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Identification of the bound residue composition derived from ^{14}C-labeled chlorsulfuron in soil by using LC-MS and isotope tracing method

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Abstract: A new method for extracting the bound residue (BR) derived from ^{14}C-labeled chlorsulfuron in soils was developed, and the technique of combining LC-MS with isotope tracing method was subsequently applied to identify the composition of the ^{14}C-BR in a loamy Fluvent derived from marine deposit. The results showed that the ^{14}C-[2-amino-4-methoxyl-6-methyl-1,3,5]-triazine, ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine and ^{14}C-chlorsulfuron parent compound constituted the main composition of the ^{14}C-BR derived from ^{14}C-labeled chlorsulfuron in the soil. The radioactive ratio of three compounds accounted for 39.8%, 35.4% and 17.9% of total recovered radioactivity, respectively. However, a small amount(3.6% of total recovered radioactivity) of the complex of ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine might have existed in the ^{14}C-BR in association with an unknown soil substrate. 2-chlorobenzenesulfonamide was also detected to be one of the components of the BR. The results could well explain the mechanism of phytotoxicity caused by the BR derived from chlorsulfuron in soil. In addition, the mechanism of BR formation in soil was also discussed in details.

Keywords: bound residue; chlorsulfuron; composition; identification; soil

Introduction

Chlorsulfuron, 2-chloro-N-[4-methoxy-6-methyl-1, 3, 5-triazine-2-yl] amino carbonyl benzenesulfonamide, is one of sulfonylurea herbicides with the property of high activity, broad spectrum and low toxicity to animals(Levitt G, 1981; Beyer, 1988; Brown, 1990). Its structure is expressed in Fig. 1.

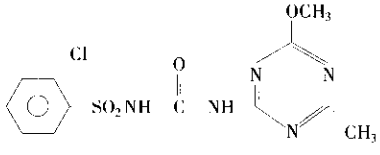

Fig. 1 Chemical structure of chlorsulfuron

Chlorsulfuron provides selective control of wild garlic and Canada thistle in small grains; broadleaf weeds in soybeans; johnsongrass, shattercane, quackgrass and wirestem muhly in corn; and weeds in conifers, hardwoods and pastures. It is used both for pre- and post-emergent application at labeled rates of 4—18.75 gAI/hm^2 (Strek, 1998). Its target enzyme is acetolactate synthase (ALS) (Sweetser, 1982; Ray, 1984; Beyer, 1988; Brown, 1990), which is the first common enzyme in the biosynthesis of branched amino acids specific to plants and microorganism. Researches on the mode of action (Ray, 1984; Beyer, 1988; Blair, 1988), degradation route(Sabadie, 1992; Hemmamda, 1994; Strek, 1998), the fate and environmental behavior (Anderson, 1985; Thirunarayanan, 1985; Fredrickson, 1986; Mersie, 1986; Beyer, 1988; Moyer, 1989; Walker, 1989; Brown, 1990) and its sensitivity(Brown, 1990; Moyer, 1990) to different crops have been well documented. While many studies have focused on the phytotoxicity of chlorsulfuron to substitution crop such as rape, rice and sugar beet (Moyer, 1990; Kotoula, 1993; Nicholls, 1998), some researchers have found that the phytotoxicity of chlorsulfuron appears to be linked to its bound residues(BR) in soil(Chen, 1996; Sun, 2000). The results by Guo *et al*.(Guo, 1998; 1999) showed that the efficiency of high-temperature distillation technique (HTD) is less than 27.8% when extracting the BR of chlorsulfuron in soil, and the structure of the BR component may be disrupted by this method, while other extracting methods such as supercritical methanol extraction technique and wet-hot extraction method can also destroy the original molecular structure of the herbicide and its degradation products(Guo, 1998; 1999). Apparently, the results based on these methods can not represent the true composition of the BR of chlorsulfuron in soil, and thus, can not be applied to explain the phenomena and mechanism of phytotoxicity caused by the BR.

The purpose of this paper was to further study the composition of BR derived from ^{14}C-labeled chlorsulfuron and mechanism of phytotoxicity caused by BR. A new method for extracting BR of ^{14}C-labeled chlorsulfuron in soil was developed, and the technique of combining LC-MS with isotope tracing method was subsequently applied to identify the composition of ^{14}C-BR derived from ^{14}C-labeled chlorsulfuron in a loamy Fluvent derived from marine deposit. The results based on the present methods could well explain the mecianism of phytotoxicity induced by BR. The mechanism of BR formation in soil was also discussed in details.

1 Materials and methods

1.1 Reagent

^{14}C-labeled chlorsulfuron(Triazine-4-^{14}C; special radioactivity, 1.8×10^{10} Bq/mol; radiochemical purity > 95%. Ye, 2002). Chlorsulfuron (purity > 98%), 2-amino-4-methoxyl-6-methyl-1,3,5-triazine(purity > 97%), 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine (purity > 97%). 0.1 mol/L Na_2CO_3-$NaHCO_3$ buffer(pH = 10.0), 0.1 mol/L citric acid-sodium citrate buffer (pH = 6.6), 0.5 mol/L KH_2PO_4, 0.5 mol/L hydrochloric acid, 0.5 mol/L sodium hydroxide, iso-propanol, n-butanol, dichloromethane, dimethylbenzene, glycol-ether, ethanolamine, 2, 5-diphenyloxazole (PPO), 1, 4-di-[2'-(5'-phenyloxazolyl)]-benzone (POPOP) and liquid scintillation cocktail (Hisafe-3, Wallac Co., Finland) etc., all the solvents above-mentioned were analytical grade. Distilled and deionized water obtained

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from a Milli-Q water purification system(Millipore Co., Milford, MA). Acetic acid, acetonitrile and methanol were HPLC grade.

1.2 Equipment

HPLC system (Waters 510, 680 and 996 etc., Waters Co., USA), solvent filtration module(Waters Co., USA), centrifugator (Universal 32, Hettich Co., Germany), decompress rotary evaporator (R-201, Institute of Shenke Machine, Shanghai, China), 4.6 × 250 mm C_{18} column(Supelco discovery C_{18}, Sigma-Aldrich Co., USA), C_{18} solid phase extraction column(10 × 150 mm, 7 μm), biological oxidizer (OX-600, Harvey Instrument Co., USA), liquid scintillation counter (Wallac 1414 LSC, Wallac Co., Finland), LC-MS(LC, Waters 2690 separation module; MS, Micromass Quattro LC, Waters Co., USA), and so on.

1.3 Incubation method for bound residues of ^{14}C-labeled chlorsulfuron in soil

Samples from seven kinds of soils and passed through a 1 mm sieve were used in the experiment. The basic properties of the seven soils are listed in Table 1. 100 g of each air-dried soil was put into 250 ml flask with a rubber plug, 5.2×10^4 Bq of ^{14}C-labeled chlorsulfuron dissolved in methanol was added into each soil sample, and stirred in a vent hood. After all the methanol were removed, distilled water was added into the soils until the water content was adjusted to 60% of soil water holding capacity(WHC), followed by stirring until uniform. In order to collect the respired ^{14}C-CO_2, a 20 ml vial filling with 10 ml 0.5 mol/L NaOH was hung under the rubber plug. During the course of incubation, the water content was kept constant by adding distilled water every day. After 60 d and 90 d incubation, a part of the soil corresponding to 20 g air-dried soils was sampled and then extracted by methanol in a vibrator for five times continuously until all the extractable residues of ^{14}C-labeled chlorsulfuron in soil was removed. The residual soil was termed as ^{14}C-BR sample.

Table 1 Properties of the soil used

No.	Types of soil	pH (H_2O)	OM, g/kg	CEC, cmol/kg	Clay, %	Silt, %	Sand, %
Soil 1	Paddy soil from quaternary red soil	5.36	15.7	13.70	39.0	41.4	19.9
Soil 2	Paddy soil from red sandstone soil	5.61	11.3	12.34	17.2	7.4	75.4
Soil 3	Paddy soil from deposit of purple mudstone regolith	5.82	20.3	15.88	22.1	50.3	27.6
Soil 4	Coast saline soil	9.04	9.5	7.13	24.3	71.1	4.6
Soil 5	Bluish clayey paddy soil	6.20	40.6	25.10	35.3	60.6	4.1
Soil 6	Silt clayey yellow mottled paddy soil	6.22	31.5	28.50	38.0	57.0	5.0
Soil 7	Fluvia marine yellow loamy	6.50	30.5	10.83	8.0	71.3	20.8

1.4 Extraction and purification method of bound residues derived from ^{14}C-labeled chlorsulfuron in soil

A 1.0-g aliquot of each ^{14}C-BR sample was combusted in the biological oxidizer and its radioactivity was detected by liquid scintillation counter(LSC), each analytical procedure was triplicated. The total radioactivity(A_T) in 10 g ^{14}C-BR sample was calculated. Another 10 g ^{14}C-BR sample was continuously extracted with 0.1 mol/L pH 10.0 Na_2CO_3 + $NaHCO_3$(50 ml × 3), 0.1 mol/L pH 6.6 citrate buffer(50 ml × 3), 0.5 mol/L potassium dihydrogen phosphate solution (50 ml × 3) and acetonitrile + 0.1% acetic acid(60:40, V/V, 50 ml × 3) in turn. All the extracting processes were performed with a vibrator. In each step, the BR sample was extracted for 1 h and subject to centrifuging (4000 r/min) for 10 min. All the supernatants were mixed and condensed under decompressed condition at 60℃ until all the acetonitrile was removed, and then the radioactivity of the extracting part(A_E) was determined. The radioactivity (A_r) of residues in soil was also determined by LSC after the residual soil was combusted. The efficiency of the extracting procedure was calculated with the formula$(A_T-A_r)/A_T$. Subsequently, the pH of the supernatants was adjusted to 3.3 with 0.5 mol/L HCl, and extracted again by dichloromethane(V/V = 1:1) for four times. The organic phase was condensed to 50 ml by decompress rotary evaporator, the water phase was subsequently extracted by the mixed solution of iso-propanol and ethyl acetate(V/V = 1:1) for four times. Combined the organic phase and vaporized it under decompressed condition at 60℃. Solution of 0.1 mol/L Na_2HCO_3-Na_2CO_3 was added into the condensed organic phase, shaked it vigorously and then transferred it to a distributary funnel in order to separate the organic phase from the water phase. This process was done in triplication. The water phase was filtered through a 0.45 μm ultra-filtrate membrane and adjusted to pH 3.3, the filtrate was passed through a C_{18} pretreatment column(10 × 150 mm, 7 μm). The component with radioactivity that can not be hold on the pretreatment column was collected(marked as fraction 1), while the retained component(marked as fraction 2) in the pretreatment column was eluted by ethyl acetate. The fraction 2 was concentrated in a condense tube until all the solvent was removed. The fraction 1 in the water phase was again extracted by chloroform(V/V = 1:1) for three times. Discarded the chloroform, then the water phase was extracted by a mixed solution of iso-propanol and ethyl acetate with volume ratio of 1:1. Repeated three times and mixed the organic phase, which was condensed to a small volume subsequently. Combined it with the fraction 2 and condensed to 2 ml. The radioactivity (A_R) of the concentrated solution was determined so as to calculate the recovery rate(A_R/A_E) over the process of the purification. The remnants were kept in 4℃(marked as BR sample).

1.4 Identification of the composition of ^{14}C-BR derived from ^{14}C-labeled chlorsulfuron with LC-MS

A 4.6 × 250 mm discovery C_{18} column was used as the separation column, the flow rate of mobile phase was 1 ml/min in a gradient elution mode, solution A was a mixture of water and 0.1% glacial acetic acid (V:V), while solution B was a mixture of acetonitrile and 0.1% glacial acetic acid(V:V). The gradient program performed is listed in Table 2. The UV detector used was a photodiode array detector. The mass spectrometer was Micromass Quattro LC. MS detection in the mode of electrospray positive ion(ES^+) and negative ion(ES^-) full scan(100—600 amu) was performed respectively, meanwhile, the selected ion with 358 and 141 of m/z for ES^+ scan and 190 of m/z for ES^- scan was also detected. The injection volume of the sample was 40 μl.

1.5 Radioactive determination of several fractions eluted from HPLC

200 μl BR sample extracted from the soil 7(90 d of incubation) was inject into the HPLC system. Elution from HPLC column was collected in several fractions with the retention time ranging in 2.5—4.1, 4.2—

5.0, 5.1—6.5, 6.5—8.1, 8.1—9.5, 9.5—10.3, 10.3—11.7, 11.7—13.2, 13.2—14.3, 14.4—15.4, 15.5—16.7, 16.8—18.4, 18.4—20.0, 20.0—22.0 and 22.0—24.0 min. 10 ml Hisafe-3 was added into every fraction mentioned above and their radioactivity was detected by LSC. Each of detection was repeated for three times. After while, 10 ml Hisafe-3 scintillation liquid was added into another 200 μl BR sample, and the radioactivity in 200 μl BR sample was also determined for the calculation of the radioactive percentage in each fraction.

Table 2 Gradient mode in HPLC separation

Time, min	Percentage of solution A in mobile phase, %
0—2	90
2—12	65
12—20	65
20—28	20
28—33	20
30—35	90

2 Results

2.1 The efficiency of consecutive extracting methods for BR and recover rate of sample pretreatment

The efficiency of the consecutive extracting methods for the BR derived from ^{14}C-chlorsulfuron in soil is listed in Table 3. It could be seen that the method has relatively high efficiency for extracting different kinds of soil sample and the sample with different incubation time. The efficiency in the overall consecutive extracting process was ranging from 76.8% to 88.6%. This rate was higher than that of high temperature distillation method and similar to the methanol-supercritical extraction method(Guo, 1998; 1999). The result also showed that there was no obvious difference in extracting efficiency among the different soil samples or the soil samples with different incubation time by using the consecutive extracting method. Thus, this method was suitable to extract the BR derived from ^{14}C-chlorosulfuron in different kinds of soil.

Table 3 The efficiency of the consecutive extracting methods for the BR derived from ^{14}C-chlorsulfuron in different soils

	Efficiency, %						
	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Soil 7
* BR_{60d}	81.9 ± 1.4	76.8 ± 0.9	81.0 ± 2.1	84.5 ± 0.9	77.1 ± 0.6	79.5 ± 0.4	78.1 ± 1.1
* BR_{90d}	84.1 ± 2.3	84.6 ± 3.6	81.5 ± 2.1	88.6 ± 3.1	78.4 ± 0.8	78.1 ± 2.3	76.7 ± 1.9

Notes: * BR_{60d} and BR_{90d} represent the BR soils after 60 d and 90 d incubation, respectively

The extracted solution of the spiked soil was very complex. It contained high content of impurity with the color of dark brown. Besides, the ^{14}C-BR of the chlorsulfuron may contain high content of degraded product with high polarity. So, the pretreatment of the sample was a very complicated and difficult procedure. It was finally found that iso-propanol could effectively extract the polar degraded products derived from the BR of ^{14}C-chlorosulfuron, with the recovery rate reached 90.5%. The method using iso-propanol was superior to that using *n*-butanol (the recovery rate was 79%). The experiment results here showed an overall recovery rate of 81.9 ± 3.3% for ^{14}C-BR derived from ^{14}C-chlorosulfuron.

2.2 Determination of ^{14}C-BR composition derived from ^{14}C-labeled chlorsulfuron in soil

As the component with radioactivity must derive from the labeled chlorsulfuron molecular, the LC-MS determination process was focused on the ^{14}C-labeled triazine ring so as to identify conveniently the BR composition derived from ^{14}C-labeled chlorsulfuron in soil. The total ion chromatogram(TIC) in the mode of electrospray positive ion full scan (100—600 amu) is shown in Fig. 2. Each peak fraction was collected in the same chromatographic condition and their radioactivity was detected, of which the results are listed in Table 4.

Table 4 The retention time of the BR component with radioactivity and their relative percentage accounted in total recovery radioactivity

Retention time, min	The relative percentage accounted in total recovery radioactivity, %
2.5—4.1	35.4
5.0—6.5	39.8
13.0—14.0	3.6
20.0—21.0	17.9

The results in Table 4 showed that the four peak with the retention

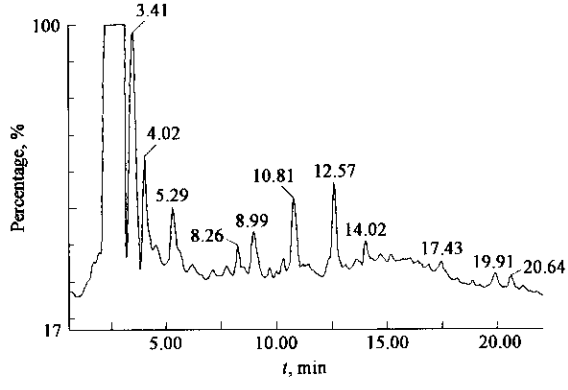

Fig. 2 LC-MS total ion chromatogram(ES$^+$) of the BR derived from ^{14}C-labeled chlorsulfuron in soil 7

time of 2.5—4.1, 5.0—6.5, 13.0—14.0, 20.0—21 min(marked as component Ⅰ, Ⅱ, Ⅲ, Ⅳ respectively) have radioactivity, and the structure of these four components was identified thereafter in details.

The mass spectra results demonstrated that the molecular weight of components Ⅰ was 126(Fig. 3). Since this component had radioactivity and derived from the triazine ring, and its retention time in HPLC was in accordance with the standard sample, 2-amino-4-hydroxyl-6-methyl-1,3,5-triazine, the possible structure of the component Ⅰ with the molecular weight of 126 should be ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine.

The mass spectroscopy and the selected ion chromatography of component Ⅱ with the retention time of 5—6.5 are shown in Fig. 4a and 4b. The molecular weight of the component Ⅱ was 140. With the radioactivity related to the triazine ring, the structure of this component could be considered as ^{14}C-[2-amino-4-methoxyl-6-methyl-1,3,5]-

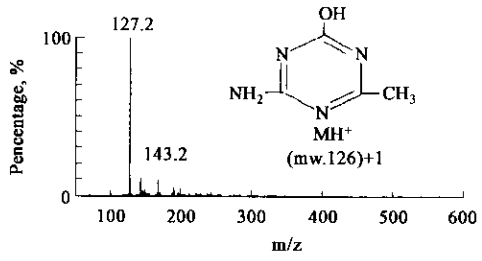

Fig.3 Mass spectra(ES^+) of the compound with the retention time of 3.4 min

triazine. In order to confirm this conclusion, the retention time of component Ⅱ in HPLC was compared with the standard sample, 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine. The coincident result was observed.

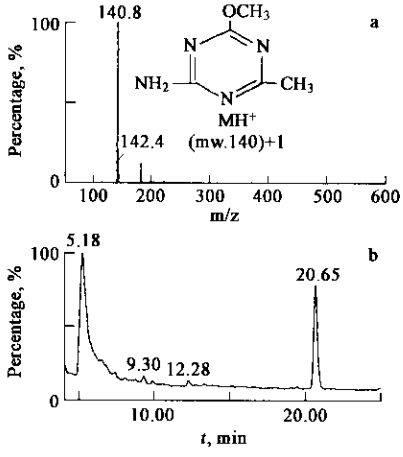

Fig.4 Mass spectra(ES^+) of component with the retention time of 5.3 min (4a) and the selected ion(m/z = 141, ES^+)chromatogram(4b)

The mass spectra (electrospray positive ion full-scan mode) of component Ⅲ with the retention time of 13.6 is illustrated in Fig.5. The MS result showed that the molecular weight (mw.) of this component should be 400(m/z = 401). It was found that there was a fragment (m/z = 126 + 1) in the BR sample but it did not present in sample blank, whereas other fragments (m/z = 143, 222, 238 and 279) presented in sample blank, indicating non-BR origin of these fragments. It must be related to the triazine ring because of the radioactivity. The structure of the fragment with 127 of m/z may be ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine. However, the fragment with the m/z of 192(mw.191 + 1) was not found in the spectra, the component Ⅲ should not contain the structure of *o*-chloro-benzene-sulfonamide(M_w = 191, the left side in the structure of chlorsulfuron). Accordingly, it could be assumed that the component Ⅲ may be a complex substance combining the ^{14}C-[2-amino-4-hydroxyl-6-methyl-1, 3, 5]-triazine with the substrate of the soils. The definite structure of this component could not be clarified since structural information such as those detected by IR and NMR were not available here.

The mass spectra and the selected ion chromatography of the component Ⅳ with the retention time of 20.60 min are shown in Fig. 6a and 6b, respectively. The MS results demonstrated that the component have molecular weigh of 357 and contain the chlorine element in its chemical formula since the chlorine isotope peaks exist in the mass

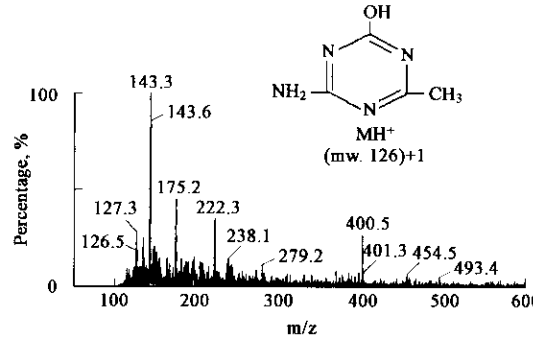

Fig.5 Mass spectra(ES^+) of component with the retention time of 13.6 min

spectra(M : M + 2 = 3 : 1). Two fragments with the m/z 141(mw.140 + 1) and m/z 167(mw.166 + 1) were found in these mass spectra. The deduced structures of the two fragments are shown in Fig. 6a. Furthermore, the retention time of the component Ⅳ was compared with that of chlorsulfuron standard sample when using HPLC, and consistent result was obtained. It is the first report that the ^{14}C-labeled chlorsulfuron parent compound can be found in the BR after long time incubation.

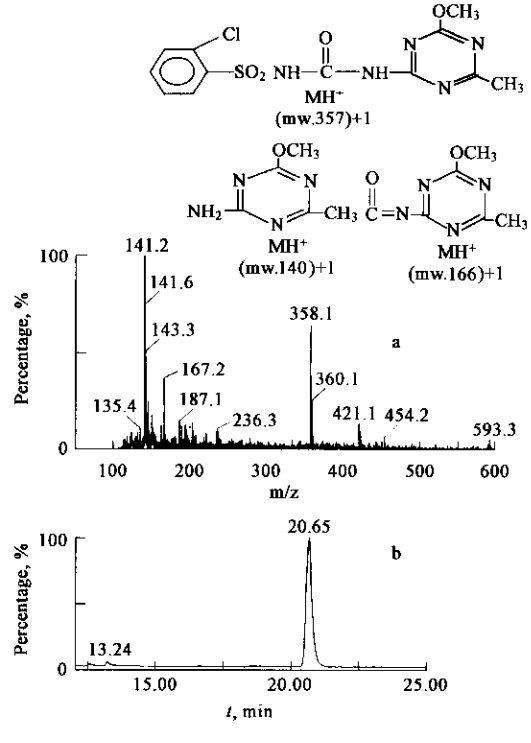

Fig.6 Mass spectra(ES^+) of component with the retention time of 20.6 min (a) and the selected ion (m/z = 358, ES^+) chromatogram(b)

The Fig.7 illustrats that the substance with the retention time of 11.7 min had the molecular weight of 191 (m/z = 190, ES^-) and also contained chlorine(the isotope peaks of which are shown in Fig. 7b). As this fraction exhibited a retention time identical to that of *o*-chloro-benzene-sulfonamide, it is fractured from the parent compound and then subject to binding by soil particles. In this paper, other components from the BR related to benzene ring without radioactivity was not further identified, since it was difficult to identify non-labeled degraded products

from the complicated BR soil sample.

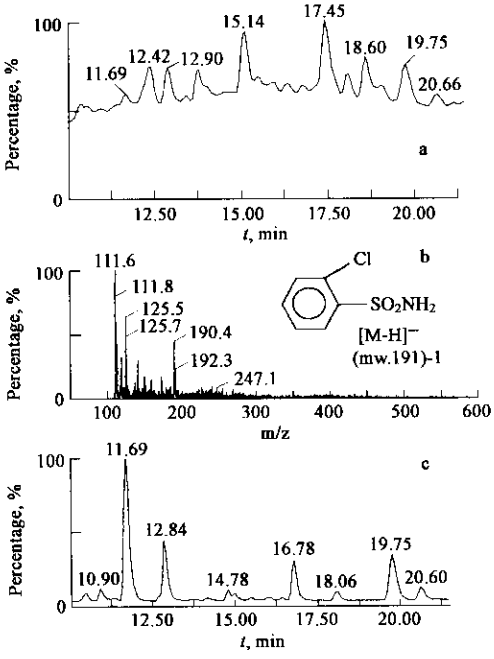

Fig.7 LC-MS total ion chromatogram(ES$^-$) of the BR(a), mass spectra(ES$^-$) of component with the retention time of 11.7 min (b) and the selected ion (m/z = 190, ES$^-$) chromatogram(c)

The final results of the BR composition derived from ^{14}C-labeled chlorsulfuron after incubation is shown in Table 5. The degraded portions of the ^{14}C-[2-amino-4-methoxyl-6-methyl-1,3,5]-triazine and ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine dominated the BR, while still a significant amount of ^{14}C-chlorsulfuron parent compound and *o*-chloro-benzene-sulfonamide constituted the main composition of the ^{14}C-BR. However, a small amount of the complex of ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine with an unknown soil substrate could exist in the ^{14}C-BR. Sabadie and Strek(Sabadie, 1992; Strek, 1998) had verified that the [2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine and [2-amino-4-methoxyl-6-methyl-1, 3, 5]-triazine were the main degradation products in extractable residues of chlorsulforon. Here, the results supported their finding.

Table 5 The BR composition derived from ^{14}C-labeled chlorsulfuron in soil 7 and the relative percentage accounted in total recovery radioactivity

Name of component in the BR	mw.	The relative percentage accounted in total recovery radioactivity, %
^{14}C-[2-amino-4-methoxyl-6-methyl-1,3,5]-triazine	140	39.8
^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine	126	35.4
^{14}C-chlorsulfuron parent compound	357	17.9
Complex of ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine with an unknown soil substrate	400	3.6
o-chloro-benzene sulfonamide	191	/

Previous studies found that chlorsulfuron parent compound would inhibit the activity of ALS in plants (Sweetser, 1982; Ray, 1984; Beyer, 1988; Brown, 1990). In our studies, determination using bioassays proved that no phytotoxic effects of 2-amino-4-methoxyl-6-methyl-1,3,5-triazine, 2-amino-4-hydroxyl-6-methyl-1,3,5-triazine and *o*-chloro-benzene sulfonamide were detected on the growths of rape seedling and rice(not reported here). Therefore, the phytotoxic effects of the BR should be caused by the released chlorsulfuron parent compound, which clearly explains the phytotoxic effect induced by the BR derived from chlorsulfuron in soil.

3 Discussions

Senesi and Testini(Senesi, 1980) have evidenced that the nitrogen atom in triazine can be easily protonated by using elementary analyses and infrared spectroscopy (IR). The protonated triazine ring can be strongly bound with humic substance in soil. Since [2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine and [2-amino-4-methoxyl-6-methyl-1,3,5]-triazine comprise the triazine ring, both of them may be protonated and interact with fulvic acid and humic acid through ion exchanging force. In a previous study (Ye, 2002), the amount of BR increased with the decreasing of soil pH. This is because the more acid the soil was, the more proponated [2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine and [2-amino-4-methoxyl-6-methyl-1,3,5]-triazine would present in soil and easier to be bound with soil matrix through the ion exchange force. In our opinion, ion exchange force may be the main interaction mode in the formation of ^{14}C-BR.

Interaction mode between pesticide and soil depends on the properties of soil and the compound. The types of mechanism involved in binding process include ionic, hydrogen and covalent bonding, charge-transfer or electron donor-acceptor mechanism, Van der waal forces, ligand exchange, hydrophobic bonding or partitioning (Bailey, 1970; Senesi, 1992; Pignatello, 1996; Gevao, 2000). Nevertheless, a chemical may be subject to different binding by several mechanism simultaneously(Calderbank, 1989).

Yang (Yang, 1998) have taken their sight into configuration, electronic structure and physico-chemical property of sulfonylurea pesticides from the point view of quantum chemistry. They proposed that the heterocyclic ring part may play a role of electron donator, and can interact with the acceptor through an electron transfer force, while the benzene ring side of the chlorsulfuron could be bound with the acceptor through hydrophobic interaction. The electron draw group on the benzene group and the sulfur atom in the bridge of the molecular can interact with acceptor through electrostatic force, and the oxygen atom in the sulfonyl group may interact with the acceptor through a hydrogen bound. According to the structural and chemical property of chlorsulfuron, humic substance and clay mineral, it could be inferred that the molecular of chlorsulfuron parent compound might also interact with the soil matrix through a mixed interaction mode. These interactions might play an important role in the BR formation of the parent compound in soil.

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