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Journal of Chromatography A, 1082 (2005) 71–80
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Discovering metabolites of post-harvest fungicides in citrus with liquid chromatography/time-of-flight mass spectrometry and ion trap tandem mass spectrometry

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Available online 7 April 2005

Abstract

In this study, we benefit from the combination of liquid chromatography (LC)/time-of-flight (TOF) MS accurate mass measurements to generate elemental compositions of ions and LC/ion trap multiple MS (MS^n) providing complementary structural information, which is useful for the elucidation of unknown organic compounds at trace levels in complex food extracts. We have applied this approach to investigate different citrus fruits extracts, and we have identified two post-harvest fungicides (imazalil and prochloraz), the main degradation product of imazalil ($[M+H]^+$, *m*/*z* 257) and a non-previously reported prochloraz degradation product ($[M+H]^+$, *m*/*z* 282). The database-mediated identification of the parent compounds was based on the generated elemental composition obtained from accurate mass measurements and additional qualitative information from the high resolution chlorine isotopic clusters of both the protonated molecules (imazalil, $[M+H]^+$ 297.0556, <0.1 ppm error, 2-Cl; prochloraz, $[M+H]^+$ 376.0381, 1.9 ppm error, 3-Cl) and their characteristic fragments ions (imazalil: *m*/*z* 255 and 159; prochloraz: *m*/*z* 308 and 266). The correlation between the structural information provided by ion trap MS/MS fragmentation pathways of the parent species and the TOF accurate mass elemental composition data of the degradation products were the key to elucidate the structures of the degradation products of both post-harvest fungicides. Finally, where standards were not available (prochloraz), further confirmation was obtained by synthesizing the proposed degradation product by acid hydrolysis of the parent standard and confirmation by LC/TOF-MS.

Keywords: Pesticide; Food analysis; Time-of-flight (TOF); Imazalil; Prochloraz; Degradates

1. Introduction

Post-harvest treatment of fungicides is a worldwide agricultural practice, which is used in a variety of crops (especially in fruits) aiming to avoid rotting and, thus, enlarge the lifetime of products on the market. Imazalil is one of the important post-harvest fungicides used in Europe, and its mode of action is thought to involve inhibition of demethylation in the biosynthesis of ergosterol [1]. In recent years, the established regulations regarding the maximum residue levels (MRLs) has prompted the development of more powerful analytical tools in order to provide enough sensitivity and selectivity to fulfil these requirements in complex samples of food. In this sense, liquid chromatography–mass spectrometry (LC/MS) and tandem mass spectrometry (LC/MS/MS) have become so far, the most widely used technique for the determination of these kinds of pesticides in environmental and food samples [2–9]. However, few studies have been

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doi:10.1016/j.chroma.2005.03.042

accomplished on the identification and monitoring of the main degradation products (either biotic or abiotic) of these compounds that may be also hazardous equal to their parent species [5]. For this task for exact mass and elemental composition, conventional mass spectrometers (single and triple quadrupoles) do not offer accuracy at the <5-ppm level using full-scan spectra for the identification of unpredicted, unknown species.

Liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) [10] benefits from the increased resolving power of signals on the *m*/*z* axis in comparison to quadrupole analysers, which facilitates the measurement of accurate masses of ions. LC/TOF-MS instruments provide elemental compositions of unknown peaks, routinely now, with better than 3 ppm accuracy [11], which gives the elemental composition if the studied unknown species shows characteristic isotopic patterns of chlorine or sulphur containing species (i.e. "A + 2 isotopes"). On the other hand, LC/ion trap MS is a useful research tool for the identification of unknown compounds because of its unique capabilities of conducting MS^n experiments with the aim of establishing the lineage and identity of individual product ions obtained from a selected precursor ion. LC/ion trap MS^n data have been used successfully for the identification of organic contaminants in complex environmental samples [12]. The combination of LC ion trap MS^n providing structural information from fragmentation studies and LC/TOF-MS providing accurate mass measurements to generate elemental compositions of ions represents a powerful analytical approach for the identification of organic compounds of trace levels in complex matrices. This combination was successfully applied for the identification and confirmation of pharmaceuticals residues in complex environmental samples, such as sediments [13].

In this work, we have used these techniques in complex fruit extracts with the aim to discover unknown pesticide residues and to characterize their main degradation products, especially post-harvest fungicides. In this paper we will show that it is possible to identify two post harvest fungicides and their corresponding degradation products without routine monitoring methods (i.e. no standards analyzed first). The parent compounds (prochloraz and imazalil) were detected by first finding the characteristic accurate mass isotopic profiles of chlorine-containing (molecular) ions and then determining the accurate mass elemental composition. The elemental composition is then searched against a database (Merck Index or ChemIndex) for preliminary identification. Then, LC/ion trap MS is used for fragmentation pathways of the unknown and compared with chemical structural software fragmentation [11]. The fourth and final step is standard identification [11].

The identification of the degradation products was accomplished basically by combining the information provided by LC/TOF-MS accurate mass analysis with that deduced from the fragmentation pathway of the parent compound and carried out by LC/ion trap MS^n experiments (typically MS/MS or MS^3) in positive ion mode (negative ion was not used in this study, but could easily be applied using the method outlined). The use of different databases, literature searches and polarity data deduced from the different retention times of the studied species were also helpful to provide the final confirmation of these unknown species. While the metabolite of imazalil has been reported [5,14–18], to the best of our knowledge, the proposed degradation product of prochloraz is a new finding.

2. Experimental

2.1. Chemicals and solvents

HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used for the analyses.

2.2. Chromatography

The separation of the different species from the whole fruit extracts was carried out using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1100, Agilent Technologies, Palo Alto, CA, USA) equipped with a reversed-phase C_8 analytical column of 150 mm × 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25 °C. Mobile phases A and B were water with 0.1% formic acid and acetonitrile respectively. A gradient elution was made using binary gradient of LC as follows: isocratic conditions for 5 min at 10% of solvent B, then linear gradient from 10 to 100% of solvent B, from 5 to 30 min. The flow-rate used was kept at 0.6 ml min^{-1} and 50 μl of citrus fruit extract were injected in each study.

2.3. Time-of-flight mass spectrometry

This HPLC system was interfaced to a time-of-flight mass spectrometer Agilent MSD TOF (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray interface operating in positive ion mode, using the following operation parameters: capillary voltage: 4000 V; nebulizer pressure: 40 psig; vaporizer temperature: 350 °C; drying gas: 9 L min^{-1}; gas temperature: 300 °C; fragmentor voltage: 190 V; skimmer voltage: 60 V; octapole DC 1: 37.5 V; octapole RF: 250 V LC/MS accurate mass spectra were recorded across the range 50–1000 *m*/*z*. The instrument worked providing a typical resolution of 9500 ± 500 (*m*/*z* 922.0098). The full-scan data recorded was processed with Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany) with accurate mass

application-specific additions from Agilent MSD TOF software.

2.4. LC/TOF-MS accurate mass measurements

Accurate mass measurements of each peak from the total ion chromatograms (TICs) were obtained using an automated calibrant delivery system. The instrument performed the internal mass calibration automatically, using a dual-nebulizer electrospray source with an automated calibrant delivery system, which introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution with contains the internal reference masses (*m/z* 121.0509 and 922.0098). The software is auto-calibrating and recording continuously the results of the internal reference masses along with the raw data and makes the accurate mass correction. This strategy provides enhanced accuracy in relation to previous TOF instruments, in which the mass calibration was external using as internal standard, a compound present in the sample, and has been found to be accurate to the mass of an electron [19].

2.5. Ion-trap mass spectrometry/mass spectrometry

This HPLC system was also connected to an ion-trap mass spectrometer Agilent MSD Trap (Agilent Technologies, Santa Clara, CA, USA) with an electrospray interface, equipped with an electrospray interface operating in positive ion mode. Ions were detected in ion charged control (ICC) (target: 50,000 ions) with an accumulation time of 200 ms, using the following operation parameters: capillary exit voltage (fragmentor): 50 V; capillary voltage: 4000 V; nebulizer pressure: 40 psig; drying gas: 9 L min^{-1}; gas temperature: 300 °C. An amplitude voltage of 1.2 V was typically used for fragmentation in the ion trap MS^n experiments.

2.6. Sample treatment

A fifteen-gram portion of sample previously homogenized was weighted in a 200 ml PTFE centrifuge tube. Then, 15 ml of acetonitrile were added and the tube was vigorously shaken for 1 minute. After this time, 1.5 g of NaCl and 4 g of $MgSO_4$ were added repeating then the shaking process again for 1 min. The extract then was centrifuged (3700 rpm) for 1 min. Five millilitres of the supernatant (acetonitrile phase) was then taken with a pipette and transfer to an 15-ml graduated centrifuge tube, containing 250 mg of PSA (primary-secondary amine) and 750 mg of $MgSO_4$, being then energetically shaken for 20 s. After this, the extract was centrifuged again (3700 rpm) for 1 min. Finally, an extract containing 1 g of sample per ml in 100% acetonitrile was obtained. The extract was then evaporated near to dryness and recomposed to the final composition (initial mobile phase composition). Prior to analysis, the obtained extract was filtered through a 0.2 μm PTFE filter (Millex FG, Millipore, Milford, MA, USA).

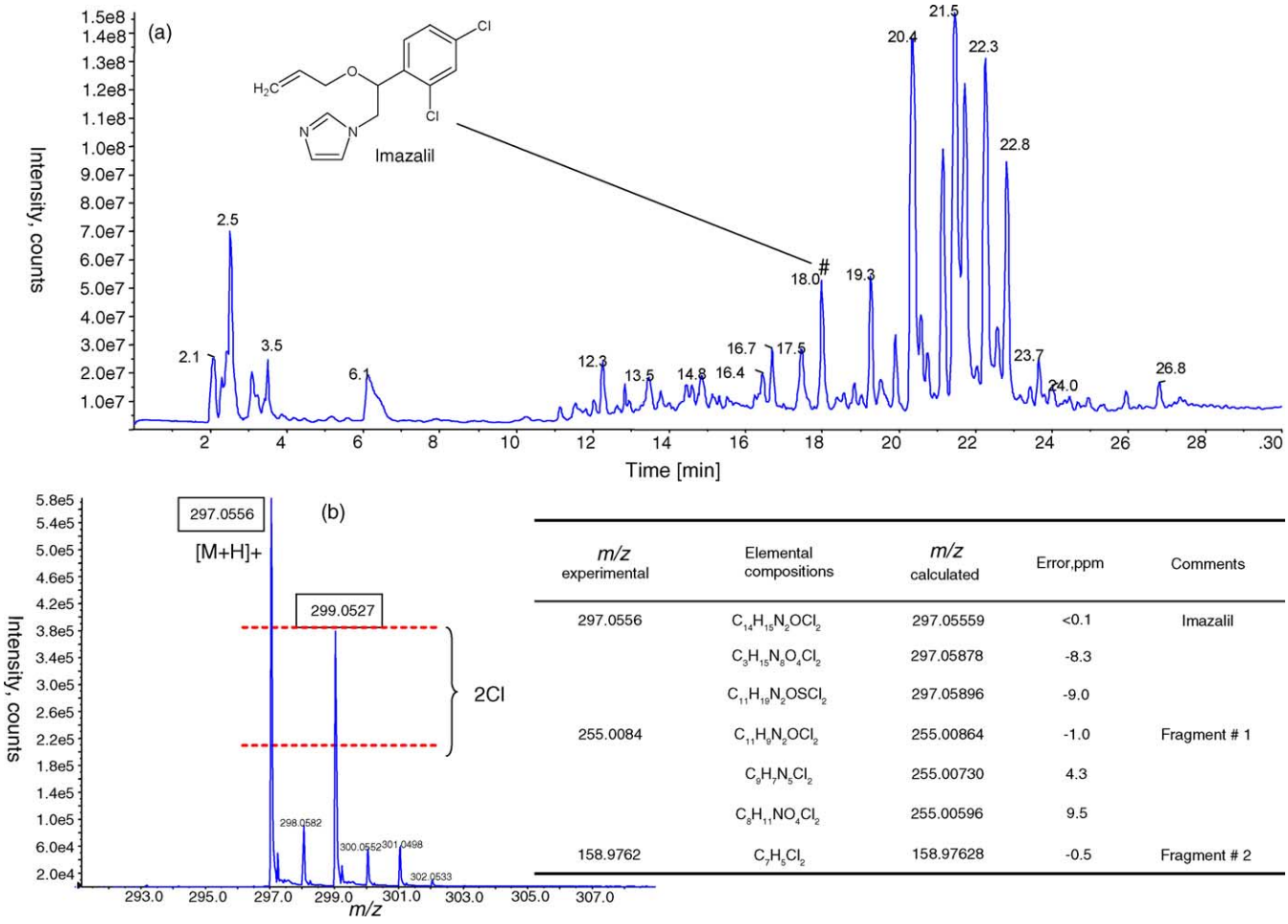
3. Results and discussion

3.1. Parent compound identification

The discovery, identification and further confirmation of the parent compounds (prochloraz and imazalil) were based on LC/TOF-MS accurate mass measurements to provide the elemental compositions of both the protonated molecules (molecular ions) and their characteristic fragment ions. Fragmentation was also checked by LC/ion trap MS to confirm the CID of the LC/TOF-MS.

3.2. Imazalil

Imazalil was identified in both citrus extracts analysed by the following technique. Fig. 1a shows the total ion chromatogram of the orange extract. The accurate mass spectrum of each peak was examined. After that, a chlorine-containing suspected species was found in this TIC at a retention time of 18.0 min. In fact, the presence and number of chlorine atoms present in the suspected species can be easily attained taking into account both the relative intensity of the $^{37}Cl/^{35}Cl$ signals and the accurate mass differences between the two masses. As can be seen in Fig. 1, the accurate mass of the *m/z* 297 peak was 297.0556 with a ^{37}Cl isotope signal of 299.0527, with a relative intensity of about two-thirds of the main peak. The mass difference between both signals is 1.9971, which is very near to the exact mass difference between ^{35}Cl (34.9689) and ^{37}Cl (36.9659) (1.9970). This evidence combined with peak area shows that the unknown unequivocally contains chlorine atom(s). The relative abundance of the isotopic signal indicates that the chlorine isotope is present with two atoms (the natural distribution of $^{35}Cl/^{37}Cl$ is slightly higher than 3:1 (75.77% ^{35}Cl; 24.23% ^{37}Cl). This fact makes much easier the assignment of an elemental composition for the suspected species. Using the calculator tool of the TOF software, we set, as a stringent criterion, the number of chlorine atoms to two. Using an accuracy error threshold of 5 ppm (standard for unknown identification), only one formula was found ($C_{14}H_{15}N_2OCl_2$, <0.1 ppm error). With an accuracy threshold of 10 ppm, two more elemental compositions matched the *m/z* input: $C_3H_{15}N_8O_4Cl_2$ (−8.3 ppm) and $C_{11}H_{19}N_2OSCl_2$ (−9.0 ppm) (see Fig. 1; Table). Sometimes the generated elemental compositions can be rejected because they are not chemically coherent (i.e. $C_3H_{15}N_8O_4Cl_2$); however, all three formulas were searched using “The Merck Index” database. It has to be taken into consideration that the additional hydrogen atom (present in the measured ions due to the positive ionization mode) must be subtracted from the elemental compositions provided by the calculator tool before entering them to the database. We found a unique match with only the first formula: imazalil.



m/z experimental	Elemental compositions	*m/z* calculated	Error,ppm	Comments
297.0556	$C_{14}H_{15}N_2OCl_2$	297.05559	<0.1	Imazalil
	$C_3H_{15}N_8O_4Cl_2$	297.05878	-8.3	
	$C_{11}H_{19}N_2OSCl_2$	297.05896	-9.0	
255.0084	$C_{11}H_9N_2OCl_2$	255.00864	-1.0	Fragment # 1
	$C_9H_7N_5Cl_2$	255.00730	4.3	
	$C_8H_{11}NO_4Cl_2$	255.00596	9.5	
158.9762	$C_7H_5Cl_2$	158.97628	-0.5	Fragment # 2

Fig. 1. (a) Total ion chromatogram (TIC) obtained from the LC/TOF-MS analysis of the studied orange extract, in which imazalil was identified (t_R 18.0 min); (b) accurate mass spectrum of the protonated molecule of imazalil; (Table: accurate mass data of imazalil and its characteristic fragment ions *m/z* 255 and 159).

This is a fungicide used for post-harvest treatment of citrus fruits.

The next step in the discovery process was to search for characteristic fragment ions of the proposed pesticide to confirm (or refute) its identity. Examining the accurate mass spectra, we found two fragment ions (*m/z* 255 and 159) with a relative abundance of respectively, 5 and 10% of that of the proposed protonated molecule. The accurate mass of fragment 1 was 255.0084, and a ^{37}Cl signal *m/z* of 257.0055. From both the relative intensity of these signals and the difference between the two masses, it can be deduced that the two chlorine atoms present in the molecular ion remain in this fragment. As can be seen in Fig. 1 (Table), the accurate mass of this fragment gave also three possible elemental compositions in the calculator tool. The first formula ($C_{11}H_9N_2OCl_2$), (−1.0 ppm error) fitted with the proposed structure, involving a loss of C_3H_6 (accurate mass loss of 42.0469 versus 42.0465 u) in relation with the proposed parent species. Moreover, the accurate mass spectrum (relative intensity and mass differences) evidenced also the presence of two chlorine atoms on fragment 2. The measured mass (*m/z* 158.9762) gave a unique elemental composition ($C_7H_5Cl_2$) which corresponds to the formation of a doubly chlorinated trophylium fragment-ion. These two fragment ions provide enough information to confirm the identity of the proposed species based on fragmentation of the parent structure. We verified this fragmentation with MS^4 using ion trap, which is shown in Fig. 4, both by standard and by looking at the suspected imazalil peak in the orange extract.

Of course, we identified imazalil by pure standard, as well. Using both LC/TOF-MS and LC/ion trap MS. For quantitation, which was approximately 1.7 mg/kg in the lemon extract and 2.1 mg/kg in the orange extract, we used LC/TOF-MS [20]. A report by Ibanez et al. [21] also uses the approach of accurate mass for unknown identification of imazalil in water samples associated with citrus production, which further states the importance of this compound in the environment. No metabolites were reported in their paper, however.

3.3. Prochloraz

Fig. 2 (inset) shows the total ion chromatogram of the lemon extract. A chlorine-containing suspected species was found in this TIC at a retention time of 22.7 min. As can

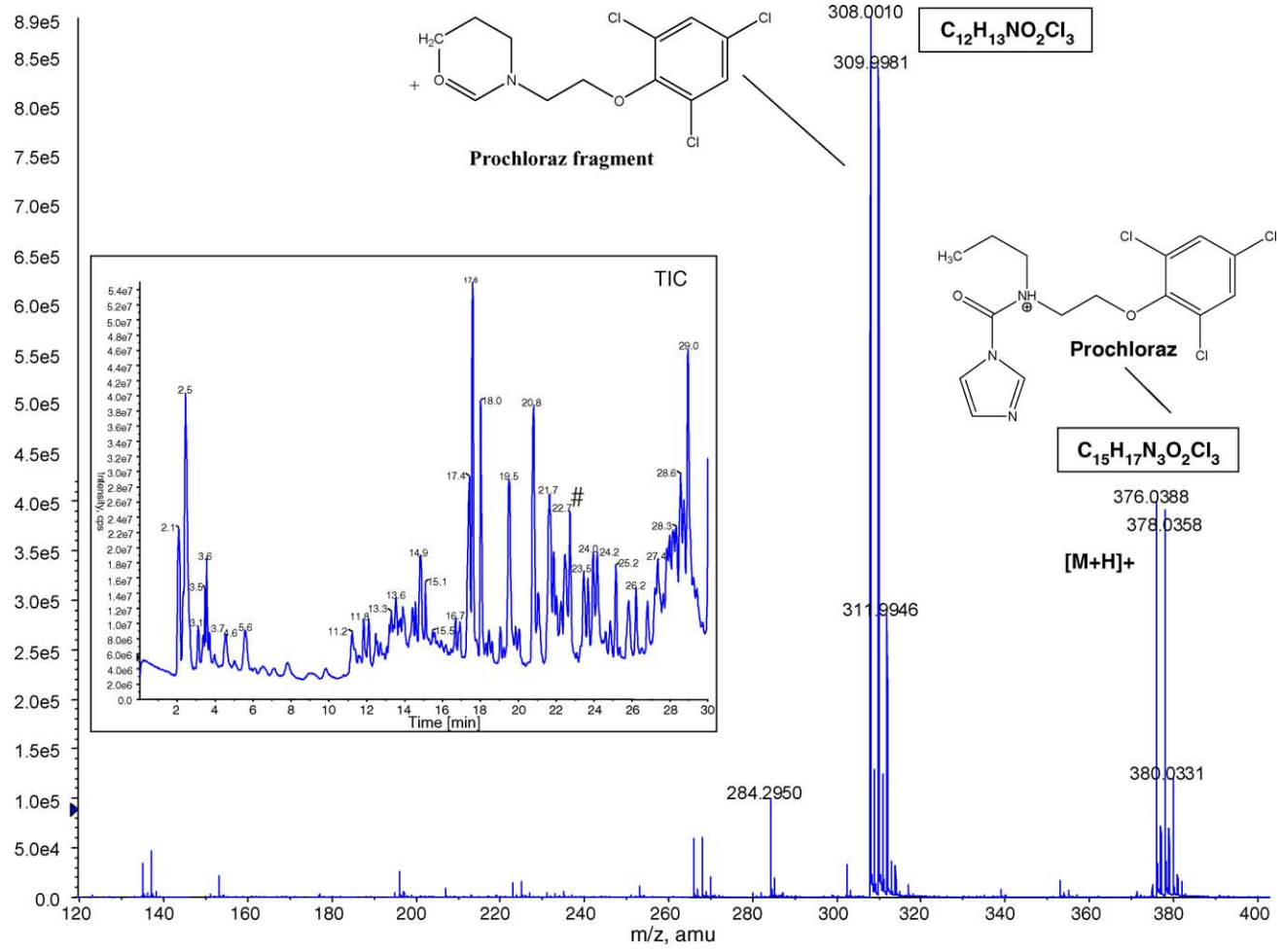


Fig. 2. Accurate mass spectrum of prochloraz, identified in the studied lemon extract at t_R 22.7 min; (inset) TIC of the lemon extract.

be seen in Fig. 2 in the accurate mass spectrum, there are two main peaks: *m/z* 376 and 308. The accurate mass of the *m/z* 376 peak was 376.0388 with a ^{37}Cl isotope signal of 378.0358, with a relative intensity of about 95% of the main peak and the difference between both signals (1.9970) evidences the presence of three chlorine atoms in the studied species. Using a large accuracy error threshold (10 ppm) and including a minimum and maximum number of chlorine atoms of three in the elemental composition calculator tool, only two elemental compositions matched the *m/z* input: $C_{15}H_{17}N_3O_2Cl_3$ and $C_8H_{17}N_9SCl_3$ (see Table 1). Using "The Merck Index" database, we found a unique match with only the second formula: prochloraz.

Examining the accurate mass spectra, we found a fragment ion with a relative intensity of two-fold of that

Table 1
LC/TOF/MS accurate mass elemental compositions of the suspected species in the lemon extract at a retention time of 22.7 min

$m/z_{experimental}$	^{37}Cl *m/z*	RA%[a]	Elemental compositions	$m/z_{calculated}$	Error (mDa)	Error (ppm)	Comments
376.0388	378.0358	44	$C_8H_{17}N_9SCl_3$	376.03877	0.026	0.07	–
			$C_{15}H_{17}N_3O_2Cl_3$	376.03808	0.7	1.9	Prochloraz
308.0010	309.9981	100	$C_{12}H_{13}NO_2Cl_3$	308.00063	0.4	1.2	Prochloraz-fragment 1
			$C_5H_{13}N_7SCl_3$	308.00132	0.7	2.2	–
			$C_9H_{17}NO_2SCl_3$	308.00401	−2.0	−6.5	–
265.9534	267.9505	< 10	$C_9H_7NO_2Cl_3$	265.95368	0.3	1.1	Prochloraz-fragment 2
			$C_7H_5N_4OCl_3$	265.95234	1.1	4.0	–
			$C_6H_9O_5Cl_3$	265.9510	2.4	9.0	–
			$C_5H_3N_7Cl_3$	265.9510	2.4	9.0	–

[a] Relative abundance.

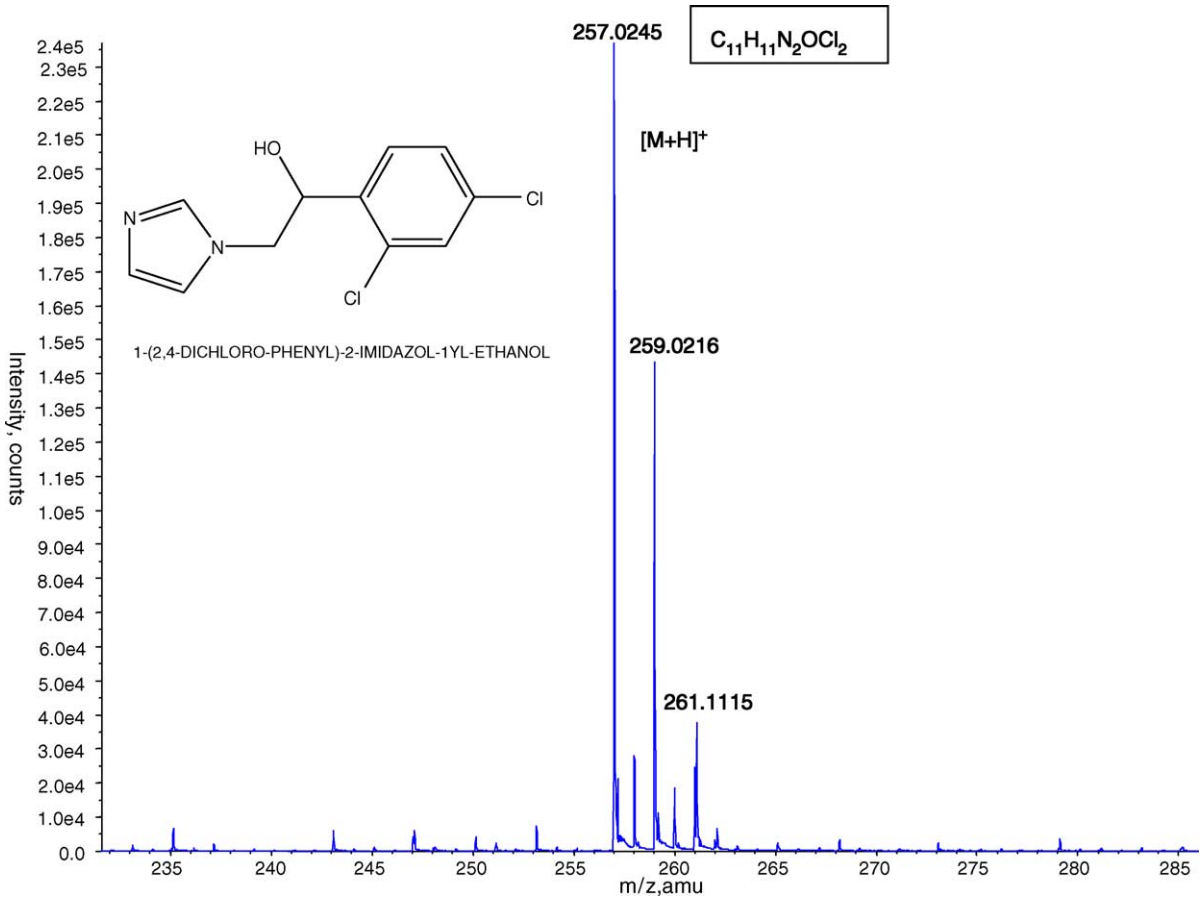


Fig. 3. Accurate mass spectrum and chemical structure of imazalil-metabolite (1-(2,4-dichloro-phenyl)-2-imidazol-1-yl-ethanol).

of the proposed molecular ion, with an accurate mass of 308.0010, and a ^{37}Cl isotopic signal of 309.9981. From both the relative intensity of these signals and the difference between the two masses, it can be deduced that all three chlorine atoms present in the molecular ion remain in this fragment. As can be seen in Table 1, the accurate mass of this fragment gave three possible elemental compositions in the calculator tool. The first formula ($C_{12}H_{13}NO_2Cl_3$, 1.2 ppm error) fitted with the proposed structure, involving a loss of $C_3H_4N_2$ in relation to the proposed species. We also found a second fragment ion with a relative abundance of about 15% of that of the proposed molecular ion, with an accurate mass of 265.9534 and ^{37}Cl isotopic signal of 267.9505. The accurate mass spectra evidenced also the presence of three chlorine atoms in this fragment. As can be seen in Table 1, the accurate mass of this fragment gave four possible elemental compositions. The first formula ($C_9H_7NO_2Cl_3$, 1.1 ppm error) also fitted with the structure of the proposed species. These two fragment ions provide enough information to confirm the identification of prochloraz. This compound was only found in the lemon extract and was later verified by standard at an approximate concentration of 1.0 mg/kg.

3.4. Discovery of degradation products

While the "parent" post-harvest fungicides were searched and identified using LC/TOF-MS, two unknown species, which showed the same isotopic patterns of the identified "parent" compounds (imazalil and prochloraz) were also found in the same extracts. The identification of these unknown degradation products were then accomplished by basically combining the information provided by LC/TOF-MS accurate mass analysis with that deduced by the fragmentation pathway of the parent compound. LC/ion trap MS experiments were used for the fragmentation pathway (typically MS/MS or MS^3). Comparing the molecular ion of the suspected degradation product with the fragment ions of the parent compound, we noticed the correspondence and a possible structure for the unknowns. Following are the discussion of the elucidation of the degradation products of imazalil and prochloraz using this method.

3.5. Imazalil-metabolite identification

In both citrus extracts, we found a peak (at a retention time of 14.6 min) with an ion with the same isotopic

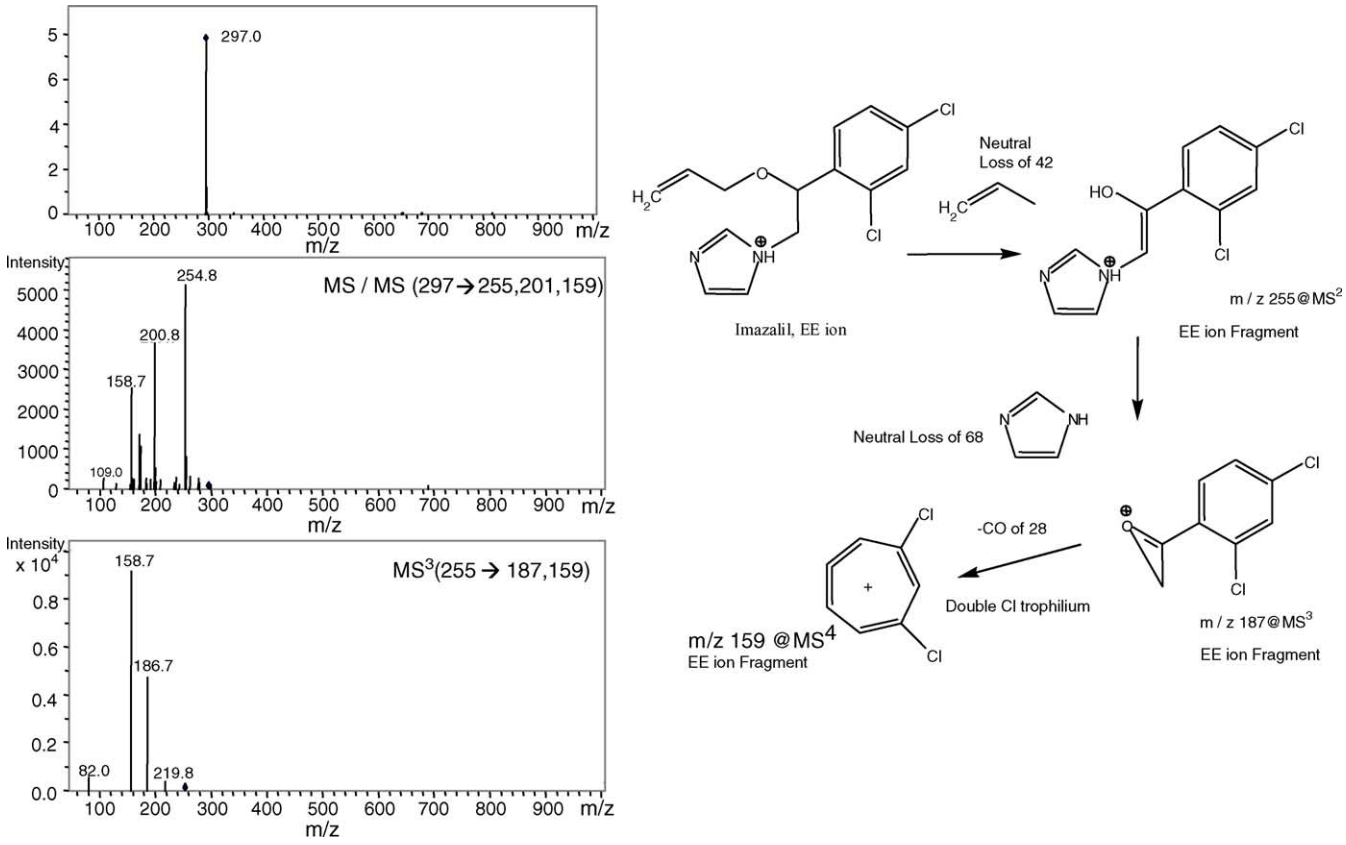


Fig. 4. Proposed fragmentation pathway of imazalil, by LC/ion trap MS^n analysis (for details, see text).

pattern of imazalil (two chlorine atoms). Taking into account that it had the same number of chlorines atoms, and it also appeared before imazalil, we considered that it could be a imazalil metabolite (metabolism often produces a more hydrophilic and less retained species). The accurate mass of the ion was 257.0245 with a ^{37}Cl signal of 259.0216 (see Fig. 3). It gave a unique elemental composition in the calculator tool: $C_{11}H_{11}N_2OCl_2$ (0.8 ppm error). For confirmation purposes, we searched for additional fragments but we did not found any characteristic fragment ion of that compound by LC/TOF-MS. The obtained elemental composition was then searched against two databases (The Merck Index and ChemIndex) with no positive results. Then, we search the elemental composition against the Sigma–Aldrich commercial electronic catalogue, and we found a unique match: "1-(2,4-dichloro-phenyl)-2-imidazol-1-yl-ethanol". The structure of this compound, shown in Fig. 3, is "compatible" with that of imazalil (see Fig. 1). This suggested that this unknown compound really was the degradation product of imazalil. Moreover, the mass and the elemental composition of this compound were also very similar to those of the fragment ion of imazalil (*m/z* 255) identified by accurate mass analysis (Fig. 4). This suggests that the degradation product is the "neutral" species corresponding to the degradation of imazalil at the same site of that the molecule that cleaves to yield the *m/z* 255 fragment.

To confirm this, we used the LC/MS ion trap to investigate the fragmentation pathways of the parent compound, which are outlined in Fig. 4. Unfortunately the signal of this compound in the sample was not intense enough for MS/MS by ion trap. The fragmentation pathway obtained in the ion trap MS/MS, consistently agreed with the LC/TOF-MS accurate mass data, providing evidence of imazalil metabolite (and parent compound too, as explained earlier). We finally made a literature search on imazalil and its degradation products, and we found data and reports that agreed with our results (Imazalil-metabolite, R14832) [5]. In fact, in the US, the sum of IMZ and IMZ-M is regulated, so the survey of residual IMZ-M is also required [5,14–18]. Finally, we confirmed the identity of the degradate by standard.

3.6. Prochloraz metabolite elucidation

In the same lemon extract where we found prochloraz, we found an ion with the same isotopic pattern (three chlorine atoms) at a retention time of 16.9 min (Fig. 5). Taking into account that it had the same number of chlorines atoms, and it also appeared before prochloraz, we considered that it could be its metabolite or degradation product. The accurate mass of the protonated molecule was 282.0218 with a ^{37}Cl signal of 284.0188. It generated a unique elemental composition using a 5 ppm accuracy threshold: $C_{11}H_{14}NOCl_3$ (1.5 ppm), which involves a loss of $C_4H_2N_2O$, in relation to

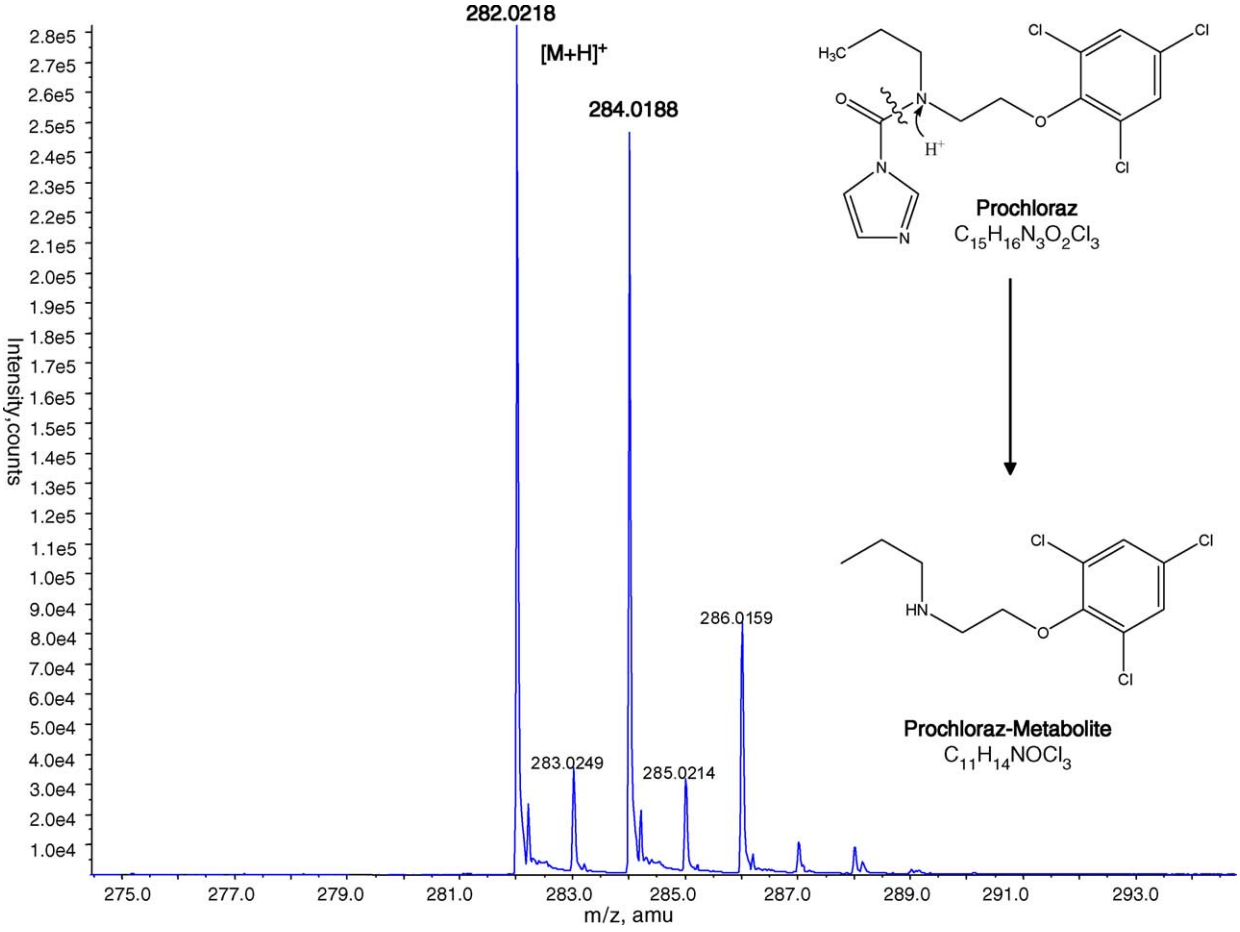


Fig. 5. Accurate mass spectrum of the proposed prochloraz degradation product, and chemical structures of both prochloraz and its metabolite.

the elemental composition from prochloraz (Fig. 5). No additional fragments ions of this species were found, in spite of increasing the fragmentor voltage to higher values in order to provide CID in-source fragmentation. The elemental composition of the suspected prochloraz degradate was matched against different databases (The Merck Index, ChemIndex and Sigma–Aldrich electronic commercial catalogue) with no positive results.

Therefore, we used the fragmentation pathway of prochloraz as a template for understanding the possible structure of the proposed unknown. For example, Fig. 6 shows the fragmentation pathway of prochloraz as determined by LC/ion trap MS. The protonated molecule looses 68 u, which is the 5 membered ring giving the *m*/*z* 308 ion, which is the major ion in the prochloraz CID spectrum by TOF. At MS^3, the *m*/*z* 308 ion fragments to a *m*/*z* 280, which is near in mass to the proposed metabolite. A reasonable degradation from this pathway is the hydrolysis of the prochloraz at the amide linkage to yield the proposed structure in Fig. 5. This structure was proposed not only on the basis of the elemental composition with a chemical structure drawing program, but also with the chemical intuition of fragmentation at the amide bond in the LC/TOF-MS!

This idea was tested quite simply by acid hydrolysis of the parent prochloraz standard by strong acid, followed by LC/TOF-MS, since no standard for prochloraz metabolite has been reported nor any standard available in the commercial catalogues. We hydrolyzed a standard solution of prochloraz, using strongly acid media at a temperature of 70–90 °C (30 min). An aliquot of the obtained extract was analysed by LC/TOF-MS, with the same chromatographic method used for the rest of experiments. We obtained a mixture of the initial parent compound (prochloraz) and the proposed metabolite. The metabolite peak (*m*/*z* 282) has the same accurate mass, elemental composition, and retention time of that which was found in the lemon extract. This provides major evidence of the proposed prochloraz metabolite. To the best of our knowledge, this is the first report which identifies and synthesizes this degradate of prochloraz. This example shows how to identify a “true unknown” by LC/TOF-MS alone. It is a true unknown in that the formula did not appear in any database nor was a standard available for confirmation. Of course, it is necessary to obtain NMR for final structural confirmation based on currently accepted protocol in organic synthesis of new standards. This we plan to do before distribution of metabolite standards to other laboratories.

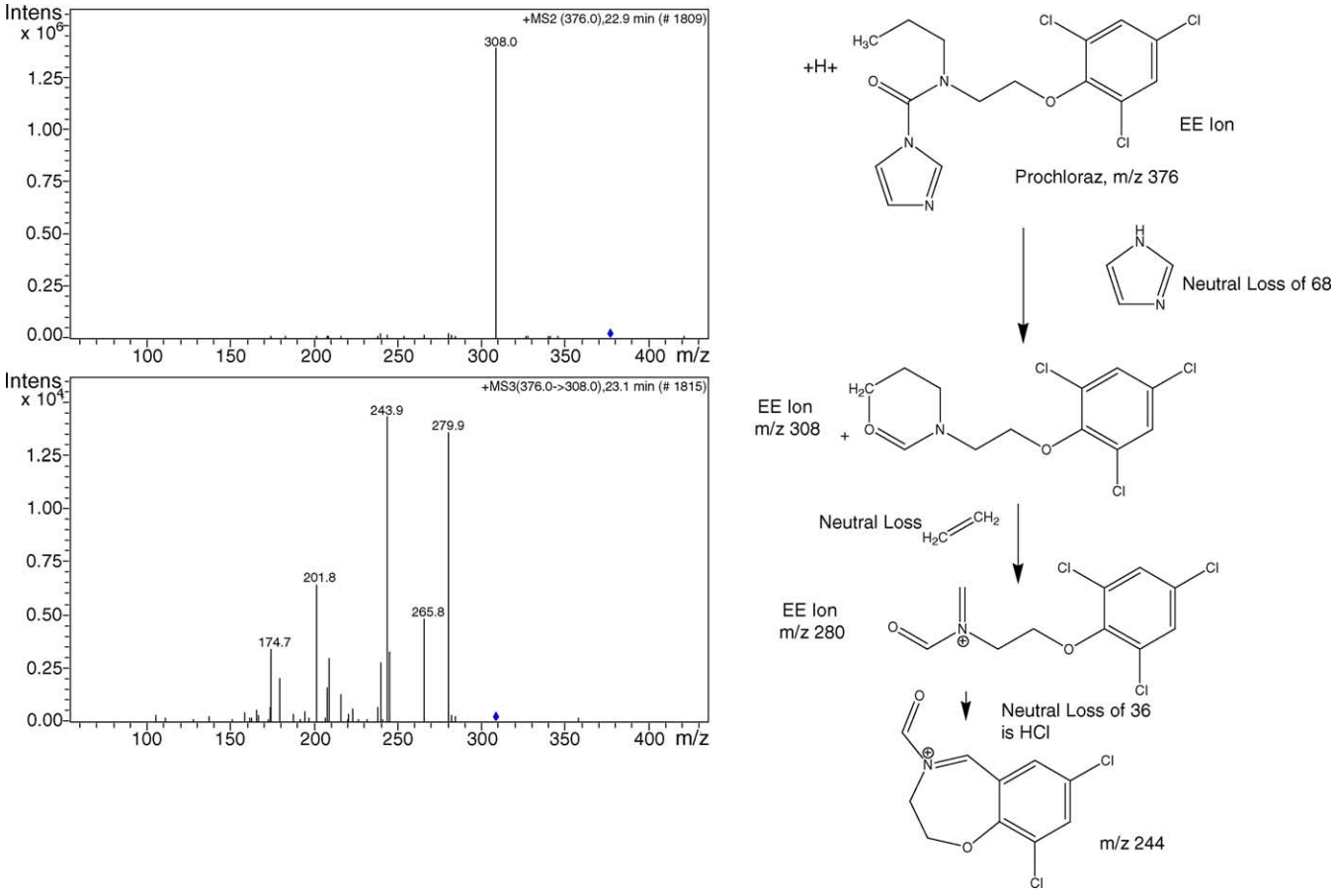


Fig. 6. Proposed fragmentation pathway of prochloraz by LC/ion trap MS^n analysis (for details, see text).

4. Conclusions

The presence of high-resolution isotopic clusters (due to i.e. Cl atoms) eases the identification of chlorine containing compounds and provides evidence for the presence and number of chlorine atoms. Since the majority of pesticides contain "A + 2" isotopes, which are frequently chlorine, this feature can be useful not only for the screening of these compounds (in the parent form) but also of the identification of their main degradation products. In this study, we have used these techniques to identify (without the initial use of standards) different post-harvest fungicides and their metabolites. In summary, the method involves accurate mass identification of "A" and "A + 2" isotopes, database searches, and MS^n pathway elucidation, followed by standard identification when possible. Where standards do not exist, synthesis may be carried out for final identification using LC/TOF-MS as a tool. Finally, it must be remembered that there may be limitations to extraction methods and ionisation of unknowns, so that this procedure is not necessarily all "encompassing" and may leave gaps, which will be studied in future work.

Acknowledgments

The authors acknowledge funding support from MEC and technical assistance from Agilent Technologies Inc. E.M.T. acknowledges funding from the Secreteria de Estado de Educacion y Universidades of the Ministerio de Educacion y Ciencia (MEC). I.F. acknowledges her contract from the "Junta de Andalucia". J.F.G.-R. also acknowledges an FPU program scholarship from MEC.

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