Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(10):168-171

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analysis of 50% Acetochlor and Clomazone EC by HPLC

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ABSTRACT

RP-HPLC was adopted for the separation and determination of acetochlor and clomazone on the BDS Hypersil C18 column (5μm, 250mm×4.6mm), acetonitrile-water (volume ratio 60:40) as the mobile phase, and the UV detector with wavelength 210nm. The results show that the retention times of acetochlor and clomazone are 9.065min and 5.598min respectively，and the linear correlation coefficients for both acetochlor and clomazone are 0.9998, the relative standard deviations are 1.25% and 1.60% respectively, the average recovery rates are 99.14% and 98.15%. This method is simple, accurate and highly selective.

Key words: acetochlor, clomazone, HPLC, analysis

INTRODUCTION

50% acetochlor and clomazone composite emulsifiable concentrate（EC）is a soil enclosed herbicide, which is widely used at present. Once the herbicide was inhaled through the plumule and radicle of weeds, the growth of weeds will be inhibited and finally resulted in death. Experiments show that the compound is more effective than the single dose of acetochlor or clomazone used for preventing and eliminating the gramineae and broad-leaved weeds of winter rapeseed field.

Although mature analysis methods have been established for single dose of acetochlor and clomazone, and some analysis methods for their compounds with other herbicides [1-4], 50% acetochlor and clomazone EC was detected only by GC [5]. It is very important to find a simple, rapid and higher selective method for the quantitative determination of acetochlor and clomazone in compounded emulsifiable concentrate（EC）, due to its wide application. Herein, a new analysis method has been established by RP-HPLC, which is simple, precise and highly selective.

EXPERIMENTAL SECTION

Instrumentation and Chemical reagents

The HPLC system consists of an Agilent 1260 chromatographic pump, UV spectrophotometer detector and the chromatographic column of BDS Hypersil C18 (250 mm×4.6mm, 5μm).

Standard sample: acetochlor (content: ≥98.0%), clomazone (content: ≥98.0%); sample: 50% acetochlor and clomazone EC ; acetonitrile: chromatography grade; methanol: chromatography grade; water: new steam double distilled water.

HPLC conditions

The mobile phase consists of acetonitrile and water with the ration of 60:40 (v/v), and flow rate 1.0mL/min. The injection volume was 5μL. The peak was determined using a UV detector set at wavelength of 210nm. The retention

times of acetochlor and clomazone were 9.065min and 5.598min respectively at the conditions.

Preparation of standard and sample solution

A stock solution of standard containing acetochlor 5.972mg/mL and clomazone 1.877mg/mL was prepared in methanol. Work solution with low concentration was prepared by the stock solution with methanol in appropriate quantities.

The sample of 50% acetochlor and clomazone EC 0.3004g was dissolved into a 25mL volumetric flask and diluted with methanol to volume, then the sample solution was prepared. It was filtered through a 0.45μm filter before HPLC analysis.

Experimental Procedure

Set the chromatographic conditions as above analyzed. After stable of the baseline (the changes of relative respond ≤2%), the standard solution and sample solution 5μL were injected respectively according to the order of standard solution, sample solution, sample solution, standard solution, taking the peak area values from the recorded chromatograms. The chromatogram of 50% acetochlor and clomazone EC was shown in Fig.1

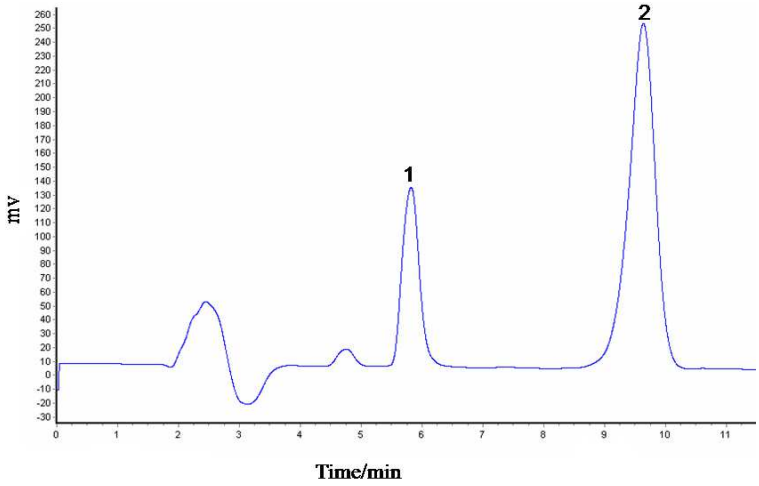

Fig.1 HPLC chromatogram of 50% acetochlor and clomazone EC 1 clomazone; 2 acetochlor

Calculation

Averaging the peak areas of twice standard solutions and sample solutions respectively, the active ingredients of acetochlor and clomazone in EC sample was calculated by the formula:

$$X = \frac{A_2 \times M_1 \times p}{A_1 \times M_2 \times 10} \times 100\ \%$$

X-mass fractions of acetochlor(clomazone) in EC sample;
A_1- peak areas average of acetochlor(clomazone) for standard;
A_2- peak areas average of acetochlor(clomazone) for sample;
M_1-weight of acetochlor(clomazone) for standard;
M_2-weight of sample
P- mass fractions of acetochlor(clomazone) in standard.

RESULTS AND DISCUSSION

Selection of detection wavelength and mobile phase

According to the UV absorption spectra of acetochlor and clomazone, it shows an absorption band located at 210nm for acetochlor and clomazone (Fig.2 and Fig.3). Therefore 210nm was selected as detection wavelength.

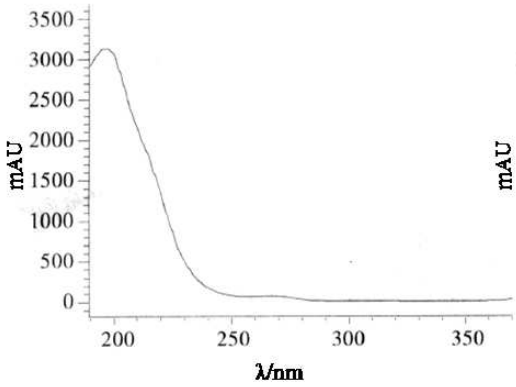

Fig. 2 the UV spectrum of acetochlor

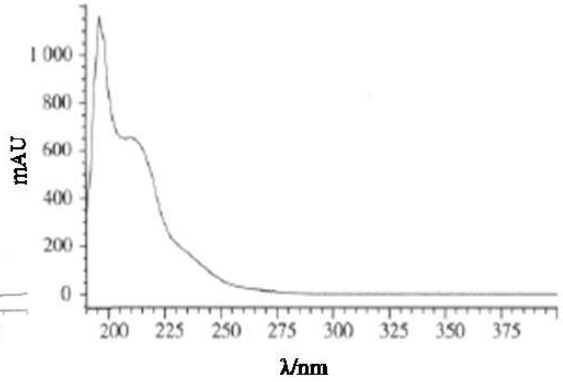

Fig. 3 the UV spectrum of clomazone

The mobile phase was selected by studying a series of mobile phase consisted of methanol-water and acetonitrile-water with different ratio. When the ration was 60:40 v/v of acetonitrile-water, it had suitable retention time, regular peak shape and good separation. Therefore, this composition of acetonitrile and water with the ration of 60:40 (v/v) was selected as HPLC mobile phase.

Construction of calibration curve

The stock solution was diluted with methanol to yield a series of appropriate concentration solutions for the construction of calibration curve. The analyzed solutions at five different concentrations were injected to the HPLC system in triplicate under above identified HPLC condition (Table 1), and the regression curve was established by plotting the peak area(A) against the concentration(C) of each constituent(Fig.4 and Fig.5). The calculated results show that the regression curve equation for acetochlor is A=23.675C+1095.9, the linear correlation coefficient is 0.9998; and for clomazone is A=27.701C+427.62, the linear correlation coefficient is 0.9998 too.

Table 1 the results of linear correlation

NO.	Acetochlor (μg/mL)	Peak area	Clomazone (μg/mL)	Peak area
1	59.72	2439	18.77	851.2
2	119.45	4132	37.54	1358
3	238.90	6725	75.09	2187
4	477.80	11973	150.18	3982
5	597.2	15559	187.72	4883

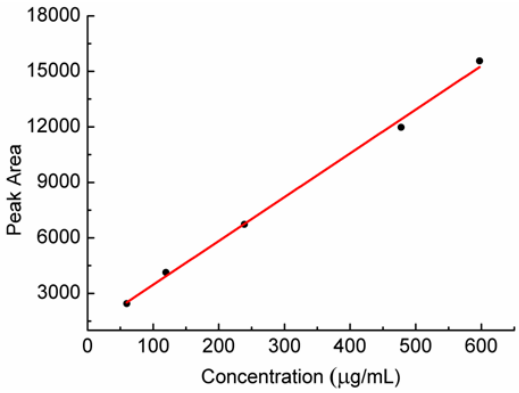

Fig.4 the standard curve of acetochor

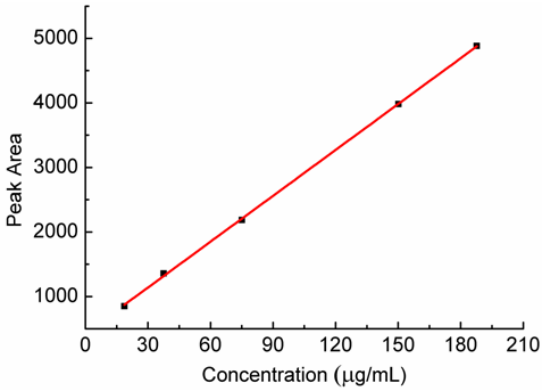

Fig.5 the standard curve of clomazone

Precision

The precision was assessed by analyzing a sample of known concentration in five replicates under above identified HPLC condition. The calculated results of peak areas, standard deviation and relative standard deviation were listed in Table 2. The results show that the RSD was 1.25% and 1.60% for acetochlor and clomazone respectively.

Table 2 the results of precision

NO.	Peak area of acetochlor	Peak area of clomazone
1	4342	1166
2	4412	1194
3	4260	1155
4	4355	1169
5	4337	1144
Average value	4341.2	1165.6
SD	54.35	18.69
RSD/%	1.25	1.60

Sample analysis

The newly established HPLC analytical method was subsequently utilized for the determination of acetochlor and clomazone in EC sample. The sample was analyzed in triplicate to calculate the content, and the content of acetochlor and clomazone were 38.03% and 8.64% respectively.

Accuracy (%recovery test)

In the recovery test, four different quantities levels of the standard samples were added to pre- analyzed sample and determined under above identified HPLC condition. The percentage recoveries obtained are between 96.75%～104.06% for acetochlor, and between 95.26%～101.08% for clomazone. Details are given in Table 3

Table 3 the results of recovery

Name	NO.	Content of sample (μg)	Added standard amount(μg)	Determined amount (μg)	Recovery amount (μg)	Recovery (%)	Average recovery (%)
acetochlor	1	137.08	119.45	255.24	118.16	98.92	99.14
	2	137.08	179.17	310.42	173.34	96.75	
	3	137.08	238.90	368.39	231.31	96.82	
	4	137.08	298.62	447.84	310.76	104.06	
clomazone	1	31.14	37.54	67.33	36.19	96.40	98.15
	2	31.14	56.31	84.78	53.64	95.26	
	3	31.14	75.08	106.10	74.96	99.84	
	4	31.14	93.85	126.00	94.86	101.08	

CONCLUSION

In this experiment, HPLC is used for the determination of acetochlor and clomazone in EC sample. The method is simple, rapid, precise and highly selective. It is worthwhile to note that the experimental result can reflect the quality of product, therefore it can be used for the monitoring of the production process.

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