SPECTROPHOTOMETRIC PROCEDURE FOR DETERMINATION OF TRIFLURALIN IN SOIL*

PROCEDIMENTO ESPECTROFOTOMÉTICO PARA DETERMINAÇÃO DE TRIFLURALINA EM SOLO

PROCEDIMIENTO ESPECTROFOTOMÉTRICO PARA LA DETERMINACIÓN DE TRIFLURALINA EN SUELOS

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Abstract: Trifluralin herbicide has been applied for a long period over agricultural fields, despite its residual persistence on non-target organisms. In this work, an alternative spectrophotometric method for trifluralin determination in soil is described. The herbicide molecule was reduced by tin (II) chloride. Diazotization was made with sodium nitrite and the product was coupled to the N-(1-naphthyl)ethylenediamine (Bratton-Marshall reagent). The reaction was made in a micellar medium of sodium dodecyl sulphate and, due to it was pH-dependent, a buffer solution had to be added. The absorbance of the coloured compound was measured at 550 nm, for herbicide concentration from 0.2 to 2.0 µg/mL of the mixture. Validation tests by comparison with liquid chromatography showed satisfactory correlations between both methods. After establishment of the analytical conditions the spectrophotometric method was employed for determination of trifluralin in soil samples from soybean culture fields. As result the not detected herbicide residues should indicates that trifluralin was not applied for a long term on those fields or was replaced by others herbicides of broad spectrum effects.
Keywords: Herbicides. Crops. Validation.

Resumo: O herbicida trifluralina tem sido usado por longos períodos em campos agrícolas, a despeito da comprovada persistência de seus resíduos em organismos não-alvo. Neste trabalho, um método espectrofotométrico alternativo para determinação de trifluralina em solo é descrito. A molécula do herbicida foi reduzida com cloreto de estanho(II). A diazotização foi feita com nitrito de sódio e o produto foi acoplado a N-(1-naftil)etilenodiamina (reagente de Bratton-Marshall). A reação foi realizada em um ambiente micelar de duodecilsulfato de sódio e, uma vez que era dependente do pH, uma solução tampão foi adicionada. A absorvância do composto colorido foi medida em 550 nm, para concentração de herbicida entre 0,2 e 2,0 µg/mL da mistura. Testes de validação pela comparação com cromatografia líquida mostraram correlação satisfatória entre ambos os métodos. Depois de estabelecidas as condições analíticas, o método espectrofotométrico foi empregado para determinação de trifluralina em amostras de solo oriundas de campos agrícolas de cultivo de soja. Não foram detectados resíduos do herbicida nas amostras de solo analisadas, o que indica que trifluralina não foi aplicado durante longo período nos campos analisados ou que foi substituído por outros herbicidas de mais amplo efeito.
Palavras-chave: Herbicidas. Culturas agrícolas. Validação.

Resumen: El herbicida trifluralina ha sido utilizado por mucho tiempo en campos de agricultura a pesar de la comprobada permanencia de sus residuos en organismos que no interesan en este estudio. En este trabajo, es descrito un método espectrofotométrico alternativo para la determinación de trifluralina en suelos. La molécula del herbicida fue reducida con clorato de estaño (II). La diazotización, hecha con nitrito de sodio y el producto fue asociado a N-(1-naftil)etilenodiamina (reactivo de Bratton-Marshall). La reacción fue realizada en un medio miscelar de duodecilsulfato de sodio y, debido a que era dependiente del pH, fue adicionada una solución tampón. La absorbancia del compuesto colorido fue medida en 550 nm, para concentración del herbicida entre 0,2 y 2,0 µg/mL de la mezcla. Ensayos de validación por la comparación con cromatografía líquida mostraron correlación satisfactoria entre los dos métodos. Después de establecidas las condiciones analíticas, el método espectrofotométrico fue empleado para la determinación de trifluralina en muestras de suelos originadas de campos de agricultura de cultivo de soja. No Fueron detectados residuos del herbicida en las muestras de suelos analizadas, lo que indica que no fue aplicado trifluralina durante largo período en los campos estudiados o que fue remplazado por otros herbicidas de más amplio efecto.
Palabras-clave: Herbicidas. Cultivos agrícolas. Validación.

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1 INTRODUCTION

The global use of pesticide is continuously expanding in diversity and intensity. Although innovations have been ocurred, such as the transgenic developments, persistent pests still attack cultures and, as a consequence, many pesticides continue to be commonly used. In Brazil this is not different and, thousands of tons of pesticides are applied and distributed in all environmental compartments, polluting atmosphere, waters sources and soils, so endangering the environment and human health. According to their fate, such chemicals can be more persistent, mobiles and toxics than expected, thus widen the contamination problem (DASGUPTA et al.,2001; CARVALHO, 2006).

The dinitroaniline herbicides are largely used (registered in more than 50 countries for use on more than 80 crop, vegetable and ornamental uses with an annual worldwide sales in 1998 were worth US$300 million, and 24,000 tonnes were produced) (TRIFLURALIN, on line, 2004). Trifluralin (Figure 1) (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is a systemic selective soil herbicide, which acts by entering into the seedling at the hypocotyl region disrupting cell division. The trade names of the commercial products include: Flurelene SE, Treflan, Tri-4, Trust, M.T.F., Trifluralina 600, Elancolan, Su Seguro Carpidor, Treficon, Trim L-36352, Crisalim, TR-10, Triflurex and Ipersan. Trifluralin is largely used for pre-emergence control of many annual grasses and broad-leaved weeds (*Brachiaria plantaginea*, *Eulesine indica*, *Portuluca oleracea* and others in Brazilians crops) in brassicas, beans, pears, carrots, parsnips, lettuces, capsicums, tomatoes, artichokes, garlic, vines, strawberries, soya beans, sunflowers, safflowers, ornamentals, cotton, sugar beet, sugar cane and in forestry. Normally applied during pre-planting by soil incorporation, trifluralin sometimes can also be used in post-planting applications in some crops (TOMLIN, 1994).

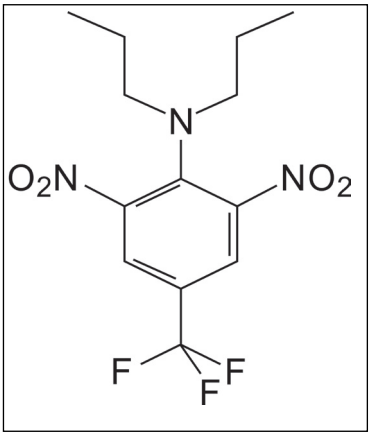

Figure 1 - Molecular structure of trifluralin

Since trifluralin is generally persistent, it remains unchanged in the soil for long periods. In agricultural fields, trifluralin degrades to half of its original concentration from 2 up to 4 months. The herbicide is photodegradable but not hydrolyzed in the soil. Under laboratory conditions, degradations are faster in soil where no air is present (DORES; DE-LAMONICA-FREIRE, 2001). Trifluralin is barely insoluble in water (0,0024%, at 27°C) but readily soluble in organic solvents such as xylene, acetone and aromatic naphtas (acetone > 50g/100 mL; methanol 2 g/100mL; xylene 81g/100 mL). Since it is very apolar (Koc = 70000g/mL), trifluralin tends to be strongly adsorbed on the soils, then leaching and ground water contamination is not expected to occur (GUS = 0,43) (AMARANTE JR. et al., 2002; FILIZOLA et al., 2002). Since higher adsorption ocurr in soil with high organic matter or clay content, and due to at the adsorbed form the herbicide is inactive, high applications rate should be required for effective weed control on such soils (SENESI, 1993). Although moderately toxic for human, the herbicide is quite toxic to cold-and warm-water fishes and also to aquatic invertebrate animals. After exposition to waters containing low concentrations of the herbicide, fishes have presented considerable bioaccumulation (LIMA et al., 2004).

Determinations of trifluralin are commonly made by using rather selective methods, such as gas chromatography (TRISKA, 1995; CARABIAS-MARTINEZ et al., 2003) or liquid chromatography (SZEKACS, 2003), both with mass spectrometry detectors, or based on immunosensor detection. Most of them, despite their advantages, have a number of requirements, in such a way that alternative methods should be searched.

This paper describe a analytical method based on the work of Escrig-Tena et al. (1998) Trifluralin was determined by using a spectrophotometric method on the visible region (550 nm). Firstly, the nitrobenzene was reduced to arylamine, diazotization of the arylamine and coupling of the diazonium ions with Bratton-Marshall reagent. The two latter reactions were performed in a micellar medium of sodium dodecyl sulfate. After the establishment of suitable analytical conditions, the results obtained by the alternative spectrophotometric method were validated by comparison with HPLC-UV.

2 MATERIALS AND METHODS

2.1 Apparatus

Spectrophotometric analyses were carried out using a model Cary 50 UV-Visible Spectrophotometer and, for liquid chromatographic

analyses, a Varian HPLC model Prostar 230, equipped with a Prostar 330 photodiode-array--detector, was used.

2.2 Reagents

The following chemicals were used: acetone, ethyl acetate, methanol, all HPLC grade (Merck, USA). Hydrochloric acid, tin (II) chloride, sodium nitrite, sulfamic acid, sodium dodecyl sulphate (SDS), potassium chloride and N-(1-naphthyl)-ethylenediamine-dihydrochloride (NED) were analytical grade, obtained from Merck (Germany). Trifluralin standard was from Dr. Ehrenstorfer (Augsburg, Germany). Aqueous solutions were prepared by using ultra-pure water (Direct Q, Millipore, USA). The stock solution of trifluralin (1000 µg/mL) was made by solubilization in methanol. Standard working solutions at various concentrations were prepared by apropriate dilution of aliquots of the stock solutions in methanol. They were stored at -18°C.

2.3 Soil samples

Soil samples were obtained from farms located at the Balsas city, (Maranhão south, Brazil), in crops of soya beans, on November 2002, and June 2003. The sampling was carried out in six points, with samples collected at 20 cm depht, packed in glass amber flaskes and protected in thermical box. Afterwards, the samples were brought to the laboratory and stored on refrigerator until their analysis.

2.4 Sample preparation and fortification

Soil samples were air-dried at 35°C, passed through a 2 mm sieve, homogenized and stored in a closed vessel. Samples of 50g of soil were fortified by adding 1.0mL of each standard solution of trifluralin. The range of fortification was 2,0 to 10,0µg/g.

2.5 Extration and clean-up procedure

Aliquots of 20g amount from each soil samples fortified was placed into 250mL erlenmeyer and spiked with standard solution of trifluralin. The extraction was made with 50mL ethyl acetate by using ultrasonic bath (ClearSonic, USC 1450) for 45 minutes (Polese et al., 2002). After shaking, the extract was decanted and the supernatant was filtered through Whatman glass microfibre filters GF/C (45µm and 47mmØ) in Millipore filtration system. The filter was washed with an additional 20mL of ethyl acetate. The procedure was repeated for the soil retained in the erlenmeyer. Since most of the environmental samples require a preliminary clean-up procedure for removal interfering co-extracting materials as much as possible before the analysis, the extracts were passed through a column containing 2g of deactivated florisil. After that, the extract was transferred to a 10mL volumetric flask with 2mL of acetone, and the reduction and derivatization was then proceeded.

2.6 Derivatisation and UV determination

Firstly, the reduction of nitrobenzene to arylamine was made by the addition of 6mL of standard trifluralin and 4mL of 10^{-2}mol L^{-1} tin(II) chloride, dissolved in concentrated HCl, into a 10mL volumetric flask. After 30 minutes, 0.4mL aliquot of the reduced compound was transferred to another 10mL volumetric flask, then the volume was completed with water (trifluralin concentration = 24µg/mL).

For derivatisation of the arylamine, different volumes of the above mentioned solution were placed into 10mL volumetric flasks, then 1mL of Clark and Lubs buffer solutions (KCl-HCl, pH 1.0) were added, followed by the addition of 2.25mL of a 0.1mol L^{-1} SDS solution and 0.4mL of a 0.2mol.L^{-1} sodium nitrite. After 5 minutes, the nitrite excess was destroyed by the reaction with 0.4mL of sulfamic acid 0.5mol L^{-1} and, 10 minutes later, the micellar medium was made by addition of 0.2mL of 0,03mol. L^{-1} NED. The volume was completed with purified water. Calibration curves were made for trifluralin concentrations from 0.24 up to 6.0µg/mL (R^2 = 0.9999), the absorbances measured at 550nm.

2.7 HPLC/UV analysis

Chromatographic analysis were made with a Zorbax C-18 analytical column (25 x 4.6mm I.D., 5 um particle size), by using gradient ellution with water (solvent A) and methanol (solvent B), starting with 30% A and 70% B, changed to 10% A and 90% B in 10 minutes, then varing to 0% A and 100% B in 5 minutes and finally returning to initial conditions in 5 minutes. The flow rate was 1mL/min and the detection was made at 280nm. Suitable aliquotes (30µL) of the standard solutions and of the extracts were injectected. To obtain the best quantitative accuracy for the analysis of the compound, concentrations of trifluralin varing from 0.25 up to 1.5µg mL^{-1} were compared with similar

concentrations, the results of spectrophotometric and chromatographic analysis being compared.

3 RESULTS AND DISCUSSION

3.1 Recoveries studies from soil samples by spectrophotometry

Spectrophotometric analysis of pesticide residues used to be uncommon due to the slightly sensibility and quite probable interferences, which occurred, since most of the pesticide molecules absorb UV or visible radiation. A way to enhance the sensibility and selectivity is to transform the compound through a specific reaction, after that to put it in a different and stable medium. Micellar medium actually is very employed in analytical chemistry due to it fulfils such requirements. In this work, the coloured compound was formed and maintened at suitable concentrations of the surfactants, thus allowing trifluralin determination from 0.2 to 20.0µg/mL (regression equation as y=0.0149x + 0.0017, with R^2 = 0.9951 and C.V. varying from 8.2 to 11.4, for 5 repetitions). The developed method also proved reasonable sensitivity (limit of detection of 0.04µg mL^{-1}) that was determined as three times the standard deviation from the blank.

The efficiency of the proposed method was evaluated through means and standard deviations of the recovery studies with soil spiked at three different levels, from 2.0 up to 10.0µg/g. When the extracts were directly compared with standard solutions, high absorbances were observed and this was attributed to matrix effect after having added the standards to an extract of the unfortified sample. However, average recoveries varying from 79 to 82%, with relative standard deviation close to 9% for 5 repetitions, could be obtained when absorbances for fortified samples were deducted from that obtained for an unfortified sample. These values are in agreement to that one obtained for analytical residue methods. Table 1 shows the results with the respective coefficient of variation.

Table 1 - Recoveries (%) of trifluralin in soil samples by spectrophotometry (n=5)

Trifluralin concentrations (µg/g)		Mean Recovery (%)	Relative Standard Deviation (%)
Spiked	Obtained		
2.0	1.60	80.2	9.3
5.0	3.97	79.3	8.3
10.0	10.97	79.7	8.8

3.2 Comparison between spectrophotometric and HPLC methods

Before the proceeding of validation studies, chromatographic conditions were obtained for trifluralin by liquid chromatography and UV detection (TRIANTAFYLLIDIS et al, 2010). The results from analysis of the calibration procedure were: linear range from 0.5 up to 50.0µg/mL, linear regression equation of y = 357590x + 20398 and correlation coefficient of 0.9999. The whole method including extraction step was performed by liquid chromatography with spiked soil samples. The recoveries values obtained for trifluralin in soil are showed in Table 2.

Table 2 - Recoveries for trifluralin in soil by HPLC (n=5)

Trifluralin concentrations (µg/g)		Mean Recovery (%)	Relative Standard Deviation (%)
Spiked	Obtained		
2.0	1.538	76.9	12.2
5.0	3.915	78.3	9.8
10.0	7.770	77.7	11.15

The results obtained by spectrophotometry and HPLC were compared. The correlation between both methods was made by ploting the values of final concentration obtained by spectrophotometry *versus* final concentration obtained by HPLC. The Figure 2 shows the satisfactory correlations both methods. As can be seen, there is suitable proportionality in the recoveries obtained by the proposed method and that one obtained by conventional determination (linear regression equation of y=0,9709x + 0,041). These studies corroborate to the powerful of the spectrophotometric method for trifluralin determination in soil samples.

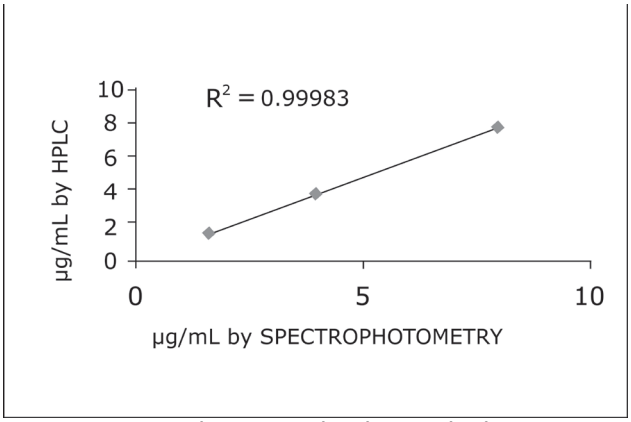

Figure 2 - Correlation study obtained when comparing trifluralin determination by spectrophotometric and liquid-chromatographic methods

3.3 Analysis of soil collected in a soybean field

Soil samples collected at Balsas, MA, were analyzed by spectrophotometric method developed was not detected the presence of trifluralin in any sample investigated. It probably occurs because at that region the herbicide was replaced by others products with wider spectrum of action.

4 CONCLUSIONS

These studies corroborate the applicability of the spectrophotometric method for trifluralin determination in soil samples. Satisfactory analytical conditions were obtained by developed method in spite of somewhat little but proper sensitivity (limit of detection of 0.04 μg mL^{-1}) for herbicide analysis in soil samples. Average recoveries varied from 79 to 82% with relative standard deviation around 9% for 5 repetitions. The correlation between developed and conventional methods could be considered very satisfactory (correlation coefficient of 0.9999). Trifluralin was analyzed on soil samples from agricultural fields of soybean culture (northeast of Brazil) and it was observed that residues were below the limit of detection.

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