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Simultaneous determination of ten herbicides residues in tobacco samples by gel permeation chromatography-gas chromatography

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ABSTRACT

In this study, gas chromatography coupled with gel permeation chromatography for analysis of the residues of 10 herbicides (atrazine, clomazone, acetochlor, metolachlor, butralin, pendimethalin, butachlor, flumetralin, oxyfluorfen, quizalofop-p-ehtyl) in tobacco samples was developed. The herbicide residues were extracted from the tobacco with 1% acetic acid in acetonitrile. The extracts were purified by gel permeation chromatography, and analyzed by gas chromatography with quantification by the external standard method. The elution time for herbicide was 15–30min in Gel Permeation Chromatography. The relativeretention time was 24.3–41.0 min in Gas Chromatography. The average recovery range for the herbicide residues was 83.8–100.1%, the relative standard deviation range was 1.93–8.91% and the detection limit range was 0.001–0.004 mg/kg. Because this method is simple, accurate, has high sensitivity, and provides good purification, it is suitable for analysis of the herbicide residues in tobacco.

KEYWORDS

Gel permeation chromatography;
Gas chromatography;
Herbicide;
Tobacco;
Residue.

INTRODUCTION

The intrinsic chemical composition of tobacco is complex, and it contains more than 20 different organic compounds, including sugars, amino acids, proteins, organic acids, alkaloids, and pigments. These compounds need to be separated from the compounds of interest, which are usually pesticides, before quantitative analysis avoiding influence. Solid phase extraction with one type of stationary phase is often used for this separation. However, with pesticide residues in tobacco, it is difficult to meet the pre-treatment requirements for analysis because of the limited packing size and capacity of the solid phase extraction cartridge. While mass spectrometry systems have become increasingly common, their widespread use in tobacco producing areas is limited because of the expense.

Gel permeation chromatography (GPC) separates solutes based on their structural differences, and can effectively remove lipids, pigments and other macromolecules from samples. Many groups have used GPC for sample purification before analysis of pesticide residues in various samples, including in vegetables[1-3], tea[4-6], and tobacco[7-9]. GPC has been used for analysis of many types of pesticides, such as 16 organochlorine pesticides[10], 48 pyrethrin pesticides[11], and 43 organophosphorus pesticides[12]. GPC could be coupled with GC, high-performance liquid chromatography and

other conventional detection equipment to effectively separate pesticides from tobacco components can quantify the levels of pesticides present in the tobacco. Such a technique could be widely used in tobacco producing areas because of inexpensive and simple.

Here, we established a GPC-GC analysis method for the analysis of 10 herbicides in tobacco. The method is simple, highly automated, and suitable for rapid, simultaneous determination of herbicide residues in tobacco.

MATERIALS AND METHODS

Reagentsand apparatus

Pesticide grade acetonitrile, glacial acetic acid, cyclohexane, and ethyl acetate, acetone were purchased along with anhydrous sodium sulfate and sodium chloride (AR grade) from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The instruments used in this study included a GC 6890N equipped with electron capture detector (Agilent Technologies, Santa Clara, CA), an Auto Prep 2000 GPC with a purification column filled with Bio-Beads® (S-X3, Bio-Rad, Hercules, California).

Sample pretreatment

One gram of each tobacco leaf sample from field trials was placed in a 50 mL centrifuge tube, and 5 mL of water was added to fully hydrate the tobacco leaves. Incubating for 10 min later, 20 mL of 1 % acetic acid solution in acetonitrile was added, followed by vortex mixing for 2 min. Then 1 g of sodium chloride was added, followed by vortex mixing for 2 min and centrifugation (4000 rpm, 5 min). The samples were dried over anhydrous sodium sulfate, and 10 mL of the dry supernatant were transferred into the sample loop of GPC for purification.

Purificationconditions

The GPC mobile phase was cyclohexane/ethyl acetate (1:1, v/v) with a flow rate of 5 mL/min and quantification loop volume of 5 mL. The eluent was collected from 15.0–30.0 min and concentrated under vacuum with a water bath temperature of 40 °C. The residue was reconstituted with acetone to a volume of 2 mL for GC analysis.

GC conditions

The GC column was a DB-1701 quartz capillary column (30 m × 0.32 mm I.D., 0.25 μm). The GC injector temperature was 210 °C and the detector temperature was 300 °C. The column temperature was initially held at 80 °C for 1 min, and heated to 260 °C at 5 °C/min. The sample (1 μL) was injected in splitless mode. The carrier gas was nitrogen (>99.999 %) with a flow rate of 2.0 mL/min.

Quantification

Standard solutions of each of the 10 herbicides (100 μg/mL) were mixed together to make stock standard solution, which was diluted to a series of different concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 μg/mL). These solutions were analyzed by GC, and a calibration curve was constructed for each herbicide and used for quantification.

RUSULTS AND DISCUSSION

Selection of GPC purification conditions

Tobacco extracts contain many compounds, such as carbohydrates, nitrogen containing compounds, organic acids and their derivatives, terpenes, and pigments. Without purification, these impurities would contaminate the instrument injection inlet, chromatography column, and the detector, and interfere with detection and analysis of the target compounds. In this study, GPC was used to purify the tobacco extracts. Most of the herbicides were eluted in 15–30 min (TABLE 1). Pigments, fats, alkaloids and other macromolecular compounds were eluted in the first 15 min, while pesticide components with relatively low molecular weights were eluted in 15 to 30 min. The column was cleaned and equilibrated in 30 to 40 min.

The GPC had advantages ofexcellentseparation efficiency, high automation, and lesser manual input. But it also has limitation of a large amount of reagents in clean balancing gel column. Through optimization elution curve in this test, the GPC could separate target pesticides from impurity in tobacco, with shorten running time and lesser reagent dosage.

Calibration curves

A calibration curve was constructed for each her-

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bicide by plotting the injection mass (x-axis) against the peak area from GC (y-axis), and a linear equation was derived from each curve. The correlation coefficients for the herbicides were between 0.99955 and 0.99995 (TABLE 2). The linear range satisfied quantitative requirement.

TABLE 1 : Elution time and recovery rate of the 10 herbicides in GPC

Herbicides	GPC elution recovery rate (%)		
	15-20 min	20-25 min	25-30 min
Atrazine	11	51	38
Clomazone	38	46	16
Acetochlor	41	39	20
Metolachlor	8	35	57
Butralin	26	55	19
Pendimethalin	48	35	17
Butachlor	8	43	49
flumetralin	59	37	4
Oxyfluorfen	18	35	47
quizalofop-p-ethyl	24	41	35

TABLE 2 : Calibration curve results for the 10 herbicides

Herbicides	Linear equation	Correlation coefficient (r)
Atrazine	y = 32063x-1183.8	0.99990
Clomazone	y = 16677x + 725.06	0.99955
Acetochlor	y = 35791x-1841.7	0.99980
Metolachlor	y = 25799x-894.4	0.99985
Butralin	y = 20483x-664.03	0.99965
Pendimethalin	y = 21500x-827.35	0.99980
Butachlor	y = 23065x-1083.7	0.99970
Flumetralin	y = 26231x-635.99	0.99995
Oxyfluorfen	y = 15778x-1066.1	0.99965
quizalofop-p-ethyl	y = 24707x-518.42	0.99980

Precision of the instrument

Within the linear range, a standard solution at a single concentration was selected for each herbicide and injected five times under the above conditions. The repeatability of five replicatesof 10 herbicides was well. The coefficients of variation were between 0.84 % and 4.61 % (TABLE 3).

Sensitivity and accuracy

The 1 gtobacco leaves were spiked with an aliquot of each herbicide standard solution, and five replicates were prepared for each standard solution concentration. The average recovery rate of the 10 herbicides was between 83.8 % and 100.1 %, the relative standard deviation range was 1.93–8.91 %, and detection limit range was 0.001–0.004 mg/kg (TABLE 4), which was accord to the request of pesticides residue analysis.

TABLE 3 : Instrument precision

Herbicide	Average peak area	Variation coefficient (%)
Atrazine	2859	2.33
Clomazone	2071	4.61
Acetochlor	3060	1.74
Metolachlor	2473	1.72
Butralin	2197	1.96
Pendimethalin	2059	2.15
Butachlor	2164	1.26
Flumetralin	2307	0.84
Oxyfluorfen	1334	1.87
quizalofop-p-ethyl	2125	2.57

TABLE 4 : Recovery rate of herbicides from spiked samples and detection limits

Herbicides	Spiked levels (mg/kg)	Average Recovery (%)	Relative standard deviation (%)	Detection limit (mg/kg)
Atrazine	0.005	90.8	6.98	0.001
	0.100	91.4	5.70	
Clomazone	0.010	83.9	8.91	0.002
	0.100	92.5	5.44	
Acetochlor	0.005	89.6	6.43	0.001
	0.100	83.8	8.14	
Metolachlor	0.008	89.6	5.82	0.002
	0.100	89.9	4.24	
Butralin	0.008	95.4	5.21	0.002
	0.100	100.1	1.93	
Pendimethalin	0.020	88.9	5.22	0.004
	0.100	98.0	4.84	
Butachlor	0.010	98.3	5.73	0.002
	0.100	85.9	3.72	
Flumetralin	0.008	91.2	5.28	0.002
	0.100	94.1	2.82	
Oxyfluorfen	0.020	88.4	4.54	0.004
	0.100	88.4	2.04	
quizalofop-p-ethyl	0.010	95.6	2.69	0.002
	0.100	86.7	3.68	

Relative retention time

Under the above conditions, the retention times for

the 10 herbicides in the GC column were between 24.3 min and 41.0 min (Figure 1-3). The separationeffect was well withoutimpurity peak, most interference could be avoided under this separating condition.

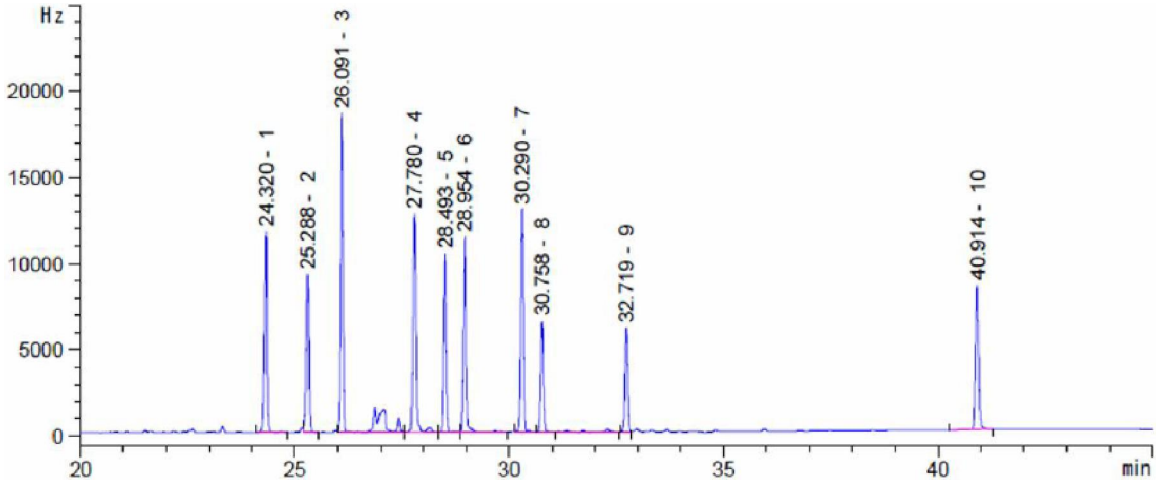

Figure 1 : Spectrum of 10 herbicides standards solution (1, atrazine; 2, clomazone; 3, acetochlor; 4, metolachlor; 5, butralin; 6, pendimethalin; 7, butachlor; 8, flumetralin; 9, oxyfluorfen; 10, quizalofop-p-ethyl)

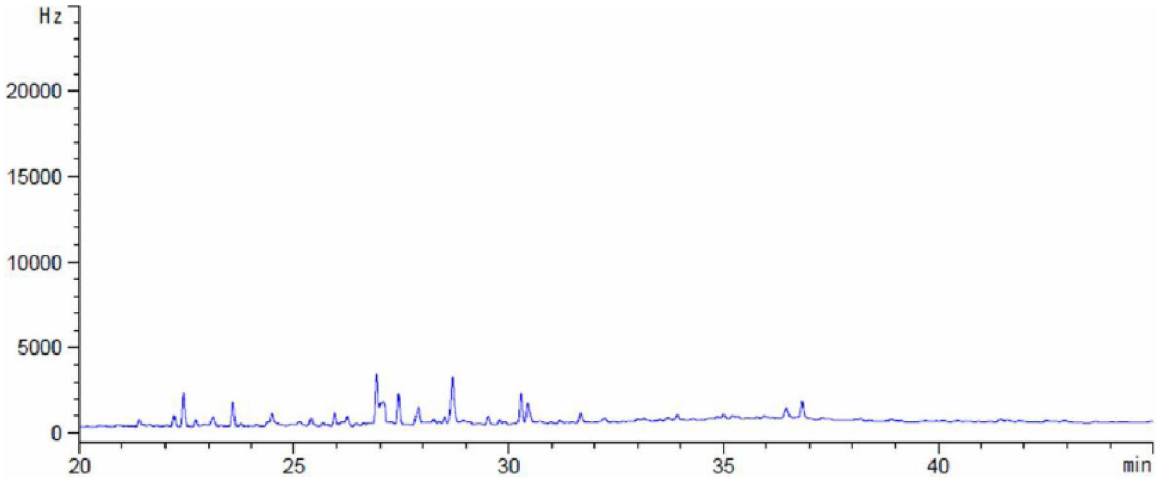

Figure 2 : Spectrum of tobacco blank

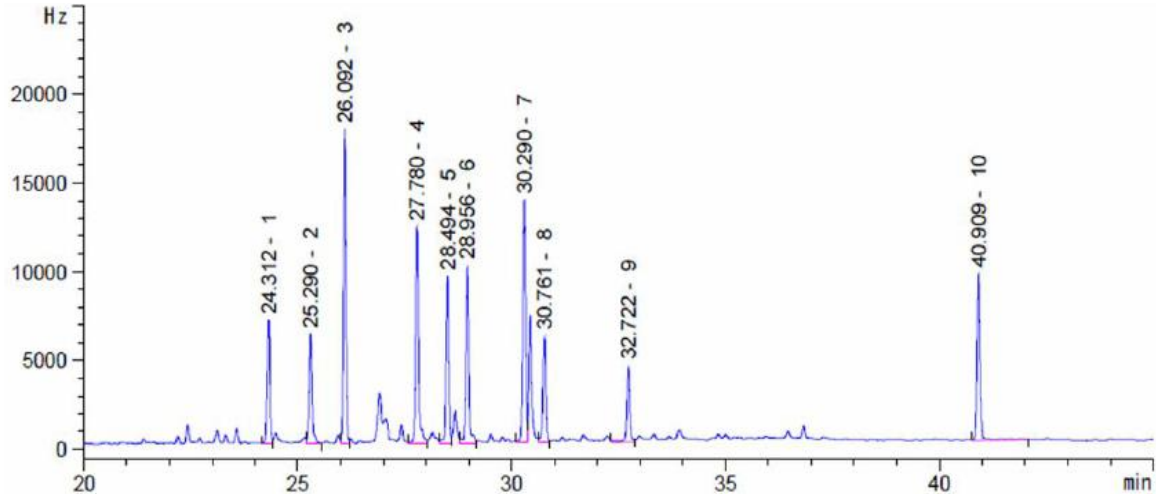

Figure 3 : Spectrum of spiked tobacco sample (1, atrazine; 2, clomazone; 3, acetochlor; 4, metolachlor; 5, butralin; 6, pendimethalin; 7, butachlor; 8, flumetralin; 9, oxyfluorfen; 10, quizalofop-p-ethyl)

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In test, it was important of initial temperature of chromatographic column and it's holding time for separationeffect. The lower initial temperature and longer holding time were useful for separating target pesticides from impurity in tobacco and reducinginterference of impurity.The advantage of purification by GPC was good separationeffect, a high degree of automation and little human input. GPC also has some defects in residue analysis on tobacco, though it was suitable for the analysis of pesticide residues in the sample purification, such as fruits and vegetable, grain and oil, tea and medicinal plants[13,14]. For the complexity and diversity of tobacco internal components, the GPC gel column must be cleaned and balanced fully after collection every sample eluentto ensure the separationeffect of the gel column good. Then it was important to resolve the obstacle of large dosage of reagent consumption in purification. In our study, we optimized the elution curve by two steps. First the eluent was collected at different time, then standard substance recovery rate and the removal of impurities in different period collection eluent was analyzed. Finally, the gel column separation effect was good, operation time was short and reagent dosage was saved.

Separation and detection by GC was achieved through vaporization sample by high temperature and elution by carrier gas in chromatographic column. In our study, the separation effect was influenced mostly by the initial temperature of chromatographic column and its holding time. We found that both the lower initial temperature and the longer appropriate holding time of initial temperature were good for separation the target pesticidesfrom tobacco impurities and induction the interference.

CONCLUSION

A GPC-GC method was developed for the analysis of 10 herbicides in tobacco. Non-target pesticides and intrinsic tobacco components were rapidly and effectively separated from the target compounds by GPC, and the concentrations of the 10 herbicides were determined by gas chromatography using the external standard method. The average recovery rate for the herbicides was between 83.8 and 100.1 %, the relative standard deviation range was 1.93–8.91%, and the method detection limit was 0.001–0.004 mg/kg. This method is very sensitive and highly automated, with good accuracy and precision, and it can simultaneously determine the concentrations of 10 herbicides in tobacco.

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