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Preparative capillary isotachophoresis as a sample pretreatment technique for complex ionic matrices in high-performance liquid chromatography

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Abstract

Preparative capillary isotachophoresis (ITP) was studied for sample pretreatment in the high-performance liquid chromatography (HPLC) of ionogenic analytes present in complex ionic matrices (urine and humic substances). Sulphanilate, methyl (4-aminobenzenesulphonyl)carbamate (asulam), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) served as model analytes. A high sample load of the ITP pretreatment was achieved by performing the preparative separations in tubes of 2.0 and 1.0 mm I.D. in the column-coupling configuration of the separation unit. The ITP separation according to ionic mobilities was combined with gradient elution HPLC in the ion-suppression mode to achieve highly dissimilar (orthogonal) separation systems in both techniques. The pretreatment provided the sample fraction for the HPLC analysis containing in addition to the analyte (sulphanilate) only ca. 2–3% of the urine matrix (spread along the complete elution profile) when a pair of discrete spacers defined the trapped constituents. Under these conditions the limit of detection for sulphanilate in urine could be reduced by more than two orders of magnitude. A high recovery of the pretreatment procedure [99 ± 1.5% for a 1.7 ppm (w/w) concentration of sulphanilate] was typical. For asulam, 2,4-D and MCPA present in a humic matrix it was shown that the ITP pretreatment may also be effective for multi-residue procedures while favourable analytical characteristics of the pretreatment such as recovery and efficient sample clean-up are maintained.

1. Introduction

Sample pretreatment techniques (SPTs) play a key role in the trace analysis of ionogenic compounds by high-performance liquid chromatography (HPLC). There is a variety of SPTs based on different separation principles and applicable for this purpose [1,2]. The actual choice depends, mainly, on the physico-chemical properties of the analyte, the concentration range within which it is to be determined and the nature of the matrix. Many of the currently used SPTs provide a group isolation of the sample constituents. Although convenient in many instances, this can be a disadvantage when a trapped group contains a large number of sample constituents of close physico-chemical properties

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as the risk of analyte peak overlap by matrix constituents may be high [3].

To solve these problems, high-efficiency SPTs are increasingly used and the analytical potential of such an approach is very convincing (see, e.g., Refs. [4–7]). SPTs based on electrophoretic principles and performed in instruments providing adequate separation efficiencies belong in this category. Convenient mainly for ionogenic analytes, they can be considered in many instances to be orthogonal to HPLC in terms of the separation principles. Zone electrophoresis (ZE) sample pretreatment as developed by Kok and co-workers [8–11] and various alternatives of preparative capillary isotachophoresis (ITP) [12–19] are especially promising. It can be deduced that from a general point of view ITP has some advantageous features as far as the sample pretreatment is concerned: (i) a high sample load capacity; (ii) the driving current is to a large extent employed for the transport of the separated constituents [20]; (iii) a well defined concentration of the separated constituents with a self-sharpening effect of the zone boundaries [21]; (iv) a predictable clean-up capability expressed in basic physico-chemical characteristics of the analytes (pK values, ionic mobilities) [22]; (v) the migration position of the analyte within the train of the ITP separands does not depend on the amount of sample once the electrolyte system for a given separation compartment guarantees its resolution [20].

Although the use of ITP for sample pretreatment in HPLC was proposed about 10 years ago [13,15], so far only limited attention has been paid to this subject [17,19]. This work was intended to study the potential of ITP pretreatment for anionic analytes present in matrices of high ionic complexity in combination with RP-HPLC with gradient elution. Such a combination was preferred as we would investigate the pretreatment of sample matrices containing constituents of highly varying hydrophobicity.

Usually, each sample type may require a specific approach as far as the sample pretreatment is concerned. Therefore, in a strict sense the use of any SPT should be evaluated in close relation with the matrix involved. However, as can be deduced from a statistical model of the peak overlap in multi-component chromatograms [3,23–26], classifications of matrices based on the saturation ratio (see below) make certain generalizations possible. In our experiments, urine, containing hundreds of acidic constituents at widely varying concentrations [27–29], was chosen as a matrix providing a discrete RP-HPLC profile under gradient elution in the ion-suppression mode with a very high sample component overlap. On the other hand, samples containing humic acids served as matrices of extremely high ionic complexity exhibiting under identical RP-HPLC conditions flat chromatographic profiles. Our study was focused on the clean-up capabilities of ITP, recoveries of the model analytes and the capabilities of the ITP pretreatment in multi-residue analysis.

2. Experimental

2.1. Instrumentation

A gradient chromatographic system consisting of two HPP 5001 pumps, a GP-2 gradient programming unit, an LCI-30 valve (20-μl sample loop) and an LCD 2040 UV–Vis detector (set at 254 or 240 nm) was obtained from Laboratorní přistroje (Prague, Czech Republic). The UV–Vis detector was connected to a CI 100 computing integrator and to a line recorder (Laboratorní přístroje). A 150 × 3 mm I.D. compact glass column packed with spherical Separon-RPS 5-μm silica-based polymeric sorbent was bought from Tessek (Prague, Czech Republic). The temperature of the column was maintained at 25 ± 1°C.

A CS isotachophoretic analyser (Villa-Labeco, Spišská Nová Ves, Slovak Republic) in a single-column mode was used for the analytical control of the fractionation. The column was provided with a 200 mm × 0.30 mm I.D. capillary tube made of fluorinated ethylene–propylene copolymer (FEP) and with a UVD-2 on-column photometric detector (Villa-Labeco). The detector was set at 254 nm. The driving current in the runs with this column was 50 μA.

Preparative experiments were carried-out using a discontinuous ITP fractionation unit [14,15] in the column-coupling configuration [30,31] constructed in this laboratory (Fig. 1). The pretreated samples were injected with the aid of an injection device [31] providing valve injection (50-μl sample loop), microsyringe injection (0–300 μl) or a combination of both. The preseparation column (II in Fig. 1) was provided with a 2.0 mm I.D. tube made of FEP. The final separation column was identical in design and was provided with a 1.0 mm I.D. tube made of FEP. The columns had on-column conductivity sensors [21] for monitoring of the separations to achieve proper timings in the switching of the columns and in the fractionation procedure (see below). The fractionation valve (V in Fig. 1) had a plunger with a 15-μl internal trapping loop. Electronic units of a CS isotachophoretic analyser (Villa-Labeco) were used in the preparative experiments without modification with the exception of the high-voltage power supply, which was reconstructed to deliver driving currents up to 1000 μA.

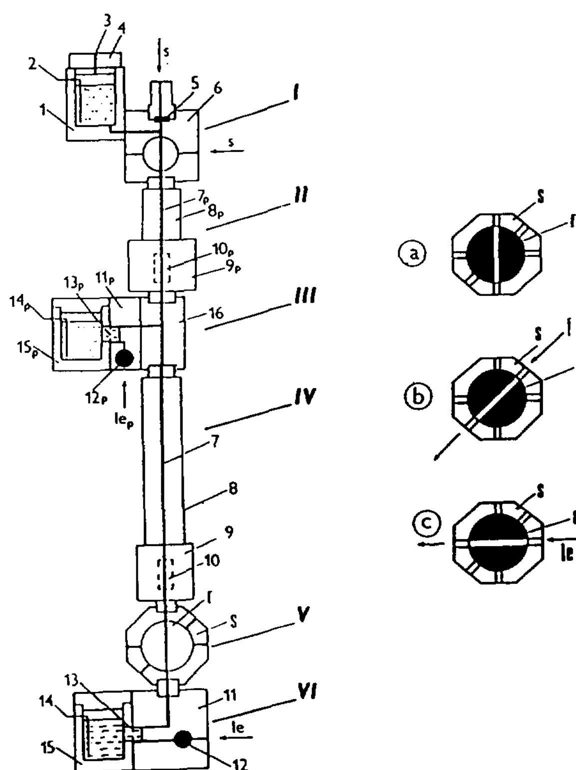

Fig. 1. ITP separation unit in the column-coupling configuration as used for the sample clean-up. I = Injection device; II = preseparation column; III = bifurcation block with the counter-electrode compartment for the preseparation column; IV = final separation column; V = fractionation (trapping) valve; VI = refilling block with electrode compartment for the final (trapping) separation column; 1 = terminating electrolyte compartment with a driving electrode (2) closed with a drilled (3) cap 4; 6 = injection valve with a septum (5) for microsyringe injection of the sample(s), s = sample introduction positions for the injection with a microsyringe (top) and with an internal sample loop in the rotor (right); 7, 7p = (capillary) tubes housed in columns (8, 8p) provided with conductivity detectors (10, 10p); 11, 11p = refilling blocks with needle valves (12, 12p); 15, 15p = leading electrolyte compartments with driving electrodes (14, 14p) separated from the channels in the refilling blocks (11, 11p) by membranes (13, 13p); 16 = bifurcation block; S, r = stator and rotor of the fractionation (trapping) valve, respectively; l_{ep}, l_e = positions for the refilling of the separation (p) and trapping columns, respectively. Operational algorithm for the fractionation valve: (a) position of the rotor of the fractionation (trapping) valve during the separation; (b) trapping of the desired fraction (f); (c) position for the refilling of the loop with the electrolyte solution (l_e).

2.2. Chemicals

Chemicals used for the preparation of the leading and terminating electrolyte solutions were bought from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA) and Lachema (Brno, Czech Republic). Methylhydroxyethylcellulose 30 000 (Serva) purified on a mixed-bed ion exchanger (Amberlite MB-1; BDH, Poole, UK) was used as an anticonvective additive in the leading electrolyte solutions for the preparation column. Water from an Aqualabo two-stage demineralization unit (Aqualabo, Brno, Czech Republic) was further purified by circulation in a laboratory-made demineralization system made of polytetrafluorethylene and packed with Amberlite MB-1 mixed-bed ion exchanger. At the outlet water was filtered through a 0.45-μm filter (Gelman, Ann Arbor, MI, USA). The solutions used in the ITP experiments were prepared from freshly recirculated water.

Water doubly distilled from a glass apparatus

and methanol for HPLC (Merck, Darmstadt, Germany) were used for the preparation of the mobile phases. Mobile phase A was acidified with sulphuric acid diluted with water (1:1) to pH 2.0 and purified by percolation through a glass column packed with a coarse fraction (60–100 μm) of Separon-RPS (Tessek) immediately before the use.

2.3. Samples

Urine samples were obtained from healthy individuals (both male and female, mid-stream fraction). They were immediately diluted (sevenfold) with demineralized water to avoid gradual precipitation of the anionic constituents. When required, the samples or aliquots were spiked at appropriate concentrations with sulphanilic acid (Lachema). No preservatives were added to the samples and they were not subjected to any additional treatment.

Humic acid (Fluka, Buchs, Switzerland) of M_r. 600–1000 was dissolved in distilled water at a 500 mg/l concentration after gradual addition of NaOH to pH 6.0 under pH-meter control. After 24 h the sample was filtered (0.45 μm) and stored for further use. When required, the samples and/or aliquots were spiked at appropriate concentration levels with the selected pesticides. Asulam [methyl (4-aminobenzenesulphonyl)-carbamate], 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxy-acetic acid) (99% purity) were kindly provided by the Residue Laboratory of the Research Institute for Chemical Technology, (Bratislava, Slovak Republic).

2.4. ITP pretreatment procedure

The injected samples after the separation were trapped in the internal loop of the trapping valve (V in Fig. 1) by disconnecting the delivery of the driving current into the separation unit by the controller of the analyser. The timing for this disconnection was derived from the signal of the conductivity detector in the final separation column (10 in Fig. 1). The rotor of the trapping valve was then turned into the isolation positon (b in Fig. 1) and the fraction of interest was flushed out with water or mobile phase into a weighed eppendorf microvial. The trapping loop was refilled with the leading electrolyte (c in Fig. 1) and the rotor was turned into the working position (a in Fig. 1) to transport a further fraction into the loop by the driving current.

3. Results and discussion

3.1. Choice of sample matrices

Once the peak capacity of the column is given it is convenient to classify sample matrices according to the frequency with which the peak overlaps of the sample constituents can be expected [3]. In this context we can distinguish the following extreme types of the matrices:

(i) $n_c \gg m$ (n_c = peak capacity, m = number of sample components), i.e., the saturation factor (m/n_c) is very low and the chance of achieving complete resolution of the sample constituents is high. In such a situation sample clean-up is not necessary unless constituents having detrimental effects on the column performance are present or preconcentration of the analyte is needed.

(ii) $n_c \approx m$, i.e., the saturation factor is close to 1 and the number of detected peaks achieves a maximum number for a given column [3]. However, the number of single-component peaks which can be expected in such samples is very low [3]. Here, the use of SPTs is usually required to achieve a reasonable certainty of identification and/or a minimum bias in the quantification.

(iii) $n_c \ll m$, i.e. the saturation factor is high and the chance of detecting a single-component peak is negligible and also the number of peaks is considerably reduced (see, e.g., Fig. 1 in [3]). The use of SPTs for this type of sample matrices is essential.

With this classification in mind, sample matrices belonging into the last group were preferred for our evaluation of the sample pretreatment capabilities of ITP. Based on preliminary experiments with various samples of biological and environmental origins (urine, extract from

various plants, fermentation broths, soil extracts and humic acids), urine and humic acids were chosen as representatives of typical complex ionic matrices. Urine, having under our elution conditions (Table 1) chromatographic profiles with many peaks of widely varying heights (Fig. 2), contains hundreds of acidic constituents [27–29] and there is a very low probability of obtaining a pure sample constituent peak [3]. On the other hand, samples of humic acids, containing extremely large numbers of acidic constituents [32] and providing a minimum number of peaks with a flat chromatographic profile (see Fig. 9), represent a good example of a complex matrix with an equal spread of peaks over the retention time interval of current analytical interest (see Fig. 2 in Ref. [26]).

3.2. ITP pretreatment of urine

The ITP pretreatment was carried out in the separation unit shown schematically in Fig. 1 (for details see Experimental). The ITP separation conditions (Table 2) differentiated the separands mainly via the differences in their ionic mobilities [21]. This was a preferred ITP separation mode as the HPLC analyses of the ITP fractions were based on ion suppression. Such a combination of the separation systems has clear orthogonal features as hardly any relationship between the ionic mobilities and hydrophobicities of the compounds can be expected. In addition, these separation systems were fully compatible and the ITP fractions could be directly injected on to the HPLC column.

The chromatogram in Fig. 2 shows the elution profile of a diluted urine sample. By using the procedure described by Nagels and Creten [26] we found that this sample type is characterized by an exponential course of the frequency distribution of the relative peak heights (see Fig. 5). From the profile it can be seen that the baseline of the detection signal within the interval of retention times of analytical interest was considerably shifted relative to that obtained in the blank run. This shift represented 60.5% of the total area of the chromatogram [12% for a 0–2.9 mAU shift and 48.5% for a 0–4.5 mAU shift (AU = absorbance units)]. Only 39.5% of the

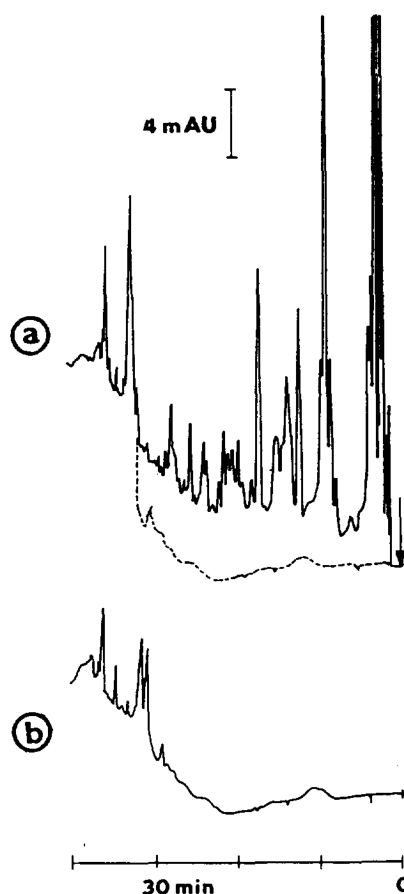

Fig. 2. Chromatographic profile of a urine sample. (a) Urine diluted (1:7 v/v) with doubly distilled water after collection (for the elution conditions see Table 1); (b) blank run under the same elution conditions as in (a). The dashed line in (a) marks the baseline as obtained in the blank run with the same detector setting.

Table 1
Elution conditions used in HPLC runs

Column	Separon RPS (5 μm) CGC, 150 × 3 mm I.D.
Mobile phase A	water (pH 2, sulphuric acid) –1% (v/v) methanol
Mobile phase B	Methanol
Gradient	Linear: 2% B in A (0–4 min); from 2%–98% B (4–36 min); 98% B (36–46% min)
Flow-rate (ml/min)	0.5

Table 2
Operational system and working conditions used in ITP runs

Parameter	Electrolyte	
	Leading[a]	Terminating[a]
Solvent	Water	Water
Anion	Cl	MES^-
Concentration (mM)	10	10
Counter ion	HIS	HIS
pH	6.0	6.0
Additive	HEC	–
Concentration (%, w/v)	0.2	–

Driving currents: preparative ITP, preseparation column (2.0 mm I.D.) = 1000 μA, trapping column (1.0 mm I.D.) = 500 μA; analytical ITP, monitoring, analytical column (0.3 mm I.D.) = 50μA.
[a] HEC = hydroxyethylcellulose; MES = morpholinethanesulphonate; HIS = histidine.

total area corresponded to the detected peaks. An estimate of the number of eluted sample constituents based on the statistical theory of component overlap is uncertain for such multicomponent chromatograms [23–25]. Nevertheless, if we assume that the detected constituents have molar absorptivities at the detection wavelength (254 nm) in the range 10^3–10^4 l mol^{-1} cm^{-1} we can state that the constituents responsible for a shifted baseline were present in the sample at 0.1–1.0 ppm concentrations whereas those forming the peaks corresponded to concentrations up to several tens of ppm. These simple estimates indicate that for the determination of the analyte present in urine at a 1 ppm concentration or less the use of a high-efficiency SPT can be very beneficial.

The isotachopherograms in Fig. 3 were obtained in the pretreatment of the same urine sample as used in the chromatography profiling (Fig. 2). The ITP fractions (marked by dashed boxes on the isotachopherogram as registered by the conductivity detector) were checked by analytical ITP (see the panels at the top) and finally separated by HPLC (Fig. 4). From the chromatograms of the fractions it can be seen that although the ITP pretreatment reduced the baseline shift relative to that of the untreated sample only fraction (a) was sufficiently clean to

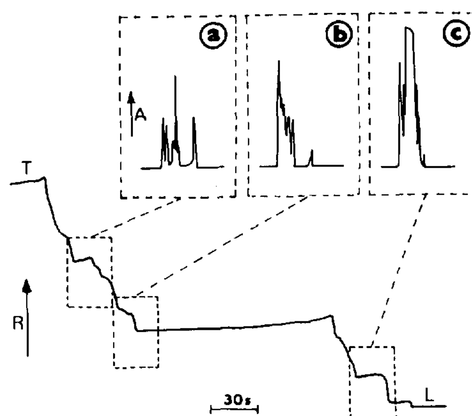

Fig. 3. ITP fractionation of urine. An isotachopherogram as obtained from the conductivity detector in the final (trapping) separation column for a 50-μl urine sample [diluted 1:7 (v/v) with doubly distilled water]. The trapped fractions (a–c) are marked by dashed boxes on the isotachopherogram. Panels at the top show isotachopherograms as obtained for the collected fractions in a single column ITP unit (see Experimental). For the electrolyte system and the driving currents, see Table 2. L, T = leading and terminating zones, respectively; A, R = increasing light absorption and resistance, respectively.

make the determinations at 0.01–0.1 ppm concentrations of the analytes possible. From the analyses of fractions (b) and (c) it is apparent that they contained separands of widely varying hydrophobicities. This suggests that the ITP pretreatment (providing the fractions within well defined effective mobility intervals) and HPLC were based on different separation principles. It is also clear that the relative peak heights of the constituents present in the fractions are exponentially distributed (Fig. 5).

Typical reproducibilities of the ITP fractionation are illustrated in Figs. 6 and 7. While the ITP control of the fractions indicates perfect reproducibility of the procedure from the HPLC runs, we can see slight differences in the peak heights for some of the separands. This can be due in part to the presence of mixed zones in the ITP pretreatment and also to some fluctuations in the migration velocities of the constituents leading to slight changes in the compositions of the trapped fractions. However, the main drawbacks of the pretreatment were in a relatively low clean-up efficiency. Whereas, for example, in

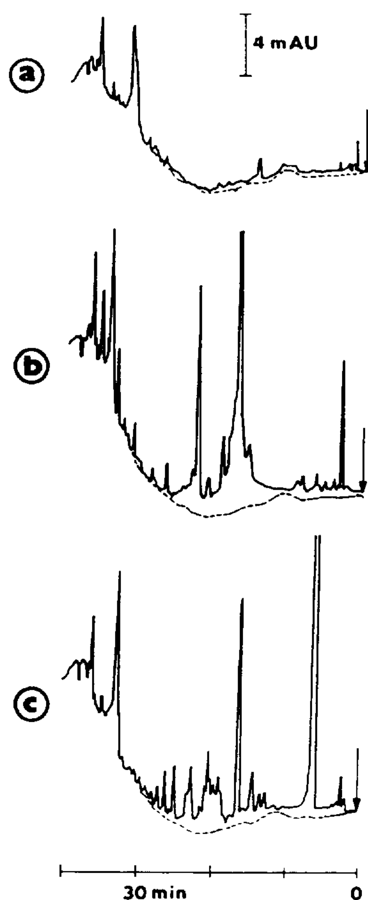

Fig. 4. Chromatograms to the separations of the ITP fractions of urine. (a)–(c) ITP fractions as obtained in the run shown in Fig. 3. The dashed lines mark actual baselines as obtained in the blank runs under identical elution conditions. For the elution conditions see Table 1.

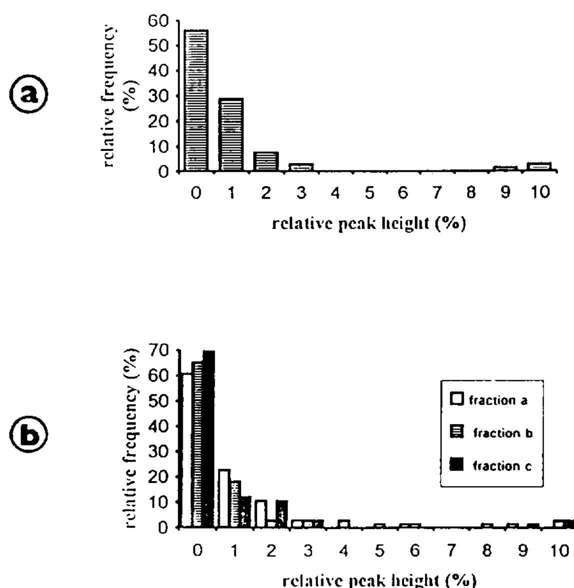

Fig. 5. Frequency distribution of relative peak heights on chromatograms as calculated for (a) urine and (b) its ITP fractions. The data were obtained for a 1% relative peak height resolution and 2σ peak density (0.0147). For further details, see the text.

fraction (a) the constituents present represented 3% of the original material, in fraction (b) it was 17% and in fraction (c) 20%. The rest of the urine components (60%) can be attributed to those migrating in the untrapped parts of the ITP train, to anionic constituents migrating with lower effective mobilities than that of the terminating anion and to cationic and uncharged sample constituents. To reduce the number of constituents present in the trapped fractions and strictly define potential interferents, experiments with appropriately chosen spacing constituents were carried out (for general information on the use of spacers in ITP see Ref. [33] and refer-

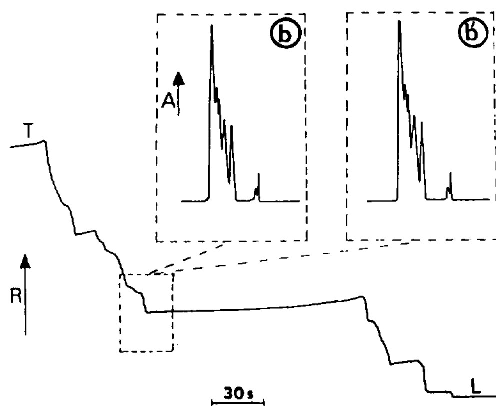

Fig. 6. Reproducibility of the ITP fractionation of urine as evaluated by the ITP monitoring. The trapped fraction is marked by a dashed box on the isotachopherogram as registered by the conductivity detector in the final (trapping) separation column. A 50-μl volume of a diluted urine sample (1:7, v/v) was taken for the fractionation. Isotachopherograms in the panels at the top (b and b′) show typical reproducibilities in the fractionation as evaluated by the ITP monitoring of the fractions. For further details concerning the ITP working conditions, see the legend to Fig. 3.

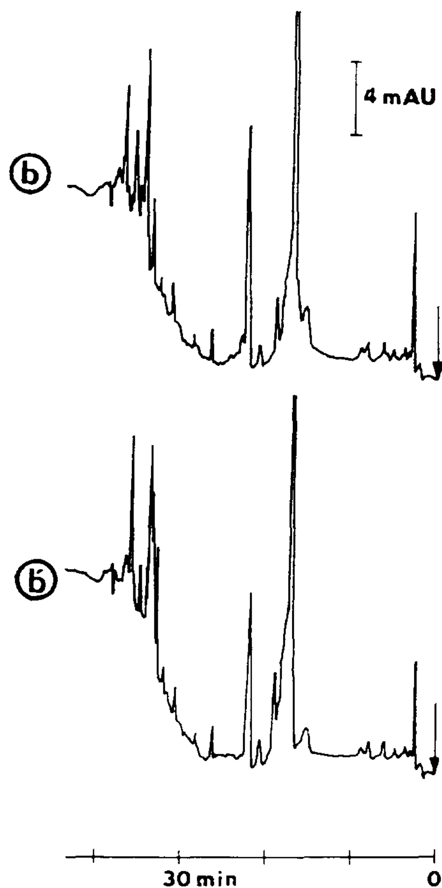

Fig. 7. Reproducibility of the ITP fractionation as evaluated by the HPLC monitoring. The same fractions as in Fig. 6 were monitored by HPLC under the elution conditions given in Table 1.

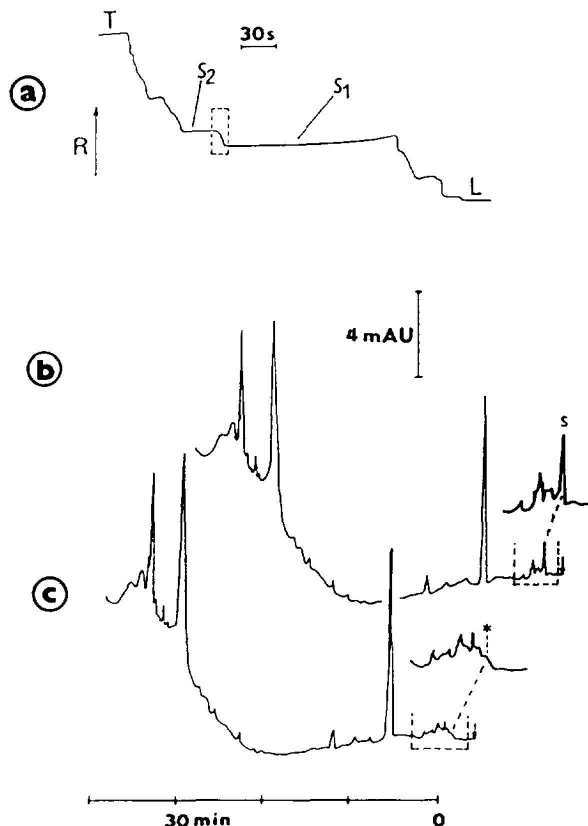

Fig. 8. HPLC analysis of sulphanilate in urine combined with the ITP sample pretreatment. (a) Isotachopherogram from the isolation of sulphanilate (present in urine at a 1.7 ppm concentration); the analyte was spaced by iminodiacetate (S_1) and β-bromopropionate (S_2) and the isolated fraction is marked by a dashed box on the isotachopherogram. (b) HPLC of the sulphanilate fraction isolated as in (a). (c) HPLC identical fraction from the unspiked urine sample. For the elution conditions, see Table 1. The ITP pretreatment was carried out under the same conditions as in Fig. 3.

ences cited therein). Sulphanilic acid, currently not present in urine [27–29], served as a model analyte in these experiments. The spacing constituents were chosen by a computer-based search [22] and iminodiacetate (S_1 in Fig. 8a) and β-bromopropionate (S_2 in Fig. 8a) were found to provide a narrow mobility gap, thus accommodating besides the analyte only a minimum number of matrix constituents. Chromatograms of the relevant ITP fractions (Fig. 8b and c) clearly illustrate a considerable improvement in the sample clean-up by using this ITP approach. For example, when the fraction from the unspiked urine (Fig. 8c) is compared with the untreated sample (Fig. 2a) and with the identical fraction without the use of spacers (Fig. 4b), the improvement achieved in the clean-up is considerable. We found that in this way the signal due to matrix constituents eluted in the position of sulphanilate was reduced ca. 150-fold. Such a background reduction enabled us to detect sulphanilate at a 0.1 ppm concentration. The use of spacing constituents in the pretreatment gave fractions with very reproducible profiles (see Fig. 8b and c) while recovery of sulphanilate as

determined at 1.7 ppm concentration was in the range 99 ± 1.5% (for three parallel runs with three different urine samples).

No disturbances due to the presence of spacers and counterionic constituents in the ITP fractions were observed in the HPLC analyses. To avoid potential problems associated with the injection of hydroxyethylcellulose on to the HPLC column, the leading electrolyte employed in the trapping column was used without this additive. Here, the electroosmotic flow was efficiently suppressed by coating the walls of the capillary tube by a high-molecular-mass derivative of methylhydroxyethylcellulose [34].

3.3. ITP pretreatment of humic matrices

Humic and fulvic acids are present in various environmental samples. As discussed above, they consist of very large numbers of components with different physico-chemical properties [32]. Under our gradient elution conditions the sample of humic acids caused a considerable shift of the baseline relative to that registered in the blank run (Fig. 9). The only peak was detected in the dead retention volume. This peak was probably due to inorganic constituents (the humic acid sample is claimed to contain 10–15% of ash) and in part also to unretained, highly hydrophilic fulvic acids.

In the HPLC analysis, humic substances of polymeric nature can precipitate at the top of the column. We found that after 5–10 runs with such samples the pressure resistance of the column increased considerably and a layer originating from humic substances was deposited on the column bed. This problem can be partially solved by washing the column with 0.1% ammonia solution after each run. This, however, negatively influences the column life owing to gradual decomposition of the bonded phase.

The distribution diagram for humic matrix shown in Fig. 10 differs significantly from that obtained for urine and its fractions (Fig. 5). Not considering the retention times between the first and sixth minutes, it resembles that expected for a sample matrix of an ideal complexity [26]. In ITP humic acids behave analogously and they

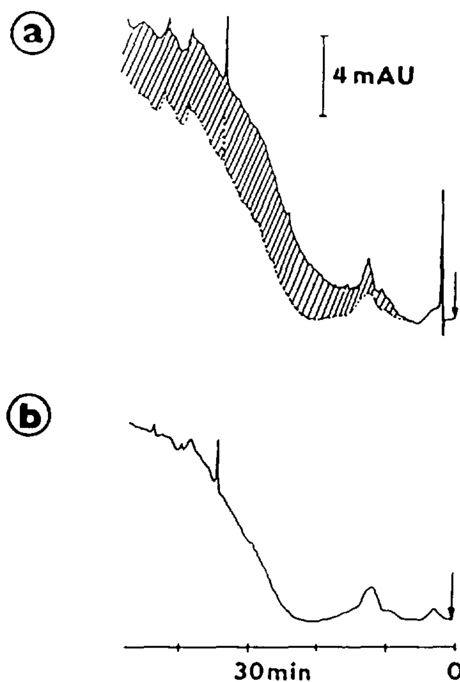

Fig. 9. HPLC profile of a humic acid sample at a detection wavelength of 240 nm. (a) 20-μl volume of the sample contained the acids at a 0.5 g/l concentration; (b) blank run under the same elution conditions (Table 1) as in (a). The shaded area in (a) indicates the shift of the detection signal on injection of the sample.

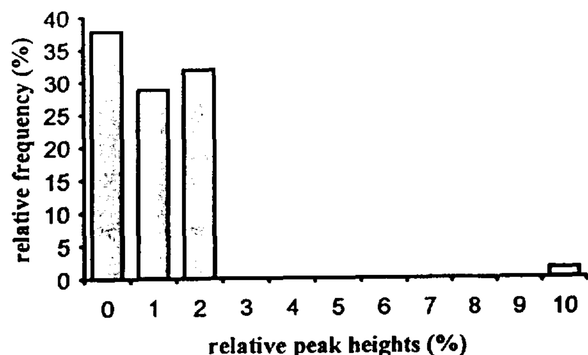

Fig. 10. Frequency distribution of relative peak heights on a chromatogram as calculated for a humic acid sample. The data were obtained for the sample as in Fig. 9 for a 1% relative peak height resolution and 2σ peak density (0.0147).

provide continuous mobility gradients [19,35]. A model sample containing acidic pesticides in a large excess of humic acids served for the evaluation of the ITP pretreatment for this type of matrix. As stated in the introduction, our intention was to carry out the pretreatment for a multi-residue analysis of anionogenic analytes of widely differing hydrophobicities. To meet these requirements, an ITP separation according to ionic mobilities was preferred. This is a favourable separation mode when analytes of close molecular masses (or, better, molecular mass/ charge number ratios) are to be isolated as a group because the ionic mobilities are related to the molecular masses of ionogenic separands [36].

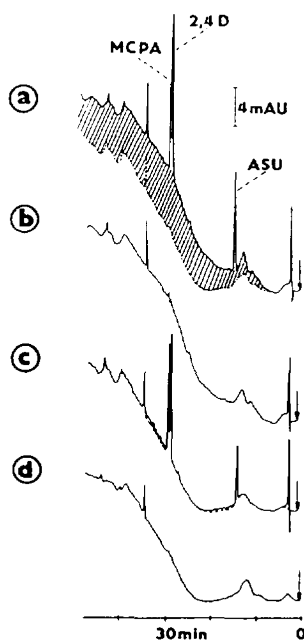

Fig. 11. ITP pretreatment for a multi-residue HPLC analysis of pesticides present in a humic matrix. (a) HPLC profile for asulam (14 ppm), 2,4-D (18 ppm) and MCPA (18 ppm) present in humic matrix (0.5 g/l of humic acids). (b) HPLC profile of the same humic acid sample as in (a). (c) HPLC profile of a humic acid sample (0.5 g/l) spiked with asulam (10 ppm), 2,4-D (14 ppm) and MCPA (14 ppm) after ITP pretreatment (the dashed line marks the baseline for a blank run). (d) HPLC profile of the corresponding ITP fraction from a blank preparative ITP run. For the ITP pretreatment conditions, see Table 2. The elution conditions are given in Table 1.

Therefore, in our particular case glutamate and pelargonate, covering a narrow mobility span under the separation conditions employed (Table 2), were suitable spacing constituents for the pesticides studied. Consequently, the trapped ITP fractions contained in addition to the pesticides only 2.5% of the original humic material (see also Fig. 11) detected within the elution window. The recoveries of the analytes under these conditions were in the range 95–100% for a 0.2–20 ppm concentration span. The chromatograms obtained in these experiments (Fig. 11) suggest that the ITP pretreatment did not significantly improve the detectability of the pesticides. This is, however, what could be expected for such a flat chromatographic profile of the matrix. Nevertheless, considering an empirical equation derived by Nagels and Creten [26], elimination of the baseline shift due to the pretreatment procedure has a positive impact on the determination limits of the pesticides. Obviously, considerable prolongation of the column life by using the ITP pretreatment should also be included in the overall analytical gain.

4. Conclusions

ITP sample pretreatment performed in tubes of 1–2 mm I.D. combined with discontinuous isolation of the analytes of interest provided a highly efficient sample preparation alternative for the HPLC of ionogenic analytes present in complex ionic matrices. Such I.D.s of the ITP separation compartment, favouring a high sample load [20], sufficiently eliminated dispersive effects of the increased heat production on the profiles of the ITP zone boundaries [21]. In addition, the ITP pretreatment could be performed in the separation mode having apparent orthogonal features relative to that used in HPLC (separations according to ionic mobilities vs. separation in the ion-suppression mode). The separation media in both ITP and HPLC were fully compatible so that no further operations with the sample fractions were needed before injection on to the HPLC column.

Optimum separation conditions for the ITP

pretreatment (the electrolyte system and spacing constituents) can be found very quickly by a computer-aided search when the migration characteristics of the analytes (p*K* values, ionic mobilities) are known [22]. From the practical point of view this is an obvious advantage as a number of experiments needed to find suitable sample pretreatment conditions can be reduced to a minimum.

ITP clean-up was effective in reducing the limits of detection in HPLC by 2–3 orders of magnitude while the recoveries of the analytes were in the range 98–100%. Our results show that multi-residue applicability of the ITP pretreatment for the ionogenic analytes is feasible. However, a perfect sample clean-up can be expected mainly for analytes with close ionic mobilities.

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