

Optimization of DLLME Extraction Parameters for Pesticides Analysis by High Performance Liquid Chromatography

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Abstract - Pesticides have been used widely all over the world for centuries to increase agricultural production and to combat harmful pests. Pesticides used for agriculture, household, and public health sectors eventually reach water bodies, posing a risk to human health and the environment even in micro concentrations. Each country legally sets its own maximum allowable limits to regulate pesticides residues. Therefore, it is necessary to monitor pesticide residues in water resources, especially if they are used for drinking water purposes. With the technological developments, nowadays sensitive analytical instruments employing complex analysis methods such as GC/MS and LC/MS have been used to analyze pesticides. Pesticide residues must be concentrated by different extraction methods before HPLC analysis to be able to detect them in micro levels with high precision and low detection limits. The aim of this study is to develop an analytical method for the analysis of acetochlor and metolachlor pesticides by HPLC instrument with dispersive liquid-liquid micro extraction (DLLME) method. In order to determine optimum conditions for DLLME extraction method, extraction solvent type (chloroform, dichloromethane and 1,2 dichloroethane), dispersive solvent type (acetonitrile, methanol and isopropyl alcohol), flowrate (1, 1.2 and 1.5 ml/min), oven temperature (20°C, 30 °C, 40 °C and 50 °C), volume of extraction solvent (300, 350 and 400 µl), and mobile phase mixture (60/40, 70/30 and 80/20 acetonitrile/water in v/v) were comprehensively investigated with Taguchi experimental design. The optimized conditions for acetochlor and metolachlor were obtained as: extraction solvent of 1,2-dichloroethane, dispersive solvent of acetonitrile and methanol, extraction solvent volume of 400 µL and 300 µL, dispersive volume of 1 ml, flowrate of 1 ml/min and 1.2 ml/min, temperature of 40°C and 50°C and mobile phase mixture of 70/30 v/v and 80/20 v/v, respectively.

Keywords: Analysis, DLLME, HPLC, optimization, pesticides, Taguchi experimental design.

1. Introduction

Pesticides are defined as substances that control the harmful effects of pests of agricultural products, food, animal feed, and weeds, from production to processing, transportation, storage and consumption [1]. Acetochlor and metolachlor, which are chloroacetanilide type herbicides, are generally used for controlling grasses and broadleaf weeds on agricultural crop land and non-crop land for general weed control [2]. Due to their polar and non-ionizable properties, highly solubility in water, moderately persistent character in the environment and moderate to high mobility in soil result in frequently detection of acetochlor and metolachlor in ground and surface waters [3].

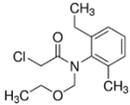
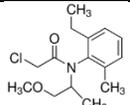
The identification and measurement of pesticides, which have serious human health and environmental effects, require complex and time-consuming methods, pre-treatment, and availability of sensitive analytical instruments due to their very different physico-chemical properties [4]. Therefore, it is important to develop methods that are reliable and sensitive, provide low detection limits, use less toxic chemicals during analysis, and do not require complex derivatization pretreatment. Although polar pesticides can be efficiently analyzed by reverse-phase liquid chromatography equipped with a UV detector [4], the procedures for isolation and enrichment of the components from the sample are required to detect non-polar pesticide residues in water and wastewater at trace levels by conventional high performance liquid chromatography (HPLC) [5]. Among the extraction methods, dispersive liquid-liquid microextraction (DLLME) method has advantages over other methods in terms of ease of processing, speed, cost, solvent requirement, recovery performance, and enrichment factors [6].

In this study, an analytical method for the analysis of metolachlor and acetochlor pesticides by HPLC instrument with DLLME method was developed. The analytical parameters were optimized by the Taguchi experimental design. Extraction solvent type, dispersive solvent type, flowrate, oven temperature, volume of extraction solvent, and mobile phase mixture were selected as experimental variables to optimize DLLME procedure by the Taguchi method.

2. Material and Methods

The standards of acetochlor and metolachlor were purchased from Sigma Aldrich with >95% purity. Table 1 presents the typical characteristics of pesticides. The chemicals of chloroform, acetone, methanol, and acetonitrile used in the pesticide analyses were of Merck grade purity (Merck, Darmstadt, Germany).

Table 1. Characteristics of the pesticides

| Pesticide | Molecular Structure | Linear Formula | CAS Number | Molecular Weight (g/mol) | Projection Area, Å ² |
|-------------|---|---|------------|--------------------------|---------------------------------|
| Acetochlor |  | C ₁₄ H ₂₀ ClNO ₂ | 34256-82-1 | 269.77 | 53.5 [7] |
| Metolachlor |  | C ₁₅ H ₂₂ ClNO ₂ | 51218-45-2 | 283.79 | 56.1 [8] |

Taguchi experimental design was applied to determine optimum conditions for DLLME extraction method. We created a Taguchi experimental design with three parameters with three levels. The experimental parameters were determined as extraction solvent type (chloroform, dichloromethane, and 1,2 dichloroethane), dispersive solvent type (acetonitrile, methanol, and isopropyl alcohol), flowrate (1, 1.2, and 1.5 ml/min), oven temperature (20 °C, 30 °C, 40 °C, and 50 °C), volume of extraction solvent (300, 350, and 400 µl), and mobile phase mixture (60/40, 70/30, and 80/20 acetonitrile/water in v/v).

The analyses of pesticides were performed using 10⁴ µg/L stock solutions. Eight levels of fortification of 5-100 µg/L were prepared in 100 mL ultrapure water. The samples were extracted according to DLLME method. A mixture of dispersive and extraction solvent (1.5 mL) was injected into 8.0 mL of standard samples in 15 mL centrifuge tube. First, the samples were mixed for 1 min at vortex mixer, then the samples were centrifuged for 2.0 min at 6000 rpm to separate organic phases from aqueous phase. A 100 µL organic phase was taken into an insert vial for analysis by HPLC instrument. Acetochlor and metolachlor were analysed at 210 and 230 nm wavelengths, respectively.

3. Results

For sensitive and accurate determination of acetochlor and metolachlor, pesticides solutions were enriched by DLLME method in an organic phase, then analysed by HPLC instrument. The analysis variances were optimized by the Taguchi experimental design with three parameters with three levels. The experimental results were presented in Fig. 1. The optimum conditions for acetochlor and metolachlor were evaluated based on the obtained highest peak area. As shown in Fig. 1, the highest peak area was observed for acetochlor with 1,2-dichloroethane and acetonitrile solvent mixtures. The extraction solvent volume and dispersive volume were 400 µL and 1 mL. The optimum flowrate was determined as 1 ml/min at 40 °C oven temperature. The mobile phase of 70/30 (v/v) acetonitrile/water was the optimum mixture.

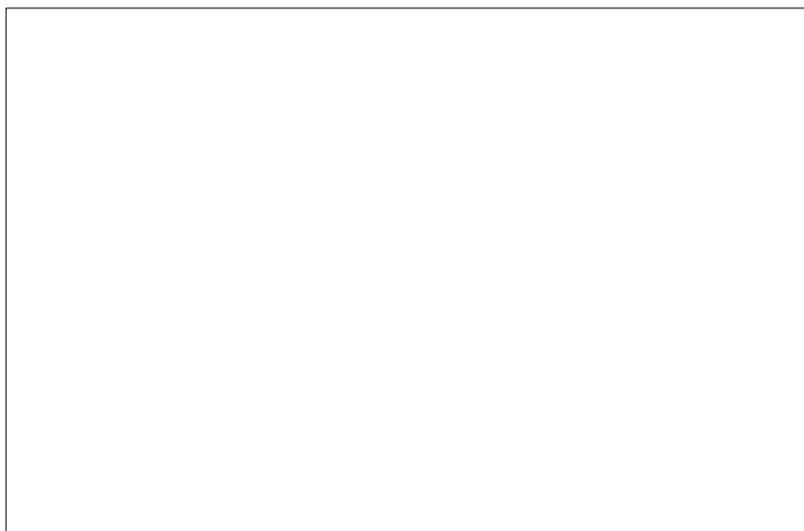


Fig. 1: Optimization of extraction and dispersive solvent type.

The highest peak area was obtained with 1,2-dichloroethane and methanol solvent mixtures. The optimized extraction volume was 300 μL and dispersive solvent volume was 1 ml. The best result was observed at flowrate of 1.2 ml/min, temperature of 50°C and mobile phase mixture of 80/20 (v/v) acetonitrile/water. The recovery rates for the acetochlor and metolachlor were determined as 90.26% and 101.54%, respectively. Calibration curves for acetochlor and metolachlor were achieved under optimized conditions for each pesticide. The calibration curves for acetochlor (a) and metolachlor (b) were shown in Fig. 2.

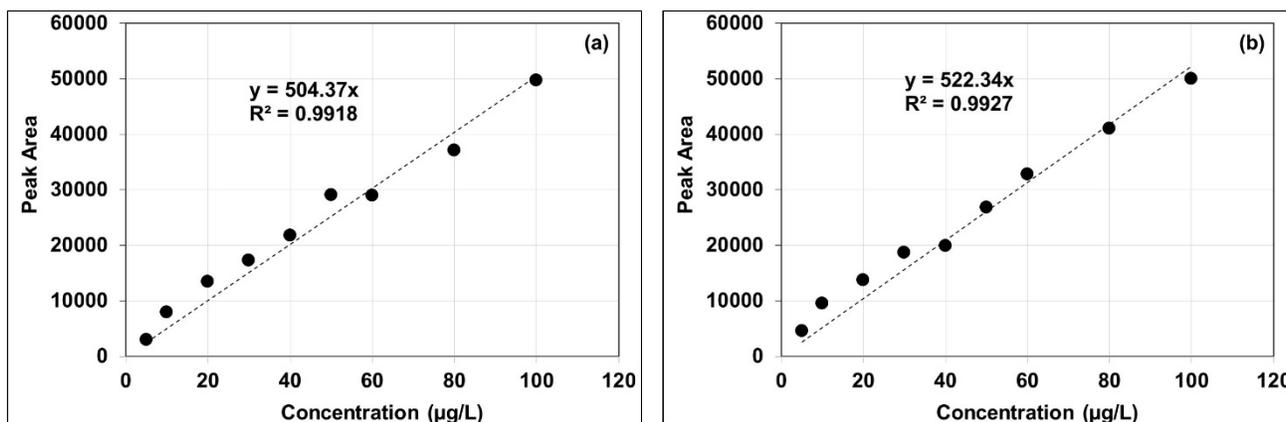


Fig. 2: Calibration curves obtained under optimized conditions: a) acetochlor, b) metolachlor.

4. Conclusion

Acetochlor and metolachlor analysis by HPLC instrument was optimized by Taguchi experimental design method. In order to detect tested pesticides in low level concentrations accurately and sensitively, the pesticide standard samples were enriched by the DLLME extraction method. The extraction parameters (dispersive and extraction solvent type, solvent volume) and operational conditions (flowrate, temperature and mobile phase mixture) were optimized. The results of this study showed that acetochlor and metolachlor can be measured by HPLC instrument in aqueous samples.

Acknowledgements

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