VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF TERBUTHYLAZINE AND S-METOLACHLOR RESIDUES IN SOIL

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Abstract

Terbuthylazine and s-metolachlor are commonly used herbicides, especially after prohibition of some other triazine and chloracetanilide herbicides. However, often used pesticides pose the risk of soil contamination due to their persistence, toxicity and bioaccumulation. The trace determination of herbicide residues, generally in environmental samples, presents a challenging analytical problem. The present work was carried out to analyze terbuthylazine and s-metolachlor residues in soil based on high performance liquid chromatography (HPLC), and QuEChERS extraction method. The method was optimized and validated according to the parameters of precision, accuracy, linearity, limits of detection and limits of quantification. Analysis was carried out using an HPLC-UV diode array detection system (Agilent 1100, USA), with an Agilent Zorbax Eclipse C18 column (50 mm × 4.6 mm, 1.8 μm) and mobile phase consisting of ultrapure water and acetonitrile (55/45, v/v). Mean recoveries obtained from soil samples fortified at three different levels ranged from 81 to 92%. The method detection limits ranged from 0.01 to 0.05 mg kg^{-1}. Obtained results completely fulfilled the SANCO/825/00 rev. 8.1 16/11/2010 criteria.

Introduction

Commonly used pesticides pose the risk of soil contamination due to their persistence, bioaccumulation and toxicity. The fate of pesticides in soil is influenced by the physico-chemical properties of pesticide, the properties of the soil (presence of clay materials, organic matter, pH), climate, biology, and other factors [1]. Some herbicides can remain active in the soil for weeks, months or years. This can be an advantage, as it ensures long-term weed control and at the same time, if the herbicide stays in the soil longer than intended, it may damage susceptible crops sown in subsequent years. E.g. chlorsulfuron is used in wheat and barley, but can remain active in the soil for several years and damage legumes and oilseeds.

A real problem represents the difficulty in identifying herbicide residues before they cause a problem. Once the crop has emerged, diagnosis is difficult because the symptoms of residual herbicide damage can often be confused with and/or make the crop vulnerable to other stresses, such as nutrient deficiency or disease.

The trace determination of herbicide residues, generally in environmental samples, presents a challenging analytical problem [2]. The low dosage used requires the application of highly sensitive analytical techniques to detect trace concentrations of residues in soil [3]. Due to the low level present and complexity of sample, clean-up and enrichment before analysis is necessary and become a crucial step for the determination of herbicides in environmental samples.

Thus, the present work was carried out to analyze terbuthylazine and s-metolachlor residues in soil based on liquid chromatography and QuEChERS extraction method.

Terbuthylazine and s-metolachlor are commonly used herbicides, especially after prohibition of some other triazine nd chloracetanilide herbicides. Terbutilazin (2-N-tert-butyl-6-chloro-4-N-ethyl-1,3,5-triazine-2,4-diamine) is a triazine herbicide and s-metolahlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl]acetamide) belong to the chloroacetanilide group of herbicide (Figure 1). They are used as a pre emergence herbicides, for control of broad spectrum weeds in maize.

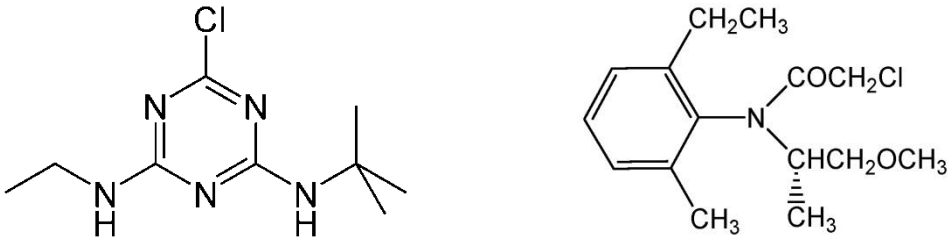

Figure 1. Structural formula of terbuthylazine and s-metolachlor

Experimental

Standards and reagents.

Certificated analytical standards of terbuthylazine (99.1%), and s-metolachlor (96%) were purchased from Dr Ehrenstorfer (Augsburg, Germany). QuEChERS dispersive SP extraction kits were from Agilent (USA). For the extraction, standard dissolution and mobile phase, acetonitrile (MeCN) (J.T. Baker) was used. Ultra pure water, used as a mobile phase, was produced by a Milli-Q system (Milli-pore, USA).

Standard solutions.

A stock solutions of terbuthylazine and s-metolachlor were prepared from the appropriate amount of analytical standards in MeCN, while working standard solutions, used for recovery and other validation parameters, were prepared by further dilution with MeCN covering the concentrations between 1.5 to 10 μg ml^{-1}.

Extraction procedure.

Soil sample used in this study was purchased from area without pesticide application. Freshly-spiked soils were prepared by weighing 10.0 g of soil into polypropylene tube 50 ml volume. Soil samples fortified with mixture of herbicides working standard solutions at three levels, 1.5, 5.0 and 10 μg/ml. The mixture was then homogenized and the sample was allowed to stand at room temperature over night until analysis. After that, 10 ml of acetonitrile was added, shaken vigorously for a 1 min and vortexed for a 1 min. In the next step, a mix of buffered salts (1000 mg of sodium citrate, 500 mg of sodium hydrogen citrate sesquihydrate, 4000 mg magnesium sulphate and 1000 mg sodium chloride) from separate pouches was added, shaken for 1 min and vortexed 1 min. The tube was placed in an ultrasonic bath for 10 min and centrifuged at 4000 rpm for 5 min (Sigma, Germany). The supernatant was transferred through Na_2SO_4 and filtered into an autosampler vial for HPLC-DAD analysis.

Chromatographic parameters.

Simultaneous determination of terbuthylazine and s-metolachlor residues in soil was performed with high-performance liquid chromatography system, equipped with a diode array detector (HPLC-DAD, 1100 Series, Agilent Technologies). The reversed phase

chromatographic separation was performed on ZORBAX Eclipse XDB-C18 (50 mm, 4.6 mm, 1.8 μm) column in an isocratic working regime with mobile phase acetonitrile/deionized water 55/45. The flow rate was 0.92 ml min^{-1}, injector volume 20 μl and the column temperature was 40 °C.

Validation of the analytical method.
The developed and optimized method for quantitative analysis of terbuthylazine and s-metolachlor in soil was validated in terms of linearity, precision, recovery, limit of detection (LOD) and limit of quantification (LOQ), according to SANCO/825/00 rev. 8.1 16/11/2010 [4].

Results and discussion
Chromatographic determination of terbuthylazine and s-metolachlor was carried out on a HPLC using reversed phase procedure and UV detection at 230 nm. HPLC/DAD chromatogram of terbuthylazine and s-metolachlor standard in acetonitrile is shown in figure 2.
The developed and optimized method for simultaneous determination of terbuthylazine and s-metolachlor herbicides in soil was validated in terms of linearity, precision, LOD, LOQ and recovery. Calibration of working standard solution was used to test the ability of procedures and instruments for determination of terbuthylazine and s-metolachlor. Linearity of calibration was assessed from a linear regression of response (area) versus concentration terbuthylazine and s-metolachlorof in solution. Result showed that procedure and instrument used had good ability in separating terbuthylazine and s-metolachlor indicated by calibration curve. Response of terbuthylazine was linear at concentrations of 1.5 to 10 μg ml^{-1}, with a correlation coefficient of 0.9996 and 0.9997 for terbuthylazine and s-metolachlor, respectively. Chromatogram of standard mixture is shown in Figure 2.

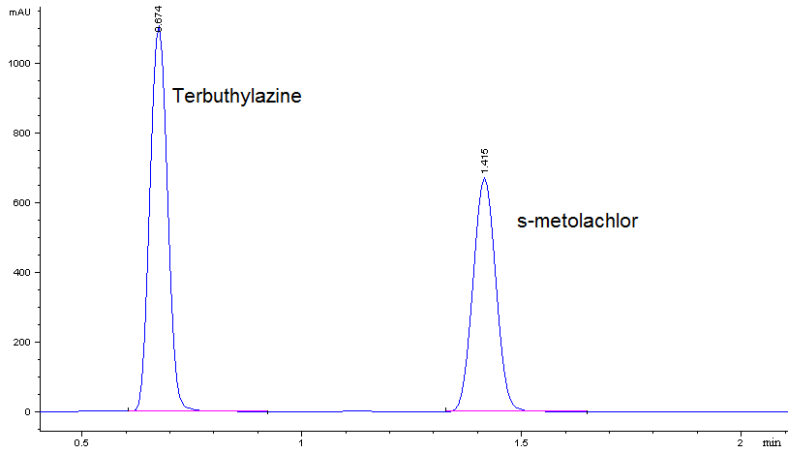

Figure 2. Chromatogram of terbuthylazine and s-metolachlor standard mixture in acetonitrile

LOD and LOQ for terbuthylazine and s-metolachlor was estimated from the fortified samples. LOD established as 0.010 mg/kg and LOQ at 0.060 mg/kg, for both analyzed herbicides.

The precision of measurement of an analyte can be evaluated as repeatability or reproducibility. In this study, precision is expressed as repeatability. Precision was checked by

matrix matched terbuthylazine and s-metolachlor standard (6.0 μg/ml) five times on the same day. Relative standard deviations (RSD) of the peak areas were 1.23 and 1.41% for terbuthylazine and s-metolachlor, respectively, fulfilling with criteria for of chromatographic measurements of RSD≤2%.

The accuracy of the method was evaluated by recovery studies. For recovery studies, a soil sample was spiked before the extraction procedure with terbuthylazine and s-metolachlor herbicides at three levels. The mean recoveries for spiked sample ranged from 81 to 92%.

Conclusion

In this study, a simple and precise method for simultaneous determination of terbuthylazine and s-metolachlor residues in soil samples was described. Reverse phase and isocratic elution based liquid chromatographic conditions are used for simultaneous determination of terbuthylazine and s-metolachlor. Considering the obtained values of analytical parameters, the proposed method proved to be an efficient and sensitive method for determination of terbuthylazine and s-metolachlor herbicides residues in soil samples.

Acknowledgements

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