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ANALYSIS OF ASULAM IN SOIL BY ISOTACHOPHORESIS AND LIQUID CHROMATOGRAPHY

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SUMMARY

Capillary isotachophoresis, preparative capillary isotachophoresis and high-performance liquid chromatography were tested for the determination of the herbicide asulam in soil. Different configurations of analytical systems based on these techniques were evaluated with respect to detection limits, recovery and reliability of quantitative analysis. While capillary isotachophoresis used alone after a simple sample pre-treatment permitted reliable quantitation at concentrations down to 0.5 ppm, the use of preparative capillary isotachophoresis for the sample cleanup before the final isotachophoretic or chromatographic analyses gave, in general, much better results. A combination of preparative and analytical isotachophoresis permitted the reliable quantitation of asulam at fortification levels of 0.1–0.2 ppm with reliable detection at a concentration of 0.02 ppm. Similar results were achieved for the combination of preparative isotachophoresis and liquid chromatography. The recoveries of the complete analytical procedures were in the ranges 81–91% (0.2 ppm) and 95–120% (0.02 ppm).

INTRODUCTION

Asulam [methyl (4-aminosulphonyl)carbamate] is effective as both a pre- and post-emergent herbicide. Its broad use for weed control requires sufficiently sensitive and selective methods for residue analysis.

The simplest procedure suitable for the determination of residues of asulam is spectrophotometry based on the coupling reaction of N-1-naphthylethylenediamine with a diazotized amino group[1]. Obviously, the final evaluation steps needs to be preceded by a sample pre-treatment. This method suffers from non-specificity, *e.g.*, for the determination of asulam in peaches[2]. On the other hand it was found to be suitable for the determination of the herbicide in heavy clays with a sample pre-treatment[3]; 90% recoveries were obtained for 1–2 ppm concentrations of asulam in the clay samples.

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A multi-stage clean-up procedure followed by thin-layer chromatography is necessary before the UV spectrophotometric determination of asulam in soil samples by the method proposed by Franci *et al.*[4]. In this instance 75–90% recoveries were determined for 0.5–10 ppm concentrations of herbicide.

Gas–liquid chromatography (GLC) with a nitrogen–phosphoric sensitive detector was used for the determination of asulam residues in peaches[2]. An extensive sample clean-up and derivatization of the hydrolytic product of asulam were performed in order to achieve recoveries of 70–80% for 1–10 ppm and 50–60% for 0.1 ppm fortification levels.

Only a simple pre-treatment was necessary for the determination of asulam in wheat by reversed-phase high-performance liquid chromatography (RP HPLC) at levels down to 0.02 ppm[5]; 78–89% recoveries were determined for the concentration 0.1 ppm and 89–94% for the concentration range 1–10 ppm.

With the exception of the last method, the procedures used for sample pre-treatment are laborious and time consuming when determinations at levels below 0.5 ppm are required. Moreover, using the same final analytical method, the sample pre-treatment usually must be changed with depending on the type of sample.

At present, many of the pesticides in current use and/or their degradation products are ionogenic in nature. Asulam (a moderately strong acid with pK_a = 4.82) also belongs to this group. With respect to its physico-chemical properties and to the nature of the matrix (soil), we decided to test capillary isotachophoresis (ITP) and RP HPLC. As the aim was to develop a procedure that would permit the reliable quantitation of asulam at a concentration of 0.1 ppm with minimal sample pre-treatment, the recently described preparative ITP technique[6] was used for the sample cleanup. The results achieved using single column analytical ITP, preparative ITP followed by analytical ITP and preparative ITP followed by RP HPLC are presented.

EXPERIMENTAL

Instrumentation

A Tachophor 2127 (LKB, Bromma, Sweden) provided with both conductivity and UV detectors (2127-140) was used for analytical ITP. Peak areas from the UV detector (254 nm) were evaluated with an HP 3390A reporting integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Preparative ITP experiments were carried out using a discontinuous fractionation unit[6] in a column-coupling configuration[7] built in our laboratory (see Fig. 1).

An isocratic chromatography system consisting of an HPP 4001 pump (Laboratorní přístroje, Prague, Czechoslovakia), an adapted Knauer sampling valve provided with a 38-μl sample loop (Knauer, West Berlin, F.R.G.) and an LCD 254 UV detector (Laboratorní přístroje) was used. The UV detector was connected to a Minigrator (Spectra-Physics, Santa Clara, CA, U.S.A.) and to a line recorder. A 250 × 6 mm I.D. stainless-steel column packed with 10-μm spherical Separon C_{18} was obtained from Laboratorní přístroje. Water–methanol was used as the mobile phase throughout.

Chemicals

Chemicals used for the preparation of the leading and terminating electrolytes

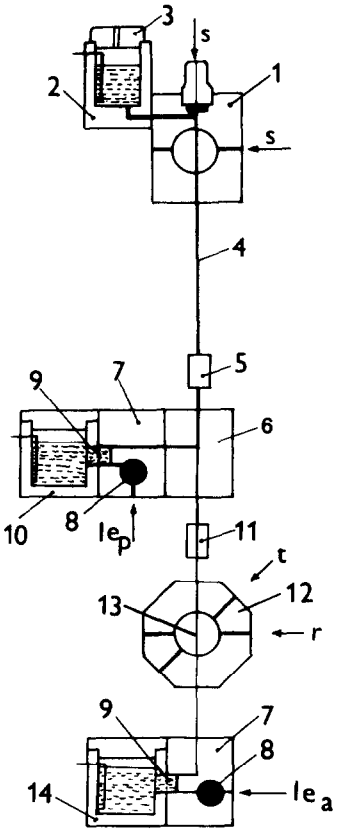

Fig. 1. ITP separation unit in a column coupling configuration[7] used for sample cleanup. 1 = Sampling valve with positions for the sample introduction (s); 2 = terminating electrolyte compartment with a cap (3); 4 = 1.6 mm I.D. pre-separation tube; 5, 11 = conductivity detectors; 6 = bifurcation block; 7 = refilling blocks with needle valves (8); 9 = membrane; 10 = leading electrolyte compartment for the pre-separation tube; 12 = fractionating valve with positions of the plunger for trapping (t) and for refilling of the channel (13); 13 = 0.85 mm I.D. trapping channel (the tube in the trapping column is of the same I.D.); 14 = leading electrolyte compartment for the trapping column. le_p, le_a = positions for the refilling of the pre-separation and trapping columns, respectively.

were obtained from Reanal (Budapest, Hungary) and Lachema (Brno, Czechoslovakia). Some of them were purified by conventional methods. α-Aminoadipic acid was bought from Serva (Heidelberg, F.R.G.) and glutamic acid from Lachema. Servalyt AG 2-11 (Serva) was used for spacing as a continuous mobility gradient[8]. Hydroxyethylcellulose (Polysciences, Warrington, PA, U.S.A.) and methylhydroxyethylcellulose 30,000 (Serva), after purification on a mixed-bed ion exchanger, were used as additives to the leading electrolytes[8] at 0.2% concentrations.

Asulam, its sodium salt and soil samples were kindly provided by the Residue Laboratory of the Research Institute for Chemical Technology (Bratislava, Czechoslovakia).

Extraction and sample pre-treatment

A 100-g amount of air-dried soil was shaken with 200 ml of 5 m*M* disodium borate solution for 90 min. The extract was centrifuged (4000 rev/min) and filtered through a paper filter. An aliquot of the filtrate was concentrated 5-fold in a vacuum rotary evaporator at 40°C, then glutamic and α-aminoadipic acids (discrete spacers[8,9]) were added at 0.2 m*M* concentrations and the volume was made up to 10 ml with doubly distilled water (1 ml was equivalent to 2.5 g of soil).

Soil samples fortified with asulam at 0.02 and 0.20 ppm concentrations were treated in the same way. Further details are given below.

RESULTS AND DISCUSSION

Analytical ITP

For the determination of asulam by analytical ITP only a UV detector operating at 254 nm wavelength was used. Of the operational systems tested, system 1 (Table I) was chosen for this investigation as the number of co-migrants within the leading–terminating mobility interval found in soil samples was acceptably low and well separated (as evaluated with a conductivity detector).

An isotachopherogram from the direct analysis of the soil extract (Fig. 2a) and extracts in which the injected volumes of the extract were spiked with 1 and 10 ng of asulam (Fig. 2b and c, respectively) clearly show that UV-absorbing co-migrants giving a simultaneous detector response with asulam do not allow its reliable qualitative and quantitative analysis. Similar detection patterns are typical, *e.g.*, in ITP separations of protein mixtures[8,9]. Therefore, an analogous means of increasing the number of constituents resolved by UV detection was followed. In protein separations both UV non-absorbing discrete spacers and mixtures of compounds forming a "continuous" mobility gradient (mostly synthetic ampholytes) are used to space UV-absorbing separands[8,9].

TABLE I

OPERATIONAL SYSTEMS

Parameter	*System No.**	
	1	*2*
Solvent	Water	Water
Leading anion	Cl^-	Cl^-
Concentration (m*M*)	10	10
Counter ion	BALA	HIS
Additive to the leading electrolyte	HEC (MHEC)	HEC (MHEC)**
Concentration (%)	0.2	0.2
pH of leading electrolyte	3.6	6.0
Terminating anion	CAPR	MES

* BALA = β-alanine; HIS = histidine; CAPR = caproic acid; HEC = hydroxyethylcellulose; MHEC = methylhydroxyethylcellulose; MES = morpholinoethanesulphonic acid.

** No additive was used in the leading electrolyte for the trapping column; before a series of experiments the capillary tube was washed with a 0.3% aqueous solution of MHEC.

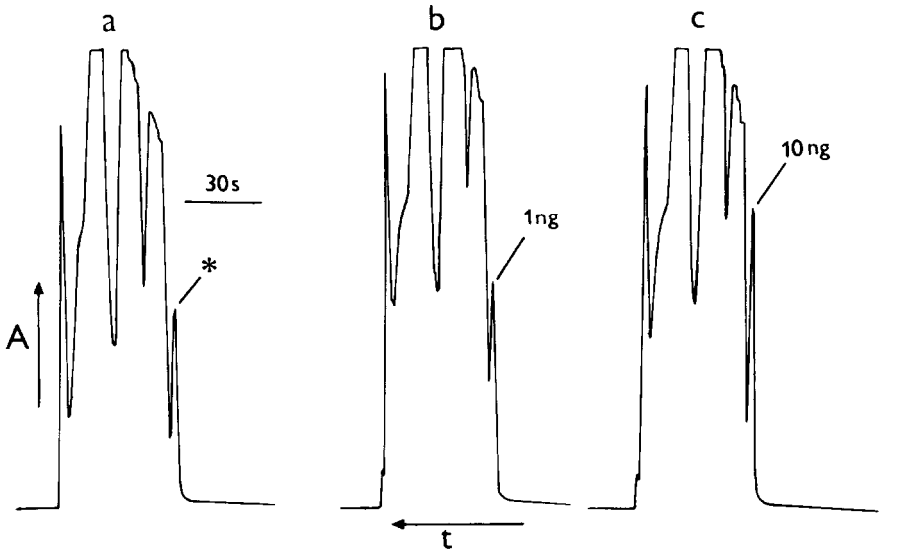

Fig. 2. Direct ITP analysis of a soil extract. (a) 20 μl of soil extract (equivalent to 50 mg of the soil sample); (b) 20 μl of the soil extract spiked with 1 ng of asulam; (c) the same sample as in (b) but spiked with 10 ng of asulam. The asterisk indicates the position of asulam. A = Increasing UV light absorption (254 nm); t = increasing time. Operational system 1 (Table I). The driving current was 100 μA.

The potential of this approach for the determination of asulam illustrate isotachopherograms, shown in Fig. 3. The isotachopherogram in Fig. 3a corresponds to a blank run in which only Servalyt and discrete spacers were injected. UV-absorbing impurities detected at the position of asulam originate from the solution of leading and terminating electrolytes, from Servalyt and probably also from the septum[10]. The corresponding peak area is equivalent to 1.6 ng of the herbicide. As can be seen from Fig. 3a and b, 1 ng of asulam can be detected with confidence, but its

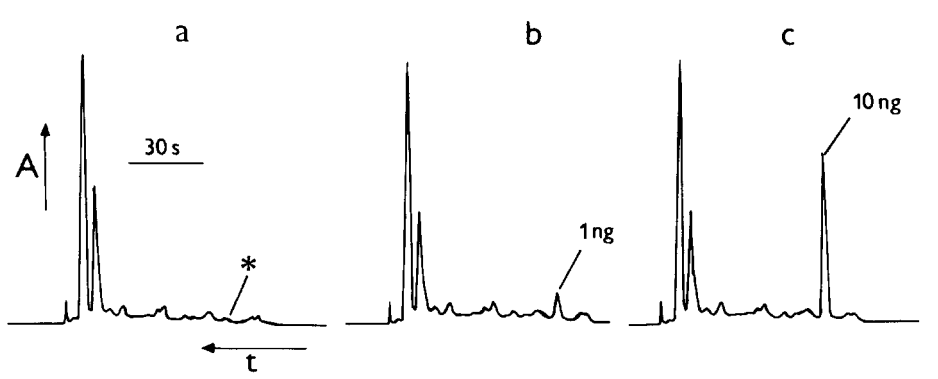

Fig. 3. Isotachopherograms illustrating the detection capabilities in the determination of asulam using a UV (254 nm) detector. (a) Blank run in which 1 μl of Servalyt AG 2-11 (diluted 1:10 with water) and discrete spacers (glutamic and α-aminoadipic acids) were injected; (b) the same as (a) except the spacers were spiked with 1 ng of asulam; (c) the same as (a) except the amount spiked was 10 ng. The asterisk indicates the position of asulam. A = Increasing UV light absorption (254 nm); t = increasing time. Operational system and driving current as in Fig. 2.

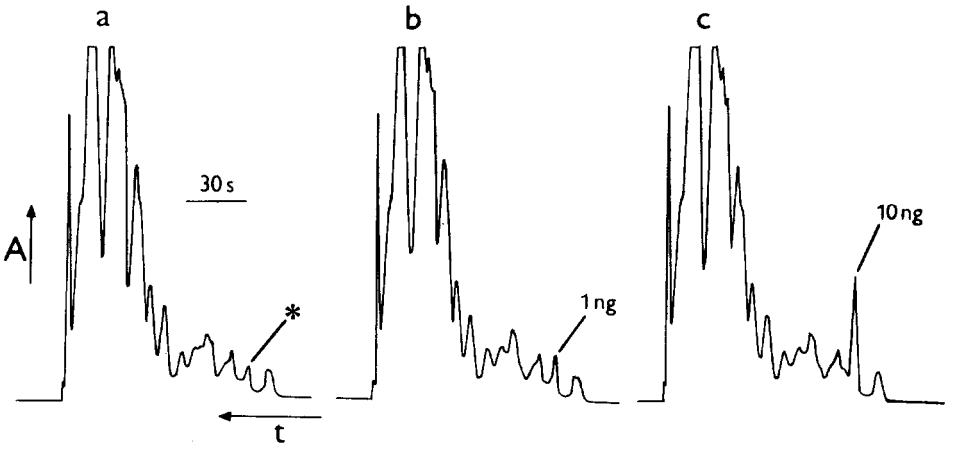

Fig. 4. Direct ITP analysis of a soil extract using discrete spacers (glutamic and α-aminoadipic acids) and continuous spacers (Servalyt AG 2-11 diluted 1:10). A 20-μl volume of the soil extract (equivalent to 50 mg of the soil sample) was injected in all instances together with 1 μl of the spacing constituents. (a) Without asulam (its position is marked with an asterisk); (b) 1 ng of the herbicide present in the injected volume; (c) 10 ng spiked. Other symbols and separation conditions as in Fig. 3.

quantitation is systematically biased when corrections for the blank run are not performed.

In the analysis of a soil extract, 20-μl volumes of both spiked and unspiked samples were injected (Fig. 4). This volume is equivalent to 50 mg of soil and the spiked amounts represent 0.02 (1 ng) and 0.2 (10 ng) ppm concentrations of asulam. When the asulam peak areas are not corrected for soil contaminants (blank run on unfortified soil extract), its determinations are biased by positive systematic errors (*ca.* 400% for 1 ng and *ca.* 35% for 10 ng). If corrections for contaminants are not possible and the above systematic errors are not tolerable, a search for better spacing constituents and/or other operational systems could be helpful in decreasing the values of systematic errors. This solution was not studied in our work. Instead, preparative ITP was used for the cleanup of the extract.

Sample cleanup by preparative ITP with evaluation by analytical ITP

In this combination of different ITP techniques the separation unit shown in Fig. 1 was used for the sample cleanup and analytical ITP in the above configuration was used for the final analysis. Operational system 2 (Table I) was used for preparative runs and system 1 for the final analysis. The former system differentiates acids of the strength of asulam mainly according to their ionic mobilities whereas the latter system differentiates the anionic constituents according to their pK values. Therefore, preparative and analytical ITP separations were performed in a "two-dimensional" manner.

Isotachopherograms from the conductivity detectors for a cleanup run are given in Fig. 5. The box on the isotachopherogram in Fig. 5b defines the constituents trapped by the fractionating valve. The content of the trapping channel in the valve (13 in Fig. 1) was washed into a 100-μl volume. This volume was identical with the

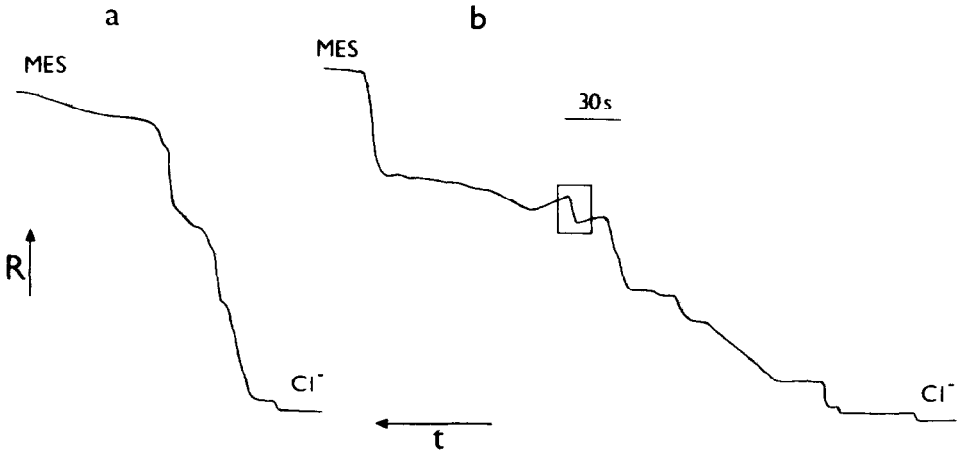

Fig. 5. Isotachopherograms from a preparative run on a soil extract. The separation unit shown in Fig. 1 was used. The box on isotachopherogram (b) defines the trapped material. A 100-μl volume of soil extract containing 5 ng of asulam (0.02 ppm) was injected in this instance. The anionic constituents present in the box were trapped in a 100-μl volume, of which 20 μl were injected into analytical unit (see Fig. 7c). Operational system 2 (Table I) was used in both columns. The driving currents were 450 and 200 μA in the pre-separation and trapping stages, respectively. (a), (b), Isotachopherograms from the pre-separation and trapping stages, respectively. R = Increasing resistance.

injection volume and neither concentration nor dilution effects were associated with preparative ITP in this instance.

Volumes of 20 μl of the samples cleaned by preparative ITP were analysed under identical conditions as used for the direct analysis (Figs. 3 and 4). Isotachopherograms from the analyses of the cleaned, unspiked soil extracts are given in Figs. 6a and 7a. The areas of the peaks at the position of asulam due to contaminants from the soil extracts were decreased in these instances to 20% of the value found in the direct analysis (Fig. 4a). The amount of these contaminants is equivalent to 1.5 ng of the herbicide and is very close to the amount of contaminants from the leading and terminating electrolytes and from Servalyt (Fig. 3a). Total removal of the UV-absorbing anionic constituents in this sample pre-treatment can be evaluated only approximately (transparency mode of the detector and, consequently, non-linear dependence of UV response on concentration at higher concentration levels) and values higher than 95% seem reasonable.

The use of preparative ITP for sample pre-treatment was also tested from the point of view of the recovery of the procedure. Amounts of asulam ranging from 5 to 50 ng injected in 100-μl volumes were separated in preparative unit, trapped in 100-μl volumes and 20-μl aliquots were analysed by ITP. In a series of 18 preparative runs the recoveries for preparative ITP varied in the range 97–103%. When the trapped volumes were corrected for the errors due to the volume measurement during the trapping (conical test-tubes of 300-μl volume were calibrated for 100-μl volumes by weighing 100 mg of distilled water and used as volumetric devices), the recoveries ranged from 98 to 102%.

An isotachopherogram from the analytical unit shown in Fig. 6b was obtained from the analysis of a soil extract fortified with 0.2 ppm of asulam (the extract con-

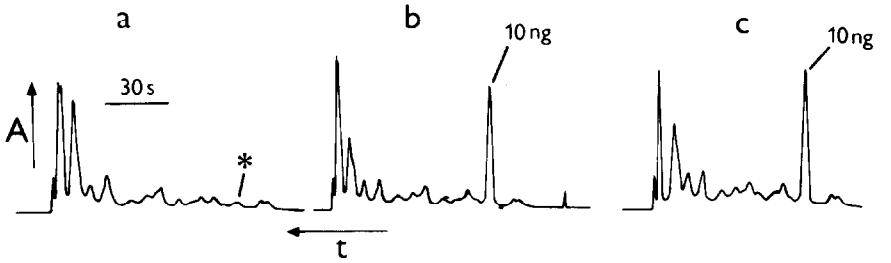

Fig. 6. Isotachopherograms from the analyses of soil extracts cleaned up by preparative ITP. (a) 20 μl of the soil extract (the asterisk indicates the position of asulam); (b) 20 μl of soil extract spiked with 10 ng of asulam (0.2 ppm); (c) 20 μl of the extract from soil fortified with 0.2 ppm of asulam. The spacing consituents were injected in the amount as in Fig. 3 in all instances. Conditions for preparative runs as in Fig. 5. For the conditions during the analytical evaluation, see Fig. 3.

taining asulam was cleaned by preparative ITP). This isotachopherogram is taken from the determination of the calibration graphs (for further details, see Fig. 8).

The determination of the herbicide in soil is illustrated by the isotachopherogram in Fig. 6c. In this instance 100 g of a soil sample were fortified with a solution containing 20 μl of asulam. The extraction procedure and further sample pre-treatment were carried out as described under Experimental and above. This isotachopherogram is taken from a series of experiments devoted to the determination of the recovery of the procedure, consisting of the extraction step, pre-concentration of the extract by partial evaporation of solvent, preparative ITP and analytical ITP. The data obtained from these experiments give the possibility of estimating the systematic bias of the determination at the 0.2 ppm concentration level. The recoveries are summarized in Table II.

In the same way the recoveries were evaluated for soil samples fortified with 0.02 ppm of asulam. Isotachopherograms illustrating the determinations of this amount of herbicide are given in Fig. 7. The recoveries of the complete procedure for this concentration level are summarized in Table II.

With respect to the direct analysis, preparative ITP pre-treatment of the extract decreases the possible positive systematic error to *ca.* 150% for 0.02 ppm and to *ca.* 13% for 0.2 ppm concentration levels. Obviously, these errors can be eliminated when parallel blank experiments can be performed. In general, the procedure described in this section provides very reproducible results with good recoveries and its

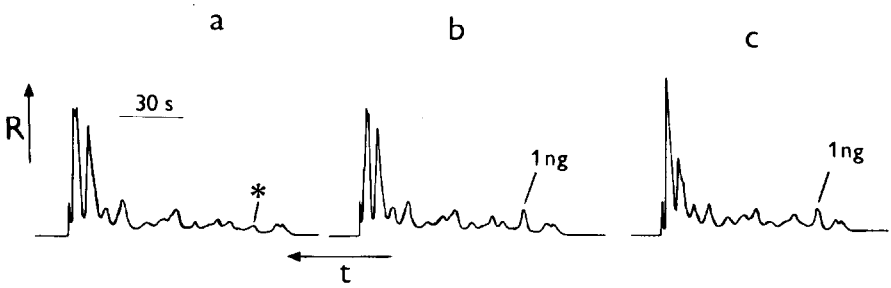

Fig. 7. Analysis of asulam present in soil samples at 0.02 ppm concentrations. Isotachopherograms refer to experiments analogous to those in Fig. 6 except the amount of asulam was 10 times smaller.

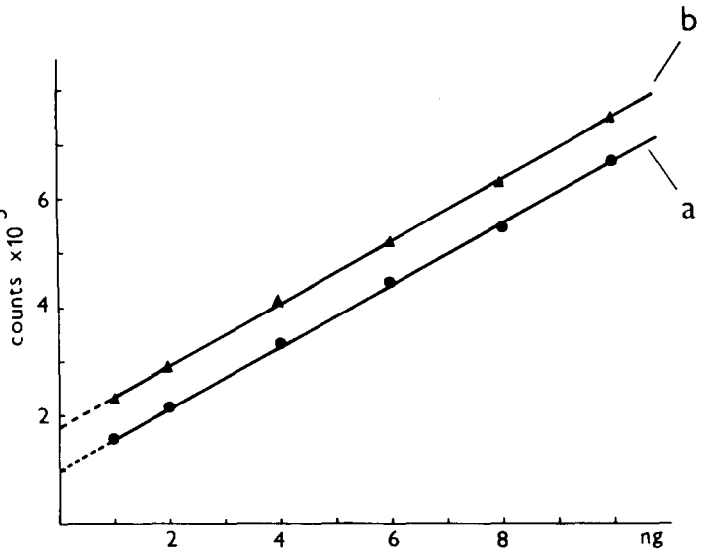

Fig. 8. Calibration graphs for the determination of asulam. (a) 1–10-ng amounts of asulam in water were injected directly into the analytical unit together with the spacing constituents. The linear relationship is described by the equation $y = 95{,}800 + 56{,}740x$ (y = peak area in counts, x = the amount of asulam) with a correlation coefficient of 0.9970 ($n = 64$). (b) 1–10-ng amounts of asulam added to the soil extract, cleaned by preparative ITP and determined by analytical ITP. The linear relationship is described by the equation $y = 169{,}400 + 56{,}630x$ with a correlation coefficient 0.9976 ($n = 63$).

use for the routine determination of asulam in soil at concentrations down to 0.1 ppm is reliable. It can be expected that further optimization of the separation conditions in preparative ITP and the use of better spacing constituents in analytical ITP will lead both to further increases in the cleaning efficiency of preparative ITP and to a decrease in the bias of the determination in analytical ITP. Improvement of the selectivity of detection is another way of fully exploiting the analytical possibilities of ITP. For example, dual wavelength UV detection as developed by Verheggen *et al.*[11] seems very promising in this respect.

TABLE II

RECOVERIES OF ASULAM FROM SOIL SAMPLES USING THE COMPLETE PROCEDURE

Parameter	*Soil sample 1 (0.2 ppm)*			*Soil sample 2 (0.2 ppm)*	
Preparative run No.	1	2	3	1	2
Recovery (%)*	87.5	90.5	87.0	88.0	82.0
	Soil sample 3 (0.02 ppm)			*Soil sample 4 (0.02 ppm)*	
Preparative run No.	1	2		1	2
Recovery (%)*	108	120		95	104

* The recoveries are given as means of three parallel determinations from a particular preparative cleanup run.

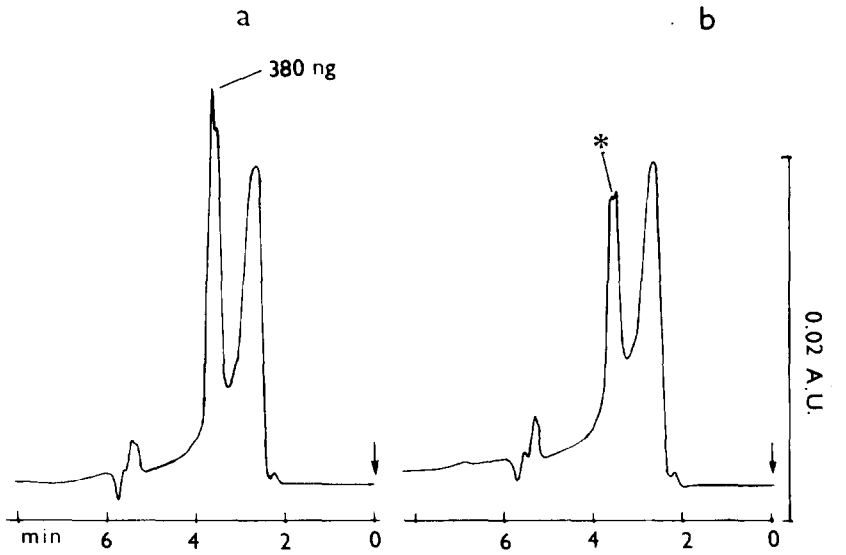

Fig. 9. Chromatograms from direct analysis of a soil extract. (a) Soil extract spiked with asulam [380 ng of the herbicide was present in the injected volume (38 μl), which corresponds to 100 mg of soil]; (b) unfortified soil extract (the position of asulam is marked with an asterisk). Eluent, water–methanol (95:5); flow-rate, 1 ml/min.

Sample cleanup by preparative ITP with evaluation by RP HPLC

Our attempts to use RP HPLC for direct determination of asulam in soil were not successful[12] when water–methanol mixtures were used as mobile phases (asulam could not be separated from some soil constituents; see also Fig. 9). Further optimization of the separation conditions was not carried out because the main disadvantage of direct chromatographic determination was associated with some soil constituents that were retained by the column, changed its selectivity and gradually decomposed, producing strongly UV-absorbing compounds. We therefore decided to use preparative ITP for sample cleanup before chromatographic determination. The pretreatment procedure was the same as that used for combined preparative–analytical ITP.

In the chromatographic experiments the volume injected (with a microsyringe) was 50 μl, of which 38 μl remained in the sample loop and was applied on to the column.

For the quantitative analyses, the characteristics of the calibration line, *i.e.*, counts given by the Minigrator *versus* amount of asulam, were determined. For chromatographic determinations the calibration points were measured for asulam in water. A linear relationship described by the equation $y = -1294 + 983x$ (where y = peak area in counts and x = amount of asulam) with a correlation coefficient 0.9965 (26 calibration points) was obtained. The response 983 counts = 1 ng is clear from this equation. A negative value of the intercept indicates a systematic error of *ca.* 1.3 ng. The source of this error was not further investigated in this work.

The chromatograms given in Fig. 10 illustrate (a) the analysis of a soil extract after ITP cleanup, (b) and (c) the determination of asulam present in the soil at 0.2

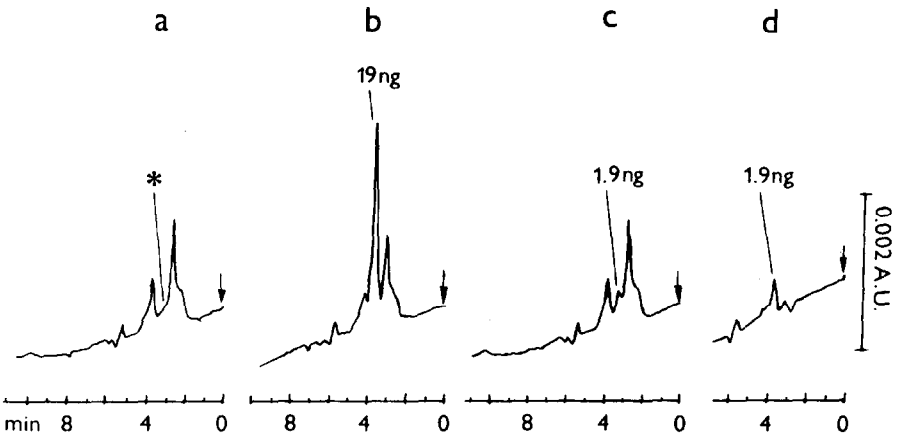

Fig. 10. Chromatograms from the analysis of a soil extract after ITP cleanup. (a) Unfortified extract; (b), (c) determination of asulam present in the soil at 0.2 and 0.02 ppm concentrations, respectively; (d) aqueous solution of asulam. Eluent, water–methanol (95:5); flow-rate, 1 ml/min.

and 0.02 ppm concentrations, respectively, and (d) the determination of pure asulam.

In this instance, combined ITP–RP HPLC led to a substantial improvement in the chromatographic determination of the herbicide because all of the components causing problems in the direct analysis were removed in the ITP cleanup step.

The recoveries for the complete analytical procedure were 90.5 ± 9.5% for 0.2 ppm of asulam (determined on two soil samples, with three ITP cleanup runs for each soil sample and one chromatographic determination for the material trapped in one ITP cleanup run). The average recovery is in good agreement with that obtained in ITP analysis (Table II). For 0.02 ppm of asulam the recovery was not determined as the number of experiments was not sufficient. A rough estimate leads to a value close to that determined for this concentration by ITP (Table II).

CONCLUSIONS

The results show that using simple sample pre-treatment asulam present in soil can be detected with confidence at concentrations down to 0.1 ppm when direct ITP analysis is carried out and when suitable spacing constituents are injected simultaneously with the sample. Using the described procedure almost unbiased quantitation at the 0.5 ppm level is possible. However, if the choice of the spacing constituents were thoroughly optimized, lower concentrations could be probably detected and/or determined. The detection limit for ITP under our experimental conditions is 0.5 ng of asulam (from the experiments with this compound injected in water).

When preparative ITP is used for the extract cleanup, asulam present in soil at a concentration of 0.02 ppm can be detected very reliably and its determination at 0.1–0.2 ppm concentrations gives very satisfactory results. Optimization of the separation conditions in preparative ITP experiments and full exploitation of the column coupling system[7] used in this work will probably lead to a further improvement in the analytical performance.

In contrast to ITP, the direct liquid chromatographic determination of asulam

in soil extracts was not possible. The use of a pre-column for the extract cleanup could be a possible solution but this was not tested in this work. Instead, preparative ITP preceded the final chromatographic determination. A substantial improvement was achieved using this combination with respect to the direct analysis by RP HPLC.

In general, preparative ITP seems to be a very promising technique for sample pre-treatment in the determination of ionogenic pesticides or their degradation products in matrices of different types as it provides high recoveries, well defined separation conditions and concentration capabilities (not exploited in this work). When optimal discrete spacers are used, almost pure components of interest can be isolated and quantified by sensitive analytical methods.

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