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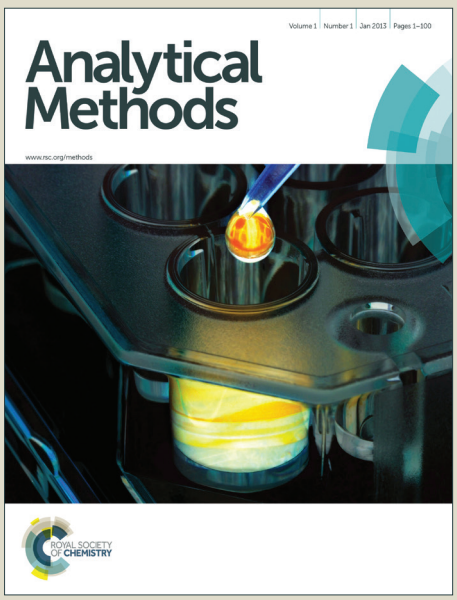

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Rapid simultaneous determination of herbicides in human serum by UPLC-ESI-MS

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A simple, rapid and reliable method was developed for multi-class and multi-residue analysis of herbicides in human serum by ultra performance liquid chromatography–electrospray ionization–mass spectrometry (UPLC-ESI-MS). Serum sample preparation was carried out by one-step protein precipitation and analytes extraction using acetonitrile. After centrifugation, an aliquot of 5 μL of supernatant was injected into a C18 column for the separation of 22 kinds of triazine and phenylurea herbicides using gradient program with water-acetonitrile as the mobile phase and the separation of 29 kinds of herbicides using gradient program with 5 mM ammonium acetate aqueous solution containing 0.1% *v/v* formic acid–acetonitrile as mobile phase. An excellent linearity of most herbicides was observed from 0.1 μg L^{-1} up to 10.0 μg L^{-1}. The limits of detection (LODs) in serum ranged from 0.03 to 6.00 μg L^{-1}, and the limits of quantification (LOQs) ranged from 0.10 to 18.0 μg L^{-1}. Intra and inter day precisions at three spiked levels were satisfactory for the 51 herbicides with the RSD of 1.02–10.0% and 1.09 –12.0%, respectively. Extraction recoveries of 51 herbicides were satisfactory and ranged from 63.6 % to 109% at the three spiked levels with the RSDs of 1.06% to 12.0%. This UPLC-ESI-MS method is simple, accurate, and useful for multi-class multi-residue determination of herbicides and benefits clinical analysis and diagnosis.

Keywords: Ultra performance liquid chromatography tandem mass spectrometry, Herbicides, Serum

Introduction

Since the Second World War, several hundred compounds have been developed as herbicides. The intensive application of herbicides has resulted in the contamination of the atmosphere, ground and

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wastewaters, agricultural products (wheat, corn, fruits, vegetables, etc.), consequently, resulting in the direct or indirect pollution of food and food products and biological systems.[1] As herbicide molecules are more or less toxic, they represent not only an environmental risk but also a health hazard.[2] Poisoning from herbicide has occurred by accidental exposure through the skin, eyes, respiratory tract irritation, and the acute poisoning case caused by herbicide has also occurred.[3] In such poisoning cases, rapid toxicological screening is necessary for correct diagnosis. Therefore, it is important to develop a method for detecting multi-classes multi-residue herbicides.

The state of the art of chromatographic methods used in the determination of herbicide residues in crops, food and environmental samples was reviewed[4, 5] Gas chromatographic–mass spectrometry can provide high sensitivity, but the analysis for some polar herbicides, including non-volatile and thermally labile herbicides, needs to preliminary derivatization step.[6-8] Liquid chromatography–tandem mass spectrometry (LC–MS/MS) can solve the problem for the determination of herbicides in many agricultural samples, such as sixteen phenylurea herbicides in soils,[9] sixteen herbicides in rice crops,[10] and phenoxy acid herbicide residues in tobacco.[11] LC–MS/MS techniques have gained increasing popularity for the analysis of herbicides in biological fluids, such as 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methylphenoxypropionic acid, and dithiopyr in the urine of pet dogs,[12] phenoxyacetic acid in human urine,[13] atrazine in urine,[14] and paraquat in plasma and urine,[15] and difenzoquat, diquat and paraquat in whole blood.[16] Wang et al. reported the simultaneous screening of highly water-soluble herbicides, including glyphosate, glufosinate, paraquat, and diquat, in serum using ion-pair liquid chromatography–mass spectrometry. [17] The herbicides were separated by solid-phase extraction, and mass spectrometry was used for analysis and was optimized for operation in the positive mode for all analytes. The Limit of quantification of glyphosate, glufosinate, paraquat, and diquat was 5, 2, 5, and 1 μg L^{-1}. However, there were few of multi-class multi-residue methods for analysis of herbicides in biological samples.

The main purpose of the present study is to develop a rapid and reliable analytical method for the determination of 51 kinds at least belonging to 8 classes herbicides. Validation parameters of the method, such as matrix effect, linearity, sensitivity, precision, and accuracy, have been determined. Finally, the newly developed method was successfully applied to quantify 51 herbicides in human serum.

Experimental

Chemicals and reagents

Fifty one herbicide standards including triazine (16), phenylurea (6), sulfonylurea (6), phenoxy acid (5), amides (6), carbamates (4), phenyl ether (2), heterocyclic (2), and other herbicides (4) were purchased from Dr. Ehrenstorfer Chemical Industries (Augsburg, Germany). Ammonium acetate, formic acid and pesticide residue-grade acetonitrile were purchased from dikma Chemical Industries (Beijing, China). Primary stock solutions of each herbicide (1.0 mg L^{-1}) were prepared in acetonitrile. Working standard solutions of the compounds were prepared by diluting the stock solutions with acetonitrile. All herbicide solutions were stored at −20℃ in the dark when not in use.

Instrumentation

UPLC-ESI-MS analyses of serum samples were performed on a Xevo Triple Quadrupole (TQ) system (Waters, USA). This system consisted of an autosampler, a binary pump, a solvent degasser, an ACQUITY UPLC BEH C18 Column (1.7 μm, 2.1 mm×100 mm) equipped with a guard column at 40°C, and a TQ mass spectrometer. The mass spectrometer used was a triple quadruple equipped with an ESI interface operating in the positive or negative mode. A centrifuge TGL-16M (Xiangyi Centrifuge Co., Hunan, China) was used in sample treatment.

Sample preparation

Human serum samples were collected from a patient in the First Central Hospital of Baoding, who consented to provide samples for this study. All experiments were performed in compliance with the relevant laws and institutional guidelines. The Ethic Committee of the First Central Hospital of Baoding has approved these experiments.

An aliquot of 100 μL of serum sample (or quality control (QC) samples with low, medium, and high spiked levels) and 200 μL acetonitrile were vortex-mixed in a polypropylene centrifuge tube under shaking for 1 min. After centrifuged at 4 ℃ and 16300×g for 3 min，the supernatant was filtered through a 0.22-μm Millex®-LH filter, and was stored at −20℃ in the dark. An aliquot of 5 μL of the filtrate was injected into the LC-MS.

Conditions of LC–MS analysis

Seperation of herbicides was carried out on the BEH C18 column at 40°C. Flow rate was 0.3 mL min^{-1} and sample injection volume was 5μL. A mobile phase for the separation of herbicides triazine and phenylurea consisted of water (A) and acetonitrile (B), and another mobile phase for the other herbicide consisted of 5 mM ammonium acetate aqueous solution containing 0.1% *v/v* formic acid (A) and acetonitrile (B). The gradient elution was as follows: 20–55% B at 0-8 min; 55–90% B at 8–12 min; 90–20% B at 12–14 min. The TQ parameters were as follows: source temperature, 150 °C; capillary voltage, 3.0 kV; desolvation temperature, 500 °C; desolvation flow, 900 L h^{-1}; collision gas flow, and 0.19 mL min^{-1}. The observed retention time, parent ions, and daughter ions as well as used cone voltage and collision energy (CE) are listed in Table 1.

Table 1

Results and discussion

Optimization of LC–MS conditions

Because of the wide variety of molecular structures of herbicides, the development of a considerable number of chromatographic separation methods was necessitated for their successful analysis. The composition and mobile phase additives not only affect the retention time and peak shape of the target compound, but also affect their ionization efficiency, thus affecting the sensitivity. Acid herbicides remain poorly in chromatographic column with water as mobile phase, showing poor repeatability and retention time shift due to their slight solubilities in water. Only using water–acetonitrile as mobile phase and gradient elution program in Table 1 a total of 22 triazine and phenylurea herbicides could be resoluted, showing good peak shape, and two pairs isomers of sebuthylazine/terbuthylazine and erbuthylon/secbumetone can be also separated. Using the mobile phase of 5 mM ammonium acetate aqueous solution containing 0.1% v/v formic acid–acetonitrile and the gradient elution program, other 29 herbicides could be well remained, but 50 mM ammonium acetate aqueous solution containing 0.1% v/v formic acid–methanol could not be used as mobile phase due to that methanol and acidic herbicides formed ester.

Using flow injection pump for continuous sampling, mass spectrometric conditions of each herbicide were optimized. The result of full scans in positive and negative ion modes showed parent $[M-H]^-$ ion of 7 herbicides and parent $[M+H]^+$ ion of 44 herbicides with high response, so that positive ion mode and negative ion mode were used. According to the different retention times and

response of compound, 4 channels and positive ion mode for triazine and phenylurea herbicides and 6 channels for the others (1 negative ion mode and 5 positive ion mode) were set up. The collision energy of different target compounds was optimized. The experiment selected higher abundance of parent/daughter ion for monitoring to ensure that each peak has at least 15 collection points. The observed retention time, parent ions, and daughter ions are listed in Table 1. Chromatograms of 22 herbicides using 4 channels and chromatograms of 29 herbicides using 6 channels including total ion chromatogram and extracted ion chromatogram are shown in Fig. 1 and Fig. 2, respectively. MS spectra with the fragmentation mechanism for three representational herbicides are shown in Fig.3, which is nicosulfuron of sulfonylurea herbicides, terbuthylazine of triazine herbicides, and oxadiazon of heterocyclic herbicides.

Fig. 1 Fig. 2 Fig. 3

Matrix effect

Matrix effects are a major concern in biological analysis. They can be a serious problem as they can severely compromise qualitative and quantitative analysis of the target compounds at trace levels as well as method reproducibility, especially when electrospray ionization is used. In the study, most of the 51 herbicides were expected that the signal responses from the compounds were slightly suppressed, or enhanced owing to the matrix effect.

In this work, serum sample preparation was carried out by one-step protein precipitation and analyte extraction. The effect of acetonitrile, methanol and acetone on matrix effect was evaluated. Two types of test solutions (A—standard solution in solvent, B—standard solution prepared with the extract from blank serum with solvent) were used to measure matrix effect. Quantitative matrix effect was delegated with Matrix Factor (MF),[18] which is defined as a ratio of peak area between B and A. The initial test showed that use of methanol as solvent showed very low recoveries for some acid herbicides possibly due to that some acid herbicides with methanol formed ester. Use of the extract obtained with acetone for most of the studied herbicides resulted in highest suppressive matrix effect probably due to the effect of co-extractives. It was known that acetonitrile is a more potent organic solvent for eliminating proteins from serum samples, which could also reduce matrix effect. MF data in Table 2 shows the MF values ranged from 0.86 to 1.09 when used 200 μL of acetonitrile for 100 μL serum sample. It is indicated that there was definite signal enhancing or suppression effect for

different analytes. A highest value of the suppression was 14% for terbuthylazine, and highest enhancing value was 9% for clodinafop-propargyl and qxyfluorfen. In order to obtain more reliable results, matrix-matched standard calibration curve was used for quantification in this work.

Table 2

Linearity

The matrix-matched standard calibration was performed using linear regression based on the peak-area of the target compounds and their at least seven concentrations in the range of 0.05–10 μg L^{-1}. The correlation coefficient (r^2) of linear calibration curves was given in Table 3. Three representational matrix-matched standard calibration curves are shown in Fig.4. A linear range for all herbicides were 0.1–10.0 μg L^{-1} with r^2 of 0.9922-0.9999, except for oxyfluorfen, oxadiazon and phenoxy acid herbicides (MCPA, CPA, CPPA, PBA, and mecoprop) linear range was 10.0–100.0 μg L^{-1} with r^2 of 0.9912-0.9999 due to lower sensitivity.

Table 3 Fig. 4

Sensitivity

The LOD was defined as the lowest concentration giving a response of three times the average baseline noise defined from five unfortified samples. The LOQ was determined as the lowest amount of a given herbicide that could be measured with an accuracy. The determined LODs and LOQs are listed in Table 3. The LODs ranged from 0.03 μg L^{-1} (dimethachlor, metazachlor, propachlor, carbamothioic, and esprocarb) to 6 μg L^{-1} (oxadiazon and oxyfluorfen). The low LOD values of these herbicides in serum can meet the requirements of clinical diagnosis.

Intra and inter day precisions

QC samples were prepared and analyzed seven times in one day and one time per one day in seven consecutive days. Table 3 shows a summary of the intra- and inter-day precisions (RSDs). The intra-day RSDs ranged from 1.02% to 10.0% at three different spiked concentrations. The inter-day RSDs ranged from 1.09% to 12.0%. These results indicate that the present method has satisfactory precision and repeatability.

Recovery

For a long time, poisoning from herbicide has not occurred in Hebei Province of China. Recently, we help hospital detect if there was herbicide in a patient's serum sample by the proposed method. The analytical result showed that no 51 herbicides studied were detected，and the contents of studied all

herbicides in the serum sample are lower than their LOQs level. To examine the method accuracy, recovery test was carried out at three spiked level. The data in Table 4 showed that the recoveries of 51 analytes were 63.6–107% with the RSD of 1.06–12.0% at the LOQ spiked level, 70.2–105% with the RSD of 1.31–9.46% at the 5 times LOQ spiked level, and 72.0–109% with the RSD of 1.18–9.01% at the 10 times LOQ spiked level. So recoveries of 51 herbicides ranged from 63.6% to 109% at the three spiked levels with the RSDs of 1.06–12.0%. It is indicated that the present procedure has good extraction recoveries.

Table 4

Conclusion

A rapid and reliable LC-MS method was developed for multi-classes multi-residues determination of herbicides in human serum using protein precipitation as the sample clean-up procedure. This method exhibited acceptable linearity, selectivity, sensitivity, precision, and recovery for the determination of 51 herbicides in the serum samples. It has a satisfactory proposal in application of simulation of poisoning samples. This method is simple, accurate, and useful for the determination of herbicides, and can be used to clinical analysis and diagnosis.

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Fig.1 Chromatograms of 22 herbicides using 4 channels and gradient elution with water–acetonitrile as mobile phase

(A) blank serum sample; (B) serum sample spiked with herbicides (5.0 μg L^{-1}); (C) total ion chromatogram of standard solution of 5.0 μg L^{-1} for each herbicide; (D) extract ion chromatogram of standard solution of 5.0 μg L^{-1} for each herbicide

1— hexazinone, 2— cyanazine, 3— tebuthiuron, 4— metribuzin, 5— atratone, 6— metamitron, 7— diuron, 8— fluometuron, 9— monoliruron, 10— isoproturon, 11— secbumetone, 12— prometon, 13— atrazine, 14— desmetryne, 15— sebuthylazine, 16— terbuthylazine, 17— prometryn, 18— linuron, 19— methoproptryne, 20— propazine, 21—terbuthylon, 22— atrazinedesethy

Fig.2 Chromatograms of 29 herbicides using 6 channels and gradient elution with 5 mM ammonium acetate aqueous solution containing 0.1% v/v formic acid–acetonitrole as mobile phase

(A) blank serum sample; (B) serum sample spiked with herbicides (5.0 μg L^{-1}); (C) total ion chromatogram of standard solution of 5.0 μg L^{-1} for each herbicide; (D) extract ion chromatogram of standard solution of 5.0 μg L^{-1} for each herbicide

Peaks: 1—4-phenoxybutyric acid, 2—4-chlorophenoxyacetic acid, 3—MCPA, 4—bromoxynil, 5—mecoprop, 6—bentazone, 7—2-(4-chlorophenoxy) propinonic acid, 8—allidochlor, 9—lenacil, 10— triasulfuron, 11—nicosulfuron, 12—propachlor, 13—clomazone, 14—dimethachlor, 15— metazachlor, 16—iodosulfuron-methyl-sodium,17—prosulfuron, 18—cyclosulfamuron, 19—primisulfuron-methyl, 20—cycloate, 21—carbamothioic, 22—thenylchlor, 23—clodinafop-propargyl, 24—diflufenican, 25—esprocarb, 26—tri-allate, 27—oxadiazon, 28—oxyfluorfen, 29—dithiopyr

Fig. 3 MS spectra with the fragmentation mechanism for nicosulfuron, terbuthylazine and oxadiazon

Fig. 4 Three representational matrix-matched standard calibration curves of nicosulfuron, terbuthylazine and oxadiazon

Table 1 MS optimization conditions

Compound	Retention time (min)	Parent ions(*m/z*)	Daughter ions (*m/z*)	Cone voltage (V)	CE(V)
Iodosulfuron-methyl-sodium	6.22	508	141, 167*	25	20,20
Triasulfuron	4.75	402	141, 167*	20	20,15
Nicosulfuron	3.41	411	182*, 213	25	20,15
Cyclosulfamuron	8.20	422	218, 261*	22	26,18
Prosulfuron	7.16	420	141*, 167	25	20,15
Primisulfuron-methyl	8.02	469	199, 254*	24	26,20
Thenylchlor	8.74	324	99, 127*	15	35,10
Dimethachlor	6.20	256	148*, 224	18	26,14
Metazachlor	6.05	278	134*, 210	16	24,10
Diflufenican	10.45	395	246, 266*	30	30,20
Allidochlor	3.69	174	41, 98*	20	20,15
Propachlor	6.03	212	94*, 134	24	28,12
Triallate	11.90	306	86, 145*	26	26,16
Carbamothioic	10.06	258	71, 124.9*	20	18,10
Esprocarb	11.04	266	71, 90.9*	25	20,13
Cycloate	10.20	216	83*, 154	20	15,10
Bentazone	3.91	239	132*, 197	-38	-28,-20
Lenacil	4.22	235	136, 153*	20	30,16
MCPA*	4.49	199	127*, 199	-18	-16,-5
CPA*	3.55	186	127*,186	-20	-12,-5
CPPA*	5.10	199	141*, 199	-18	-16,-5
PBA*	4.30	179	93*, 179	-10	-10,-5
Mecoprop	6.20	213	141*, 213	-18	-14,-5
Dithiopyr	10.93	402	340, 354*	30	20,18
Clodinafop-propargyl	9.80	350	91.1, 266*	25	25,15
Oxyfluorfen	11.15	362	316*, 334	25	13,10
Clomazone	6.35	240	89.1, 125*	25	40,20
Oxadiazon	11.34	345	220, 303*	20	18,13
Bromoxynil	5.03	275.7	78.9, 80.9*	-40	-25,-25
Sebuthylazine	7.94	230	96, 174*	30	25,20
Cyanazine	4.46	241	104, 214*	30	30,15
Terbuthylazine	8.56	230	96, 174*	30	25,10
Atrazinedesethyl	9.31	200	85, 158*	45	25,15
Propazine	8.13	230	146, 188*	30	20,15
Desmetryne	5.84	214	124*, 144	35	20,20
Methoproptryne	7.53	272	198*, 240	30	20,25
Prometryn	9.31	242	158, 200*	35	20,25
Prometon	6.78	226	170, 184*	30	25,20
Terbuthylon	7.39	226	114, 170*	30	25,18
Secbumetone	6.57	226	114, 170*	30	25,18

Atratone	4.90	212	100, 170.1*	30	25,20
Metamitron	2.03	203	145, 175*	30	15,15
Metribuzin	4.55	215	131, 187*	30	20,20
Hexazinone	3.85	253	71, 171*	20	30,15
Atrazine	6.11	216	96.1, 174*	35	25,18
Linuron	8.74	251	162*, 182	20	18,15
Tebuthiuron	3.68	229	116, 172*	25	25,18
Monolinuron	6.40	215	126*, 148	20	15,15
Fluometuron	6.09	233	46.2, 72*	25	18,18
Isoproturon	6.61	207	46, 71.9*	25	15,15
Diuron	6.63	235	46.1, 72.1*	25	15,15

Note: Quantification ion pair is composed of the daughter ion (*m/z*) with (*) sign and its parent ion;
MCPA: 2-methyl 4-chlorophenoxyacetic acid, CPA: 4-chlorophenoxyacetic acid,
CPPA: 2-(4-chlorophenoxy)propinonic acid, PBA: 4-phenoxybutyric acid

Table 2 Matrix effects of serum for 51 herbicides

Serum (μL)	100			200		
Acetonitrile (μL)	100	200	300	200	400	500
Matrix factor	0.49–1.11	0.86–1.09	0.36–1.28	0.55–1.20	0.76–1.02	0.8–1.08

Table 3 Intra- and inter-day precisions of determination of 51 herbicides (n=7)

Compound	r^2	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	Spiked at LOQ level		Spiked at 2.5 LOQ level		Spiked at 7.5 LOQ level	
				Intra-day RSD %	Inter-day RSD %	Intra-day RSD%	Inter-day RSD %	Intra-day RSD %	Inter-day RSD %
Iodosulfuron-methyl-Sodium	0.9974	0.42	1.26	6.51	9.43	5.55	8.34	5.45	8.82
Triasulfuron	0.9948	0.06	0.20	1.99	2.56	1.82	3.01	2.09	1.89
Nicosulfuron	0.9959	0.04	0.10	5.11	6.63	4.32	5.22	4.12	4.99
Cyclosulfamuron	0.9998	0.20	0.60	7.11	10.02	6.92	9.06	7.01	6.29
Prosulfuron	0.9984	0.30	0.90	2.61	2.76	1.90	2.03	1.81	2.39
Primisulfuron-methyl	0.9983	0.40	1.20	5.03	4.95	5.01	5.32	4.29	4.10
Thenylchlor	0.9999	0.04	0.15	7.44	8.46	6.43	7.09	5.03	6.74
Dimethachlor	0.9940	0.03	0.10	6.23	11.50	5.98	9.10	5.09	8.89
Metazachlor	0.9972	0.03	0.10	10.0	9.71	8.23	8.41	5.99	6.58
Diflufenican	0.9998	0.04	0.10	7.11	9.59	7.25	8.42	7.77	9.00
Allidochlor	0.9922	0.05	0.15	6.89	11.00	6.99	7.31	7.01	6.92
Propachlor	0.9998	0.03	0.10	9.11	9.46	7.12	6.06	6.09	7.46
Tri-allate	0.9996	0.56	1.70	3.92	7.24	3.02	4.40	2.89	3.99
Carbamothioic	0.9923	0.03	0.10	4.09	6.71	4.00	3.45	1.99	2.66
Esprocarb	0.9991	0.03	0.10	4.95	8.99	4.09	6.10	3.19	3.20
Cycloate	0.9958	0.15	0.50	2.12	3.08	1.98	2.21	1.67	1.86
Bentazone	0.9996	0.73	2.20	6.33	8.36	5.01	4.45	3.29	4.90
Lenacil	0.9980	0.18	0.60	3.44	7.59	3.09	6.09	3.00	6.31
MCPA*	0.9997	1.92	5.76	2.11	3.44	2.09	4.44	2.31	5.01
CPA*	0.9977	4.00	12.0	7.01	11.61	6.87	9.20	4.03	6.55
CPPA*	0.9993	5.75	17.0	2.11	3.06	2.31	4.11	2.10	2.80
PBA*	0.9912	2.50	7.50	7.36	9.69	4.02	6.07	3.11	5.88
Mecoprop	0.9985	2.54	7.50	3.21	3.93	2.21	2.01	2.00	1.78
Dithiopyr	0.9995	0.30	0.90	4.59	9.84	4.09	6.98	3.76	7.34
Clodinafop-propargyl	0.9980	0.06	0.20	6.00	9.11	4.30	8.00	4.71	8.21
Oxyfluorfen	0.9995	6.00	20.0	1.21	1.09	1.22	2.00	1.02	1.62
Clomazone	0.9979	0.06	0.20	3.32	5.42	3.11	6.09	2.78	4.79
Oxadiazon	0.9999	5.90	18.0	2.76	4.08	2.36	4.77	2.22	4.01
Bromoxynil	0.9971	0.20	0.50	3.01	8.32	3.11	4.71	2.89	7.22
Sebuthylazine	0.9993	0.10	0.30	4.78	9.76	4.60	8.48	2.11	1.67
Cyanazine	0.9984	0.50	1.50	1.11	1.06	2.18	3.15	2.01	3.09
Terbuthylazine	0.9983	0.10	0.30	3.49	5.88	3.21	7.23	2.77	6.89
Atrazinedesethyl	0.9983	0.05	0.20	4.66	8.75	4.09	8.78	3.68	7.71
Propazine	0.9990	0.10	0.30	3.21	4.76	4.09	9.42	3.10	6.42
Desmetryne	0.9988	0.05	0.20	3.77	6.31	3.02	5.57	3.12	1.84
Methoproptryne	0.9986	0.05	0.20	2.12	3.33	2.01	1.31	1.89	2.96
Prometryn	0.9993	0.05	0.20	2.22	4.02	2.44	6.66	2.12	3.11
Prometon	0.9997	0.10	0.30	3.92	7.56	3.38	7.09	2.66	3.3
Terbuthylon	0.9993	0.06	0.20	4.00	8.00	3.66	3.76	3.29	5.82

Secbumetone	0.9995	0.06	0.20	2.32	2.00	2.48	5.45	2.54	7.54
Atratone	0.9980	0.05	0.20	3.67	8.60	3.27	4.34	3.00	3.86
Metamitron	0.9983	1.00	3.00	5.77	12.02	4.17	6.84	3.66	4.23
Metribuzin	0.9995	1.00	3.00	1.62	1.86	2.53	5.40	2.66	4.74
Hexazinone	0.9984	0.05	0.20	3.98	5.16	3.79	9.46	3.12	4.18
Atrazine	0.9979	0.10	0.30	3.22	8.89	3.00	3.44	2.67	1.75
Linuron	0.9999	1.20	4.00	3.01	5.99	2.76	3.78	3.43	7.08
Tebuthiuron	0.9980	0.08	0.30	2.11	3.03	3.01	7.25	2.01	2.18
Monolinuron	0.9998	0.30	1.00	3.01	6.41	3.33	6.67	3.13	3.15
Fluometuron	0.9999	0.12	0.50	4.77	9.23	3.26	3.92	3.02	4.12
Isoproturon	0.9998	0.10	0.30	4.00	6.29	4.09	9.24	2.11	1.18
Diuron	0.9983	0.10	0.30	5.12	7.48	4.31	6.85	3.09	3.89

*MCPA: 2-methyl 4-chlorophenoxyacetic acid, CPA: 4-chlorophenoxyacetic acid, CPPA: 2-(4-chlorophenoxy)propinonic acid, PBA: 4-phenoxybutyric acid

Table 4 Recoveries and RSDs of the herbicides (n=3)

Compound	Spiked at LOQ level		Spiked at 5 LOQ level		Spiked at 10 LOQ level	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
Iodosulfuron-methyl-sodium	85.1	9.43	81.9	8.34	91.4	8.82
Triasulfuron	92.0	2.56	96.0	3.01	95.2	1.89
Nicosulfuron	89.5	6.63	92.8	5.22	96.1	4.99
Cyclosulfamuron	89.6	10.0	92.8	9.06	103	6.29
Prosulfuron	82.9	2.76	88.3	2.03	90.5	2.39
Primisulfuron-methyl	92.5	4.95	91.2	5.32	98.8	4.10
Thenylchlor	95.0	8.46	94.3	7.09	96.6	6.74
Dimethachlor	91.2	11.5	90.8	9.10	89.2	8.89
Metazachlor	89.2	9.71	90.1	8.41	92.2	6.58
Diflufenican	71.0	9.59	72.5	8. 42	77.7	9.01
Allidochlor	86.0	11.0	93.3	7.31	90.9	6.92
Propachlor	91.2	9.46	102	6.06	109	7.46
Tri-allate	90.1	7.24	89.0	4.40	92.3	3.99
Carbamothioic	88.1	6.71	93.2	3.45	91.0	2.66
Esprocarb	94.5	8.99	96.0	6.10	95.1	3.20
Cycloate	90.9	3.08	91.1	2.21	92.0	1.86
Bentazone	83.9	8.36	88.1	4.45	87.2	4.90
Lenacil	88.1	7.59	90.9	6.09	92.1	6.31
MCPA*	78.6	3.44	76.1	4.44	73.5	5.01
CPA*	88.1	11.6	86.7	9.20	92.3	6.55
CPPA*	78.9	3.06	73.7	4.11	90.1	2.80
PBA*	63.6	9.69	70.2	6.07	72.0	5.88
Mecoprop	69.2	3.93	77.3	2.01	80.0	1.78
Dithiopyr	92.0	9.84	93.1	6.98	101	7.34
Clodinafop-propargyl	89.0	9.11	95.1	8.00	92.3	8.21
Oxyfluorfen	71.5	1.09	77.2	2.00	79.0	1.62
Clomazone	79.1	5.42	83.1	6.09	89.0	4.79
Oxadiazon	89.3	4.08	88.9	4.77	90.0	4.01
Bromoxynil	87.5	8.32	83.6	4.71	91.4	7.22
Sebuthylazine	89.9	9.76	87.7	8.48	88.4	1.67
Cyanazine	83.7	1.06	85.4	3.15	93.4	3.09
Terbuthylazine	88.7	5.88	91.8	7.23	91.7	6.89
Atrazinedesethyl	104	8.75	91.3	8.78	96.8	7.71
Propazine	88.0	4.76	96.2	9.42	93.8	6.42
Desmetryne	89.7	6.31	91.0	5.57	94.6	1.84
Methoproptryne	96.8	3.33	90.8	1.31	93.5	2.96
Prometryn	90.7	4.02	84.4	6.66	94.1	3.11

Prometon	88.1	7.56	93.8	7.09	96.6	3.30
Terbuthylon	93.1	8.00	86.3	3.76	93.8	5.82
Secbumetone	90.9	2.00	89.8	5.45	96.6	7.54
Atratone	92.0	8.60	86.6	4.34	97.2	3.86
Metamitron	69.4	12.0	71.4	6.84	82.8	4.23
Metribuzin	107	1.86	83.9	5.40	88.6	4.74
Hexazinone	103	5.16	105	9.46	101	4.18
Atrazine	77.4	8.89	93.6	3.44	93.8	1.75
Linuron	72.1	5.99	90.6	3.78	103	7.08
Tebuthiuron	87.2	3.03	89.0	7.25	97.1	2.18
Monolinuron	70.1	6.41	91.9	6.67	92.5	3.15
Fluometuron	103	9.23	92.0	3.92	95.6	4.12
Isoproturon	91.3	6.29	90.1	9.24	95.2	1.18
Diuron	80.4	7.48	88.4	6.85	93.8	3.89

*MCPA: 2-methyl 4-chlorophenoxyacetic acid, CPA: 4-chlorophenoxyacetic acid, CPPA: 2-(4-chlorophenoxy)propinonic acid, PBA: 4-phenoxybutyric acid

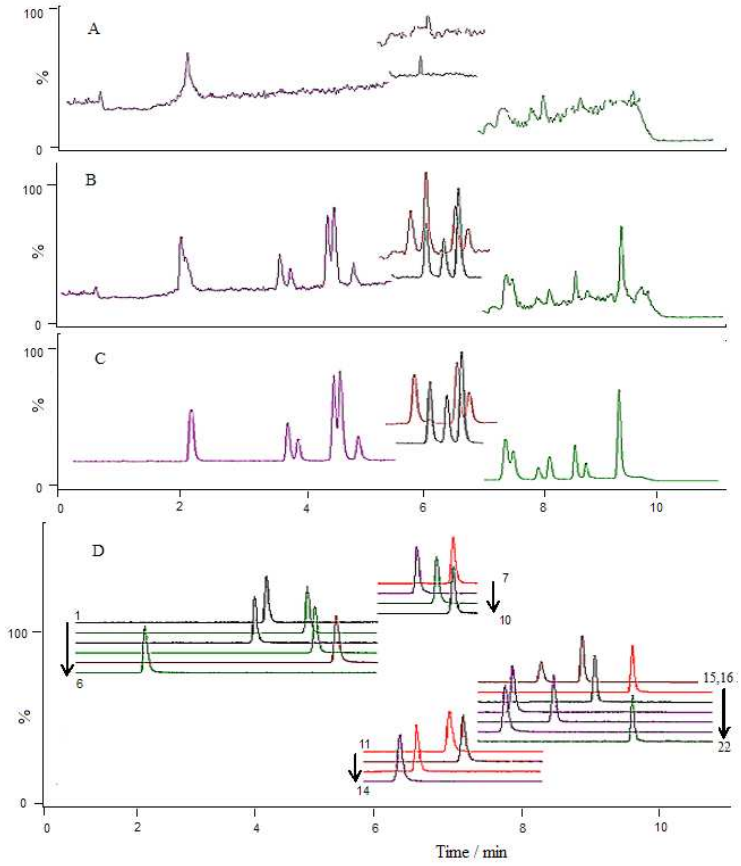

Fig. 1

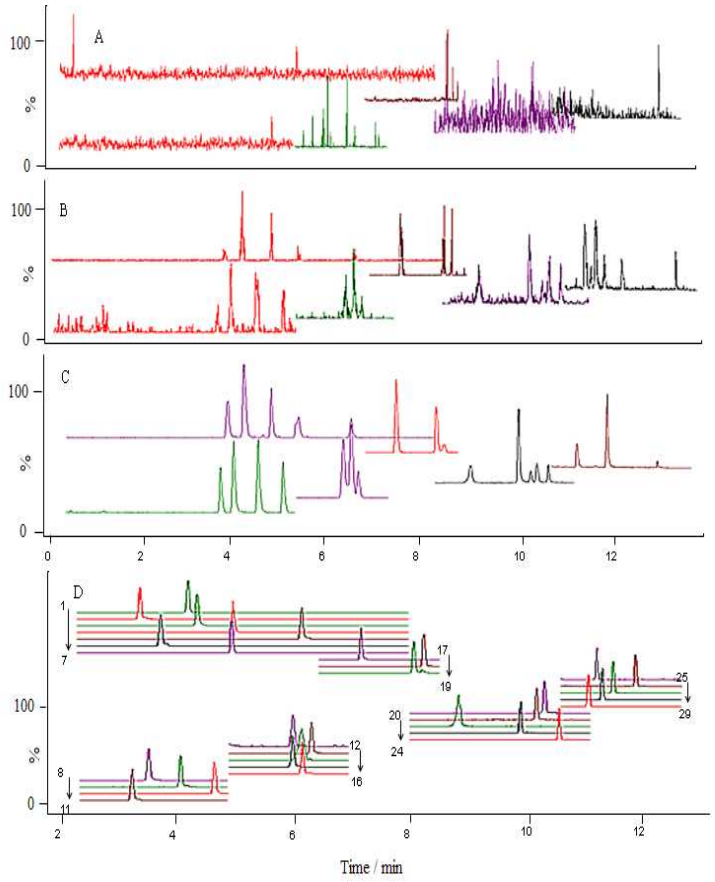

Fig. 2

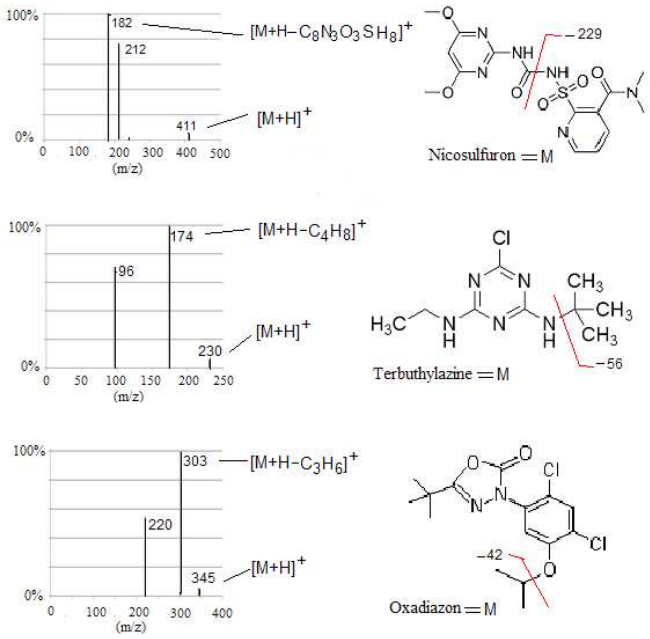

Fig. 3

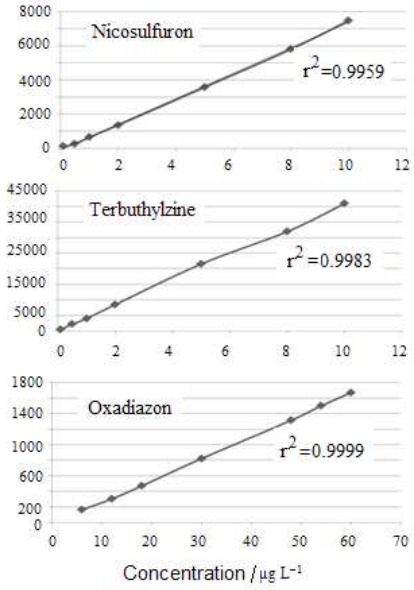

Fig. 4