Determination of the Fluroxypyr Ester by HPLC with Internal Standard

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Abstract. An HPLC method to determine fluroxypyr esters was established. Methods The SinoChrom ODS-BP (5μm, 4.6mm×200mm) column was used. The mobile phase was consisted of methanol-water(volume ratio 85:15). The flow rate was 1.0mL/min. The column temperature was room temperature. The wavelength was 235nm. Results The o-nitroaniline was selected as internal standard for the determination. The linear range of fluroxypyr esters was 0.02～0.5mg/mL, r=0.9994, the standard addition recovery was 99.5% ~100%,and RSD was 0.2%(n=6). Conclusion This method was proved to be accurate and reliable, so it could be applied to the quality control of measuring fluroxypyr esters.

Introduction

Fluroxypyr esters as a kind of the systemic conductive herbicide was developed by the Dow Chemical Company, which is widely used a variety of crops such as wheat, barley, maize, orchard [1 ~ 2]. The technology for detecting pesticide was limited to the chemical, colorimetric and bioassay method at initial and these methods had a poor performance in determination of related substances because the purity of the original pesticide was not high [3]. Gas chromatography was widely used for analysis of pesticides in the 1960's, thus the detection level of the pesticide was greatly improved. Since the 1980's, high-performance liquid chromatography (HPLC) began to be widely used in the analysis of the thermal instability and ionic pesticide and its metabolites. Because of its high separation efficiency, high detection efficiency, high analysis speed, and high sensitivity analysis, chromatography became broad and important means in the modern analysis field [4].

Experiment

Instrument and Reagent

High performance liquid chromatograph: Dalian Elite Analytical Instruments 230+ with P230+ pump, Uv 230+ detector and EC2000 Chromatography workstation; Ultrasonic cleaning machine: KQ3200B; The filter: GM-0.33Ⅱ diaphragm vacuum pump with 0.45μm membrane; Trace sampler (flat): 25μL.

Methanol: chromatographic grade; Water: fresh distilled water; Fluroxypyr esters: known mass fraction 99% or higher; Internal standard substance: picloram, triclopyr, 3, 6 - dichloro picolinic acid, and o-nitroaniline, known mass fraction 99% or higher.

Standard solution of fluroxypyr esters: the amount of fluroxypyr esters was accurately weighed, and was solved in methanol, so that the solution of 2.0 mg mL^{-1} was obtained.

Internal standard solution of o-nitroaniline: the amount of o-nitroaniline was accurately weighed, and was solved in methanol, so that the solution of 2.0 mg mL^{-1} was obtained. When it was used as internal standard solution, this solution was needed to dilute to 0.2 mg mL^{-1}.

Chromatographic Conditions

The chromatographic column: SinoChrom ODS-BP (5μm, 4.6mm×200mm); Mobile Phase: methanol-water (volume ratio 85:15); The velocity: 1.0mL/min; Column temperature: at room temperature; Wavelength: UV235mm; Sample quantity: 5μL.

Results and Discussions

Selection of Internal Standard Substance

Internal standard materials which are similar physical and chemical properties, such as triclopyr, o-nitroaniline, 3, 6 - dichloro picolinic acid, picloram, were optimized, the result is shown in figure 1:

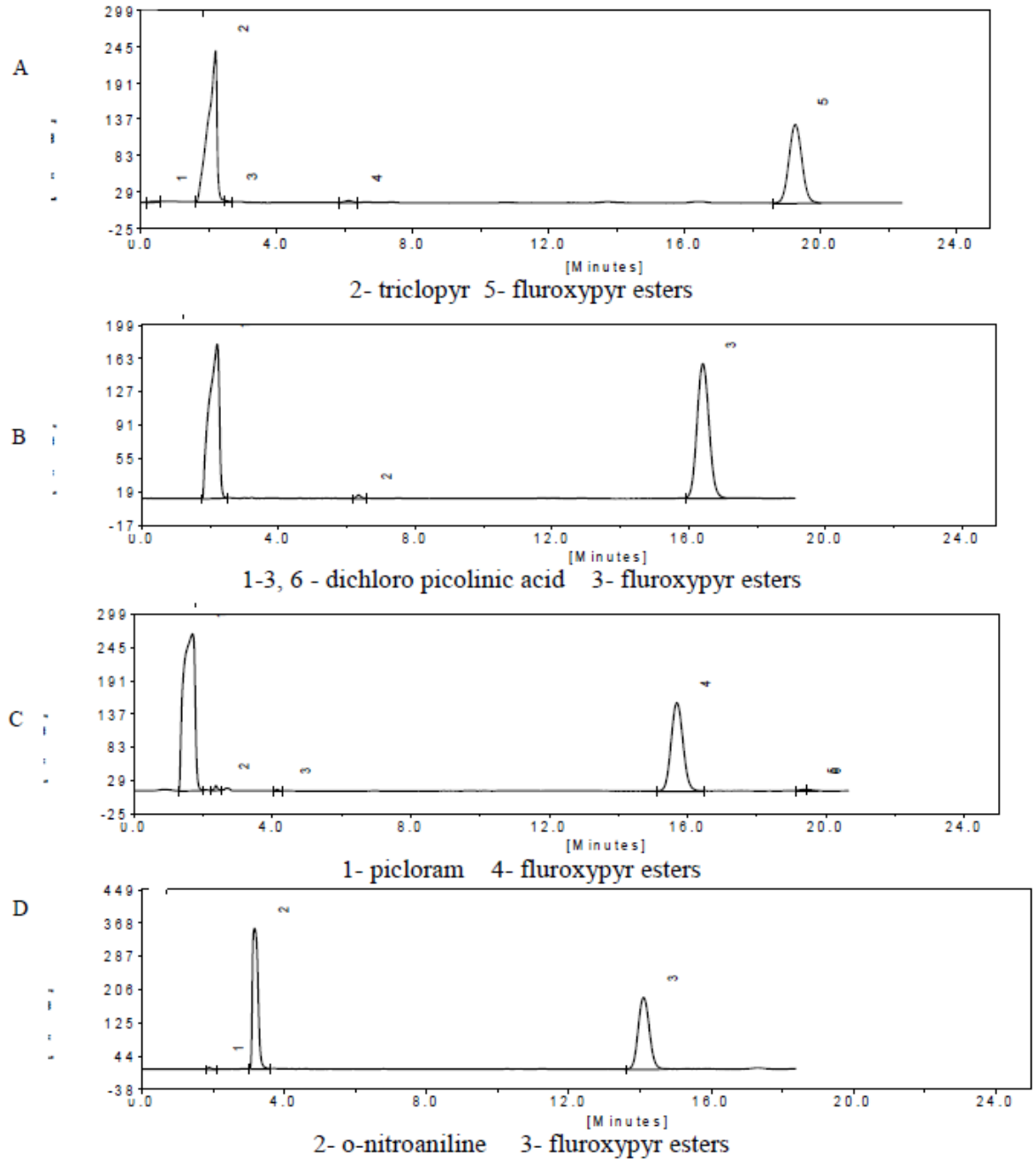

Fig.1 HPLC chromatograms

The experimental results show that the leading peak was found when the former three kinds of internal standard substance was added into the sample, and the shape of the peak was symmetrical and the separation was good when the sample was mixed with o-nitroaniline.

Determination of Linear Equation

Use a burette to accurately measure out a series of different volumes of the standard fluroxypyr esters solution into each of five 100mL volumetric flasks, and add 25.0mL 0.2mg mL^{-1} of internal standard solution of o-nitroaniline to each flask, thus the concentrations of these standard sample solutions were 0.02,0.1,0.2,0.3,0.5mg mL^{-1}. Then in the same HPLC chromatographic conditions,

inject 10.0μL of sample, respectively, and corresponding to the peak height and peak area can obtained from the chromatography workstation. The standard curve was constructed by plotting the ratio of fluroxypyr esters peak area to o-nitroaniline peak area versus mass concentration of fluroxypyr esters(mg mL^{-1}), and regression equation can be for y =3.727x - 0.026, r = 0.9994. Results show that the linear relationship is good at the range of 0.02 ~ 0.5 mg mL^{-1}.

Determination of Standard Addition Recovery Rate

An amount of known standard fluroxypyr esters was added to the blank sample, then these solutions were treated and determined according to the above analysis method, and the results are shown in table 1. The recovery rate of this method was between 99.5% ~ 100.5, the average recovery was 99.9%, and RSD was 0.20%.

Tab.1 The Standard Addition Recovery Rate of Fluroxypyr Esters

Sample no.	Amount of Standard/g	Measured Results/g	Recovery Rate /%	Average Recovery /%	RSD/%
1	0.0234	0.0233	99.6	99.9	0.20
2	0.0365	0.0367	100.5		
3	0.0458	0.0456	99.6		
4	0.0532	0.0533	100.2		
5	0.0613	0.0611	99.7		

Precision Test of Analysis Methods

Prepared a standard solution with a certain amount standard fluoride tobacco ester, then sampled 6 times repeatedly in the same HPLC chromatographic conditions, and every time took 10μL. The results are shown in table 2.

Tab.2 The Determination Results of Precision for Fluroxypyr Esters

No.	Quality of the Standard/g	Quality of the Internal Standard /g	A_s/A_{is}	Mass Fraction /%	Average $\bar{x}$ / %	RSD/%
1	0.0576	0.0564	0.79870	96.0	96.0	0.15
2			0.80058	96.3		
3			0.79586	95.7		
4			0.80017	96.2		
5			0.79920	96.1		
6			0.79321	95.4		

Repetitive Experiment

Weighed 6 fluroxypyr ester samples (content> 95.0%), then determined them in the same HPLC chromatographic conditions. The results are shown in table 3.

Tab.3 The Determination Results of Repetitive Experiment

No.	Quality of the Sample /g	Quality of the Internal Standard /g	A_s/A_{is}	Mass Fraction /%	Average $\bar{x}$ / %	RSD/%
1	0.0556	0.0530	0.76834	95.8	95.7	0.10
2	0.0563	0.0547	0.77895	95.9		
3	0.0579	0.0546	0.80043	95.7		
4	0.0617	0.0550	0.85634	96.0		
5	0.0557	0.0546	0.76648	95.4		
6	0.0559	0.0542	0.77093	95.6		

Determination of Samples

Weighed three copies of fluroxypyr ester sample, and putted a certain amount of nitroaniline as internal standard substance, respectively, then made into 100 mL of methanol solution, determined five times for each sample solution with 10μL. Calculated the content of fluroxypyr ester in the sample within the standard curve method, and figured out the RSD values of five content determinations, respectively.

Tab. 4 The Content of Fluroxypyr Esters in the Samples

No.	Quality of the Sample/g	Quality of O-nitroaniline/g	RSD/%
1	0.0230	0.0226	0.24
2	0.0234	0.0229	0.18
3	0.0231	0.0225	0.37

Conclusion

Fluroxypyr ester is a kind of the systemic conductive herbicide which is used for treating the late of seeding stem. O-nitroaniline was selected as internal standard which is the similar properties and structure. It is favor to improve the accuracy of the method for measuring the content of fluroxypyr ester by using high performance liquid chromatography (HPLC) standard curves of internal standard method. This method is easy to operate, and the linear relationship between the 0.02～0.5mg/mL is good, and the recovery rate is high, and the precision and repeatability is good, so this method can be used for quality control of fluorine tobacco ester herbicides.

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