of the effect of the dwell time on the sensitivity and precision of the method was performed. Our results showed

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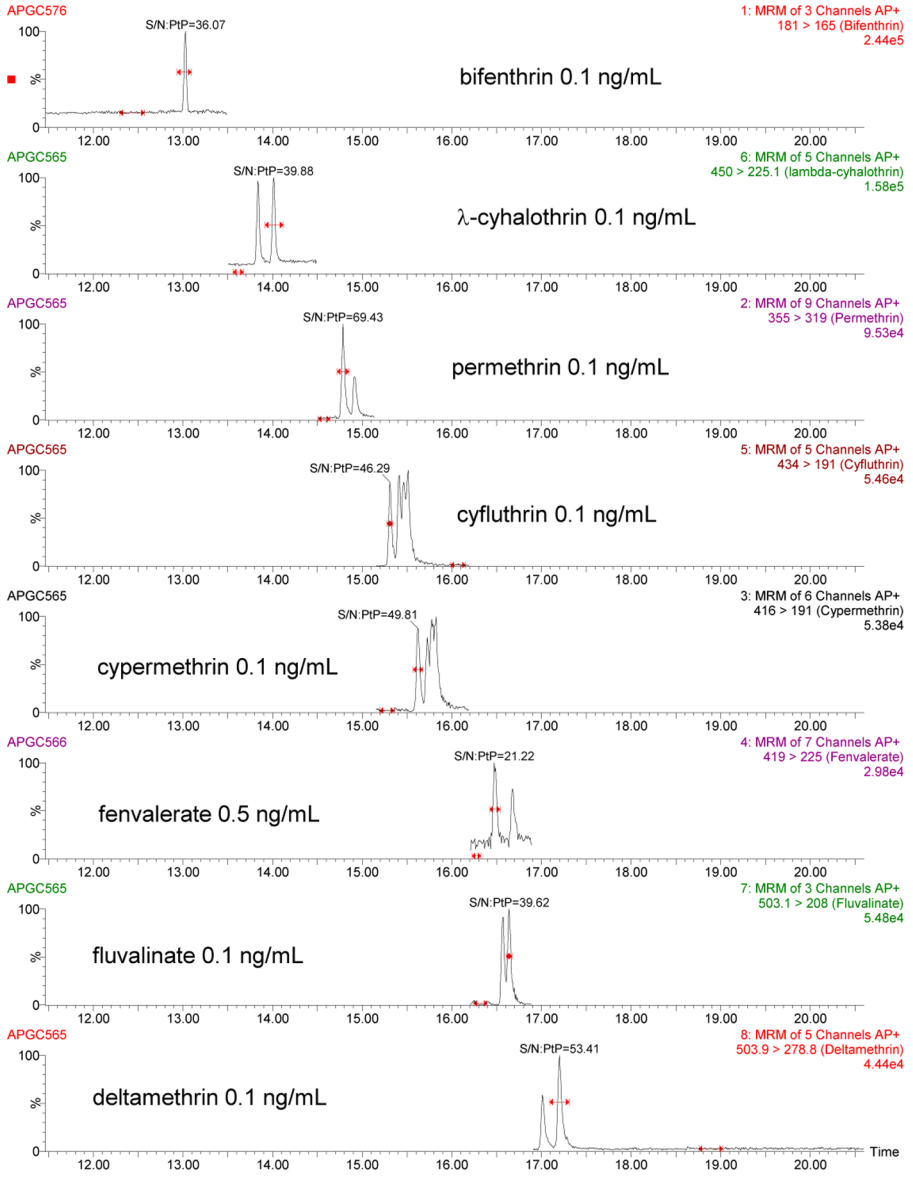

Figure 4. GC/(APCI)MS/MS chromatogram of all pyrethroids for the lowest calibration point under proton transfer conditions. S/N−PtP: peak-to-peak signal-to-noise ratio.

that, although this was an important parameter to be optimized in the past,[37] nowadays, it can be given automatically by the new triple quadrupole instruments, facilitating the work to the analyst. See Supporting Information for more details.

Improving Detection Capabilities in Real Samples and Study of the Matrix Effects. The developed methodology was applied to the determination of pyrethroids in different kind of vegetables and fruit samples. Samples that had been previously analyzed in our laboratory by GC/(EI)MS/MS[12] were run again by GC/(APCI)MS/MS. Figure 5 shows a positive finding of λ-cyhalothrin detected in nectarine that had been previously reported as a negative sample (<0.01 mg/kg) under GC/(EI)MS/MS conditions. Reanalysis by GC/(APCI)-MS/MS allowed detection at concentrations as low as 0.001 mg/kg (corresponding to 4 μg/L in the extract), clearly illustrating the improved method sensitivity for real samples.

It is well-known that atmospheric pressure ionization may be affected by matrix constituents. To gain insight in possible matrix-induced suppression (or enhancement) of the response of the pyrethroids in GC/APCI-MS/MS, sample extracts of various vegetables and fruits were spiked and analyzed, and the response was compared with that of solvent standards. For this purpose, QuEChERS extracts were prepared according to a previous work.[38] An aliquot of the acetonitrile extracts obtained were evaporated and reconstituted in toluene before GC analysis (final extract, 3 g/mL). Extracts of apple, orange, tomato, and carrot were spiked at 10 ng/mL (0.003 mg/kg in sample) and were analyzed together with their respective blanks and a reference standard mixture in solvent (10 ng/mL). From the chromatograms obtained, it was noticed that partial isomer conversion occurred (either in the extracts and/or in the GC inlet) for certain pyrethroid/matrix combinations.[2,33] This was most pronounced for deltamethrin (all matrixes; see Figure S-4, Supporting Information), but conversion was also observed for fenvalerate (carrot, tomato) and λ-cyhalothrin (mostly in orange). For evaluation of matrix effects, the sum of isomers was used. Signal suppression was observed for all matrixes, although in a different degree. The highest suppression was

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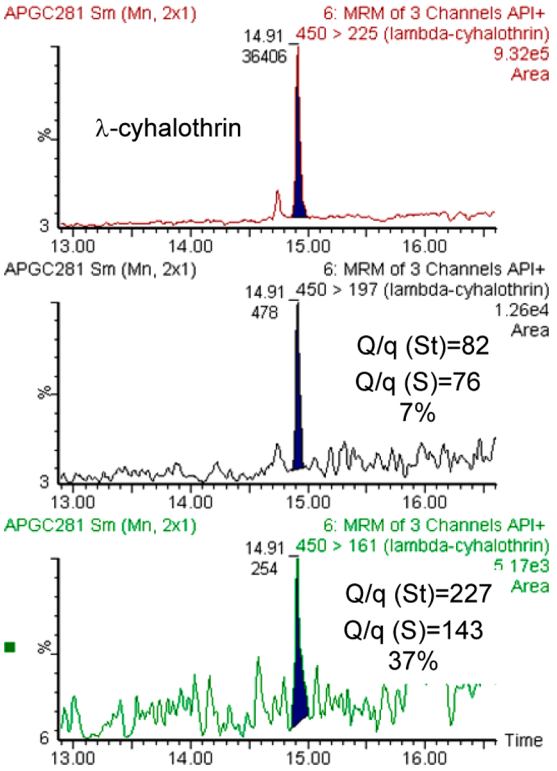

Figure 5. Positive finding of λ-cyhalothrin (~0.001 mg/kg) in a nectarine sample detected by applying GC/(APCI)MS/MS previously reported as a blank by GC/(EI)MS/MS.

observed in apple, for which the signal decreased to approximately 40% for most compounds. In general, for the different pyrethroids/matrix combinations, the response relative to the solvent standard varied between 25% and 100% (see Table 3). It should be remarked that in GC/APCI-MS/MS matrix effects may also originate from the GC inlet and that the observations here are the combined result of both effects, those derived from the injection (typically signal enhancement) and those derived from ionization in APCI (commonly suppression). The results obtained indicate that, although the signal suppression was not very pronounced in some cases, for a correct quantification of a positive finding, matrix-matched calibration should be used.

Table 3. Matrix Effects[a]

	apple	orange	tomato	carrot
bifenthrin	32	54	60	69
λ-cyhalothrin	42	97	97	100
permethrin	43	68	54	75
cyfluthrin	33	43	30	39
cypermethrin	35	49	35	44
fenvalerate	31	46	24	67
τ-fluvalinate	27	40	74	102
deltamethrin	37	66	72	90

[a]Response in matrix (%) relative to that in solvent standard.

CONCLUSIONS

The use of APCI has been evaluated as an alternative source for GC/MS/MS analysis of pyrethroids, which are highly fragmented under EI conditions. In contrast to EI, quasi-molecular ions ($M^{+\bullet}$ and/or $[M + H]^+$) were obtained. Addition of water as modifier in the source favored formation $[M + H]^+$ for all pyrethroids except fenvalerate. The quasi-molecular ions were highly favorable as precursor ion in MS/MS and strongly improved sensitivity and selectivity compared to GC/EI-MS/MS. Instrumental LODs lower than 20 fg were achieved. The response was repeatable (<15%) and linear in the range of 0.1−500 ng/mL. Matrix was found to affect ionization for certain pyrethroid/matrix combinations. Suppression up to a factor of 4 was observed in certain cases; in other cases, matrix effects were not significant. Method LODs below 0.003 mg/kg could easily be achieved in real samples. This is well below the legislative requirements and allows dilution of the final extracts which would reduce instrument maintenance needs. The results obtained convincingly demonstrate the advantages and applicability of APCI as new source for GC/MS(/MS), which is considered to be very promising for other applications apart from the examples shown in this work.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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