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USE OF CONVENTIONAL INSTRUMENTATION WITH MICROBORE COLUMNS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A method to determine extra-column band broadening in microbore high-performance liquid chromatography (HPLC) (column diameter, *ca.* 1 mm) is developed. The extra-column band broadening of several commercially available UV detectors is compared to that of a home-made electrochemical detector. A trace-enrichment method for microbore HPLC is presented and applied to the determination of a series of aromatic nitro compounds. The effect of miniaturization of HPLC on detection limits is discussed.

INTRODUCTION

In the work on miniaturization of high-performance liquid chromatography (HPLC) several approaches can be discerned according to the type of columns used: open tubular capillary columns, with an internal diameter of 30–120 μm (refs. 1 and 2); packed columns with a diameter of *ca.* 0.1 mm, which are called packed capillary[3–5] or ultra-micro[6] columns, depending on their length; and microbore columns, which have an internal diameter in the range of 0.5–2.0 mm (refs. 7–11).

Various advantages of miniaturized HPLC have been mentioned, *e.g.* the reduction of the cost of packing material and of solvents used as mobile phase[9], the possibility of a direct coupling of HPLC and mass spectrometry (MS)[2,8], the generation of very high plate numbers[3,4,9], high-speed separation[9,11], and a reduction of detection limits[10]. Lower detection limits can be reached because a reduction of column dimensions leads to smaller peak volumes. However, since the maximum allowable sample volume is proportional to the column cross-section, a reduction of the column diameter is only significant in this respect when the amount of sample available is limited.

In all studies on miniaturized HPLC much attention is paid to the construction

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of suitable equipment in order to reduce the extra-column band broadening, which can be considerable when conventional instrumentation is used. We have examined the potential and limitations of the use of conventional instrumentation for the separation of aromatic nitro compounds with miniaturized HPLC. Since it is evident that, on further reducing column dimensions, the instrumental requirements will increase, we have opted for the use of microbore columns with an internal diameter of 1.1 mm. Special attention has been given to the comparison, for microbore HPLC, of a home-made dropping mercury electrode (d.m.e.) detector with that of a number of commercially available UV detectors.

A simple trace-enrichment method has been elaborated which allows the use of relatively large sample volumes. The method has been applied to the determination of nitrobenzene in river water and of 4-nitrophenol in urine.

EXPERIMENTAL

Apparatus

Mobile phase flow-rates between 50 and 250 μl/min were delivered by an Altex (Berkeley, CA, U.S.A.) Model 100 A pump or a one-stroke (300 ml) syringe pump made in our workshop. The samples were injected with a Rheodyne (Berkeley, CA, U.S.A.) Model 089-0932 valve with a 175-μl loop and an appropriate restrictor, through which 12% of the mobile phase flows in the "inject" position of the valve. All connections were made of stainless steel capillary of 1/16 in. O.D. and 0.25 mm I.D.

In Table I the UV detectors used are listed, together with their cell volumes. In order to facilitate comparison, all detectors were used at 254 nm. Though this is not the most suitable wavelength for all the aromatic nitro compounds studied, nitrobenzene has an absorption maximum at 251 nm and all other compounds have molar absorptivities of over 10^3 at 254 nm (ref. 12).

TABLE I

UV DETECTORS EXAMINED

Manufacturer	*Model*	*Cell volume (μl)*
Perkin-Elmer (Norwalk, CO, U.S.A.)	LC 55	8
Pye Unicam (Cambridge, Great Britain)	LC 3	8
Waters (Milford, MA, U.S.A.)	440	12.5
Zeiss (Oberkochen, G.F.R.)	PM 2 DLC	8

For electrochemical detection use was made of a flow-through polarographic cell with a horizontally placed mercury capillary, which has been described earlier[13]. Drop times were between 0.1 and 0.2 sec. The electrical currents were measured with a home-made potentiostat/amplifier. Electronic dampening with a time constant of 1 sec was applied. Potentials were measured against a Ag/AgCl/1 *M* LiCl, methanol–water (50:50) reference electrode.

Reagents

Distilled demineralized water and analytical-grade methanol (Baker, Deventer,

The Netherlands) were used as solvents. Concentrated stock solutions of aromatic nitro compounds, obtained from various sources, were prepared in methanol, and samples of the desired composition were made by diluting aliquots of these stock solutions. Sample solutions were degassed in an ultrasonic bath.

Mobile phases, containing 10^{-2} *M* potassium nitrate and 10^{-3} *M* nitric aid, were filtered over a 0.8-μm Millipore (Bedford, MA, U.S.A.) filter and deaerated by purging with nitrogen at 35°C.

Packing of the microbore columns

Microbore columns were made of stainless steel tubing of 1/16 in. O.D. and 1.1 mm I.D. The inside of the tubing was polished with a wet cotton thread and polishing powder. The ends of the column were sealed with 1/16 in. × 0.13 mm frits, made of 2-μm stainless steel gauze, which fitted in Swagelok zero dead volume unions. In some cases it was more convenient to use male unions; then, a Swagelok 1/16–1/16 in. union was made "low dead volume" by inserting into the central hole of the union a piece of 1/16 in. O.D. and 0.3 mm I.D. PTFE capillary, which had been stretched until it just fitted.

The columns were packed with LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.) with a mean particle diameter of 7.7 μm. For a 250 mm × 1.1 mm I.D. column 0.3 g of packing material was wetted with 0.3 ml of methanol, and a slurry was made with 3 ml of tetrachloromethane. The slurry was stirred in an ultrasonic bath for 5 min and injected into a 250 mm × 4.6 mm I.D. slurry reservoir. This reservoir, which was connected to the column by a short 1.1 mm I.D. precolumn, was filled with methanol. The column was packed downwards by pumping through methanol for 20 min at 500 bar.

RESULTS AND DISCUSSION

Measurement of the extra-column band broadening

Several methods have been used to calculate extra-column band broadening, *e.g.*, (1) measuring the band width in a system without column[14], (2) measurements on the peak in an actual chromatogram[15], and (3) measuring the band width as a linear function of the retention volume[16]. The first method does not take into account the extra-column band broadening in frits, column ends and connectors. For the second method purely gaussian on-column band broadening is a prerequisite. The third method lacks a theoretical basis. We therefore developed another approach.

The width of a peak due to solute *i* in a chromatogram is determined by on-column and extra-column band broadening according to

$$\sigma_i^2 = \sigma_{c,i}^2 + \sigma_{ec}^2 \qquad (1)$$

where σ_i is half the band width at 0.607 of the peak height, and $\sigma_{c,i}$ and σ_{ec} are the on-column and extra-column contributions, respectively, in volume units.

The on-column band broadening is given by

$$\sigma_{c,i}^2 = \frac{H_i}{L} \cdot V_{R,i}^2 \qquad (2)$$

where L is the column length, $V_{R,i}$ and H_i are the retention volume, corrected for the extra-column volume, and the plate height for compound i, respectively. The plate height H_i can be evaluated from a Van Deemter equation such as[17]

$$H_i = \frac{2\,D_{m,i}}{T_m u} + \frac{2\,\lambda_2 d_p}{1 + \lambda_1\,(D_{m,i}/u d_p)^{1/2}} + \frac{1}{5.7}\cdot\frac{k_i'^2}{(1 + k_i')^2}\cdot\frac{\varepsilon_m^{1/2} d_p^{3/2} \nu^{1/6} u^{1/2}}{(1 - \varepsilon_m)\,D_{m,i}^{2/3}} + \frac{1}{30}\cdot\frac{k_i'}{(1 + k_i')^2}\cdot\frac{(1 - \varepsilon_m)\,T_s\,d_p^2\,u}{\varepsilon_s\,D_{s,i}} \quad (3)$$

Assuming equal diffusion coefficients for the compounds studied, for a particular chromatographic system and a given flow-rate, eqn. 3 can be written as

$$H_i = H_a + \frac{k_i'^2}{(1 + k_i')^2}\cdot H_b + \frac{k_i'}{(1 + k_i')^2}\cdot H_c \quad (4)$$

where k_i' is the capacity factor of solute i and H_a, H_b and H_c are constants. Calculation shows that in eqn. 4 (under normal chromatographic conditions) H_b and H_c are of the same order of magnitude, while H_a is the predominant term. Moreover, the second term on the right-hand side of eqn. 4 increases and the third term decreases with k_i' for $k_i' > 1$. The dependence of H_i on k_i' in this range can therefore be expected to be small.

In order to verify the above conclusion, chromatograms were run for mixtures of aromatic nitro compounds at flow-rates of 0.5–2.5 ml/min on a 120 mm × 4.6 mm I.D. column. An adapted Waters Model 440 UV detector was used; the extra-column band broadening of this detector can be neglected in comparison to the on-column band broadening of 4.6 mm I.D. columns (*cf.* below).

Plate heights of the compounds were calculated according to eqn. 2. It appeared that, although the plate heights depended on the column and flow-rate used, the ratios of the plate heights of different compounds did not. This is illustrated in Table II where the plate heights for a number of aromatic nitro compounds are expressed relative to that of the last eluting compound, 3-chloronitrobenzene. For most compounds the differences in plate height are well within experimental error. That is, the tentative conclusion that H_i does not depend strongly on k_i' is fully confirmed. Only the phenolic compounds show significantly different plate heights. Since, for these solutes, relatively large band broadening is accompanied by strong tailing of the peaks, the deviating result is possibly caused by a specific physico-chemical interaction between solute and stationary phase.

Since the plate heights of different compounds are equal or have a constant ratio, we can write for H_i, for all compounds studied

$$H_i = \beta_i\,H_0 \quad (5)$$

where H_0 is a constant for a particular column and flow-rate, and the plate height ratio β_i is unity for the majority of the compounds tested. The values of β_i used for further calculation are listed in Table II.

TABLE II

CHROMATOGRAPHIC CHARACTERISTICS OF MODEL COMPOUNDS

Plate heights expressed relative to that of 3-chloronitrobenzene. Column: 120 × 4.6 mm I.D., packed with 7.7-μm LiChrosorb RP-18. Mobile phase: methanol–water (50:50) + 10^{-2} *M* potassium nitrate + 10^{-3} *M* nitric acid. Flow-rate: between 0.5 and 2.5 ml/min.

Compound	k_i'	*Relative plate height ± S.D.*	*No. of expts.*	β_i
4-Nitroaniline	1.10	0.97 ± 0.14	16	1.0
4-Nitrophenol	2.25	1.17 ± 0.07	11	1.2
1,2-Dinitrobenzene	3.00	1.03 ± 0.12	9	1.0
2,4-Dinitrophenol	3.05	3.1 ± 0.4	4	−*
1,3-Dinitrobenzene	3.80	1.04 ± 0.13	12	1.0
2-Nitrophenol	4.20	1.30 ± 0.11	11	1.3
Nitrobenzene	4.45	0.88 ± 0.10	14	0.9
2-Nitrotoluene	8.25	1.03 ± 0.07	11	1.0
4-Nitrotoluene	9.10	1.02 ± 0.14	9	1.0
3-Nitrotoluene	9.80	1.02 ± 0.09	10	1.0
3-Chloronitrobenzene	11.9	1		1.0

* Not used for calculations.

The extra-column band broadening can now be easily evaluated from a chromatogram. Combining eqns. 1, 2 and 5 yields

$$\sigma_i^2 = \frac{H_0}{L} \cdot \beta_i V_{R,i}^2 + \sigma_{ec}^2 \qquad (6)$$

In other words, σ_{ec}^2 is the intercept obtained from a plot of σ_i^2 *vs.* $\beta_i V_{R,i}^2$ constructed from relevant data of a number of peaks in a chromatogram.

The validity of the method is illustrated in Fig. 1 where several graphs of σ_i^2 *vs.* $\beta_i V_{R,i}^2$ are seen to yield straight lines ($R \geqslant 0.995$). Moreover, the value of the intercept apparently does not depend on the length of the column used. This proves that on-column band broadening cannot have contributed significantly to this intercept or, in other words, that the said intercept can safely be assumed to represent σ_{ec}^2.

The standard deviation of the σ_{ec} values calculated from different chromatograms run under the same conditions was *ca.* 1 μl (n = 6–8).

Detector performance

The extra-column band broadening of four commercially available UV detectors was examined. The detectors were used without modification and the microbore columns were connected to the cell as prescribed by the manufacturer. Chromatograms were run of 1-μl samples of 5–8 nitro compounds and σ_{ec} was calculated as described above. The calculated σ_{ec} includes the contribution to band broadening of the injection side of the system. The contribution of the sample volume itself can, however, be assumed to be negligible, since no significant changes in σ_{ec} were found on varying the sample volume from 0.5 to 2 μl.

Fig. 2 shows that large differences in σ_{ec} occur from one detector to another

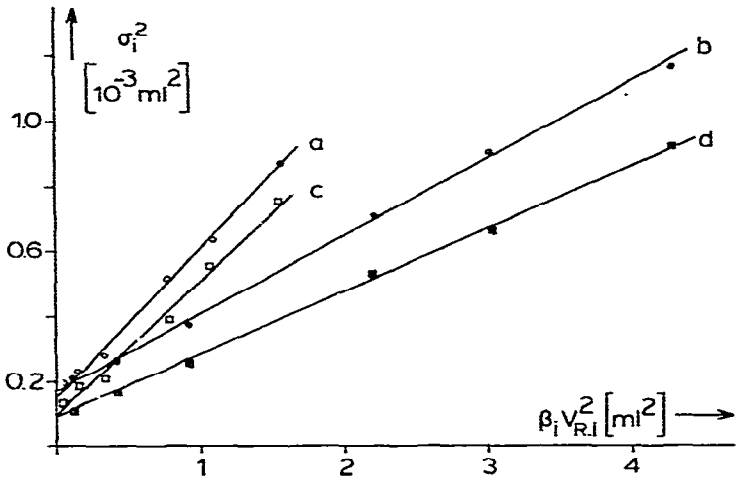

Fig. 1. Plots of σ_i^2 *vs.* $\beta_i V_{R,i}^2$ used for determination of extra-column band broadening. Columns: 1.1 mm I.D. packed with 7.7-μm LiChrosorb RP-18. Mobile phase: methanol–water (50:50) + 10^{-2} *M* potassium nitrate + 10^{-3} *M* nitric acid. Detector, Waters 440. Column length (*L*) and flow-rate (*F*) were as follows: (a) $L = 150$ mm; $F = 190$ μl/min; (b) $L = 250$ mm, $F = 190$ μl/min; (c) $L = 150$ mm, $F = 90$ μl/min; (d) $L = 250$ mm, $F = 90$ μl/min.

which can not be explained by differences in cell volume. For all detectors σ_{ec} increases with flow-rate, which indicates that the laminar flow profile in the flow-through cell and the connecting capillaries is an important source of band broadening[18]. The large σ_{ec} of the Perkin-Elmer LC55 detector is probably caused by the long thermostatting capillary inside the cell housing of this detector.

Since the Waters Model 440 detector combines the highest sensitivity with the smallest extra-column band broadening, this detector was adapted for use with microbore columns in the way described by Hermansson[19]. The optical cell volume was

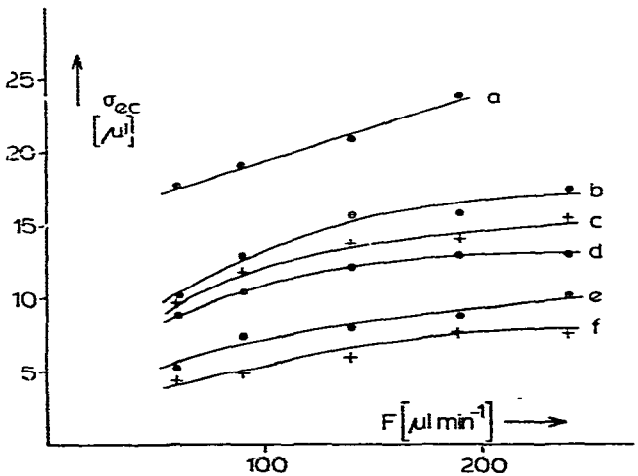

Fig. 2. Dependence of extra-column band broadening on flow-rate for various detectors. (a) Perkin-Elmer LC 55; (b) Zeiss PM 2 DLC; (c) Pye Unicam LC 3; (d) Waters 440; (e) adapted Waters 440; (f) d.m.c.

decreased to 0.7 μl by inserting conically ground pieces of PTFE capillary (0.3 mm I.D.) into the sample and reference cell, and the column was connected as close as possible to the cell. Fig. 2 shows that these adaptations considerably reduce the extra-column band broadening. The consequent gain in resolution was, however, accompanied by a deterioration of the detection limits: although modifying the cell did not influence the detector sensitivity much, the noise increased from $0.05 \cdot 10^{-3}$ to $0.15 \cdot 10^{-3}$ a.u. (see Table III). Therefore the improvement of detection performance which can be obtained by changing from conventional (4.6 mm I.D.) to microbore columns is partially lost when, for resolution's sake, a detector adaptation as described above is required.

TABLE III

DETECTION LIMITS OF NITROBENZENE

Column: 250 × 1.1 mm I.D. packed with 7.7-μm LiChrosorb RP-18. Mobile phase: methanol–water (50:50) + 10^{-2} *M* potassium nitrate + 10^{-2} *M* nitric acid. Sample volume: 1 μl.

Detector	*Signal/ng*	*Noise*	*Detection limit* (ng)*
Waters 440	$1.28 \cdot 10^{-3}$ a.u.	$0.05 \cdot 10^{-3}$ a.u.	0.08
Adapted Waters 440	$1.08 \cdot 10^{-3}$ a.u.	$0.15 \cdot 10^{-3}$ a.u.	0.28
D.m.e.**	7.88 nA	0.5 nA	0.13

* At signal-to-noise ratio of 2:1.

** The signal per ng nitrobenzene and the noise, but not the detection limit, depend on the mercury capillary used.

Relative to UV detection, electrochemical detection has a greater potential in miniaturization of HPLC since there is no need for a "cell path volume" as with spectrometric detection. With, for instance, the d.m.e. detector used in the present work, the column effluent flows via a short capillary directly onto the mercury drops. As indicated in Fig. 2, the extra-column band broadening of this detector is smaller than that of any of the UV detectors studied. As for sensitivity towards the aromatic nitro compounds the d.m.e. detector is comparable to the Waters Model 440 UV detector. As a typical example, detection limits for nitrobenzene with these detectors are given in Table III.

Trace enrichment

The maximum sample volume that can be used without significantly increasing band broadening is proportional to the cross-sectional area of the chromatographic column. With microbore columns this maximum is only one or a few microlitres[20]. Since in practice the amount of sample available is generally much larger the introduction of a trace-enrichment step will often be advantageous.

Scott and Kucera[10] have developed a trace-enrichment method that is based on the use of two (one open and one packed) sample loops; here, an additional (sampling) pump and an extra six-port valve are required. Ishii *et al.*[21] used an off-line method: samples are concentrated on a pre-column which is subsequently inserted prior to the analytical column.

In the present work we have modified our previously published[22] on-line trace-enrichment procedure, which combines a minimum need for extra apparatus with easy handling.

Set up. The design of the pre-column used for trace enrichment is shown in Fig. 3. The pre-column, which has the same internal diameter as the analytical column, was hand-packed with 7.7 μm LiChrosorb RP-18. The length of the plug of packing material is easily varied by changing the length of the PTFE capillary inside the pre-column. The pre-column and analytical column are connected by a Swagelok T-piece (1/16 in.) which is made "low dead volume" by inserting a piece of 0.3 mm I.D. PTFE capillary with a V-shaped cut in the middle. The third branch of the T-piece is connected to a valve.

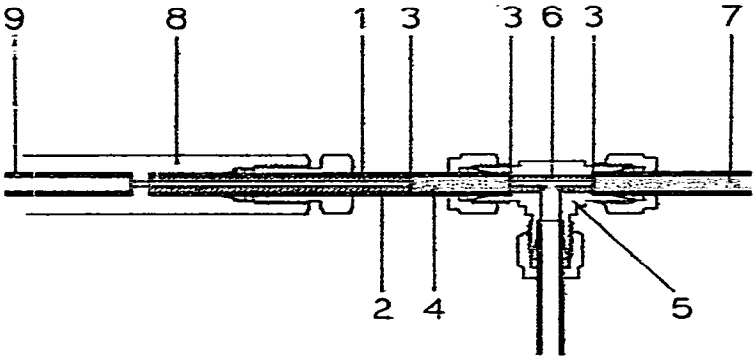

Fig. 3. Construction of pre-column for trace-enrichment studies. 1 = Pre-column, 35 mm × 1/16 in. O.D. (1.1. mm I.D.); 2 = PTFE capillary, 0.3 mm I.D.; 3 = frits; 4 = packing material; 5 = Swagelok T-piece; 6 = PTFE capillary with V-shaped cut; 7 = analytical column; 8 = Swagelok zero dead volume union (1/16–1/16 in.); 9 = to injection valve; 10 = to valve V.

Procedure. A scheme of the trace-enrichment procedure is presented in Fig. 4. The 175-μl loop on the injection valve is rinsed with deaerated water containing 10^{-2} *M* potassium nitrate and 10^{-3} *M* nitric acid. Next, a 100-μl sample is injected (Fig. 4a), the valve V connected to the T-piece is opened, the pump switched on, and the sample loop connected on-line with the mobile-phase flow. Since 12% of this flow is passing through the restrictor of the injection valve, the sample is eluted through the pre-column as a 6% solution in methanol if the mobile phase contains 50%-methanol (Fig. 4b). The presence of 75 μl of water in the last (*i.e.*, the left-hand) part of the sample loop causes non-retarded components present in the sample to be rinsed automatically from the pre-column. The volume of effluent from the pre-column is measured in a syringe connected to valve V. After the passage of 200 μl of effluent, valve V is closed, and the solutes concentrated on the pre-column are eluted and separated on the analytical column (Fig. 4c).

At regular intervals, the pre-column is rinsed with 175 μl methanol to remove strongly retained compounds.

Results. Recoveries of the trace-enrichment method were determined by comparing peak areas with those obtained in the normal injection mode. The results are given in Table IV. As can be seen from this table, if a 10 mm long pre-column is used, compounds with $k' \geq 2$ in methanol–water (50:50) are recovered for over 90%.

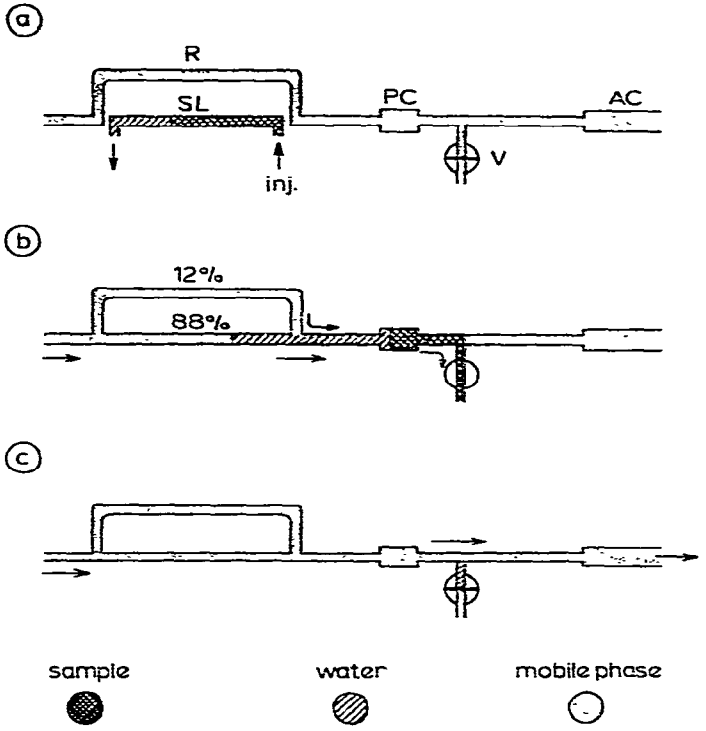

Fig. 4. Scheme of trace-enrichment procedure. SL = sample loop; R = restrictor; PC = pre-column; AC = analytical column; V = valve.

Application of the trace-enrichment method affects the performance of the analytical column negatively. After even a few injections made in the trace-enrichment mode, the plate number of the analytical column was found to drop from.

TABLE IV

RECOVERIES OF MODEL COMPOUNDS IN TRACE-ENRICHMENT STUDIES

For experimental details, see text. Each result is the average of 2–3 experiments.

Compound	k_i' *in methanol–water (50:50)*	*% Recovery on*	
		5-mm pre-column	*10-mm pre-column*
4-Nitroaniline	1.10	9	60
4-Nitrophenol	2.25	70	94
1,2-Dinitrobenzene	3.00	96	103
1,3-Dinitrobenzene	3.80	94	–
2-Nitrophenol	4.20	96	96
Nitrobenzene	4.45	95	108
2-Nitrotoluene	8.25	103	99
4-Nitrotoluene	9.10	97	104
3-Nitrotoluene	9.80	107	100
3-Chloronitrobenzene	11.9	99	99

typically, 5000 to 3500 (flow-rate, 140 μl/min). Fortunately, the plate number then remained stable for a period of a few weeks.

Extra-column band broadening does not materially increase when using a pre-column for trace enrichment. Although the total extra-column volume now is larger, band broadening at the injection side becomes less important, since the solutes are concentrated on top of the pre-column. Strictly speaking, eqn. 6 cannot be applied to calculate σ_{ec}, since the retention volumes, $V_{R,i}$, are not defined exactly in the trace-enrichment mode. If, however, for $V_{R,i}$ the values of the normal injection mode are substituted, straight lines ($R \geqslant 0.994$) are obtained in a σ_i^2 *vs.* $\beta_i V_{R,i}^2$ plot. With a flow-rate of 140 μl/min a value for σ_{ec} of 8.4 μl was found for the adapted Waters Model 440 detector (as against a value of 8.0 μl in the normal injection mode) with a σ_{ec} value of 6.1 μl for the d.m.e. detector (as against a value of 6.0 μl in the normal injection mode).

Applications

Nitrobenzene in river water. Nitrobenzene is a pollutant frequently occurring in river water. In the river Rhine, concentrations of up to 30 ppb have been found[23].

Water samples from the river Amstel were filtered over a Millipore filter and potassium nitrate (10^{-2} *M*) and nitric acid (10^{-3} *M*) were added. Samples of volume 100 *ul* were concentrated as described above and, next, eluted with methanol–water (50:50). The adapted Waters Model 440 UV detector and the d.m.e. detector were used.

The chromatograms of river water samples spiked with nitrobenzene show that the detection limit both the UV and the electrochemical detector is *ca.* 2 ppb. The nitrobenzene level of the Amstel water obviously is below this detection limit (Fig. 5).

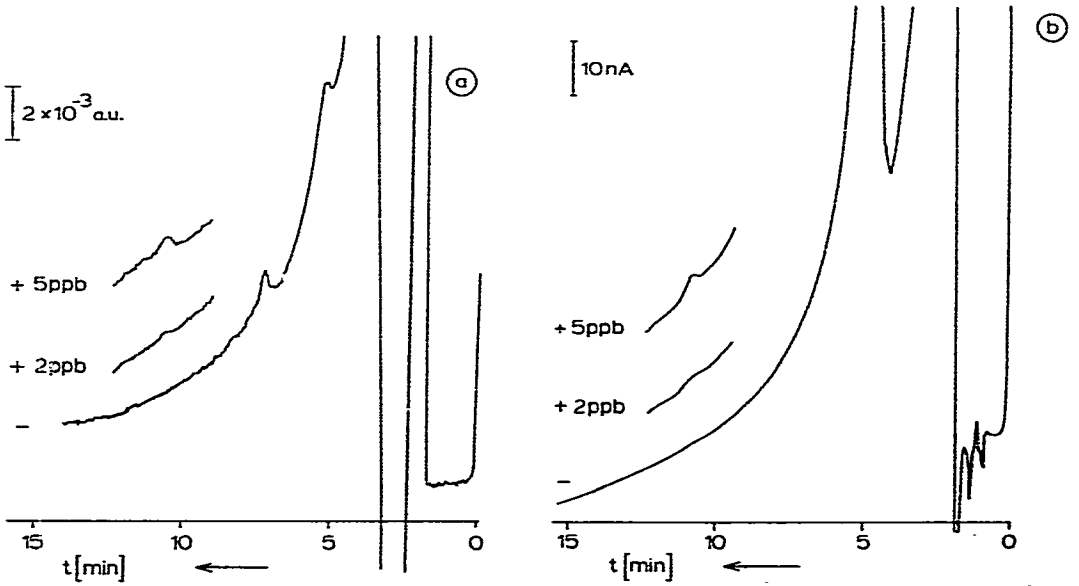

Fig. 5. Determination of nitrobenzene in river water. Column: 250 × 1.1 mm I.D. packed with 7.7-μm LiChrosorb RP-18. Mobile phase, methanol–water (50:50) + 10^{-2} *M* potassium nitrate + 10^{-3} *M* nitric acid; flow-rate, 80 μl/min; 100-μl sample without (–) and with (2 or 5 ppb) added nitrobenzene. (a) Adapted Waters 440 detector (254 nm); (b) d.m.e. detector; $E = -800$ mV.

Interferences due to the tailing solvent or oxygen peak can be eliminated by changing the mobile phase composition to methanol–water (40:60). Nitrobenzene now has a k' value of 8.0; however, since the peak height is considerably reduced, the detection limit does not improve.

4-Nitrophenol in urine. Exposure to the pesticide parathion can be detected by the occurrence of its degradation product 4-nitrophenol in urine. The average concentration of 4-nitrophenol in human urine (U.S.A.), as measured with a derivatization–gas chromatographic technique[24], is 12–26 ppb. After exposure to parathion, concentrations of 18–67 ppb were found. With another derivatization technique even larger differences between exposed and non-exposed subjects have been recorded[25].

In the present study urine samples were filtered over a Millipore filter, 10^{-2} M potassium nitrate was added and nitric acid to pH = 3. After ultrasonic degassing, 100-μl samples were pre-concentrated and, next, eluted with methanol–water (40:60). Detection by means of UV absorption at 254 nm was not possible because of very strong interferences. Fig. 6 shows the chromatogram of a urine sample with d.m.e. detection. The concentration of 4-nitrophenol in this sample is apparently less than the detection limit of 25 ppb.

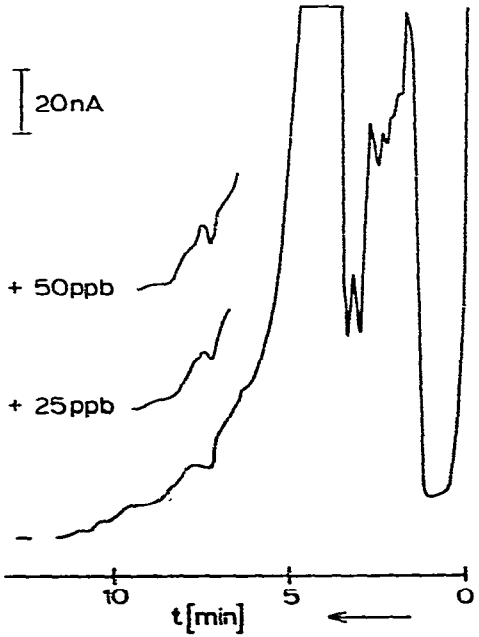

Fig. 6. Determination of 4-nitrophenol in urine. Column, 250 × 1.1 mm I.D. packed with 7.7-μm LiChrosorb RP-18. Mobile phase, methanol–water (40:60) + 10^{-2} M potassium nitrate + 10^{-3} M nitric acid; flow-rate, 100 μl/min; 100-μl sample without (–) and with (25 or 50 ppb) added 4-nitrophenol. D.m.e. detector; E = −800 mV.

For the present determination, the peak-height precision in the range 50–200 ppb of added 4-nitrophenol was ±10% (n = 9).

CONCLUSIONS

The use of conventional UV detectors for HPLC on microbore (*ca.* 1 mm I.D.) columns causes considerable extra-column band broadening. Fortunately, this detrimental effect can largely be suppressed by a simple modification of the detector cell, and by reducing the length of connective tubing as much as possible. The electrochemical detector studied is well suited for use in microbore HPLC, as is evident from the σ_{ec} and calculated α_{min} (minimum required relative retention) data summarized in Table V.

TABLE V

BAND BROADENING (σ_{ec}) AND MINIMUM REQUIRED RELATIVE RETENTION (α_{min}) DATA FOR VARIOUS DETECTORS IN NORMAL-INJECTION AND TRACE-ENRICHMENT EXPERIMENTS

Column: 250 × 1.1 mm I.D.; column dead volume, 160 μl; flow-rate, 140 μl/min.

Detector	σ_{ec} (μl)	α_{min} (R_s = 1.0) *for peaks with* k_i' =		
		1	*3*	*10*
Normal-injection mode (N = 5000)				
Theoretical minimum	–	1.11	1.075	1.062
Perkin-Elmer LC 55	20.9	1.53	1.19	1.081
Pye Unicam LC 3	13.8	1.36	1.14	1.071
Waters 440	12.1	1.32	1.13	1.069
Zeiss PM 2 DLC	15.7	1.41	1.15	1.074
Adapted Waters 440	8.0	1.23	1.10	1.065
D.m.e.	6.0	1.19	1.09	1.064
Trace enrichment (N = 3500)				
Theoretical minimum	–	1.14	1.09	1.074
Adapted Waters 440	8.4	1.25	1.11	1.077
D.m.e.	6.1	1.20	1.10	1.076

A decrease of detection limits is often put forward as one of the advantages of miniaturized HPLC. One should realise, however, that modifying the flow cell of a conventional UV detector (*cf.* above), which may be necessary to improve resolution, increases the baseline noise and, thus, effects a decrease of signal-to-noise ratios. Improved detection performance can therefore only be expected if the amount of sample volume available is small. In other cases, the use of conventional (*i.e.*, 4.6 mm I.D.) columns will lead to lower limits of detection.

The simple trace-enrichment procedure presented in this work allows the injection of samples of up to *ca.* 100 μl. Consequently, for compounds displaying sufficient retention on the stationary-phase material selected, detection limits (in concentration units) can be lowered by two orders of magnitude compared with a direct 1-μl injection. With larger (*e.g.*, 1-ml) sample loops, and for more strongly retained solutes, a three-order-of-magnitude effect can probably be realized. One should keep in mind, however, that with flow-rates of around 100 μl/min, trace enrichment in such a case already takes some 10 min. Handling of significantly larger volumes will, therefore, not be feasible. In other words, leaving apart aspects such as material savings and the

use of special techniques such as on-line HPLC–MS, one can state that HPLC on microbore columns is used to its fullest advantage if the available sample volume is of the order of 0.5–1 ml or less.

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