Analysis of Herbicide Atrazine and Its Degradation Products in Agricultural Soil by Ultra-Performance Liquid Chromatography–Mass spectrometry

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***Abstract*—** *A novel ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) method was developed for the determination of herbicide atrazine (ATR) and its principal metabolites namely deisopropylatrazine (DIA), deethylatrazine (DEA) and hydroxyatrazine (HA) in soils. The limit of detection ranged from 0.06 μg kg^{-1} (DEA) to 0.25 μg kg^{-1} (HA). Recoveries for the four target analytes at three spiked levels ranged from 73.2 to 110% with relative standard deviation of 5.1–8.1%. In the cases of the three control soil samples spiked with ATR were treated for 60d, the sum content of the three degraded products is 3, 6.4, and 6.8 times greater than ATR residue, respectively. Analyzing 80 soil samples from four counties evaluated this method. ATR of 1.1–125 μg kg^{-1} in 80 of 80 samples, ATR of 0.5–7.8 μg kg^{-1} in 39 of 80 samples, and DIA of 0.5 and 0.6 μg kg^{-1} in 2 of 80 samples were found. The proposed method can ensure the rapid and highly sensitive analysis of atrazine and its degradation products in soil, and can provide a direction for proper application of atrazine and a base for evaluating their hazards to the environment.*

Keywords*— *Atrazine residue, degradation products, soil, ultra performance liquid chromatography–mass spectrometry.

I. INTRODUCTION

Herbicide atrazine (ATR) is a pollutant of environmental concern due to acute toxicity, relatively stable nature in environments, Several epidemiological cancer studies concerning ATR and its possible association with carcinogenic effects in humans are being reviewed.[1] The acute toxicity of its metabolites such as deethylatrazine (DEA) and deisopropylatrazine (DIA) was found to be twice as that of ATR.[2] Their presence in the environment has caused public concerns owing to their toxicity to human health. Therefore, it is important to develop a sensitive and accurate analytical method for detecting ATR residue and its degradation products in the environment.

Chromatography was an effective analytical tool for single residue and multi-residue triazine in the environment.[3,4] Currently, gas chromatography (GC) methods[5] and high performance liquid chromatography (HPLC)[6-8] have been used for the determination of triazines in environment and food samples. However, GC or HPLC is not enough to match the routine analysis of interested trace herbicides in complex matrix due to the lack of high detection sensitivity and high identification certainty. In comparison with GC/HPLC, GC/HPLC–mass spectrometry (GC-MS/HPLC–MS) was more frequently used for multi-residue analysis of triazine in the environment. However, there was relative lack of reports for studying the degradation products of ATR herbicide.

ATR and its some degradation products were determined in water.[9-13] A few of works were reported for the determination of ATR and its some degradation products in soil. Hrdlička and Dolinová [14] presented an automated hot solvent extraction and HPLC method for the determination of atrazine and its degradation products in soil from maize fields, with detection limit (LOD) of 4 μg kg^{-1} for ATR and 2 μg kg^{-1} for DEA and DIA. Min et al. [15] reported a sensitive GC-MS method for the determination of ATR in soil, with quantification limit (LOQ) of 0.3 μg kg^{-1} for ATR, 1.0 μg/kg^{-1} for DIA, and 0.8 μg kg^{-1} for DEA. The content of ATR ranged from 0.77 to 10.83 μg kg^{-1}, but DEA and DIA were not detected. Recently, Amadori et al.[16] reported a HPLC method with LOQ of 50 μg kg^{-1} for investigating the content change of ATR, DEA and DIA in the soil spiked with an initial ATR content of 2500 μg kg^{-1} after 15, 30, 45 and 60 days of treatment time under controlled conditions, but no data for content of DEA and DIA in real agricultural soil were reported.

In our previous work, an UPLC–MS method was described for the determination of 50 hebicides in soil sample,[17] but it is unsuited to analyze ATR and its degradation products. The purpose of this paper is to develop a new sensitive UPLC–MS method for effective separation and simultaneous determination of ATR, HEA, HIA and HA. Linearity, sensitivity, precision, and accuracy of the method were validated. To assess its applicability, the proposed method was applied for the analysis of

soil. The detection limits, repeatability, and recoveries achieved meet the needs of trace level monitoring of these compounds in soil.

II. MATERIALS AND METHODS

2.1 Chemicals and Materials

Atrazine (ATR), deethylatrazine (DEA), deisopropylatrazine (DIA), and atrazine-2-hydroxy (HA) (>98.5% for all) were purchased from Dr. Ehrenstorfer Chemical Industries (Augsburg, Germany). Pesticide residue-grade acetonitrile, methanol were purchased from Dikma Science and Technology Co., Ltd. (Beijing, China). Analytical-grade phosphate, dipotassium hydrogen phosphate, and potassium dihydrogen phosphate were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Primary stock solutions of each analyte (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 standard solutions were stored at –20°C in the dark when not in use.

2.2 Instrumentation

UPLC-ESI-MS/MS analyses of soil samples were performed on a Xevo Triple Quadrupole (TQ) system (Waters, USA). This system consisted of an autosampler, a binary pump, a solvent degasser, a BEH C18 stainless-steel cartridge column (100 mm×2.1 mm i.d., 1.7 μm; Waters) 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), an RE-2000A rotary evaporator (Shanghai Yarong Biochemistry Instrument Co.), and a PHS-3C pH meter (Shanghai Precision & Scientific Instrument Co., Shanghai, China) were used in sample treatment.

2.3 Sample Collection and Preparation

A total of 80 soil samples were collected at harvest time in 2014 from 4 counties in Hebei Province of China. All samples were placed in polyethylene bags and transported to the laboratory. The control soils were prepared from the fresh agricultural soil without ATR-applied history.

The initial soil samples were spiked with 10 μg kg^{-1} of ATR and 10% of moisture content. After stir to mix well, the control soil samples were placed in a constant temperature incubator to investigate the degradation within 60d under treatment at 35°C. Soil moisture content was maintained constant throughout the incubation by weighing and correcting for any weight loss by adding sterile deionized water.

A portion of 3 g dried sample (ground-up sieved 40 mesh) was accurately weighed and added into a 50-mL centrifuge tube. The spiked samples were vortexed for 1 min and allowed to stand for 2 h at room temperature to distribute the herbicides evenly and to give them time to interact with the sample matrix. Then, ATR, DEA, DIA and HA were extracted with 12 mL acetonitrile. After vortex-mixing for 1 min and shaking for 10 min, the extracts were centrifuged at 9980×g for 6 min. Then, the supernatant was transferred into a heart-shaped bottle and the residue was again extracted. The extracts were concentrated to just dryness by a rotator evaporator at 25°C. The dried residue was re-dissolved into 0.5 mL acetonitrile/water (20: 80, v/v). The final solutions were filtered through a 0.22 μm filter membrane before LC–MS analysis.

2.4 Conditions of LC–MS Analysis

A mobile phase consisted of acetonitrile and water (v/v, 80:20). Following injection of 5 μL of the sample onto the column, the analytes were eluted using an isocratic mode with a flow rate of 0.3 mL min^{-1}. The mass spectrometer used was a triple quadruple equipped with an ESI interface operating in the positive mode. Quantification was carried out by using matrix matched standards with the external calibration. 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, 0.19 mL min^{-1}. Table 1 shows the ion and collision energy for all analytes.

TABLE 1
MS PARAMETERS FOR DETECTING ATR, DEA, DIA, AND HA

Compound	Retention time (min)	Parent ions(m/z)	Daughter ions (m/z)	Cone voltage(V)	CE (eV)
ATR	4.82	216	96,174	35	25,18
DIA	2.19	174.1	96, 132	25	20,17
DEA	1.51	188.1	104, 146	45	25,15
HA	1.16	198.2	86, 114	30	24,22

III. RESULTS AND DISCUSSION

3.1 Optimization of Extraction and Cleanup Conditions

To achieve the objective for monitoring ATR, DEA, DIA and HA in soil, a sample preparation procedure was investigated. Using acetone as an extraction solvent resulted in a higher suppressive matrix effect due to the highest co-extractives, while using acetonitrile, higher recoveries of triazine, DEA, DIA and HA were observed. The ratio of acetonitrile volume to sample mass was optimized for the extraction of the four analytes by using 3 g blank sample spiked at the 5 LOQ level. The feasible ratio was 4:1 (v/m), over that, the recoveries could not be increased. Thus, 12 mL acetonitrile was enough to quantitatively extract the analytes from 3 g of samples.

In general, the cleanup is required. SPE can be used in the pesticide residue analysis. In this study, efficiencies of cleanup were compared on a ProElut C18 cartridges (1000mg/6mL) using acetonitrile as eluting solvent. In the case of soil samples, recoveries for the four analytes were similar both with and without SPE cleanup step. So the extract of soil samples did not clean up further.

3.2 Optimization of UPLC Separation

Chromatographic conditions were optimized to obtain good resolution, stronger herbicide signal, and shorter analysis times. The mobile phase composition has a significant effect on peak shapes and the retention behavior of the analytes in the LC column, as well as on the MS response. To find suitable conditions for the separation of these compounds using the C18 column. several mobile phases were evaluated. The final results demonstrated that the mobile phase composed of acetonitrile–water (v/v, 80:20) provided the determination of DIA, DEA, AT, and HA using the isocratic mode. The influence of the flow rate was investigated at 0.2, 0.3, and 0.45 mL min^{-1}. At 0.45 $mLmin^{-1}$, the signal peak of all analytes was the highest, but this flow rate led to poor retention time and resolution of coexistent ions. A flow rate of 0.2 mL min^{-1} showed the lowest peak height and a longer analytical time. Thus, a flow rate of 0.3 mL min $^{-1}$ was selected. The data in Table 1 show that the retention times (min) of HA(1.16), DEA(1.51), DIA(2.19) and ATR(4.82) were lower due to their high polarity.[16] Figure 1 shows the chromatograms of atrazine and its degradation products in the control soil with 10 μg kg^{-1} atrazine and 10% moisture under treatment for 60d at 25 °C. Under the selected condition, all analytes could be separated within 5 min, and appropriate resolution between the HA, DIA and DEA peaks was obtained. It is shown that using a BEH C18 stainless-steel cartridge column and isocratic elution mode with acetonitrile–water as a mobile phase can achieve effective chromatographic separation.

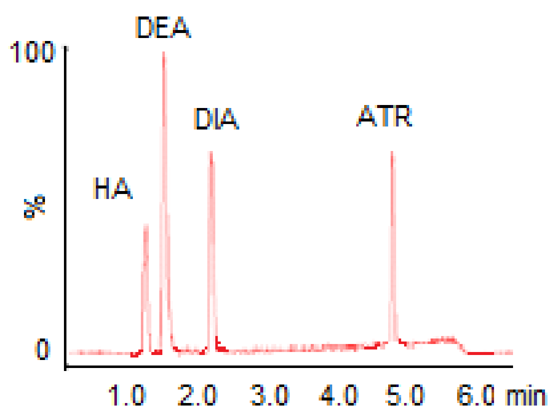

FIG. 1. CHROMATOGRAMS OF ATRAZINE AND ITS DEGRADATION PRODUCTS IN THE CONTROL SOIL WITH 10 mg kg^{-1} ATRAZINE AND 10% MOISTURE UNDER TREATMENT FOR 60d at 25 °C

3.3 Optimization of MS Detection

Electrospray ionization–MS is a low energy ionization technique that is ideal for easily ionizable biological molecules, as molecular ions can be formed in the source of the mass spectrometer without fragmentation. Electrospray ionization (ESI) involves the dispersion of a liquid containing analytes of interest into a fine aerosol by applying a high potential difference to the sample solution with respect to a counter electrode. Both electrospray ionization ESI^+ and ESI^- modes were tested for the best ionization. The triple quadruple analyzers, which provided high mass resolution and high mass accuracy, were used for

multi-residue screening. Multiple reaction monitoring (MRM) parameters for the analytes were systematically optimized. The parent ions $[M+H]^+$ and $[M–H]^−$ were observed by infusing a 0.1 mg $L^{−1}$ individual standard solution in water/acetonitrile (50:50, v/v) at a flow rate of 5 mL $min^{−1}$. The result of full scans in positive and negative ion modes showed a parent $[M+H]^+$ ion of the analytes with high response, so that positive ion mode were used. In addition, the MRM was optimized to achieve the highest sensitivity and the optimum value of normalized collision energy. Parent ions and daughter ions for the analytes are listed in Table 1. In general, no daughter ion was observed at low collision energy; only the parent ion was observed. However, the daughter ion's signals were increased and the parent ion signal was decreased at high collision energy. Qualification was performed with the exact mass of the parent ion together with the exact mass of the daughter ion with highest signal.

3.4 MS Spectra Analysis

Fragment ion of ATR, HA, DEA and DIA were observed. Their mass spectra with main fragmentation pathways are shown in Fig. 2.

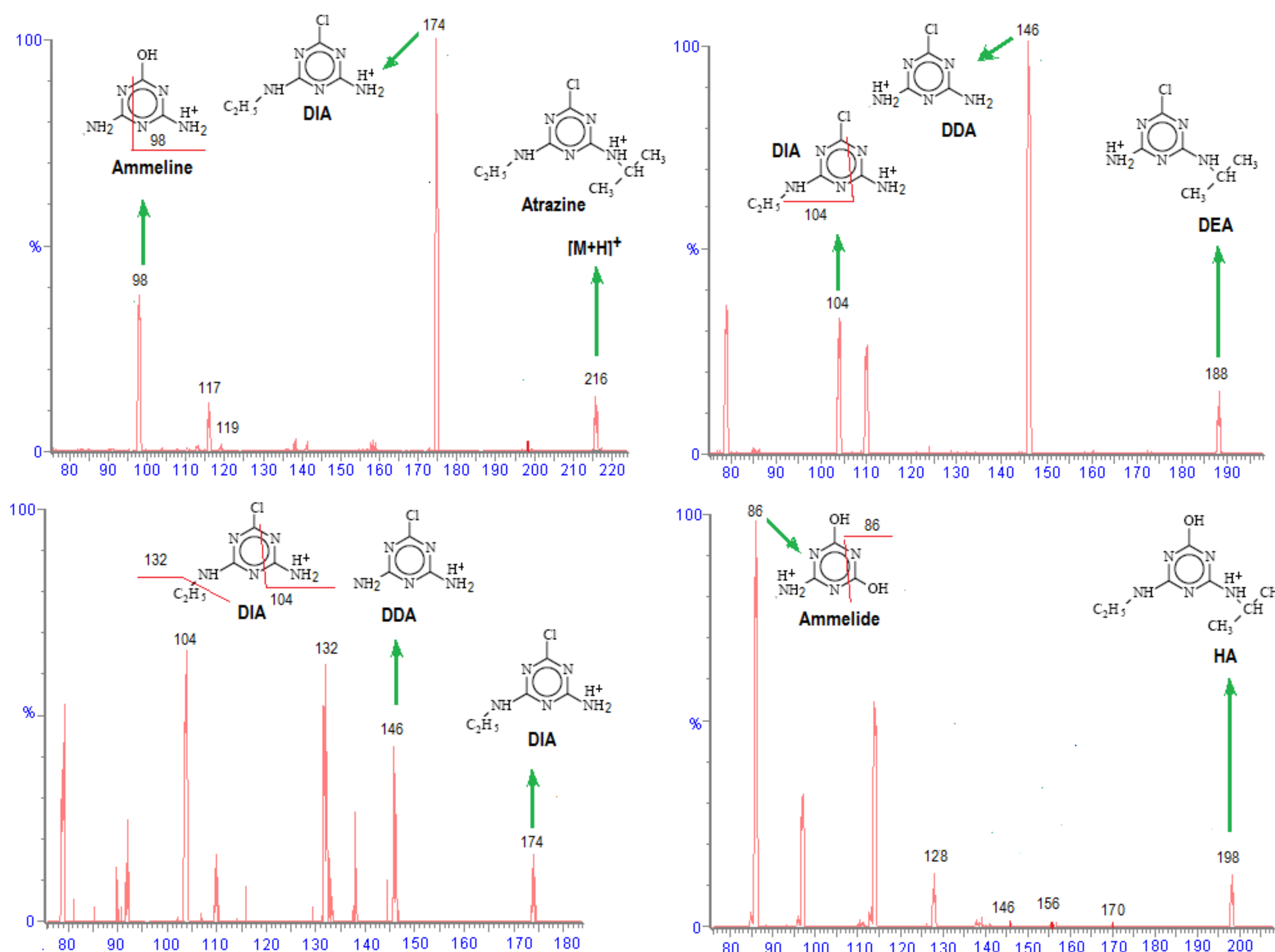

FIG. 2. MS SPECTRA WITH THE FRAGMENTATION MECHANISM FOR ATRAZINE AND ITS DEGRADATION \ PRODUCTS

Their parent ion $[M+H]^+$, m/z=216 for ATR, m/z=198 for DEA, m/z=188 for DIA, and m/z= for HA were observed, showing higher response. A daughter ion $[M+H–Cl+OH]^+$ (m/z 198) of ATR was found with OH (17 u) instead of Cl (35.5 u), which corresponded to the parent ion $[M+H]^+$ of HA; a daughter ion $[M+H–C_2H_4]^+$ ion (m/z 188) of ATR was observed with the loss of C_2H_4(28u) from the parent ion(216u), which corresponded to the parent ion $[M+H]^+$ of DEA; and another daughter ion $[M+H–C_3H_6]^+$ (m/z 174) was also found, which corresponded to the parent ion $[M+H]^+$ of DIA. It is shown in Figure 2 that these daughter ions of atrazine could be further degraded to some fragment ions, for example, DEA degraded to dealkylated hydroxymetabolites of didealkylatrazine (DDA, m/z 146), and DIA degraded to fragment ions m/z 132 and m/z

104. Otherwise, fragment ions m/z 98 and m/z 86 were suggested from the degradation of ammeline and ammelide, respectively.

3.5 Matrix Effect

Matrix effects in LC–MS with electrospray ionization source are very important for the determination of herbicides in different matrices. They can severely compromise quantitative analysis of the compounds at trace levels, as well as greatly affect the method reproducibility and accuracy. To evaluate this possible effect, two types of test solutions (A: Analyte standard solution prepared with solvent; B: Analyte standard solution prepared with the extract from blank matrices) were used to measure matrix effect. The results show that no interference was detected at the expected retention time. However, the analyte concentration changed, showing enhancing. Figure 3 summarizes the matrix effect for each analyte.

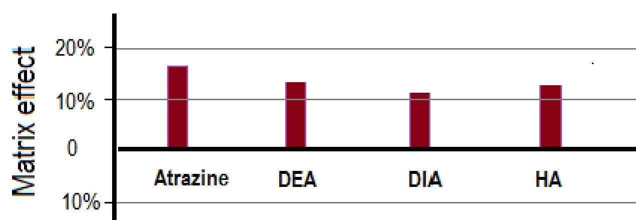

FIG. 3. MATRIX EFFECTS OF ATRAZINE AND ITS DEGRADATION PRODUCTS IN SOIL

Enhancing effect for ATR, DEA, DIA and HA in soil was observed, and a highest enhancing effect for ATR was 15%. To obtain high accuracy, quantification was performed using matrix-matched external standards calibration. In addition, the matrix effects on the accuracy of mass measurements were also evaluated at the concentration of 0.1 mg L^{-1} of each analyte. No significant differences were observed in the mass accuracies obtained in the matrix-matched standards compared to those prepared with pure solution. The results demonstrated that the quantification of these analytes using a matrix-matched external standard calibration curve is the most effective method of reducing indirect matrix effects present in this method.

3.6 Validation of the UPLC-MS Method

Linearity and detection limit. Under the optimized conditions, the linearity of matrix-matched external standards calibration curves was evaluated by determining the four analytes. The linearity range, linear regression equations, and correlation coefficients (r^2) were obtained (Table 2) The response was linear over two orders of magnitude with a correlation coefficients (r^2) of 0.9921–0.9984.

TABLE 2
ANALYTICAL PERFORMANCE OF THE METHOD

Analyte	Linearity range (μg kg^{-1})	Regression equation	Correlation coefficient (r^2)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
ATR	0.4–40	A=9670.3C–2769	0.9975	0.10	0.4
DEA	0.2–20	A=21282C–3476	0.9937	0.06	0.2
DIA	0.4–40	A=873.68C+162.8	0.9984	0.10	0.4
HA	0.8–80	A=4905.5C–2881	0.9921	0.25	0.8

The limit of detection (LOD) was determined as the sample concentration that produced a peak with a height three times the level of the baseline noise, and the limit of quantification (LOQ) was calculated as the sample concentration that produced a peak with a height 10 times the signal to noise. The method LOD for ATR, DEAQ, DIA, and HA was 0.10, 0.06, 0.10, and 0.25 μg kg^{-1}, and LOQ was 0.4, 0.2, 0.4, and 0.8 μg kg^{-1}, respectively. The sensitivity of the method is higher than that in recent literatures for the determination of ATR and its degradation products in soil.[14-16]

Precision. The intra-day precision (RSD) was measured by analyzing spiked samples with 10 μg kg^{-1} for each analyte for seven times within a day, and the inter-day precision was determined by analyzing the spiked samples for a time per a day within seven days. The intra- and inter- day RSDs for the four analytes were 5.2–8.3% and 8.7–16.6% for soil sample, respectively.

Recovery. Evaluation of recoveries and RSDs was performed to validate the UPLC-MS method by blank soil samples at three spiked levels.

TABLE 3
COVERANGE RECOVERY AND RSD OF ATR,DEA, DIA, AND HA IN SOIL

Analyte	Spiked level (μg kg^{-1})	Coverage recovery(%)	RSD,n=3 (%)
ATR	1	87.1	7.8
	5	99.4	6.2
	10	96.0	5.1
DEA	1	73.2	8.2
	5	88.0	7.7
	10	112	5.1
DIA	1	84.2	8.1
	5	89.9	6.8
	10	97.7	5.9
HA	1	77.8	7.9
	5	86.2	7.7
	10	110	5.7

Table 3 compared that the recoveries of ATR, DEM, DIA, and HA from soil at three spiked levels varied from 73.2 to 112 % with the RSD of 5.1–8.2%.

It is indicated the method has high sensitivity, repeatability, and accuracy for the quantification of ATR, DEA, DIA and HA in soil. The validated method can ensure the rapid and highly sensitive analysis for atrazine and its degradation products in soil.

3.7 Sample Analysis

To assess the applicability of the validated method, the effectiveness of this method in measuring trace levels of the analytes was evaluated by analyzing 3 control soil samples, and 80 soil samples from 4 counties.

ATR residue in the control soil samples with 10% moisture at different treatment times under 35°C was determined in triplicate. Three degraded products, DEA, DIA, and HA, were found firstly when treatment for 5d, 10d, and 15d, respectively. When treatment for 60d, the contents of ATR, DEA, DIA, and HA in the control soils were 440, 385, 601, and 336 μg kg^{-1} for Soil 1#, 370, 410, 1430, and 529 μg kg^{-1} for Soil 2#, and 410, 388, 1420, and 691 μg kg^{-1} for Soil 3#. In the cases of the three control soils, the sum content of the three degraded products is 3, 6.4, and 6.1 times greater than ATR residue, respectively. This should be caused for more concern due to that these degraded products have similar or higher toxicity than ATR residue.

The found positive soil samples were identified by comparing retention time and the exact mass of the parent ion together with the exact mass of the daughter ion with highest signal. The quantification of these analytes was carried out using a matrix-matched standard calibration curve. The result is given in Table 4.

TABLE 4
DETERMINATION OF ATR, DEA, DIA, AND HA IN SOIL

Region	ATR		DEA		DIA		HA	
	Positive sample number	Content range (μg kg^{-1})	Positive sample number	Content Range (μg kg^{-1})	Positive sample number	Content range (μg kg^{-1})	Positive sample number	Content range (μg kg^{-1})
ZanHuang county	20	1.1–125	6	0.5–7.8	1	0.6	20	nd
LuQuan county	20	2.5–10.9	8	0.7–4.9	1	0.5	20	nd
GaoCheng county	20	1.1–8.5	9	0.5–2.3	20	nd	20	nd
Wuji county	20	4.6–63.4	16	0.9–3.1	20	nd	20	nd

Note: 20 samples for each county; mean content (n=3, RSD≤20%); .nd, not detected

It is shown that ATR residue of 1.1 to 125 μg kg^{-1} was detected for all 80 soil samples from 4 counties. DEA residue of 0.5–7.8 μg kg^{-1} was detected for 39 of 80 soil samples; and DIA residue of 0.5 and 0.6 μg kg^{-1} was detected for 2 of 80 soil samples.

IV. CONCLUSION

Using acetonitrile can effectively extract atrazine and its degradation products from soil. Using a BEH C18 stainless-steel cartridge column and isocratic elution mode with acetonitrile–water (v/v, 80:20) as a mobile phase can achieve effective chromatographic separation within 5min. The validated method can ensure the rapid and highly sensitive analysis for atrazine and its degradation products in soil. The sum content of the three degraded products (DEA, DIA and HA) is 3–6.4 times greater than atrazine residue. This should be caused for more concern due to that these degraded products have similar or higher toxicity than atrazine residue. The developed method provides an effective, sensitive and accurate analytical base for investigating their distribution and degradation as well as evaluating their hazards to the environment and human health.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 21375032).

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