

A Rapid Multi-Residue Determination Method of Herbicides in Grain by GC–MS–SIM

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Abstract

In this study, a gas chromatography–mass spectrometry method is successfully developed for the determination of 11 herbicide residues (alachlor, acetochlor, butachlor, pretilachlor, metolachlor, dimethenamid, propachlor, napropamid, propanil, atrazine, and metribuzin) in rice and soybeans. The sample is extracted with acetone–water, degreased by liquid–liquid partition, and purified through solid-phase extraction with Florisil. Experiments on 5 fortification concentrations are carried out, and the limit of determination is 0.02 mg/kg. The average recoveries of soybean samples range from 63.3% to 96.0%, and the relative standard deviations are from 2.14% to 11.2%. The average recoveries of rice samples range from 76.8% to 102% and the relative standard deviations are from 2.2% to 9.08%. The results indicate that the method developed is fast, accurate, and easy to operate. It also demonstrates that the method can meet the requirements of simultaneous determination of 11 herbicides in rice and soybeans.

Introduction

The herbicides alachlor, acetochlor, butachlor, pretilachlor, metolachlor, dimethenamid, propachlor, napropamid, propanil, atrazine, and metribuzin have been widely used in oilseeds and crop planting (1). Like other pesticides, these herbicides have an inevitable dangerous effect on human health and the environment. In recent years, countries all over the world and the Food and Agriculture Organization of the United Nations have regulated the maximum residue limits for herbicides. Up to now, there have been some reports on determining similar herbicides (2–6), but there are no reports about the simultaneous determination of these herbicides in cereals. The reported methods by liquid chromatography or gas chromatography (GC) were mostly for two to four kinds of these 11 herbicides, and

liquid–liquid partition, gel-permeation chromatography, and column chromatography were used for sample pre-processing (3,4,7–10). In our country, there are no corresponding national or industry standard methods for the determination of herbicide residues, and what we used was all based on the standards of EU (11,12). As for the traditional chromatographic determination method, it is difficult to completely remove impurities, and it is easy to arrive at the wrong estimation. All new methods demand careful validation (13,16).

This paper shows a method developed for the determination of 11 herbicides (alachlor, acetochlor, butachlor, pretilachlor, metolachlor, dimethenamid, propachlor, napropamid, propanil, atrazine, and metribuzin) by GC–mass spectrometry (MS). It meets the technical requirements of foreign trade and imports/exports inspection (14).

Experimental

Apparatus

An Agilent 6890-5973N GC–MS (Hewlett-Packard, Palo-Alto, CA) was used, equipped with 6890 series auto-injector, 5973MSD and quadrupole mass analyzer; Minishaker MS1 vortex oscillator; UNIVERSAL 32R low temperature centrifuge; ZYMARK LV nitrogen gas concentrator; HITACHI TF-123 pulverizer; POLYGRON PT3000 homogenizer; 10 μ L micro-injector; and 10–1000 μ L pipette. The solid-phase extraction (SPE) column (10 \times 12 mm, internal volume of 6 mL) was purchased from International Sorbent Technology (Hengoed, UK).

Reagents and materials

Alachlor, acetochlor, butachlor, pretilachlor, metolachlor, dimethenamid, propachlor, napropamid, propanil, atrazine, and metribuzin were provided by Riedel-de Haën Company (Seelze, Germany), and the purity was 99%.

Organic solvents were acetone, *n*-hexane, acetonitrile, and ethyl ether, and they were all analytical grade. Standard samples were accurately weighed (25 ± 0.1 mg) and diluted into 500

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$\mu\text{g/mL}$ with acetone. The solution can be stored for 3 months under $0\text{--}4^\circ\text{C}$. If needed, the solution can be diluted into desired concentrations with acetone and the diluted solution can be stored for 1 month under $0\text{--}4^\circ\text{C}$. Anhydrous sodium sulfate was obtained from Baker (pesticide residue quality, Deventer, The Netherlands) and heated for 4 h at 650°C , and then stored in a drier after cooling.

Extractant: some *n*-hexane was spiked into acetonitrile and mixed completely. *N*-hexane-ether (85 + 15) was used, as was sodium chloride solution (10%, *m/v*).

Rice and soy were all purchased from a market. They were ground into powders, filtrated through a 20-mesh sieve, divided into two parts, and placed in clean vessels for use.

Florisil was filtered through a 60–100 mesh; before use, it was pretreated with an *n*-hexane-ether (5 mL, 85 + 15, *v/v*) solution and *n*-hexane (5 mL) in sequence.

Sample processing

Extraction

A sample (10 ± 0.01 g) was soaked in 10 mL water for a night, and then acetone (20 mL) was added to extract the residues in samples by agitating for 3 min. After centrifugation for 4 min at 4000 *r/min*, the supernatant was moved into a flask (250-mL). The remainder was extracted with acetone (30 mL, 2 times) again, and the supernatant was collected into a flask. The acetone in the flask was removed through reduced pressure distillation, and the raffinate was transferred into a centrifuge tube (50 mL) and extracted with sodium chloride solution (10%, 10 mL) and

n-hexane (15 mL) for 3 min. After centrifugation for 3 min at a speed of 2500 *r/min*, the remainder was re-extracted with *n*-hexane, and all of the *n*-hexane phase was collected.

Liquid-liquid partition

The *n*-hexane phase was dewatered with anhydrous sodium sulfate and then evaporated under reduced pressure until dry. The remainder was dissolved in *n*-hexane (2×5 mL) and extracted with extraction solution I (10 mL \times 3 times). The acetonitrile phase was collected and the remainder was extracted again with extraction solution II. All of the acetonitrile phase was collected and distilled. The remainder was dissolved with *n*-hexane (5 mL).

SPE purification

The previously mentioned *n*-hexane solution was purified by the Florisil solid-phase column extractor with an eluant of *n*-hexane-ether (15 mL) at a flow rate of 0.5 mL/min. The eluant collected was dried with a nitrogen gas drier, and the remainder was dissolved with 1.0 mL *n*-hexane, which was ready for GC analysis.

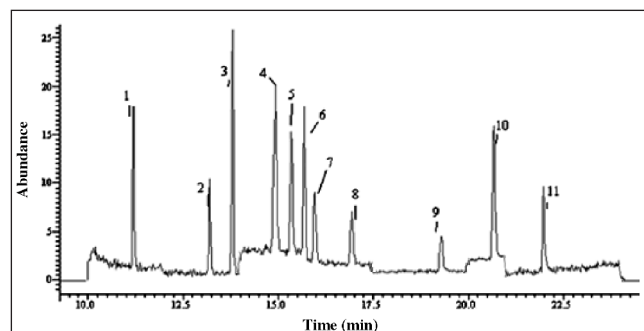


Figure 1. The chromatogram of the 11 herbicides (0.1 mg/kg). Propachlor, 1; atrazine, 2; acetochlor, 3; dimethenamid, 4; alachlor, 5; metribuzin, 6; metolachlor, 7; propanil, 8; butachlor, 9; pretilachlor, 10; napropamid, 11.

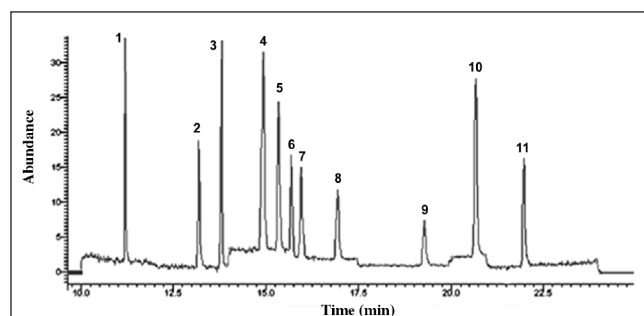


Figure 2. The chromatogram of the 11 herbicides (0.2 mg/kg). 1; atrazine, 2; acetochlor, 3; dimethenamid, 4; alachlor, 5; metribuzin, 6; metolachlor, 7; propanil, 8; butachlor, 9; pretilachlor, 10; napropamid, 11.

Table I. Relative Ion Abundance Ratio and Monitoring, Quantitation Ions of the 11 Herbicides

Compound	Quantitative ions (<i>m/z</i>)	Monitoring ion (<i>m/z</i>) and relative abundance
Propachlor	120	120(100), 176(37), 212(8)
Atrazine	200	200(100), 215(62), 173(35)
Acetochlor	146	146(100), 223(53), 174(48), 162(83)
Dimethenamid	154	154(100), 203(42), 230(58)
Alachlor	160	160(100), 188(93), 237(24)
Metribuzin	198	198(100), 144(14), 214(4)
Metolachlor	162	162(100), 238(47), 211(7)
Propanil	161	57(68), 161(100), 217(18)
Butachlor	176	176(100), 160(86), 188(49)
Pretilachlor	238	202(55), 238(100), 262(40)
Napropamid	128	72(100), 128(63), 271(26)

Table II. The Regression Equation, Correlation Coefficient, and Low Limit of Detection of the 11 Herbicides

Compounds	Quantitation Ions (<i>m/z</i>)	Regression equation	Correlation coefficient	LOD ($\mu\text{g/kg}$)
Propachlor	120	$Y = 1.97 \times 10^5 x - 5.46 \times 10^3$	0.9999	20
Atrazine	200	$Y = 2.15 \times 10^5 x - 1.76 \times 10^2$	0.9940	20
Acetochlor	146	$Y = 1.52 \times 10^5 x - 2.20 \times 10^2$	0.9968	20
Dimethenamid	154	$Y = 4.18 \times 10^5 x - 4.24 \times 10^2$	0.9960	20
Alachlor	160	$Y = 2.28 \times 10^5 x - 4.25 \times 10^3$	0.9958	20
Metribuzin	198	$Y = 1.77 \times 10^5 x - 6.83 \times 10^3$	0.9989	20
Metolachlor	162	$Y = 1.71 \times 10^5 x - 6.94 \times 10^3$	0.9921	20
Propanil	161	$Y = 1.46 \times 10^5 x - 6.27 \times 10^3$	0.9937	20
Butachlor	176	$Y = 2.11 \times 10^5 x - 4.73 \times 10^3$	0.9964	20
Pretilachlor	238	$Y = 3.09 \times 10^5 x - 2.56 \times 10^3$	0.9926	20
Napropamid	128	$Y = 2.55 \times 10^5 x - 1.08 \times 10^3$	0.9962	20

Equipment parameters

Chromatographic conditions: the chromatographic column was a DB-1701, 30 m × 0.25 mm i.d. × 0.25 μm, and the carrier gas was nitrogen (purity > 99.995%). It was operated in continuous flow mode at a flow rate of 1.0 mL/min. The injector temperature was 270°C and splitless sampling was used (1 μL). Temperature programming conditions were set as follows: the initial temperature was 70°C (for 1 min), and then was raised to 200°C at a speed of 15°C/min (for 1 min); finally, it was programmed to 280°C at a speed of 50°C/min (for 7 min).

Mass analyzer conditions: The ion source temperature, tetrapolar temperature, and transfer line temperature were 230°C, 150°C, and 280°C, respectively. EI was chosen as the ionization mode, and the mass scanning range was 50–400 amu. The electron multiplier tube tension was 200 V, and the detector was in selected ion monitoring mode.

Results evaluation

The qualitative analysis of mass spectrum was based on more than 3 ions, and the relative abundance of the ions

should alter within 20%. It was quantified with the external standard method. The blank value must be deducted from the results.

Results

Optimization of MS conditions

The DB-1701 column was selected for GC–MS analysis, and the results of the optimization of chromatographic separation conditions are shown in Figures 1 and 2. SPE was used, and the acetone–water was firstly concentrated by rotary evaporation, and then extracted with *n*-hexane after the addition of sodium chloride solution. Characteristic ions of relative high intensity and strong anti-turbulence were selected as monitoring and quantitative ions (Table I).

Linearity and limit of detection

The standard stock solution was diluted gradually and analyzed by GC–MS–SIM, and the linear range was 0.05 mg/L

Table III. The Recovery and Relative Standard Deviation (RSD) of the 11 Herbicides in Soybeans

Compound	Fortified concentrations (mg/kg)	Measured concentration (mg/kg)	Average Recovery (%)	RSD (n = 10) (%)	Compound	Fortified concentrations (mg/kg)	Measured concentration (mg/kg)	Average Recovery (%)	RSD (n = 10) (%)
Propachlor	0.02	0.016	78.1	10.2	Metolachlor	1.00	0.901	90.1	6.42
	0.05	0.043	84.9	8.96		2.00	1.830	91.5	2.21
	0.50	0.442	88.3	5.50		0.02	0.016	81.6	10.4
	1.00	0.920	92.0	6.14		0.05	0.043	85.5	4.64
	2.00	1.790	89.2	6.14		0.50	0.427	85.3	6.41
Atrazine	0.02	0.016	80.8	8.19	1.00	0.877	87.7	4.90	
	0.05	0.043	85.1	8.07	2.00	1.810	90.3	3.11	
	0.50	0.445	89.0	5.72	Propanil	0.02	0.017	82.9	6.76
	1.00	0.928	92.8	5.44		0.05	0.044	88.1	5.49
	2.00	1.810	90.2	2.14		0.50	0.454	90.8	5.35
Acetochlor	0.02	0.016	80.8	6.51		1.00	0.911	91.1	4.10
	0.05	0.041	82.4	6.50		2.00	1.850	92.5	2.43
	0.50	0.425	85.0	3.70	Butachlor	0.02	0.013	63.3	10.6
	1.00	0.876	87.6	5.77		0.05	0.036	72.8	8.11
	2.00	1.780	88.6	2.56		0.50	0.396	79.1	7.33
Dimethenamid	0.02	0.016	78.7	6.52		1.00	0.836	83.6	4.36
	0.05	0.042	84.4	7.04		2.00	1.670	83.3	3.39
	0.50	0.443	88.5	5.59	Pretilachlor	0.02	0.018	88.7	5.00
	1.00	0.908	90.8	5.34		0.05	0.043	86.4	4.75
	2.00	1.820	90.9	3.61		0.50	0.450	90.0	5.17
Alachlor	0.02	0.016	78.6	6.30		1.00	0.910	91.0	3.67
	0.05	0.043	84.9	6.85		2.00	1.860	92.7	2.66
	0.50	0.427	85.3	4.42	Napropamid	0.02	0.018	88.4	7.69
	1.00	0.888	88.8	6.78		0.05	0.045	90.2	6.72
	2.00	1.780	88.8	2.58		0.50	0.459	91.7	4.26
Metribuzin	0.02	0.016	81.7	11.2		1.00	0.960	96.0	5.00
	0.05	0.044	87.8	6.02		2.00	1.880	93.9	2.05
	0.50	0.443	88.5	5.18					

to 1.0 mg/L. The regression equation, coefficient correlation, and limit of detection (LOD) are listed in Table II. For the real samples, the detection linear range was 0.01 mg/kg to 0.1 mg/kg and the LOD was 0.02 mg/kg. Chromatograms of the 11 herbicides (0.1 mg/kg) are shown in Figure 1.

Recovery and precision

Recovery experiments of 5 fortified concentrations were carried out on soy and rice samples which contained none of the 11 target compounds, each concentration 10 parallels. The data of recovery and precision are shown in Tables III and IV, and the blank, rice, and soybean extract chromatograms are represented in Figures 3–6. The total average recovery was between 63.3% and 102%, and the average recovery in soy and rice samples, respectively, was 63.3%~96.0% and 76.8%~102%. The total average relative standard deviation was 2.14%~11.2%, and the average relative standard deviation of soy and rice, respectively, was 2.14%~11.2% and 2.2%~9.08%. From the results, this method could meet the requests of residue analysis.

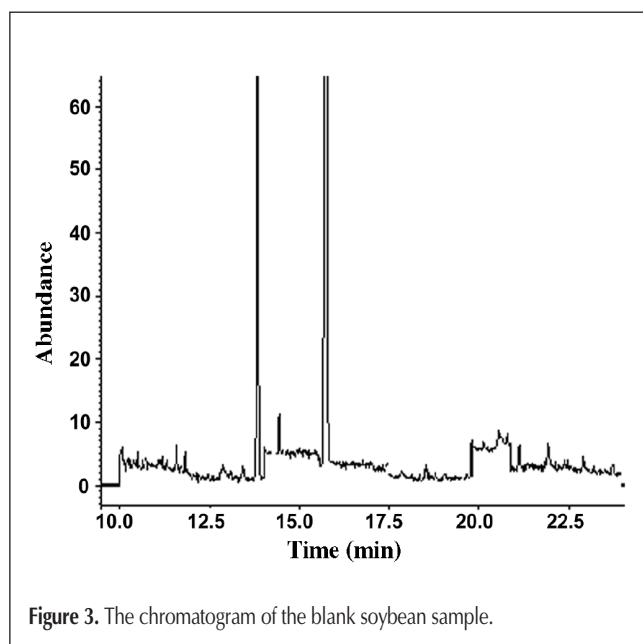


Figure 3. The chromatogram of the blank soybean sample.

Table IV. The Recovery and Relative Standard Deviation (RSD) of the 11 Herbicides in Rice

Compound	Fortified concentrations (mg/kg)	Measured concentration (mg/kg)	Average recovery (%)	RSD (n = 10) (%)	Compound	Fortified concentrations (mg/kg)	Measured concentration (mg/kg)	Average recovery (%)	RSD (n = 10) (%)
Propachlor	0.02	0.019	93.2	2.63	Metolachlor	0.50	0.450	90.0	5.78
	0.05	0.047	94.5	4.52		1.00	0.936	93.6	4.19
	0.50	0.452	90.3	4.97		2.00	1.920	95.6	2.20
	1.00	0.941	94.1	3.49		0.02	0.018	88.1	9.08
	2.00	1.850	92.1	2.67		0.05	0.045	90.1	4.10
Atrazine	0.02	0.018	91.9	4.23	0.50	0.450	90.0	5.78	
	0.05	0.045	89.8	5.60	1.00	0.931	93.1	4.87	
	0.50	0.464	92.8	4.14	2.00	1.880	93.8	3.28	
	1.00	0.919	91.9	3.39	Propanil	0.02	0.017	83.7	6.70
	2.00	1.910	95.5	2.28		0.05	0.046	91.4	3.80
Acetochlor	0.02	0.017	85.2	4.99		0.50	0.457	91.4	4.23
	0.05	0.042	84.5	5.82		1.00	0.958	95.8	4.47
	0.50	0.436	87.1	3.35		2.00	1.910	95.5	3.87
	1.00	0.904	90.4	3.48	Butachlor	0.02	0.013	66.6	7.12
	2.00	1.870	93.5	2.20		0.05	0.038	76.8	7.35
Dimethenamid	0.02	0.017	84.6	4.56		0.50	0.421	84.2	4.13
	0.05	0.042	84.5	5.82		1.00	0.894	89.4	3.81
	0.50	0.447	89.4	2.79		2.00	1.790	89.5	2.74
	1.00	0.938	93.8	2.59	Pretilachlor	0.02	0.017	86.9	3.61
	2.00	1.850	92.1	3.55		0.05	0.045	90.6	5.65
Alachlor	0.02	0.017	83.7	4.38		0.50	0.463	92.5	4.03
	0.05	0.043	86.0	5.09		1.00	0.944	94.4	4.40
	Napropamid	0.50	0.440	88.0		5.05	2.00	1.950	97.5
		1.00	0.901	90.1	4.69	0.02	0.019	96.1	6.08
		2.00	1.880	93.9	2.82	0.05	0.048	95.9	6.63
Metribuzin		0.02	0.018	88.3	4.72	0.50	0.471	94.2	5.60
		0.05	0.045	90.1	4.92	1.00	1.020	102.0	4.56
	Metribuzin	2.00	1.920	95.9	2.82	2.00	1.920	95.9	2.82

Discussions

A few kinds of columns could be selected for pesticide separation, including DB-5, DB-1, DB-1701, and DB-35 columns. As the herbicides in our research were low pole, a low-pole DB-1701 column was selected for the GC-MS analysis, which could be effective. Compared with SIM, SIS sometimes generates $[M+1]^+$ while determining high-concentration samples, which can distort the mass chromatogram. Also, the space-charge effect of the ion trap can make the abundance ratio of target ions variable (15), which results in a loss of sensitivity of some ions and difficulty in searching the standard chromatograms library. SIM was chosen in this paper.

Cereal, especially rape seed, contains a lot of lipid, protein, and phospholipids, which makes the pre-processing hard, results in low recovery, and brings serious interference to chromatographic analysis. Tedious liquid-liquid extraction, column chromatography purification, and gel-permeation chromatography

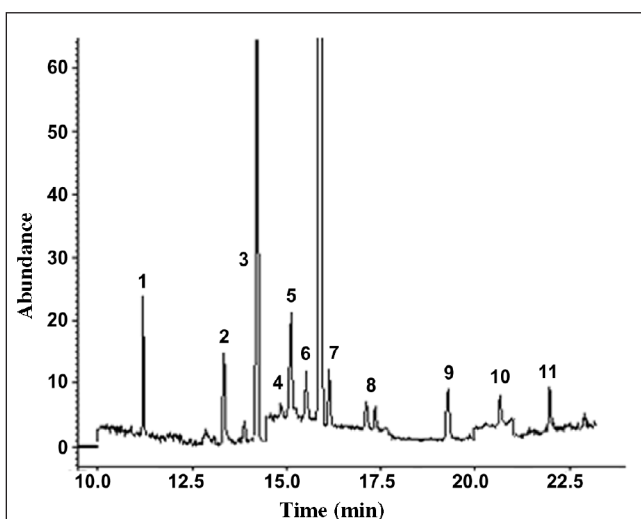


Figure 4. The chromatogram of the spiked soybean sample (20 $\mu\text{g}/\text{kg}$). Propachlor, 1; atrazine, 2; acetochlor, 3; dimethenamid, 4; alachlor, 5; metribuzin, 6; metolachlor, 7; propanil, 8; butachlor, 9; pretilachlor, 10; napropamid, 11.

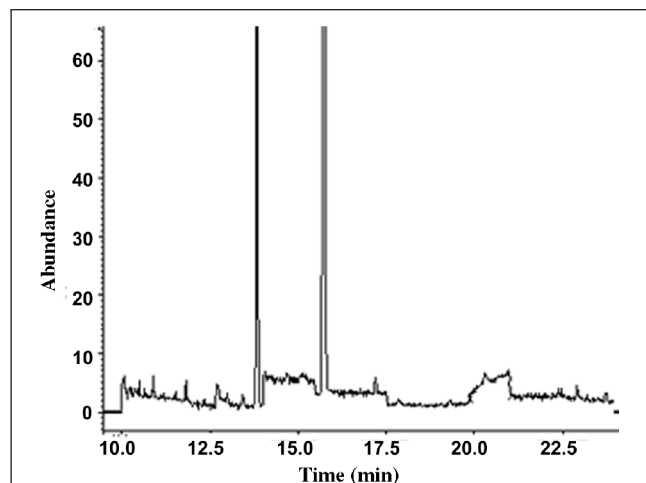


Figure 5. The chromatogram of the blank rice sample.

organic, which is solvent-consuming, are the main methods reported. However, they are time- and effort-consuming and employ a lot of poisonous and harmful reagents (16,17). SPE is rapid and high-performance. Amides herbicides all have a good solubility in acetone, *n*-hexane, and acetonitrile. *n*-Hexane, acetonitrile, acetone, and acetone-water were used as extraction solvent, respectively, and the results showed that acetone-water had the best effect. In order to ensure further purification, the acetone-water was first concentrated by rotary evaporation, then extracted with *n*-hexane after the addition of sodium chloride solution.

Conclusions

In this paper, a method was developed and described for the determination of 11 herbicide residues in rice and soybean seed. Condition selection of each step and optimization is an important part in the study, and the selectivity, linear range, recovery, precision, and limit of quantitative analysis were all evaluated and verified. The sample was first extracted with an acetone-water solution, then degreased by liquid-liquid partition, purified with a Florisil SPE column, and finally, detected by GC-MS. The method is accurate, sensitive, and convenient. The limit of detection is 0.02 mg/kg, and it keeps pace with the advances of international technology.

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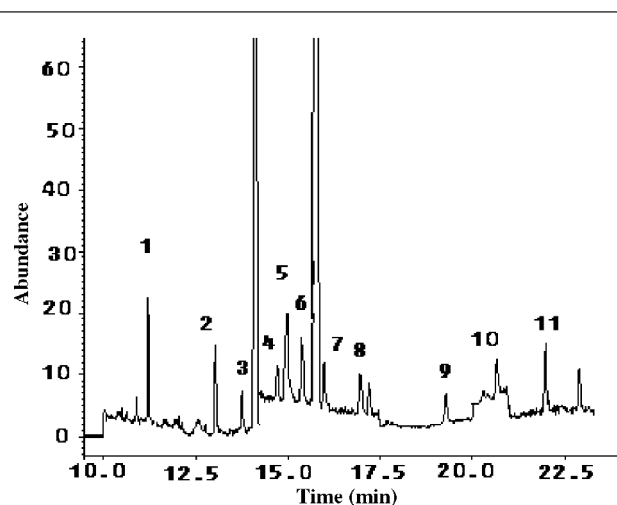


Figure 6. The chromatogram of the spiked rice sample (20 $\mu\text{g}/\text{kg}$). Propachlor, 1; atrazine, 2; acetochlor, 3; dimethenamid, 4; alachlor, 5; metribuzin, 6; metolachlor, 7; propanil, 8; butachlor, 9; pretilachlor, 10; napropamid, 11.

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