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Determination of Imazaquin and its Metabolite by Liquid Chromatography-Quadrupole-Time of Flight Tandem Mass

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Abstract A method consisting of solvent extraction followed by liquid chromatography-quadrupole-time of flight-tandem mass spectrometry analysis was developed for the identification of Imazaquin and its metabolite. The relationships between detector response and sample concentrations showed a high degree of linearity ($r > 0.998$) over the range 0.03–10 μg/g. The recoveries obtained were in the acceptable range of 86%–104% between spiked. The relative standard deviation of this method was 6.4%–17.1%. A 35-day study of Imazaquin degradation was taken in agricultural soil from Binzhou, China. The degradation followed first order kinetics ($C = 0.7672e^{-0.0774t}$), with half-life of less than 8.5 days. Investigation of the by-products from liquid chromatography-quadrupole-time of flight-tandem mass spectrometry has shown that there were four important metabolites 4-methylene-2-(quinolin-2-yl)-1H-imidazol-5(4H)-one, quinoline-3-carbaldehyde, 1-amino-2,3-dimethyl-1-oxobutan-2-ylium and 1H-[1,2]oxazino[4,5-b] quinolin-1-one in the degradation process. The accurate mass measurements error was 5 ppm in this study. The method was successfully applied to the analysis of imazaquin and its metabolite residues in soil.

Keywords Imazaquin · Degradation · LC-Q-TOF · Transformation products

In recent years, mass spectrometry has become a key technology for qualitative and quantitative analysis. As an efficient analytical technique, LC-tandem mass spectrometry is developing very fast these years. With powerful qualitative ability and accuracy, 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 pesticides in environmental and food samples. Time-of-flight (TOF) mass spectrometry is the simplest and fastest mass spectrometers (Yolanda et al. 2004), which is used for accurate mass measurements and the quantitation of polar pesticides. This technique is characterized by operating at a resolving power of 10,000 or more. Therefore, it gives accurate masses for both parent and fragment ions and enables the measurement of the elemental formula of a compound achieving compound identification. In addition, the combination of quadrupole-TOF permits tandem mass spectrometry, provides more structural information, and enhances selectivity (Lacorte and Fernandez-Alba 2006). This has become an important method in the pesticide residue analysis for rapid structural elucidation of unknown pesticides (Ferrer et al. 2005a, b).

Imazaquin is a member of imidazolinones class herbicide, which were designed to control a wide spectrum of broadleaf weeds, developed by the American Cyanamid Company (Gary et al. 1987). This compound has both carboxylic acid and basic quinoline and pyridine functional groups, respectively (Stougaard et al. 1990). The structure of imazaquin was showing in Fig. 1. And it is a new of low-use-rate, reduced environmental risk herbicides for the protection of a wide variety of agricultural commodities. Capillary Zone Electrophoresis (CZE) (Yi et al. 2007) and high-performance liquid chromatography (HPLC) (Gary et al. 1987; Stougaard et al. 1990; Chen et al. 2007) methods had been reported the determination of this compound. Though, CZE and HPLC were sensitive enough in determining of imazaquin, LC-Q-TOF–MS had its great

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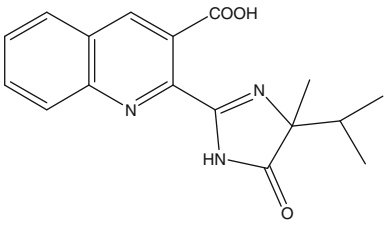

Fig. 1 Structure of Imazaquin

advantage in studying the dissipation and rapid structural elucidation of imazaquin metabolites.

The goal of this work is to study the potential of Q-TOF for pesticide residue analysis in samples in regard to quantification and confirmation of imazaquin. In addition, the capability of Q-TOF for elucidation of unknowns, such as other nontarget pesticides or transformation products, and the use of the accurate mass data provided by this instrument to establish fragmentation pathways in soil.

Materials and Methods

Imazaquin (99.9%) was purchased from Wako Pure Chemical Industries Ltd. (Osako, Janpan), stock standard solution (1,000 mg/mL) of Imazaquin was prepared in methanol. A MeCN, methanol and dichloromethane were high purity solvents for pesticide residue analysis from Supelco (Bellefonte, PA, USA), and Merck (Darmstadt, Germany), respectively, and the formic acid was HPLC grade from Sigma–Aldrich (St. Louis, MO, USA). Dehydrated $MgSO_4$ and PSA was obtained from Penta (Dikma, Beijing, China), $MgSO_4$ were heated for 7 h at 600°C in a muffle furnace to remove water and phthalates.

LC–MS/MS analyses were performed on ESI-Q-TOF mass spectrometer (Micromass Manchester, UK) connected to a HPLC system (Waters Alliance 2695 HPLC) consisted of an on-line degasser, solvent delivery module, auto-injector, column oven, photo-diodearray (PDA) detector (Waters996 PDA).

Chromatographic separation for Imazaquin and its metabolites were performed on a Luna C-18 (150 mm × 4.6 mm i.d., particle size 5 μm) column (Phenomenex, CA, USA) with the following solvent system: A = 0.1 vol.% formic acid–water, B = 0.1 vol.% formic acid–ACN. For the column, the elution was performed at a flow rate of 0.8 mL/min (splited by a valve into 0.2 mL/min before Q-TOF) using:45% B while on-line ESI–MS and MS/MS analysis.

Drying gas as well as nebulizing gas was nitrogen. The nebulizer gas flow was set to approximately 15 L/h and the desolvation gas flow to 600 L/h. A cone voltage of 30 V and a capillary voltage of 2.5 kV were used in negative ionization modes. The nitrogen desolvation temperature was set to 200°C and the source temperature to 80°C. TOF MS resolution was approximately 5,000 (fwhm). MS and MS/MS spectra were acquired over an m/z range 40–500. For MS/MS operation, collision energies of the different product ions were chosen depending on the method purpose. The MCP detector potential was set to 2,500 V. Scan times of 0.5 s/spectrum were chosen. A suitable MS profile was used. Data station operating software was MassLynx v 4.1. (Micromass, Manchester, UK).

Field trials were carried out in an experimental farmland located at Binzhou, Shandong province, China. The area of the field was 300 m^2. A random block scheme was used, with three replications for each test, and each block contained 30 m^2. The control plots were localized, tagged and separated by guard rows to avoid contamination by drift, etc. Soil was applied of 10% of imazaquin aqueous solutions (from Jilin Bada Pesticide Co., Ltd. Jilin, China) at two recommended dosages (112.5 and 150 g of active ingredient per hectare, set by the manufactory of Jilin Bada Pesticide Co., Ltd. Jilin, China).

Results and Discussion

Q-TOF micro combines the simplicity of a quadrupole [MS 1], the high ion conductance of a hexapole collision cell and the high efficiency of a TOF mass analyzer [MS 2]. Q-TOF micro exploits TOF MS to achieve the simultaneous detection of ions across the full mass range. This is in contrast to conventional instruments that must scan over one mass at a time. Q-TOF micro offers up to 100 times more sensitivity than tandem quadrupole instruments when acquiring full product ion (MS/MS) mass spectra essential to metabolism studies and peptide characterization (Junko et al. 2004). But there was few applications in studying pesticide degradation so far.

In order to detect the presence of possible pesticide product ions in real samples, the instrument was operated first in TOF–MS scan mode. Full scan experiments in positive and in negative ionization mode at different cone voltages were performed to establish the optimum cone voltage for each compound. MS/MS experiments at different collision energies were performed in order to obtain the more sensitive response for each product ion. At each collision energy tested, a product ion with both known exact mass and suitable abundance. The accurate mass obtained for each product ion was used to confirm the proposed fragmentation pathway. Additionally, relative intensity abundances were evaluated.

Guo et al. (2008) and Giuseppe et al. (1998) studied imazaquin by LC/MS in positive mode. But the spectrum obtained from Q-TOF micro in positive mode showed too

much background interferences. At the same time, there was few background interferences found in spectrum in negative ionization mode, which appears to be more sensitive and suitable technique for both the parent compound and the most of the photogenerated products was very clearly showed that $[M–H]^-$ ions was at $m/z = 310.1{,}220$ (Fig. 2). According to the above results, negative ionization was selected to study imazaquin.

A 35-day study of degradation was taken to study the mechanism of imazaquin by analyzing second hour soil samples to the 35th day samples. The relationships between detector response and sample concentrations showed a high degree of linearity ($r > 0.998$) over the range 0.03–10 μg/g. The recoveries obtained were in the acceptable range of 86%–104% between spiked. The relative standard deviation of this method was 6.4%–17.1% (Table 1). From Table 2, we studied the degradation of imazaquin in soil showing simple first-order kinetics. The degradation kinetics equation in this study was $C = 0.7672e^{-0.0774t}$, the Half-live of imazaquin was less than 8.5 days.

The study of the mechanism of imazaquin was conducted on TOF/MS. Total of five fragments were observed by MS/TOF from the soil sample. The line spectrum is shown in Fig. 3, where the fragments are labelled from a to f, while imazaquin is labelled e. The major fragments of the drug had accurate *m/z* values of 249.0971, 325.1449, 147.1667, 266.1390 and 223.0763. Using the data, the best possible molecular formula for each fragment was generated with the help of elemental composition calculator. The same are listed in Table 3. For the elucidation of structures of fragments, multi-stage mass studies (MS/MS) were carried out by taking the fragments in a sequential order. In

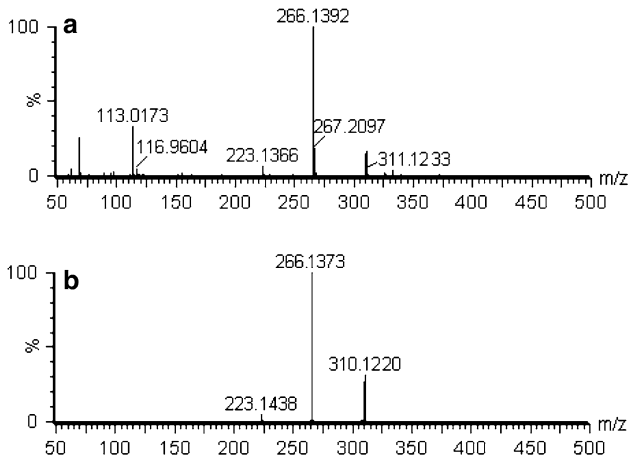

Fig. 2 **a** TOF–MS spectrum of imazaquin; **b** TOF–MS/MS spectrum of imazaquin

Table 1 The recoveries of imazaquin in soils (=5)

Spiked level (μg/g)	Recoveries (%)						RSD (%)
	1	2	3	4	5	Average	
0.03	72	75	86	109	92	87	17.1
0.5	85	82	93	94	75	86	9.2
10	98	107	96	112	105	104	6.4

Table 2 The degradation of imazaquin in soil

Sample time (*d*)	Residues in soil (mg/kg)	RSD (%)	Degradation (%)
0.08	1.2681	1.4	–
1	0.7868	1.5	38
3	0.6906	1.2	46
7	0.3832	0.8	70
14	0.2321	1.7	82
21	0.1643	0.6	87
28	0.0807	0.9	94
35	0.0616	1.3	95

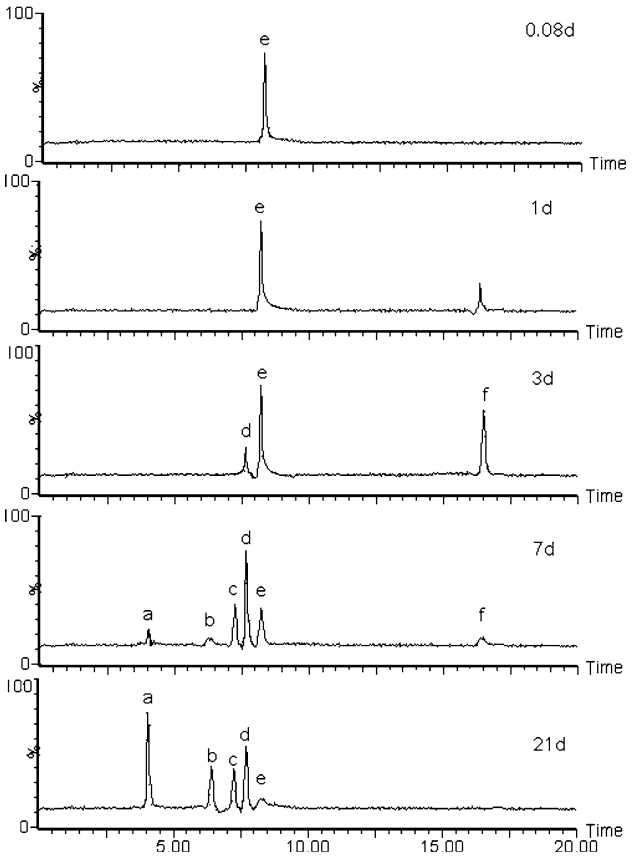

Fig. 3 The TOF–MS Tic spectrum of imazaquin degradation in 0.08, 1, 3, 7, 21 days

Table 3 Accurate mass measurement results for components found in spectrum

Peak	RT (min)	Fragment (*m/z*)	Calculated	Composition	Differences (ppm)
a	4.6	223.0763	223.0746	$C_{13}H_9N_3O$	1.7
b	6.9	249.0971	249.0980	$C_{15}H_{11}N_3O$	−0.9
c	7.3	266.1390	266.1372	$C_{16}H_{16}N_3O$	1.8
d	7.7	147.1667	294.3385	$C_{17}H_{16}N_3O_2$	−5.1
e	8.3	311.1250	311.1270	$C_{17}H_{17}N_3O_3$	−2.0
f	16.5	325.1449	325.1426	$C_{18}H_{19}N_3O_3$	2.3

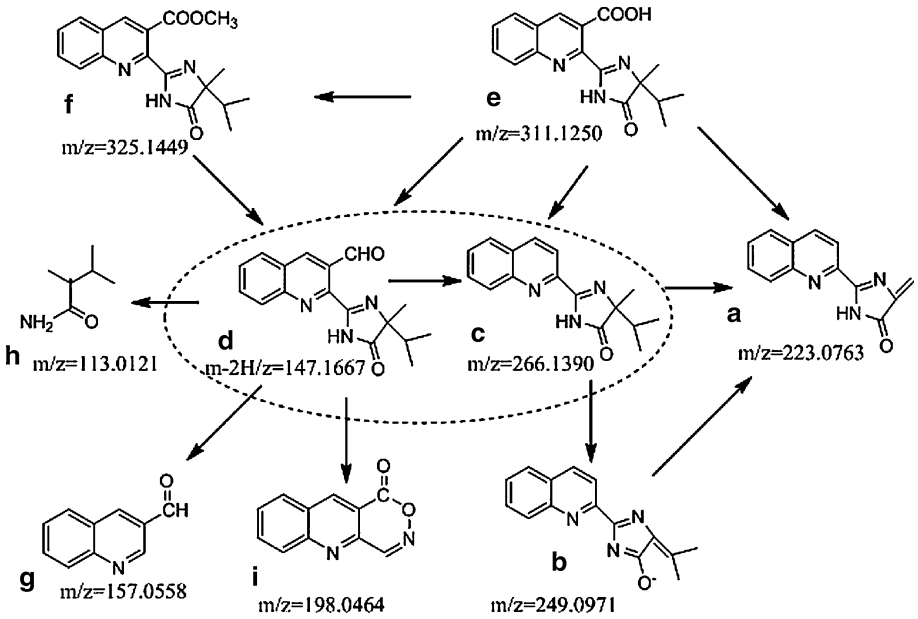

Fig. 4 Reaction pathway of imazaquin. *a* 4-methylene-2-(quinolin-2-yl)-1H-imidazol-5(4H)-one, *b* 4-(propan-2-ylidene)-2-(quinolin-2-yl)-4H-imidazol-5-olate, *c* 4-isopropyl-4-methyl-2-(quinolin-2-yl)-1H-imidazol-5(4H)-one, *d* 2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)quinoline-3-carbaldehyde, *e* imazaquin, *f* methyl 2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)quinoline-3-carboxylate, *g* quinoline-3-carbaldehyde, *h* 1-amino-2,3-dimethyl-1-oxobutan-2-ylium, *i* 1H-[1,2]oxazino[4,5-b]quinolin-1-one

MS/MS studies, *m/z* (M–H) = 311.1220 was fragmented into two major fragments (Fig. 2b).

The specification of the Q-TOF-Micro states a maximum mass error of 5 ppm for the range of masses discussed in this paper. In the 0.08th day, the "e" ion was found in TOF–MS Tic spectrum. The MS spectrum and MS/MS spectrum confirmed which would be imazaquin itself. In the first day sample, another ion "f" was detected. The MS spectrum showed that the accurate mass was 325.1449 Da, while the imazaquin was 311.1250 Da. The difference between them was only a CH_2 group. Mean while, ion "c" and "d" were found in both MS/MS spectrums of "e" and "f". Thus, it can be concluded that "f" was the esterified production of imazaquin. In the third day, "d" ion was found. The "d" was broken down into three fragments in d's MS/MS spectrum, the "g", "h" and "i". Those fragments were not found in next days study. So those fragments could not be the productions of imazaquin degradation. In the seventh and twenty-first days, ion "a", "b" and "c" were found. The MS/MS spectrum of "c" showed that the ion was broken down into "a" and "b". So the "a", "b" and "c" must be the productions of imazaquin degradation. From these information, the degradation pathway of imazaquin was drawn in Fig. 4.

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