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Determination of herbicides by reductive amperometric detection in liquid chromatography

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Abstract

A liquid chromatographic method has been developed for the determination of the herbicides metamitron, metribuzin, isometiozin and nitralin in soil samples. Reductive amperometric detection using a hanging mercury drop electrode has been selected because of the selectivity of the technique. The separation has been performed in a Spherisorb CN, 5 μm (250×4.0 mm) column. A mobile phase consisting of acetonitrile–phosphate buffer (32:68, v/v) pH 2.5 was used with the detector operated in direct current mode at $E=-1350$ mV. The analysis time was 9 min. The mean relative standard deviations (R.S.D.) of the results were <4%. Conditions for extraction of herbicides from soil samples have been established. The method allows the determination of 3.4, 2.9, 12.1 and 6.9 ng g^{-1} of metamitron, metribuzin, isometiozin and nitralin in soil, respectively.

Keywords: Detection, LC; Amperometric detection, reductive; Environmental analysis; Pesticides

1. Introduction

The commercial use of chemicals for pest control in agriculture was first developed in the forties. Recent surveys have shown that the world-wide use of agrochemicals continued to increase in the last decades [1]. Herbicides are much more heavily used in Europe or the USA than either insecticides or fungicides, and estimates of use from 1993 show that herbicides represent 70–80% of the total amount of pesticides used on crops in developed countries [2]. The environmental risk from any given herbicide is determined by its toxicity to wild-life, its mobility and its persistence in the environment, and the time, frequency and rate of application. In order to evaluate all of these parameters, sensitive and selective analytical methods are required.

Chromatographic methods for the determination of herbicide residues in crops, food and environmental samples have been reviewed [3]. Specific reports of gas chromatography (GC) or liquid chromatography (LC) multiresidue triazine herbicide analysis have been published [4–9]. A variety of detectors, mainly nitrogen–phosphorus (NPD), mass spectrometry (MS) and electron-capture (ECD) for GC and UV, diode-array (DAD) and fluorescence for LC analysis, have been used for the identification and quantification of all classes of herbicide residues. However, in spite of the fact that electrochemical detection (ED) is one of the most sensitive and selective detection methods in LC, it has not been used very often for the determination of these compounds. However, ED has been used for trace determination of phenylurea herbicides in water [10] and dual electrochemical reductive–oxidative detection has been used for the determination of organophosphorus pesticides in water [11].

ED can be operated in the oxidative or reductive

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amperometric mode. LC assays using reductive amperometric detection have been described using mercury pool [12], gold amalgamated [13], bubble electrode [14] or dropping mercury electrodes (DMEs) [15–18]. The DME has the distinct advantage of presenting a continuously renewable fresh surface during chromatographic analysis. Unlike conventional voltammetry, the potential of the working electrode is not scanned. In direct current (d.c.) mode, the electrode potential is held constant at a predetermined value. The use of a short life-time drop increases the background signal, so that electrodes like the hanging mercury drop electrode (HMDE) or the static mercury drop electrode (SMDE) with large life-time drops (several min) are preferred. Although the drop may be renewed during a chromatogram, this is generally not necessary as the baseline shift within a single chromatogram is normally acceptably small.

When spectroscopic detectors are used, the extraction and clean-up methods must be very efficient to avoid interferences with organic materials in the soil. Reductive amperometric detection has such great selectivity that clean-up and extraction processes can be simplified and shortened.

The present paper studies the use of the reductive amperometric detection mode, in the determination of the herbicides metamitron, metribuzin, isometiozin and nitralin, using a LC system. The selected herbicides are generally used on extensive potato cultures that are traditionally grown in the Alava Plain region (Basque Country, Spain).

2. Experimental

2.1. Reagents and chemicals

Acetonitrile (BDH, Poole, UK), Methanol (BDH) and all the other chemicals were of analytical-reagent grade. Aqueous solutions were prepared with ultra-high-quality water obtained with a Milli-Ro and Milli-Q system (Millipore, Milford, MA, USA). The supporting electrolyte used in the mobile phase was prepared by mixing $5 \cdot 10^{-3}$ mol l^{-1} sodium dihydrogenphosphate (Riedel-de Haën, Hannover, Germany) and orthophosphoric acid to yield the desired pH. The resulting buffer solution was mixed with methanol and acetonitrile to obtain the desired eluent composition. Tridistilled mercury (Merck, Darmstadt, Germany) was used in the working electrode. Helium, used to purge the column eluent, was of oxygen-free grade (Air Liquide, Madrid, Spain).

The herbicides metamitron and nitralin were supplied by the Laboratory of Dr. Ehrenstorfer (Angsburg, Germany) with purities of 99.8%. Isometiozin (Tantizon) and metribuzin (Sencor) were obtained from Bayer Hispania Commercial and were further purified to 99.4% before use.

2.2. Apparatus

A Metrohm E-506 polarograph (Metrohm, Herisau, Germany), a function generator E-612, a VA-663 stand and a Yokogawa X-Y recorder (Yokogawa Europe, Amersfoort, Netherlands) were used for cyclic and adsorptive stripping voltammetry.

The LC system consisted of a Waters Model 510 pumping system with a pulse dampener to obtain a constant flow-rate, a Model U6K injector (Waters) and a Knauer on-line degasser. A 25.0 cm×4.1 mm I.D. 5 μm Spherisorb CN column (Tracer Anal., Barcelona, Spain) was used. The polarographic detector consisted of a Model 400 polarographic analyzer (EG&G Princeton Applied Research, Princeton, NJ, USA). The potential was applied in the d.c. mode. The working electrode was a PARC SMDE Model 420. All the potentials were reported relative to a silver–silver chloride electrode. The electrochemical data were processed by means of a Metrohm 714-IC Metrodata workstation.

The instrumental system used in LC and steps taken to reduce noise have been described in detail [19]. Special caution is necessary for removing the oxygen from the mobile phase; the eluent reservoir was continuously purged with helium and all eluent lines were of stainless steel to avoid oxygen infusion.

2.3. Extraction and clean-up in soil

A 20-g sample of homogeneized soil, sieved to 2 mm, was extracted with 25 ml of acetonitrile by shaking in a capped centrifuge tube for 30 min, centrifuged for 5 min at 1000 *g* and then filtered under reduced pressure. The filtrate was concentrated by evaporation using a nitrogen stream. The residue

was made up to 1 ml with mobile phase and aliquots of 25 μl were injected into the LC system.

3. Results

Electrochemical properties of the metamitron, metribuzin, isometiozin and nitralin herbicides have been studied in other papers [20–24]. The cyclic voltammograms obtained with the optimum mobile phase as the supporting electrolyte and using an HMDE are shown in Fig. 1. All the compounds exhibited irreversible reduction waves. For the triazines, the maximum current peak is due to reduc-

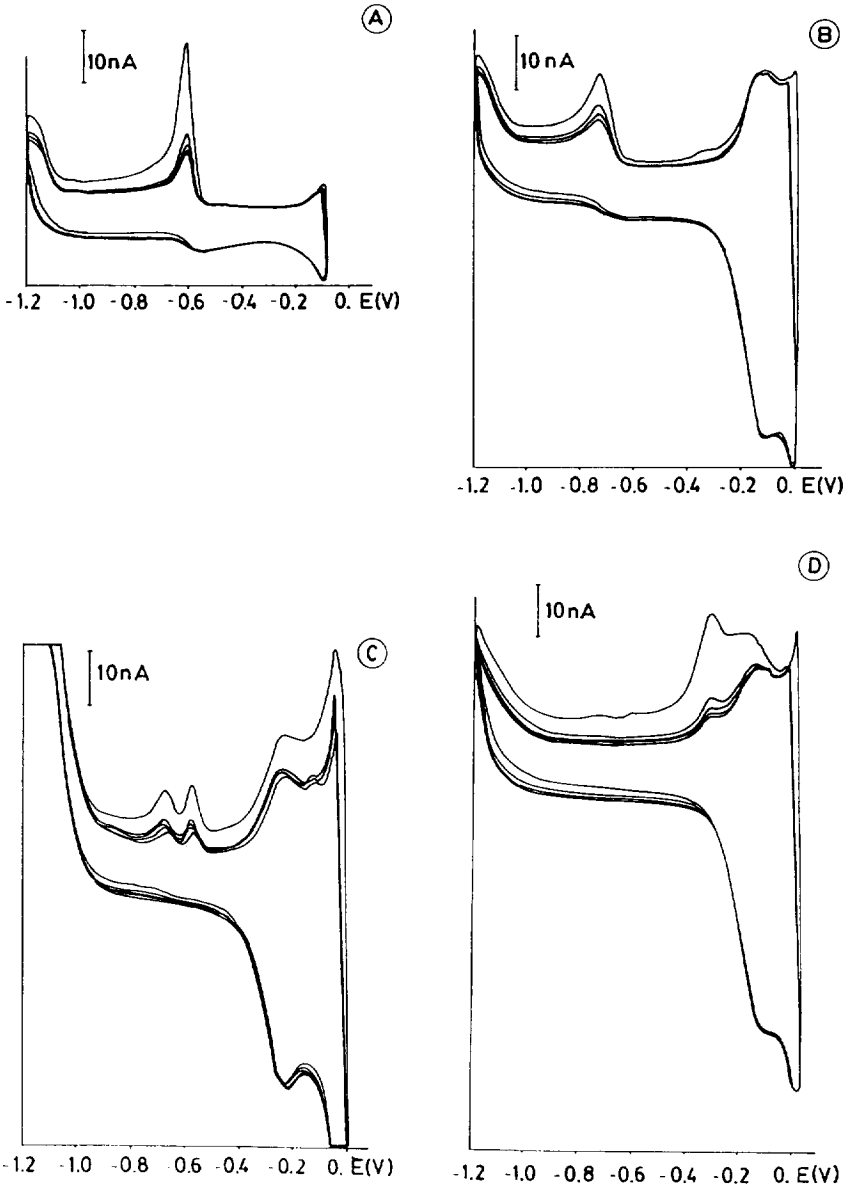

Fig. 1. Cyclic voltammograms of: (A) metamitron; (B) metribuzin; (C) isometiozin; (D) nitralin, on HMDE. Concentration: $1 \cdot 10^{-5}$ mol l^{-1}. Scan rate: 200 mV s^{-1}. Solvent: 68% phosphate buffer (pH=2.5), 32% acetonitrile.

tion of the azometine group, whereas reduction of the nitro group causes the main peak in the case of nitralin.

Following the second scan, a decrease in the reduction peaks of the four substances is observed, which means that an adsorption process of these molecules on the electrode takes place. The existence of electro-adsorptive processes on the HMDE can provoke a diminution of the intensity of chromatographic peaks. This could be avoided by renewing the mercury drop during the chromatogram, but the 2 min delay time per drop, which is necessary to stabilize the baseline, makes this procedure impractical. Moreover, the applied potentials in the chromatographic detection procedure, as shown below, are too negative for the electro-adsorption process to take place.

3.1. Direct current reductive amperometric detection

For this detection technique, the HMDE (or SMDE) electrode is kept at a constant potential. Normally this potential would be chosen near the most negative reduction potential of all of the substances studied. However, a very negative potential cannot be used without some sacrifice in terms of the signal-to-noise ratio. The fact that the pH affects the electrochemical response and the potential limits, makes it necessary to study both

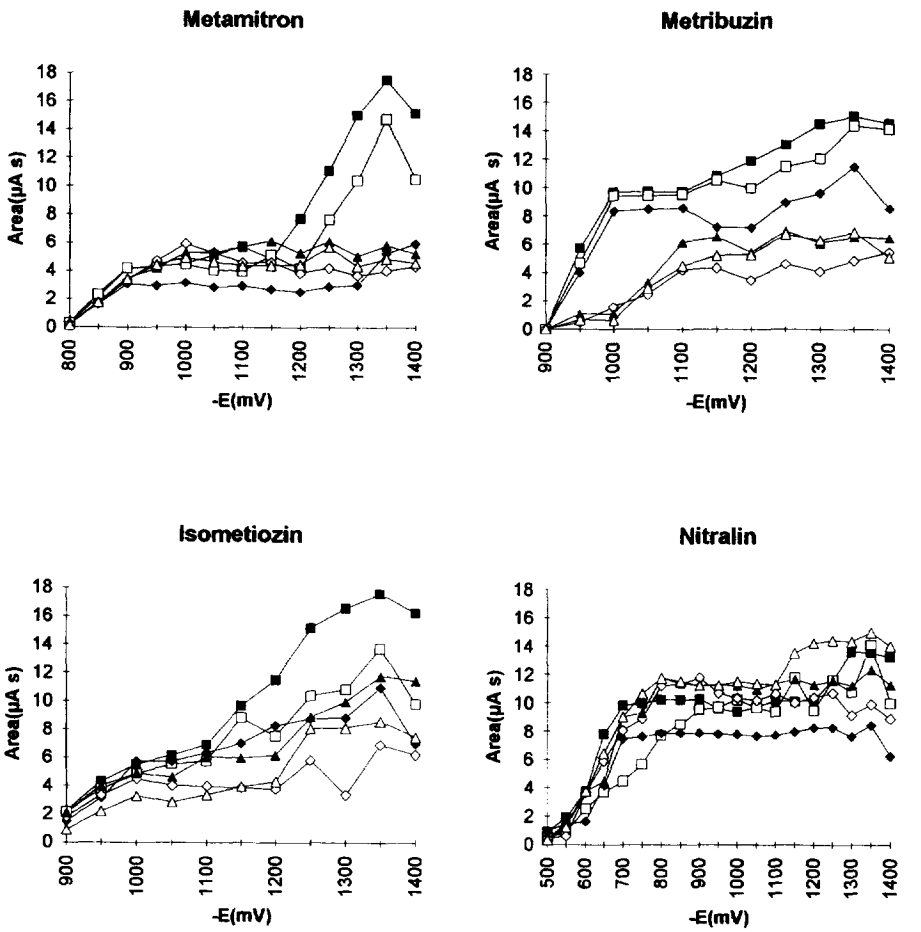

Fig. 2. Hydrodynamic voltammograms of metamitron, metribicin, isometiozin and nitralin. Amounts injected, 1000 ng. Flow-rate, 1.0 ml min^{-1}; injection volume, 25 μl. ◇, pH=2.5; □, pH=3.0; ♦, pH=3.5; ◊, pH=4.0; △, pH=5.0; ▲, pH=6.0.

parameters, i.e., potential and pH, together. The hydrodynamic voltammograms used to determine the optimum pH and potential conditions for amperometric detection are shown in Fig. 2. The diffusion plateau for all of the substances was observed from a potential of −1350 mV and the optimum pH values were 2.5 for metribuzin, metamitron and isometiozin and 6.0 for nitralin, although the latter has a response almost constant over the whole pH range. Higher potentials provoke high background noise and impede the measurements with the available cell. Hence, a potential of −1350 mV and a pH of 2.50 were selected.

The mobile phase composition was optimized with respect to ionic strength and organic solvent. Two organic modifiers, methanol and acetonitrile, were used to optimize the time of analysis, the peak width and the resolution. Table 1 shows the influence of the content of methanol and acetonitrile in the mobile phase. The conditions for selection are $R_s > 1$ and a value of K' for the run time that is as short as possible. Hence, acceptable results were obtained with a phosphate buffer–acetonitrile (68:32, v/v) mobile phase, pH 2.50. The retention times obtained for metamitron, metribuzin, isometiozin and nitralin were 3.44, 3.83, 5.51 and 7.86 min, respectively, with a flow-rate of 1.0 ml min^{-1} (Fig. 3). The influence of ionic strength supplied by the buffer on the chromatographic response is shown in Fig. 4. The peak heights of each of the herbicides increased with increasing buffer concentration up to $5 \cdot 10^{-3}$ mol l^{-1} and then remain practically constant. This is why a buffer concentration of $5 \cdot 10^{-3}$ mol l^{-1} was selected as the optimum value.

The flow-rate and drop size are important parameters in LC with reductive amperometrical detection. The flow cell geometry is based on the wall-jet configuration with a distance between the delivery tip and the capillary tip of 0.5 mm. Drop sizes of 1.0, 1.6 and 2.5 mm^2 can be used. Greater peak areas and smaller peak heights are obtained when slower flow-rates are used, with a subsequent decrease in peak resolution. When the drop size is smaller, the resolution decreases because the adsorption processes on it are reinforced. The experimental relationship between peak area, flow-rate and drop size was similar for all herbicides studied. As an example, Fig. 5 shows the behaviour of metamitron. The selected conditions were 2.5 mm^2 drop size and 1.0 ml min^{-1} flow-rate.

The chromatographic response can be expressed in terms of area or peak height. A behaviour more regular and homogeneous is observed when the response of the detector is quantified as peak area

Table 1
Resolution (R_s) of two adjacent peaks related to organic modifier composition

Buffer	Acetonitrile	Methanol	Meta-metri R_s	Metri-iso R_s	Iso-nitra R_s
80	20	–	0.83	3.39	7.09
77	23	–	0.97	2.78	5.99
74	26	–	1.001	3.128	3.65
72	28	–	0.92	2.7	3.17
70	30	–	1.003	2.41	2.10
68	32	–	1.058	3.05	2.91
65	35	–	0.98	2.68	1.86
63	37	–	0.53	1.72	1.97
60	40	–	0	1.64	1.44
70	25	5	0.72	3.29	3.18
70	20	10	0.74	3.09	3.86
70	15	15	0.28	2.34	3.27
75	20	5	0.71	2.23	5.06
75	15	10	0.85	4.59	5.41
75	5	20	0.43	4.12	5.55
65	25	10	0.27	1.45	1.63
65	30	5	0.79	2.43	2.52
70	–	30	0.79	2.25	4.33

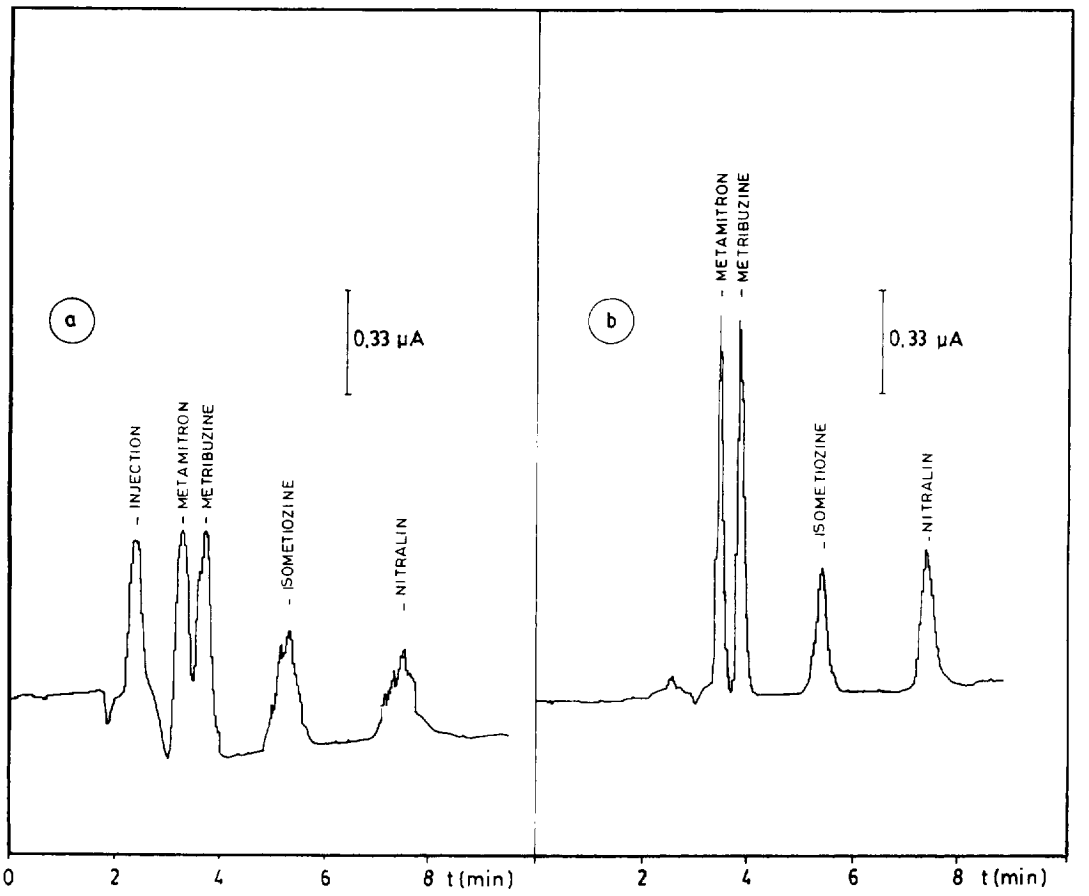

Fig. 3. Chromatograms obtained in d.c. detection mode. Standard mixture containing 500 ng of each herbicide, (a) herbicides disolved in acetonitrile and (b) herbicides disolved in phosphate buffer–acetonitrile (68:32, v/v; pH=2.5). Electrode potential, −1350 mV; flow-rate, 1.0 ml min^{-1}. Metamitron, t_R=3.44; metribuzin, t_R=3.83; isometiozin, t_R=5.51; nitralin, t_R=7.86 min.

rather han peak height, h. Under the conditions described above, linear relationships were found between "$h_{herbicide}/h_{internal\ standard}$" and herbicide concentration. The concentration range examined extended to 5–10 times the limit of detection. One of the herbicides, isometiozin, was used as the internal standard for the others. On the other hand, when isometiozin had to be determined, the selected

Table 2
Calibration fits for optimum overall performance

Herbicide	Intercept	Slope×10^{-3} (ng^{-1})	Correlation coefficient	R.S.D. (%)			D.L. (ng)
				A	B	C	
Metamitron	0.106±0.066	9.282±0.183	0.9996	3.81	2.95	3.38	1.70
Metribuzin	0.044±0.102	11.42±0.249	0.9995	3.80	2.63	3.56	1.38
Isometiozin	0.049±0.028	2.801±0.080	0.9991	4.12	2.23	3.89	5.63
Nitralin	0.026±0.034	4.618±0.095	0.9996	1.98	1.35	1.83	3.42

R.S.D.=relative standard deviation (n=10).
D.L.=detection limit, (A) 6 ng injected of each herbicide, (B) 30 ng injected of each herbicide, (C) 300 ng injected of each herbicide

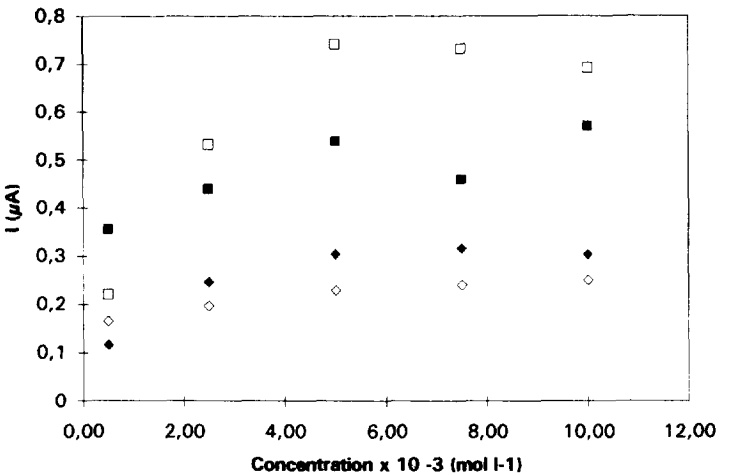

Fig. 4. Influence of ionic strength supplied by the buffer on the chromatographic response. ◇, metamitron; □, metribuzin; ♦, isometiozin; ◊, nitralin. Other conditions as in Figure 3.

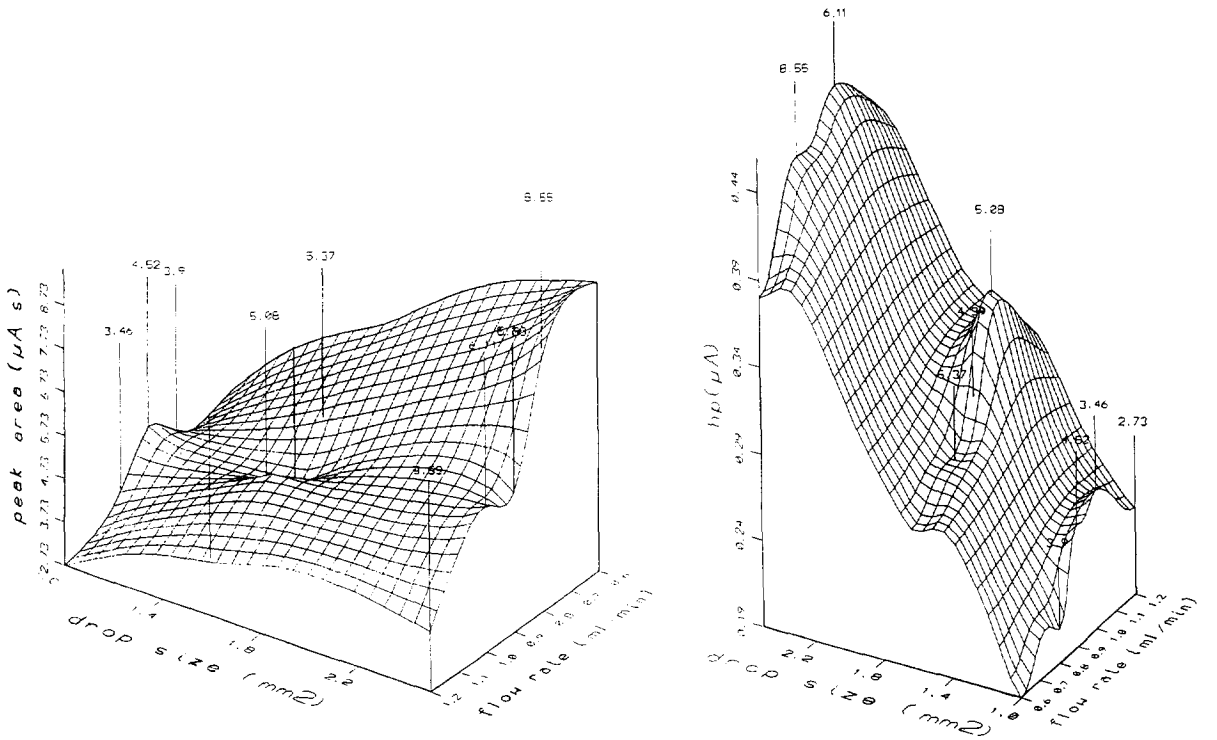

Fig. 5. Dependence of the detector response on the drop size and flow-rate. Metamitron, 500 ng. Rotation about the Z axis, 45°. Other conditions as in Figure 3.

internal standard was metribuzin. The equations obtained by the least squares regression method are reported in Table 2. The limits of detection defined as $2N/m$, where N is the noise and m is the slope of the calibration graph, are also reported in Table 2.

The relative standard deviations ($n=10$), at a concentration level of 30 ng injected, were in the range 1.8–3.6%; the values decreased to 1.3–2.9% when 300 ng of each herbicide were injected. The R.S.D.s were in a similar range, 2.0–4.1%, at concentration levels close to the limits of detection (6 ng of each herbicide).

3.2. Determination of the herbicides in soils

The selectivity and sensitivity of d.c. reductive amperometric detection allows quantitative measurements of these herbicides without the necessity for extract clean-up to remove interfering soil co-extractives.

Although not necessary for some LC detectors, it is very important that the extraction solvent be replaced by mobile phase prior to analysis of the soil extracts by reductive amperometric detection. Fig. 3 illustrates the chromatographic separation of the herbicides when injected in the extraction solvent (acetonitrile) and when injected in the mobile phase. In the former situation, the chromatogram shows a large peak at 2.3 min. This peak is not due to dissolved oxygen because nitrogen gas was used to degas the extract, but it is due to a change in the conductivity of the mobile phase, with subsequent modification of the electrodic double layer configuration, when acetonitrile was eluted.

In order to check the applicability of the proposed method to real matrices, soil samples from Alava (Basque Country, Spain) were analysed. This soil was selected for study because it was representative of that generally used for potato production. The characteristics of the soil selected for study can be summarized in the parameters: texture, clay; humidity, 2.80%; conductivity, 109 $\mu S\ cm^{-1}$; total organic matter, 4.02%; pH (H_2O), 7.73; pH (KCl), 7.20.

None of the herbicides were detected in the soil samples. Soil samples were then fortified with the usual rates used in potato production: 1.54 mg kg^{-1} of metamitron, 0.18 mg kg^{-1} of metribuzin, 2.40 mg kg^{-1} of isometiozin and 0.25 mg kg^{-1} of nitralin. Samples were analysed following the procedure described above (Fig. 6). The recoveries of these concentration levels are shown in Table 3. Extraction efficacy was not found to vary significantly among different concentrations of herbicides.

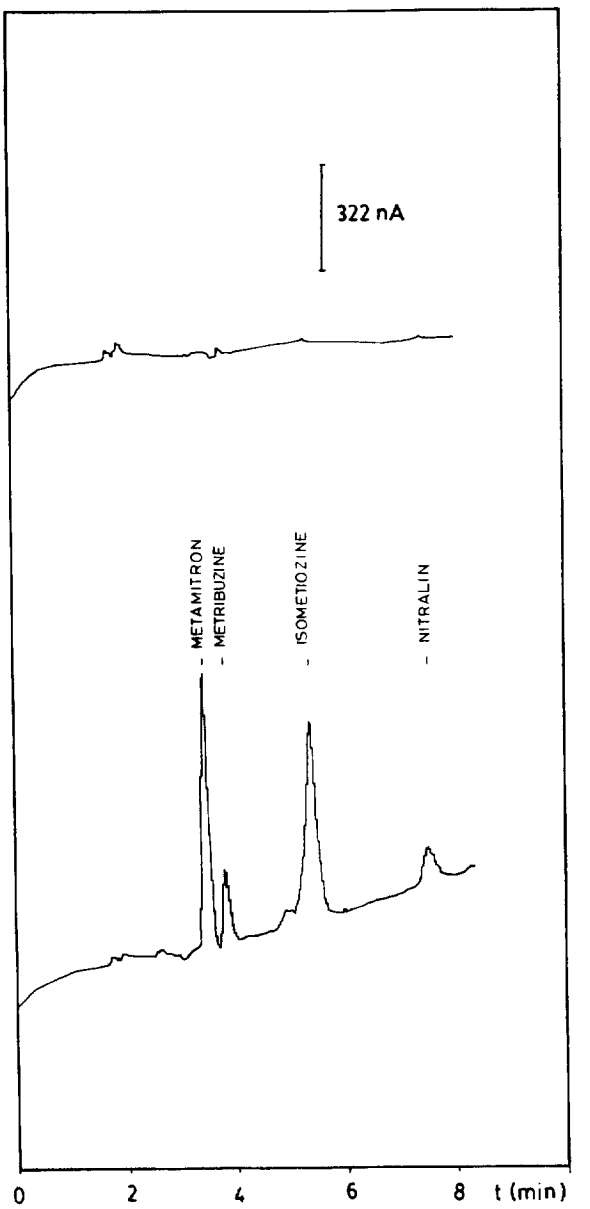

Fig. 6. Chromatogram of extract of fortified soil samples with the usual doses: 1.54 mg kg^{-1} of metamitron, 0.18 mg kg^{-1} of metribuzin, 2.40 mg kg^{-1} of isometiozin and 0.25 mg kg^{-1} of nitralin. (Top) blank soil.

Table 3
Recovery data ($n=5$) for several soil fortification levels

Herbicide	A		B		C	
	Recoveries	R.S.D. (%)	Recoveries	R.S.D. (%)	Recoveries	R.S.D.(%)
Metamitron	103.2±3.5	3.8	99.8±3.9	4.0	100.6±4.2	4.3
Metribuzin	94.3±4.8	4.1	94.8±4.4	3.9	93.9±4.6	3.7
Isometiozin	93.1±3.7	3.6	95.0±4.0	3.9	93.5±3.2	3.6
Nitralin	97.9±4.3	4.4	98.5±3.1	4.2	98.0±4.8	4.0

(A) usual doses (see text); (B) doses close to the level of quantification: metamitron, 6.0 μg kg^{-1}, metribuzin, 6.0 μg kg^{-1}, isometiozin, 24.0 μg kg^{-1} and nitralin, 14.0 μg kg^{-1}.

This method allowed the determination of these herbicides in soils of about 3.40 μg kg^{-1} for metamitron, 2.93 μg kg^{-1} for metribuzin, 12.09 μg kg^{-1} for isometiozin and 6.98 μg kg^{-1} for nitralin. No interference by other organic substances present in the soil was observed.

4. Conclusions

A LC system with reductive amperometric detection was developed for the determination of metamitron, metribuzin, isometiozin and nitralin herbicides. The method was applied to the analysis of soil samples. The method requires only minimal sample extract clean-up and has a short chromatographic run time. The precision and limits of detection are satisfactory and comparable with UV detection.

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