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Simultaneous Residue Measurement of Pendimethalin, Isopropalin, and Butralin in Tobacco Using High-Performance Liquid Chromatography with Ultraviolet Detection and Electrospray Ionization/Mass Spectrometric Identification

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A simultaneous residue analysis of pendimethalin, isopropalin, and butralin in tobacco was developed with high-performance liquid chromatography with ultraviolet monitoring and electrospray ionization mass spectrometry (HPLC-UV-ESI/MS). The herbicide residues of pendimethalin, isopropalin, and butralin in tobaccos were extracted by ultrasonication with ethyl acetate, followed by a cleanup procedure with gel permeation chromatography. The three herbicides were separated within 15 min using a LiChrosorb 18 column with methanol−10 mmol/L ammonium acetate (85:15, v/v) as the eluent. The limits of quantitation, using HPLC-UV, were ca. 0.05, 0.08, and 0.06 mg/kg for pendimethalin, isopropalin, and butralin, respectively, whereas the overall recoveries ranged from 77.5 to 91.8%. The proposed method has been successfully applied to measure 300 real samples, and the residue profiles of three herbicides in tobacco samples were obtained and evaluated.

KEYWORDS: HPLC; LC-MS; tobacco; residue; pendimethalin; isopropalin; butralin

1. INTRODUCTION

Pendimethalin, isopropalin, and butralin are selective, preemergence, dinitroaniline herbicides. They have been widely used to control annual grasses and broadleaf weeds in a large variety of fruit trees, nuts, vegetables, green crops, etc. For food and environmental safety, the detailed investigation into the residue and metabolism of these herbicides is very important. Pendimethalin, isopropalin, and butralin in environmental water, air, and soil have been widely analyzed by gas chromatography (GC) (*1*−*5*) and high-performance liquid chromatography (HPLC) (*6*−*8*). Other methods, such as enzyme-linked immunosorbent assays, spectrophotometry, and electrochemistry, have also been reported (*9*−*11*). However, the simultaneous residue analyses of pendimethalin, isopropalin, and butralin in plant species such as tobaccos have not been extensively documented (*12*).

Tobacco is greatly consumed by smokers throughout the world. The pesticide residue in tobaccos might be potentially harmful to smokers' health. With this in mind then, the residue determination and control of pendimethalin, isopropalin, and butralin in flue-cured tobacco leaves are very important for tobacco products and consumers. For the complex tobacco samples, GC with photometric detection and HPLC with UV detection are subjected to matrix interference, which makes the quantification and identification of these herbicides difficult. In such cases, the removal of the matrix effects and the identification of the target compounds are of great importance. The present paper reported the extraction and cleanup procedures, as well as the chromatographic conditions developed for the simultaneous determination of pendimethalin, isopropalin, and butralin residues in the flue-cured tobacco leaves from different sources, using HPLC-UV-electrospray ionization mass spectrometry (ESI/MS).

2. MATERIALS AND METHODS

2.1. Chemicals and Solutions. Unless specified otherwise, all chemicals were residue grade and were obtained from Siyou Biomedical and Chemical Factory (Tianjin, China). Methanol was HPLC grade and purchased from Beijing Chemical Factory (Beijing, China). Water was purified using a Milli-Q system. Pendimethalin, isopropalin, and butralin (>99.0%) were purchased from Dr. Ehrenstorfer Ltd. (Augsburg, Germany). All of the samples were filtered through a cellulose acetate membrane filter (0.45 μm) before HPLC runs.

Stock solutions of each herbicide were prepared in methanol, and the final concentrations were 100 μg/mL. Quantification of samples

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was made using calibration curves of the three herbicides at the final concentrations of 0.5, 1, 2, 5, 10, 25, and 50 μg/mL. Each determination was performed in triplicate.

2.2. Apparatus and HPLC-ESI/MS Conditions. HPLC conditions were optimized using a Shimadzu LC-6A apparatus equipped with a UV/vis detector and CR-4A processor. The HPLC-ESI/MS was performed on an Agilent 1100 series HPLC (Agilent Co., Germany) and an Esquire 3000 ESI/MS system with an ion trap mass spectrometer (Bruker Daltonik Gmbh, Germany).

The separation was carried out on a Supelco LC-18 column (250 mm × 4.6 mm i.d., 5 μm). The mobile phase was CH_3OH−10 mmol/L NH_4Ac (85:15, v/v), at a flow rate of 0.8 mL/min, and the detection wavelength was at 235 nm. The injection volume was 20 μL. The mass spectrum was performed with positive ESI (+ESI). The spray temperature was 300 °C. The voltage was −4.0 kV. The nebulizer pressure was 20 psi. The nitrogen flow rate was 9 L/h. Before the eluate from HPLC was introduced into the mass spectrometer, the flow rate split was 25:1. The software used included Bruker Daltonics EsquireControl 5.xx, DataAnalysis 2.00, and Agillent ChemStation A.07.

2.3. Tobacco Pretreatment. Two hundred grams of the flue-cured tobacco leaves (without peduncle) was chopped and crushed to produce the tobacco powder at 40 mesh. Pendimethalin, isopropalin, and butralin were extracted with 100 mL of ethyl acetate by ultrasonic extraction (UE) for 30 min. Then, a 50 mL volume of the filtrate was transferred to a 250 mL flask with a 10 mL graduated bottom and was evaporated to 10 mL under vacuum. After it was filtered through a Nylon membrane filter (0.45 μm), a 5 mL filtrate was injected and cleaned by gel permeation chromatography (GPC). GPC was performed on a 400 mm × 25 mm i.d. Bio-Bead S-X3 column with cyclohexanes−ethyl acetate (1:1, v/v) at 5 mL/min. The fraction between 25 and 40 min was collected and transferred, and it was evaporated to near dryness under vacuum. The residue was dissolved with 1 mL of methanol−water (1:1, v/v) solution under ultrasonication. The 1 mL solution was filtered with a 0.45 μm membrane for HPLC analysis.

2.4. Recovery. The described method for the sample pretreatment was validated by recovery investigation. The standards of pendimethalin, isopropalin, and butralin were added to 10 g of tobacco leaf powder to fortify tobacco containing 0.5, 2.0, and 20 mg/kg of pendimethalin, isopropalin, and butralin, respectively. The recovery was determined by the HPLC-UV method according to the complete pretreatment procedures described above.

3. RESULTS AND DISCUSSION

3.1. Selection of Extraction and Cleanup Methods. Three extraction methods were used to extract pendimethalin, isopropalin, and butralin from tobacco leaf powder. These methods were UE, accelerated solvent extraction (ASE), and high-speed homogenizer extraction (HHE). Results showed that recoveries for these herbicides by three extraction methods were over 95%. However, the matrices by ASE and HHE were more complicated, making the subsequent cleanup procedure difficult. In contrast to ASE and HHE, UE was preferential for providing both high extraction recovery and a relatively low level of matrix interference.

The matrices of tobacco were too complex for direct analysis by HPLC. Thus, a further cleanup procedure was necessary. Over the years, several methods, such as liquid−liquid partition (LLP), supercritical fluid extraction (*3*), GPC, and solid phase extraction (SPE), have been conducted to lower the detection limit and remove the matrix interference with high recovery. In this research, we tried to achieve these aims with SPE (C_8 and C_{18} column), LLP, and GPC methods. Using SPE, the recovery was less than 50% and the low capacity of the SPE column also made the matrix removal insufficient. The results demonstrated that it is difficult to find an optimal LLP system for simultaneously obtaining high recoveries for three herbicides. With the GPC method, three herbicides with similar molecular weights can be coeluted within 15 min from 25 to 40 min. It produced >95% recovery for pendimethalin, isopropalin, and butralin. Lipids are eluted in the volume up to approximately 100 mL before the first 20 min, which was discarded to avoid seriously affecting elution behaviors of the three herbicides in HPLC analysis.

Table 1. Chromatographic Behaviors of Three Herbicides[a]

mobile phase	composition (v:v)	retention time (min)	resolution among herbicides	resolution from the matrix
A	40:60	>40		
B	50:50	>40		
	70:30	30–45	√	
	80:20	23–34	baseline separartion	√
	85:15	10–14	baseline separartion	√
	90:10	7.8–8.5	overlap	×

[a] A, CH_3OH/H_2O; B, CH_3OH/10 mmol/L NH_4Ac; column, 250 mm × 4.6 mm i.d. C_{18}; flow rate of mobile phase, 0.8 mL/min; detection wavelength, 235 nm; apparatus, LC-6A HPLC.

Several extraction solvents (in UE), e.g., acetonitrile, acetone, methanol, dichloride methane, and ethyl acetate, were tested. The optimized sample pretreatment procedures (see section 2.3) provided the overall recoveries >77% for the three herbicides without interference.

3.2. HPLC-UV. In our research, we chose CH_3OH as an organic modifier. NH_4Ac was added to the mobile phase to improve peak symmetry. The chromatographic behaviors of the herbicides using different mobile phases were investigated in detail, and the results are shown in **Table 1**. In **Table 1**, the reasonable retention time for three herbicides could be obtained between 10 and 14 min by adjusting the ratio of the CH_3OH and 10 mmol/L NH_4Ac at the ratio of 85:15 (v/v). Under the optimal conditions, three herbicides could be completely separated from the matrices of the tobacco. The typical chromatograms of them on LC-6A HPLC are shown in **Figure 1**.

The linear ranges of the UV response at 235 nm were observed over the concentration range from 0.5 to 50 μg/mL for each pendimethalin, isopropalin, and butralin herbicide. The regressions between peak area (A) and concentration (C, μg/mL) yielded the following equations:

$$\text{for pendimethalin, } A = 87303\,C - 2571 \quad (n = 7,\ R^2 = 0.9998)$$

$$\text{for isopropalin, } A = 30792\,C - 1055 \quad (n = 7,\ R^2 = 0.9996)$$

$$\text{for butralin, } A = 80748\,C + 2661 \quad (n = 6,\ R^2 = 0.9952)$$

The limits of detection (LODs) for pendimethalin, isopropalin, and butralin were found to be 0.13, 0.20, and 0.15 μg/mL by calculating a signal-to-noise ratio of 2 (S/N = 2). These LODs allow pendimethalin, isopropalin, and butralin in a tobacco sample to be detected out at their limits of quantitation of ca. 0.05, 0.08, and 0.06 mg/kg, respectively.

The reproducibility of the method was checked with concentrations of 2.0 and 20 μg/mL for each herbicide. The relative standard deviations (RSDs) of the peak area were 1.1−2.6% (n = 3). RSDs of the retention times ranged from 0.5 to 0.9% (n = 3). The overall recovery of the method was examined by determining three fortified samples by adding each herbicide to the blank tobaccos at three concentration levels, 0.5, 2.0, and 20 mg/kg, respectively. These fortified samples were pretreated according to the procedures described in section 2.3, and their

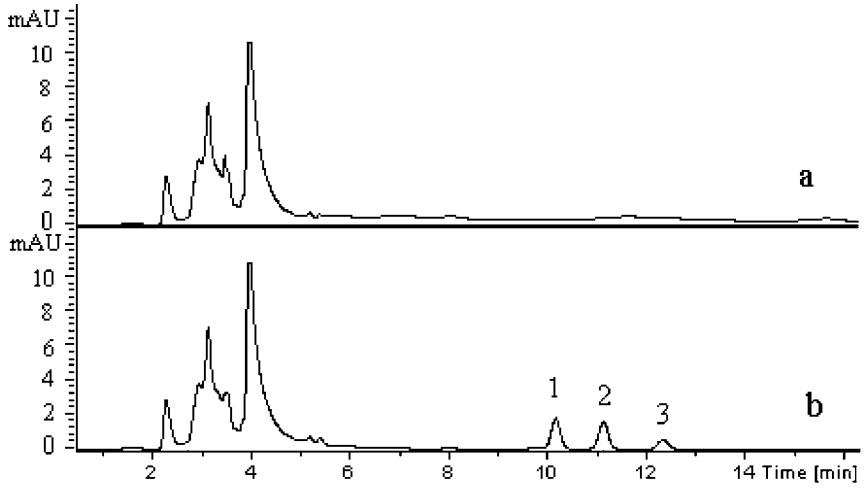

Figure 1. Typical UV chromatograms of pendimethalin, isopropalin, and butralin. Chromatograms: (**a**) Tobacco blank; (**b**) fortified sample. Peaks: 1, pendimethalin (2 mg/kg); 2, butralin (2 mg/kg); and 3, isopropalin (2 mg/kg).

Table 2. Recovery Results of the Method

herbicide	spiked concn	average recovery (%, $n = 3$)	RSD (%, $n = 3$)
pendimethalin	0.5	82.3	2.3
	2.0	80.8	3.1
	20	89.1	1.9
isopropalin	0.5	86.1	2.6
	2.0	83.2	2.1
	20	91.8	2.3
butralin	0.5	79.6	3.3
	2.0	77.5	2.6
	20	84.3	2.4

Table 3. Molecular and Product Ions and Their Relative Abundance Obtained by +ESI/MS and +ESI/MS^2 for Pendimethalin, Isopropalin, and Butralin

herbicide	ions	*m/z*	relative abundance (%)	MS
pendimethalin	$[M + H]^+$	282	33	ESI/MS
(M_W 281.3)	$[M + Na]^+$	304	30	ESI/MS
	$[M + H - C_5H_{10}]^+$	212	100	ESI/MS
parent ion	$[M + H]^+$	282	30	ESI/MS^2
	$[M + H - C_5H_{10}]^+$	212	100	ESI/MS^2
isopropalin	$[M + H]^+$	310	100	ESI/MS
(M_W 309.3)	$[M + H - C_3H_6]^+$	268	10	ESI/MS
parent ion	$[M + H]^+$	310	30	ESI/MS^2
	$[M + H - C_3H_6]^+$	268	100	ESI/MS^2
	$[M + H - 2C_3H_6]^+$	226	20	ESI/MS^2
butralin	$[M + H]^+$	296	15	ESI/MS
(M_W 295.3)	$[M + Na]^+$	318	15	ESI/MS
	$[M + H - C_4H_8]^+$	240	100	ESI/MS
parent ion	$[M + H]^+$	296	16	ESI/MS^2
	$[M + H - C_4H_8]^+$	240	100	ESI/MS^2

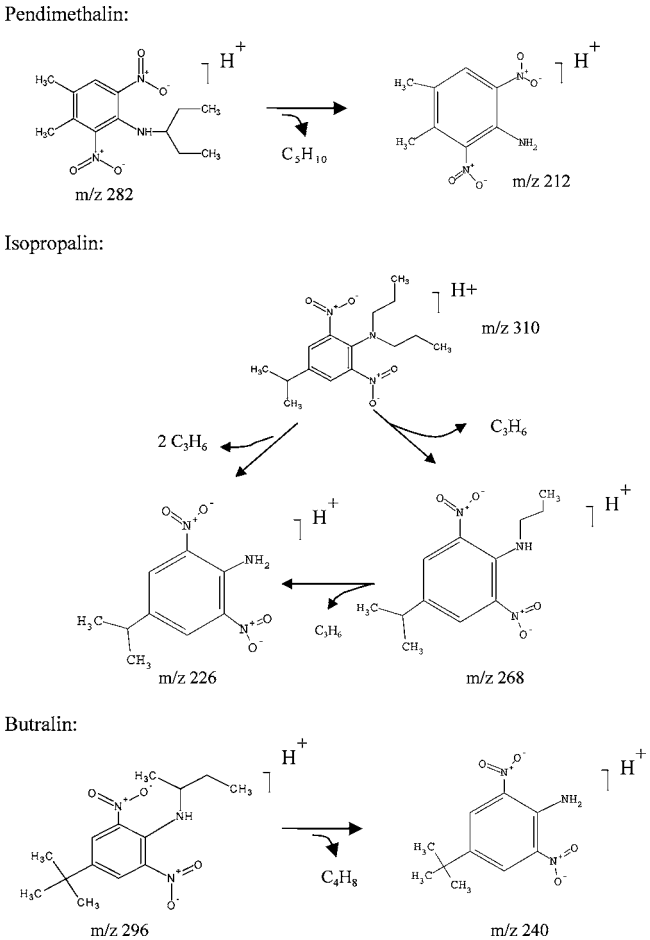

Figure 2. MS cleavage pathways of pendimethalin, isopropalin, and butralin.

overall recoveries ($n = 3$) were obtained and listed in **Table 2**. These data demonstrate that this method is sufficiently accurate, reproducible, and satisfactory for residue analysis of pendimethalin, isopropalin, and butralin in the tobacco samples.

3.3. ESI/MS and HPLC-ESI/MS of Pendimethalin, Isopropalin, and Butralin. Table 3 summarizes the base peak and the most abundant ions (with the relative abundance) of the ESI/MS and ESI/MS^2 of pendimethalin, isopropalin, and butralin. The possible cleavage pathways of these three herbicides are given in **Figure 2**. It can be seen from **Table 3** that the base peak of isopropalin was its protonated molecule, and base peaks of the other two were their fragment ions. Thus, in HPLC-ESI/MS, the ions at *m/z* 212, 310, and 240 were chosen as the specific ions for identification of three herbicides.

Figure 3 shows a total ion chromatogram (TIC) and MS of the fortified sample (containing each herbicide at 2 mg/kg). The symmetric peak shapes of three herbicides can be seen in the TICs. In addition, the responses (intensity) for three herbicides are relatively high. The MS corresponding to their retention times are given in the bottom of **Figure 3**. The specific ions for three herbicides can be clearly observed. For a complex

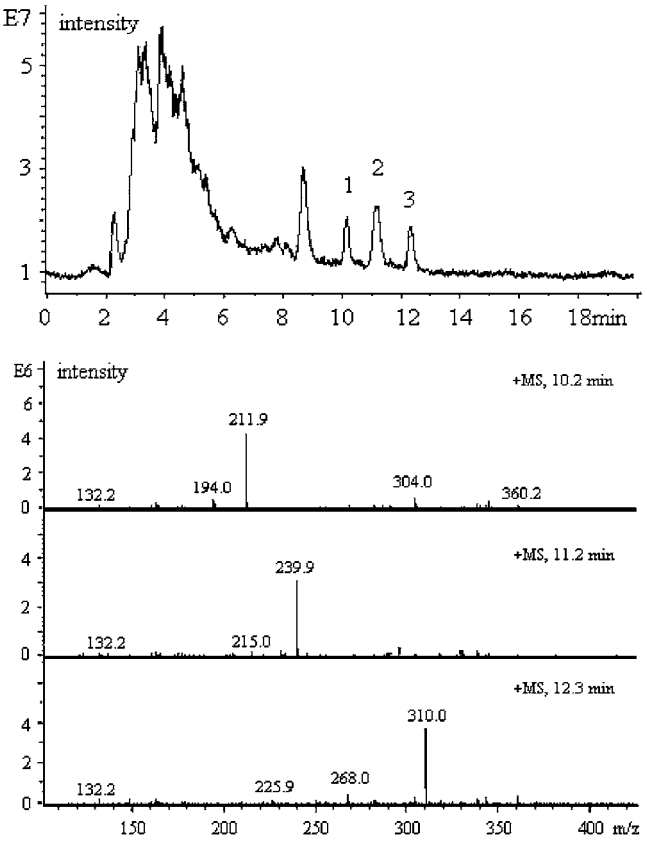

Figure 3. TIC and MS of the fortified sample. Conditions are as described in section 2.2, and the sample is the same as in **Figure 2**. Peaks: 1, pendimethalin (2 mg/kg); 2, butralin (2 mg/kg); and 3, isopropalin (2 mg/kg).

sample like tobacco, the MS identification for the target compounds ensured the determination to be reliable.

3.4. Application to Real Samples. Using the developed HPLC-UV-ESI/MS method, 300 samples, including 239 national and 61 foreign tobacco leaf samples, were determined. Pendimethalin was detected in 138 samples, ranging from 0.08 and 4.23 mg/kg at the average of 2.71 mg/kg. Butralin was found in 76 samples, ranging from 0.07 to 4.83 mg/kg at an average of 0.92 mg/kg, and isopropalin was obtained in nine samples, ranging from 0.07 to 1.24 mg/kg at the average of 0.45 mg/kg. Pendimethalin and butralin have been simultaneously found in 47 samples. These residual herbicides might come from environmental soil and water, as well as from the pesticide administration for control of the field weeds. These residue data near or exceed the tolerable maximum residue limits (0.05 mg/kg for pendimethalin, 1.0 mg/kg for isopropalin, and 5.0 mg/kg for butralin) of these three herbicides in tobaccos in European countries (*13*). It clearly demonstrated that the residue levels of pendimethalin and butralin in the tobacco samples would be expected to pose health risks to the consumers.

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