An Analytical Method for the Simultaneous Determination of Butachlor and Benoxacor in Wheat and Soil

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Butachlor is a chloroacetanilide herbicide successfully employed in weeding some important crops, and benoxacor is a safening compound able to induce the enzymatic mechanism of chloroacetanilide detoxification in plants. A practical method for a simultaneous detection of butachlor and benoxacor residues in wheat and in soil is described. The procedure can be performed by GC and HPLC. They were extracted with methanol and cleaned up by solid phase extraction (SPE). The analytes were satisfactorily separated via both GC and HPLC techniques, and no interferences were observed coming from plant or soil matrixes or reagents. The limit of quantitation was found to be 5.0 ng by GC and 20.0 ng by HPLC for butachlor and 2.5 ng by GC and 15.0 ng by HPLC for benoxacor. Butachlor recovery tests ranged from 85.4% to 91.7% in wheat shoots and 84.0% to 93.2% in soil; benoxacor recovery tests ranged from 86.5% to 90.8% in wheat shoots and 85.7% to 90.7% in soil. The reproducibility and the accuracy make this method a selective and sensitive tool for routine analyses.

KEYWORDS: Butachlor; benoxacor; analytical method; wheat; soil

INTRODUCTION

As the tolerance of some crops to some herbicides is marginal, research is oriented toward developing some synthetic compounds, termed herbicide safeners, which increase the herbicide tolerance (*1, 2*). This strategy has facilitated the selective use of some herbicides, which are now supplied in a mixture with specific safeners (*3−5*). A widely used safener, dichloroacetanilide benoxacor [(±)-2,2-dichloro-1-3,4-dihydro-3-methyl-2H-1,4-benzoxazin-4-yl) ethanone], was found to induce the activity of glutathione *S*-transferases, which are enzymes involved in the detoxificative pathway of some chloroacetanilide herbicides in maize and wheat (*6−8*). Among chloroacetanilides, butachlor (*N*-butoxymethyl-2-chloro-2′,6′-diethylacetanilide) is commonly applied either at pre-emergence or at early post-emergence to control a wide spectrum of annual grasses and some broadleaf weeds in wheat and transplanted rice fields (*9−13*).

The effect of benoxacor as a safener in protecting wheat and maize plants against chloroacetanilide herbicides is known; nevertheless, no analytical procedures for evaluating the residues and the persistence of the butachlor-benoxacor used in combination are available in the literature; a GC method for butachlor is reported, which is standardized for aqueous samples (*14*).

The introduction of new compounds requires adequate analytical tools to control the herbicidal pollution in the plant−soil system as well as to perform further insights into the safening mechanism. Therefore, the aim of this research was to develop a reproducible and easily applied analytical method,

Table 1. Physical-Chemical Properties of Soil Used

pH (H$_2$O)	8.10
organic carbon (%)	1.22
C.E.C.^{a} (mequiv 100 g^{-1})	16.5
sand (%)	23.5
silt (%)	47.5
clay (%)	29.0

^{a} C.E.C. = cation exchange capacity.

for the sensitive and simultaneous determination of butachlor and benoxacor residues in wheat plants and soil samples.

To enhance the laboratory feasibility of the method, it was standardized for both GC and HPLC determinations of the two analytes following the same procedure for extraction and purification from possible matrix interferences. The purification was performed by solid-phase extraction (SPE).

MATERIALS AND METHODS

Chemicals and Apparatus. Standard butachlor (95.8%) and benoxacor (99.8%) were supplied by Sigma Aldrich (St. Louis, MO). Stock solutions of the two analytes were prepared in acetone at 1 mg/mL and stored in the dark at 4 °C; working standard solutions were prepared by appropriate dilutions.

SPE Florisil cartridges were obtained from Chemtek Analytica (UK).

Acetone, acetonitrile, ethyl acetate, methanol, *n*-hexane, phosphoric acid, and water were all of analytical grade and were purchased from BDH (Poole, UK).

A Perkin-Elmer Auto System XL gas chromatograph, equipped with a capillary column ELITE-35 (30 m length; 0.53 mm i.d.; 0.50 μm film thickness) (Perkin-Elmer, USA) and a nitrogen-phosphorus detector

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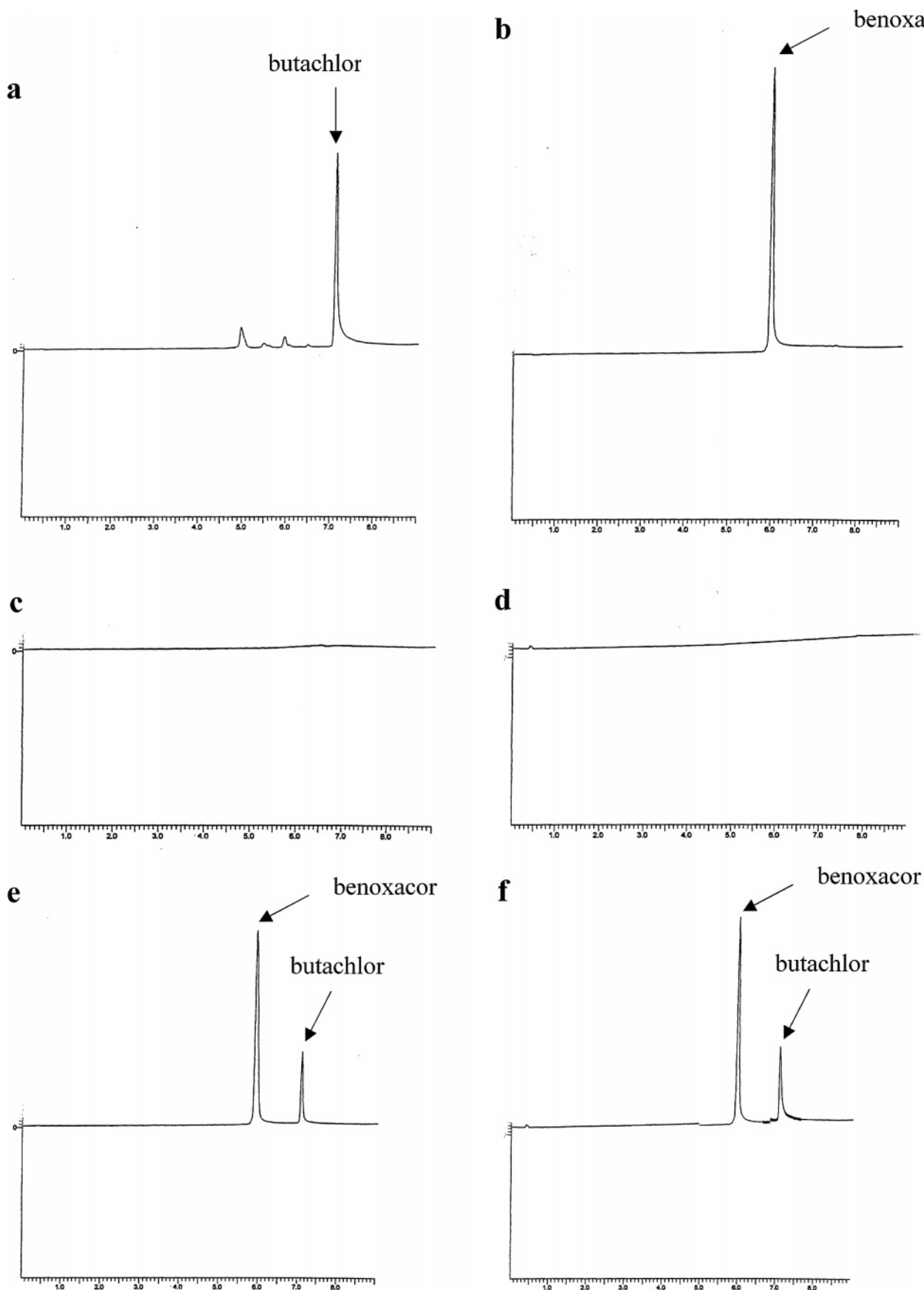
Figure 1. GC chromatograms of standard butachlor (50 ng – **a**) and benoxacor (50 ng – **b**), of extracts from untreated samples of wheat (**c**) and soil (**d**), and of fortified samples of wheat (1000 μg kg^{-1} – **e**) and soil (500 μg kg^{-1} – **f**). (The figures are differently scaled.)

(NPD), was employed for the GC determinations of butachlor and benoxacor residues.

A Perkin-Elmer Series 410 HPLC, equipped with an LC 95 UV detector set to monitor absorbance at 215 nm wavelength and with an
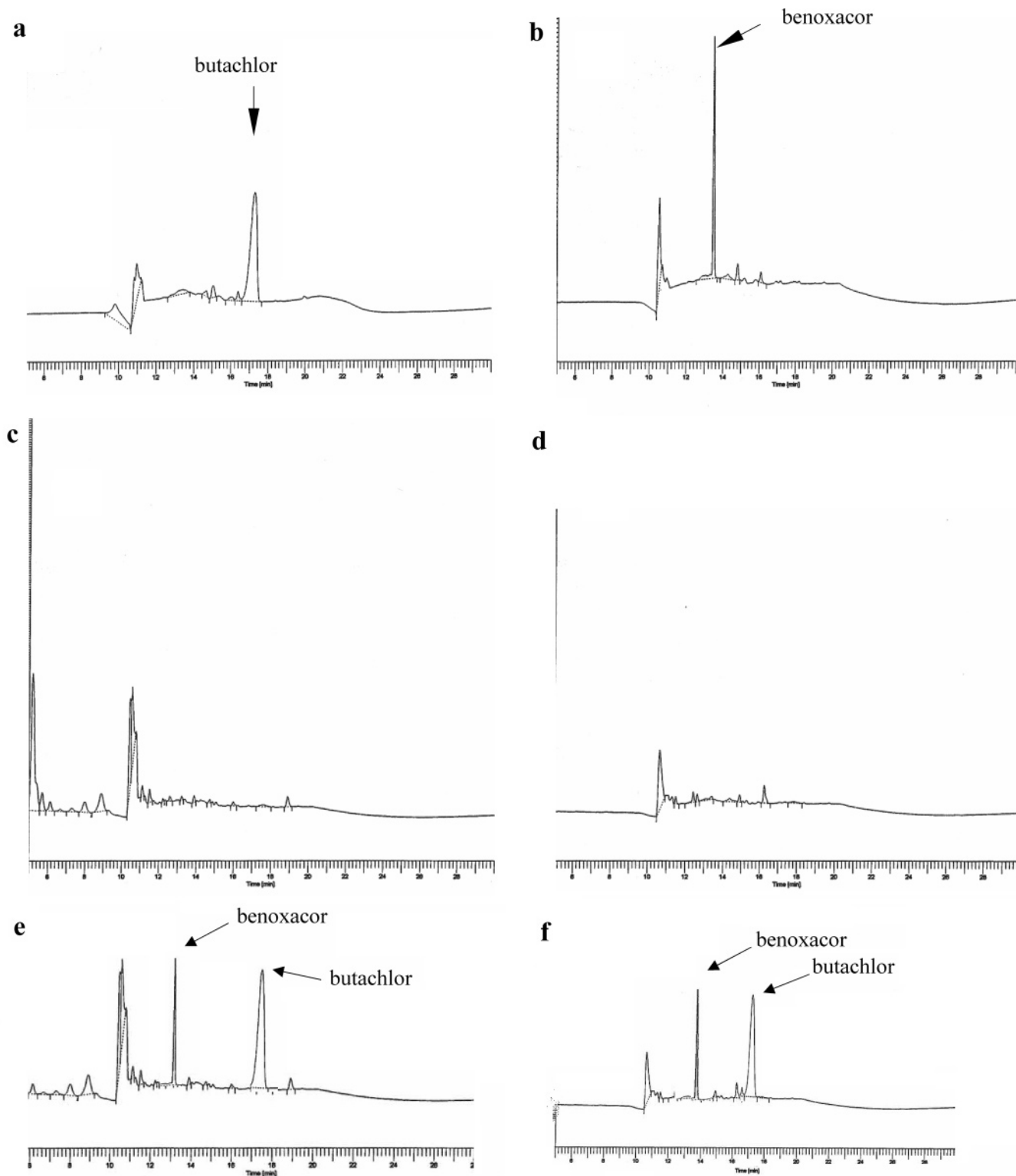
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Figure 2. HPLC chromatograms of standard butachlor (100 ng − **a**) and benoxacor (100 ng − **b**), of extracts from untreated samples of wheat (**c**) and soil (**d**), and of fortified samples of wheat (1000 μg kg^{-1} − **e**) and soil (500 μg kg^{-1} − **f**). (The figures are differently scaled.)

Alltima C-18 column (25 cm length; 4.6 mm i.d.; 5 μm mean particle size) (Alltech, USA), was employed for the HPLC determinations of butachlor and benoxacor residues.

Sample Preparations and Analyses. *Plant Samples.* Wheat seeds (hybrid Palesio) were germinated in plastic flats containing quartz sand prewashed with a 10% hydrochloric acid solution and sterilized with a 5% NaClO solution. Seedlings were grown in the dark at 18 °C (relative humidity of 80%). After 4 days, the seedlings were subjected to day-night conditions (12 h of light at 21 °C, at light intensity of 300 μmol m^{-2} s^{-1}, and 12 h of darkness at 18 °C). Distilled water (50 mL) was

applied daily to each flat. When the seedlings were 6 days old (two foliar stage), shoot samples (20.0 g) were collected and suitable amounts of butachlor and benoxacor were added to the samples so as to obtain concentrations of 100−500−1000−2000 and 10 000 μg kg^{-1} of fresh tissue. The samples were powdered in liquid nitrogen using a mortar and pestle and extracted with methanol (w/v, 1:5); the resulting extract was filtered, dried under vacuum, and redissolved into 2 mL of *n*-hexane. The extract solution was applied to a SPE cartridge Florisil column (1000 mg/6 mL, 170 μm), preactivated with 15 mL of *n*-hexane, washed with 5 mL of *n*-hexane, and analytes eluted with 4 mL of an

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Table 2. Means[a] of Recovery Percentages of Butachlor and Benoxacor Fortified Wheat and Soil Samples (Standard Deviation in Brackets)

fortification concentration μg kg^{-1}	wheat		soil	
	butachlor	benoxacor	butachlor	benoxacor
100	86.8 (0.6)	86.7 (0.6)	89.9 (2.1)	85.7 (0.6)
500	86.5 (1.6)	86.5 (1.8)	84.0 (3.0)	86.7 (2.0)
1000	85.4 (2.2)	86.6 (0.8)	89.2 (2.0)	87.4 (1.3)
2000	91.7 (4.2)	89.3 (0.4)	93.2 (1.0)	88.4 (1.1)
10 000	91.5 (1.3)	90.8 (0.4)	92.9 (1.2)	90.7 (0.7)

[a] Each datum is the mean of triplicate analyses ($n = 3$).

ethyl acetate/*n*-hexane solution (v/v, 2:3). The sample, wash, and elution solvents were allowed to flow through the cartridges under vacuum. The recovered fractions were evaporated to dryness, using a stream of nitrogen gas, redissolved in 1 mL of methanol, and subjected to GC and HPLC analyses. Each analytical determination, for each concentration, was run in triplicate.

For the GC-NPD analyses, the injection volume was 2 μL and the instrument inlet was set at 220 °C. The column oven temperature was held at 100 °C for 1 min, raised at 20 °C min^{-1} to 240 °C followed by a 5 °C min^{-1} ramp to 250 °C. The temperature was maintained at 250 °C for 10 min to bake out any remaining matrix interferences. The carrier gas (helium) flow was 5 mL min^{-1}. Under these conditions, the retention time was 7.1 min for butachlor and 6.0 min for benoxacor.

For the HPLC-UV analyses, the injection volume was 20 μL. The LC separation was performed using water containing 1% phosphoric acid (mobile phase A) and acetonitrile (mobile phase B); the gradient profile was as follows: 1 min 80% A:20% B, followed by a linear increase in 1 min to 100% B, and then the column was maintained with 100% B for 8 min; finally, the column was raised to 80% A:20% B for 20 min. Under these conditions, the retention time was 17.3 min for butachlor and 13.5 min for benoxacor.

Soil Samples. A typical soil of central Italy (Papiano, Perugia) taken from the 0–20 cm layer was employed. For the soil sampling, a suitable amount of moist soil (2.0 kg) was air-dried and sieved (<2 mm) to remove plant material, soil macrofauna, and stones. After sieving, the soil was homogenized for 3 h in a rotary cylinder and stored at 20 °C in the dark for 3 days (preincubation). Determination of pH, cation exchange capacity, organic carbon content, and particle size distribution was performed according to ASA-SSSA methods (*15*) (**Table 1**).

Soil samples (50 g) were fortified, in triplicate, with methanolic solutions of butachlor and benoxacor to obtain concentrations of 100–500–1000–2000 and 10 000 μg kg^{-1} for both of the compounds. Each sample was extracted with methanol (w/v, 1:5) and then filtered, dried under vacuum, and redissolved in 2 mL of *n*-hexane. The solution was applied to a SPE cartridge Florisil column, which was preactivated and

eluted as previously described for the plant samples. The eluted fractions were evaporated to dryness, redissolved in 1 mL of methanol, and submitted to GC and HPLC determination as described above.

Each analytical determination, for each concentration, was run in triplicate.

RESULTS AND DISCUSSION

The matrixes of soil and wheat samples were too contaminated by interfering substances for direct analyses by GC and HPLC; therefore, the samples required further purifications. A purification procedure based on solid-phase extraction (SPE) was standardized, which caused the elimination of many interfering substances without significant loss in the recovery yield. The extraction and purification procedure did not require high temperatures, avoiding the possibility of decomposition for both of the analytes.

Figure 1 shows original GC chromatograms of standard butachlor (**a**) and benoxacor (**b**), of the extracts of untreated samples of wheat (**c**) and soil (**d**), and of the analytes extracted from fortified samples of wheat (**e**) and soil (**f**). The HPLC chromatograms of standard butachlor (**a**) and benoxacor (**b**) and of the extracts of untreated samples of wheat (**c**) and soil (**d**), and of the analytes extracted from fortified samples of wheat (**e**) and soil (**f**), are given in **Figure 2**.

The rapid and simple solid-phase extraction procedure (SPE) developed for the cleanup step greatly reduced the interfering substances in the elution areas for both GC and HPLC analyses; in fact, the peaks of the two analytes were well separated from the peaks of plant and soil matrixes, and no interferences were shown between the two analytes for both GC and HPLC analyses.

The limits of quantitation (LOQ), defined as the amount at twice the signal/noise ratio (*16*), were 5.0 and 20.0 ng for butachlor following the GC and HPLC procedures, respectively, and 2.5 and 15.0 ng for benoxacor following the GC and HPLC procedures, respectively.

Due to the higher sensitivity shown by the GC analyses when compared to HPLC analyses as well as the relatively lower level of matrix interferences resulting from the selectivity of the nitrogen-phosphorus detector, the linearity, recovery tests, reproducibility, and accuracy were performed only for the GC procedure.

The linear regression between peak area and the concentrations of butachlor and benoxacor, observed over the 0.5 to 500 μg mL^{-1} range both for plant and for soil samples, gave

Table 3. Reproducibility and Accuracy for GC Determination of Butachlor and Benoxacor in Fortified Samples of Wheat and Soil ("Within Day" Assay)[a]

a.c. (μg kg^{-1})	c.d. (μg kg^{-1}) mean (S.D.)		RSD		accuracy (as relative error)	
	butachlor	benoxacor	butachlor	benoxacor	butachlor	benoxacor
	wheat					
100	86.8 (0.6)	86.7 (0.6)	0.7%	0.7%	−13.2%	−13.3%
500	432.5 (8.0)	432.5 (9.0)	1.8%	2.1%	−13.5%	−13.5%
1000	854 (22)	866 (8.0)	2.6%	0.9%	−14.6%	−13.4%
2000	1834 (84)	1786 (8.0)	4.6%	0.4%	−8.3%	−10.7%
10 000	9150 (130)	9080 (40)	1.4%	0.4%	−8.5%	−9.2%
	soil					
100	89.9 (2.1)	85.7 (0.6)	2.3%	0.7%	−10.1%	−14.3%
500	420 (15)	433.5 (10)	3.6%	2.3%	−16.0%	−13.3%
1000	892 (20)	874 (13)	2.2%	1.5%	−10.8%	−12.6%
2000	1864 (20)	1768 (22)	1.1%	1.2%	−6.8%	−11.6%
10 000	9290 (120)	9070 (70)	1.3%	0.8%	−7.1%	−9.3%

[a] a.c. = actual concentration; c.d. = concentration determined; S.D. = standard deviation; RSD = relative standard deviation. Each datum is the mean of triplicate analyses ($n = 3$).

calibration curves showing an average correlation coefficient (r^2) of 0.999, indicating a good linearity of the response.

The recovery percentage of the two compounds from wheat and soil samples, obtained for the five fortification levels, is reported in **Table 2**. They ranged from 85.4% to 91.7% in wheat and from 84.0% to 93.2% in soil for butachlor and from 86.5% to 90.8% in wheat and from 85.7.% to 90.7% in soil for benoxacor.

To ascertain the reproducibility and accuracy of the method, analyses "within day" (*16*) were performed, and the results are shown in **Table 3**. Reproducibility was quantified as the relative standard deviation (RSD), which is the percent ratio between the standard deviation of the data and the mean determined concentration. The RSD values found in the tests of butachlor recovery ranged from 0.7% to 4.6% for wheat samples and from 1.1% to 3.6% for soil samples. The RSD values found in the tests of benoxacor recovery ranged from 0.7% to 2.1% for wheat samples and from 0.7% to 2.3% for soil samples. The accuracy of the measurements is reported in **Table 3**, and it is defined in terms of percent relative error obtained from the difference between mean recovery and expected recovery, divided for the expected recovery. The accuracy data show a good degree of constancy in the recoveries for both of the analytes.

In conclusion, the method described in this paper allows rapid and quantitative detection of butachlor and benoxacor in both wheat and soil samples. The use of solid-phase extraction (SPE) cartridges caused a reduction in the presence of interfering substances both in GC and in HPLC analyses. The GC technique gave the higher sensitivity. In addition, the selectivity of the nitrogen phosphorus detector (NPD) brought about a further reduction of matrix interferences.

The linearity, reproducibility, and accuracy, coupled with good peak resolution and low matrix interferences, make this method particularly suitable for routine analyses of butachlor and benoxacor in plant or soil systems.

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