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OPTIMIZATION OF PRECOLUMN DESIGN IN LIQUID CHROMATOGRAPHY

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SUMMARY

The influence of precolumn design on the efficiency of liquid chromatographic separation systems which involve the use of an on-line trace-enrichment step has been studied. For most practical purposes, precolumns having an inner diameter of 2–4.6 mm and a length of 10–2 mm can be recommended; these allow sampling flow-rates of up to at least 10 ml min^{-1} at a back pressure of 1–10 MPa. Within this range, the precolumn geometry does not appear to be critical in the on-line trace enrichment of either strongly retained or more weakly retained analytes. A new precolumn design is presented which allows rapid slurry packing with a micro-spatula or via syringe loading, and easy replacement of low-cost precolumn cartridges.

INTRODUCTION

The use of precolumns in liquid chromatography (LC) has increased considerably in the past five years. Precolumns are being used as guard and as enrichment columns. A guard column is placed between the injector and the analytical column and serves to protect the latter against contamination by sample (and mobile phase) constituents and grit released by the chromatographic equipment, including the injector. A precolumn can also be used for trace enrichment, either in an on-line or off-line sampling mode, followed by on-line desorption of the enriched analyte onto an analytical column. We will use the term precolumn for enrichment columns applied in on-line systems, since such a precolumn, when used for trace enrichment, generally at the same time serves for sample clean-up and thus also acts as a guard column.

As regards guard columns, the negative influence that they can have on system efficiency has been recognized in several studies. They should be chosen such that, during chromatography, the retention on them is lower than on the analytical column and ideally zero. This can be achieved by selecting a packing material with different surface characteristics or using pellicular materials[1]. Also, guard columns should preferably be coupled directly to the analytical column, without diameter changes[2].

The above solutions to minimize extra-column band broadening usually can-

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not be employed with precolumns intended for trace enrichment. An exception to this is the use of a precolumn with off-line loading followed by direct coupling and on-line desorption onto an analytical column. On-line trace enrichment on a precolumn and desorption to the analytical column preferably take place with the aid of a switching valve, to which the precolumn is attached by means of connecting capillaries. The use of a switching valve is recommended, since it ensures an almost continuous flow over the analytical column. Such connectors unavoidably involve diameter changes, unless the precolumn inner diameter is of the same magnitude (0.1 0.25 mm) as that of the capillaries.

The precolumn packing material should display high retention for the analyte of interest during the sampling step[3,4] and have high loadability, *i.e.*, pellicular sorbents are not recommended. High retention during sampling must be combined with negligible retention during desorption in order to minimize extra-column band broadening during elution. In practice, one generally uses the same stationary phase material in pre- and analytical column and chooses all other conditions (flow-rate during sampling, sampled volume and eluent composition) such that extra-column band broadening is negligible.

A precolumn should contain an amount of sorbent sufficient to allow trace enrichment of the desired amount of solute from a given volume of sample solution; the void volume should, however, be low to prevent extra-column band broadening and to minimize the trapping of matrix constituents which may contaminate the analytical column or interfere with the detection. Secondly, the precolumn should not present back-pressure limitations during sampling at high flow-rates. Finally, the precolumn should be easy to replace and repack and, especially with disposable ones, should be inexpensive; its design should be such that field sampling and automated sample handling are feasible.

The present paper deals with general aspects of precolumn size and geometry, and their influence on the performance of the total chromatographic system in the case of analytes weakly retained during the precolumn loading step. Finally, a newly designed precolumn is described, and several applications are summarized.

GENERAL CONSIDERATIONS

Precolumn geometry

From earlier results it is known[3–5] that an amount of 10–30 mg of packing material, *i.e.*, a geometric (column) volume of 30–60 μl, is generally sufficient for the concentration of trace amounts of organics from 1–100 ml aliquots. As for the contribution to extra-column band broadening of a precolumn packed with a material identical to that in the analytical column, several authors[1,6,7] have shown that coupling of columns of equal efficiency by means of short pieces of capillary or zero-dead-volume unions can be carried out with only a small loss of overall efficiency and slightly increased peak asymmetry. Others[8,9] have demonstrated that excellent linearity between the average plate count and column length can be obtained by coupling 1 mm I.D. columns, in contrast to the situation with wider bore columns. This different behaviour must be attributed to differences in either heat effects or radial inhomogeneity of the packed bed; these effects are much less serious with narrow-bore columns.

On the basis of the above, it may seem attractive for on-line trace enrichment either to use a completely narrow-bore system or, when conventional-size analytical columns have to be used, to couple a narrow-bore precolumn to a wider bore analytical column in order to reduce extra-column band broadening. In the latter case the length and, for a given volume, the inner diameter of the precolumn will be limited by the pressure drop, ΔP. From Darcy's law (*cf.*, ref. 10) one easily derives for the minimum acceptable inner diameter

$$\text{I.D.}^4_{\text{min}} = 8000 \cdot V \cdot F \cdot \eta/\pi^2 \cdot \Delta P \cdot d_p^2 \cdot \varepsilon_T \quad (1)$$

where F = flow-rate (ml sec^{-1}), η = fluid viscosity (Poise), ε_T = total column porosity, V = precolumn geometric volume (ml), d_p = mean particle diameter (cm) and ΔP = pressure drop (dyne cm^{-2}).

As an example, Table I summarizes data for relevant precolumn loading conditions at four pressure drops. Obviously, with pressure drops of 1–10 MPa which may be assumed to indicate a range acceptable in practice, precolumns of about 2–4 mm I.D. and a length of around 0.4–2 cm should be used, the actual values of course being determined by the preferred precolumn volume. It should be emphasized that the contribution of the frit(s) in the precolumn to the pressure drop has been neglected here.

Precolumn design

The home-made precolumns used in this study were initially constructed from pieces of stainless-steel tubing and commercially available column terminators or

TABLE I

CALCULATION OF PRECOLUMN DIMENSIONS FOR VARIOUS PRECOLUMN VOLUMES AND PRESSURE DROPS

Conditions: flow-rate of water at 25°C, 10 ml min^{-1}; precolumn packed with 8-μm particles; ε_T = 0.7.

*Pressure drop (MPa)**	*Precolumn*		
	Volume (μl)	*I.D. (mm)*	*Length (mm)*
1	30	3.0	4.2
	60	3.6	5.9
	100	4.0	8.0
5	30	2.0	9.5
	60	2.4	13.2
	100	2.7	17.4
10	30	1.7	13.2
	60	2.0	19.0
	100	2.3	24.0
15	30	1.5	16.9
	60	1.8	23.5
	100	2.0	31.8

* 1 MPa = 10^7 dyne cm^{-2}.

zero- or low-dead-volume unions. With such systems very short columns cannot be produced due to the space necessary for the attachment of the end fittings. This problem was first solved by Van Vliet *et al.*[11] who filled the superfluous space by means of a drilled-through PTFE rod. Some of the columns used in this study were prepared according to this design. However, such columns are too expensive for field sampling; besides, they are not very flexible as regards column length, and the column terminators are not explicitly designed for frequent opening and closing which is often necessary. Further, the necessity of on- and off-line coupling of the precolumn by means of wrenches was found to be too laborious and time-consuming.

Initial experiments were performed using the precolumns referred to above as well as several further home-made and commercially available precolumns (types I–VII; *cf.*, Table II and *Columns* section below). At a later stage an improved precolumn design (types VIII and IX, Table II) was developed and tested, the emphasis being on ease of handling and general usefulness. Construction details are presented in the pertinent section below.

EXPERIMENTAL

Apparatus

Automated preconcentration and LC analysis were carried out with an LC system (Kontron, Zürich, Switzerland) consisting of two 410 pumps, a Uvikon 720 LC variable-wavelength UV detector, set at 220 nm, a 7120 injection valve (Rheodyne, Berkeley, CA, U.S.A.), a Kontron Model 200 programmer and a prototype of the Model MCS 670 column-switching unit. One low-pressure selector valve and one high-pressure switching valve of the column-switching unit were used, the former for selection of the sample solution to be preconcentrated or to flush the precolumn, the latter for on- and off-line switching of the precolumn. The dead volume between the valves is 0.1 ml. Chromatograms were recorded on a BD 40 recorder (Kipp, Delft, The Netherlands) at a chart speed of 3 cm min^{-1}.

TABLE II

SURVEY OF PRECOLUMNS USED IN THE PRESENT STUDY

Column terminators: V = Valco zero dead volume; S = Swagelok (drilled through) low dead volume.

Type	*Size (mm)*	*Geometric volume (µl)*	*Specifications*
I	1.45 × 4.6	24	Home-made, V (ref. 11)
II	2.0 × 4.6	33	LiChroCart
III	4.0 × 4.6	66	Home-made (ref. 11)
IV	30 × 4.6	500	Valco
V	30 × 4.6	500	Brownlee
VI	33 × 2.0	105	Home-made, S
VII	41 × 1.1	33	Home-made, V
VIII	11 × 2.0	36	Home-made (Figs. 1 and 2)
IX	2.0 × 4.6	33	Home-made (Figs. 1 and 2)

(Pre)columns

A 250 × 4.6 mm I.D., 8-μm CP Spher C_{18} column (Chrompack, Middelburg, The Netherlands) was used as analytical column.

Nine precolumn designs were tested, which differed in geometry, construction and/or packing (Table II). LiChroCart precolumns are prepacked cartridges filled with 7-μm LiChrosorb RP-18 (Merck). The cartridges from Brownlee (Santa Clara, CA, U.S.A.) are prepacked with 10-μm LiChrosorb RP-18. The prepacked Valco and all the home-made precolumns were packed with 8-μm CP Spher C_{18} (Chrompack). Precolumn types I, III and IX were hand-packed with a micro-spatula using a dense slurry in methanol. Types VI and VII were slurry-packed under high pressure (40 MPa) with 3% (w/w) packing material in tetrachloromethane–methanol (95:5). Type VIII precolumns were slurry-packed by means of a syringe, as will be explained below. Precolumns of types VIII and IX are now commercially available from Chrompack.

Chromatography

3,5-Dichlorophenol was selected as test compound and chromatographed on CP Spher C_{18}, with methanol 10^{-3} *M* phosphoric acid (80:20) as eluent at a flow-rate of 1 ml min^{-1}; its capacity factor was 1.7. For trace enrichment, 5 ml of an aqueous solution containing 90 ng of 3,5-dichlorophenol per ml were sampled at a flow-rate of 5 ml min^{-1}. Before sampling, the precolumns were conditioned with 10 ml of 10^{-3} *M* phosphoric acid. After loading, desorption and subsequent analysis were done with the eluent mentioned above, using UV detection at 220 nm.

Chemicals

3,5-Dichlorophenol was purchased from Aldrich Europe (Beerse, Belgium) and was of analytical grade. Analytical-grade methanol and 85% phosphoric acid were obtained from Baker (Deventer, The Netherlands). Demineralized water was purified in a Milli-Q filtration system (Millipore, Bedford, MA, U.S.A.). Eluents were degassed under vacuum and filtered prior to use.

Efficiency measurement

The efficiency of the various systems was expressed in terms of the plate number, N, calculated from $N = (t_R/0.5w_{0.6})^2$, with t_R being the retention time of the test solute and $w_{0.6}$ the peak width at 60% peak height, and the asymmetry factor at 10% peak height, $A_{0.1}$.

RESULTS AND DISCUSSION

Measurement of system efficiency

The system efficiency was measured for 10-μl loop injections on a precolumn placed in series with the analytical column and for 5-ml sample aliquots. The efficiency of the analytical column itself, measured for a 10-μl loop injection of 3,5-dichlorophenol, was $N = 8300$ and $A_{0.1} = 1.2$. The 5-ml volume was chosen because it is almost equal to the breakthrough volume of 3,5-dichlorophenol (7 ml) on the smallest precolumn (1.45 × 4.6 mm I.D.). In this case the concentrated zone is dispersed over nearly the entire precolumn volume, which presents the most un-

favourable injection profile. It should therefore be stressed that with solutes that are more strongly retained and, thus, concentrated as a narrow zone, the results in terms of system efficiency will be better than those obtained with the rather weakly sorbed 3,5-dichlorophenol.

The precision of the determination of N and $A_{0.1}$ for a single precolumn was 4% and 7% (n = 7), respectively. A precision of 4–9% was obtained when testing the repeatability of the packing procedure for five home-packed 11 × 2.0 mm I.D. and 2.0 × 4.6 mm I.D. precolumns (types VIII and IX) both when making loop injections onto a precolumn coupled on-line with the analytical column and when doing the actual trace-enrichment experiments. With the 1.1 mm I.D. precolumn, repeatability was worse; this is in accord with the well known fact that narrow-bore columns are rather difficult to pack efficiently.

Performance of type I–VII precolumns

Typical results of measurements of system efficiency after on-line insertion of the various precolumns are given in Table III; all data are the means from two to five experiments. Plate numbers were calculated using a simplified (manual) method, *i.e.*, assuming Gaussian peak shapes. The influence of asymmetry on plate count has been pointed out by Kirkland *et al.*[1], who showed that the calculation of plate numbers by means of moment analysis, *i.e.*, taking into account peak skew, resulted in, for example, a 30% decrease in plate count compared to the results obtained by measurements using the peak width at 60% peak height for peaks with $A_{0.1}$ = 1.3–1.4 and in a 75% decrease for peaks with $A_{0.1}$ = 1.7. Since, besides, the number of experimental results per precolumn is limited, it is evident that conclusions based on the data in Table III will be of a qualitative rather than a quantitative nature.

TABLE III

DEPENDENCE OF SYSTEM EFFICIENCY ON PRECOLUMN TYPE

System efficiency recorded after trace enrichment of 5 ml of 3,5-dichlorophenol; for further conditions see text. BF = Backflush desorption.

Type	*Precolumn (mm)*	*System efficiency*	
		N	$A_{0.1}$
Analytical column: N = 8300, $A_{0.1}$ = 1.2			
I	1.45 × 4.6	7000	1.4
II	2.0 × 4.6	7800	1.6
III	4.0 × 4.6	6900	1.3
IV	30 × 4.6	7200	1.3
IVBF	30 × 4.6	9600	1.4
V	30 × 4.6	7100	1.6
VBF	30 × 4.6	7300	1.4
VI	33 × 2.0	7700	1.4
VIBF	33 × 2.0	7700	1.3
VII	41 × 1.1	8200	1.2
Analytical column: N = 9700, $A_{0.1}$ = 1.0			
VIII	11 × 2.0	9900	1.1
IX	2.0 × 4.6	9100	1.1

The main conclusion is that, even for trace enrichment of 5 ml of the weakly retained 3,5-dichlorophenol, all type I–VII precolumns tested have a rather similar, and not too negative, influence on system performance: in the trace-enrichment experiments, the original values of $N = 8300$ and $A_{0.1} = 1.2$ change to $N = 7500 \pm 750$ and $A_{0.1} = 1.45 \pm 0.15$ in all but one case. The results so obtained do not significantly differ from those obtained with loop injections on to the system consisting of a precolumn in series with the analytical column (data not shown).

The stability of the packed bed in the various precolumns during trace enrichment was tested by determining the system efficiency after a prolonged series of experiments. With short home-made (types I–III) precolumns the efficiency did not noticeably change even after six to eight concentration experiments. However, during similar series of experiments on a long (type IV) precolumn, a rapid decrease of system efficiency was frequently observed. In one such series, after five consecutive experiments the initial plate number of 7200 had dramatically dropped to 4200, while $A_{0.1}$ simultaneously increased from 1.4 to 1.7. This strongly suggests that the precolumn packing was distorted during the experiments as a result of the repeated sampling of purely aqueous solutions. In a subsequent series, in which desorption was done in the backflush mode, no such decrease of system efficiency was observed. Similar results were obtained with other high-pressure packed precolumns, such as the Brownlee (type V) precolumn.

In order to demonstrate that the distortion of the packed bed in the relatively long precolumns actually occurs throughout the whole cartridge, one such precolumn was reversed and the trace-enrichment/backflush desorption experiments repeated. A dramatic 40% loss in efficiency was then recorded. In agreement with the above observations, with short precolumns no difference in results between forward and backflush desorption was found. Finally, not unexpectedly, the performance of the high-pressure packed narrow-bore type VII precolumns also decreased rapidly upon trace enrichment and forward-flush elution. Probably, in this case, the decrease in efficiency was at least partly due to the high linear velocity (17.5 cm sec^{-1}) used during sampling. In several cases upon opening the precolumn a 2–3 mm deep hole was seen to have formed at the top.

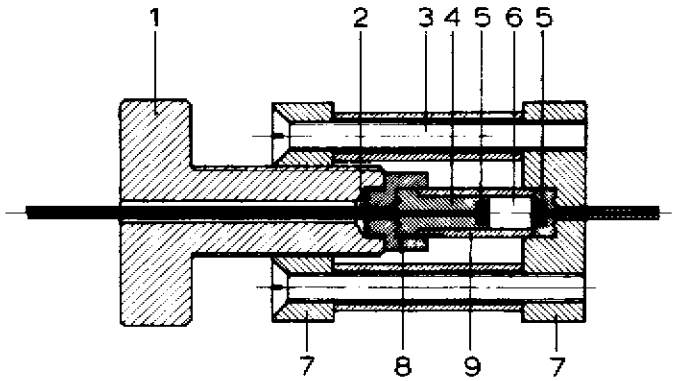

Fig. 1. New precolumn design. 1 = Hand-tightened screw; 2 = stainless-steel capillary, 1/16 in. O.D., 0.25 mm I.D.; 3 = bolt (of column holder); 4 = PVDF rod, 4.6 mm O.D.; 5 = stainless-steel frit (variable diameter, dependent on precolumn diameter); 6 = plug of stationary phase; 7 = column holder; 8 = stainless-steel cap; 9 = precolumn (stainless-steel tube, length 11–20 mm, 1/4 in. O.D., 2.0–4.6 mm I.D.).

Improved precolumn design

A more flexible precolumn system was developed in cooperation with Merck (Darmstadt, F.R.G.) who incorporated it in their LiChroCart Autofix column system, which consists of a holder with a prepacked disposable (pre)column cartridge[12]. At a later stage, we developed a system consisting of a cartridge holder and manually replaceable hand-packed precolumns (see Fig. 1). The precolumns are pieces of stainless-steel tubing of variable length and diameter with a porous frit pressed into a polyvinylidenefluoride (PVDF) plug on one end. The other end is open and sharp-edged. Upon column installation this end is pressed into a PVDF seal which contains a frit and is part of the column holder. Precolumn sealing is performed by means of a hand-tightened screw. The precolumn sealing is leak-tight up to at least 35 MPa. The frit sealings ensure that packing material cannot leak out of the precolumn along the frits. Contrary to the former design, it can therefore be safely used in combination with switching valves both in the forward- and back-flush mode. The precolumn proper can be rapidly exchanged, without using tools since the column holder remains connected to the switching valve and the cartridges are inserted by means of a single hand-operated screw. The precolumn cartridges are inexpensive and can easily be hand-packed, either with a micro-spatula or using a syringe (see Fig. 2). The latter procedure has also been used successfully for loading the precolumn in off-line sampling of relatively small (1–30 ml) volumes.

Results for trace-enrichment experiments with 3,5-dichlorophenol carried out

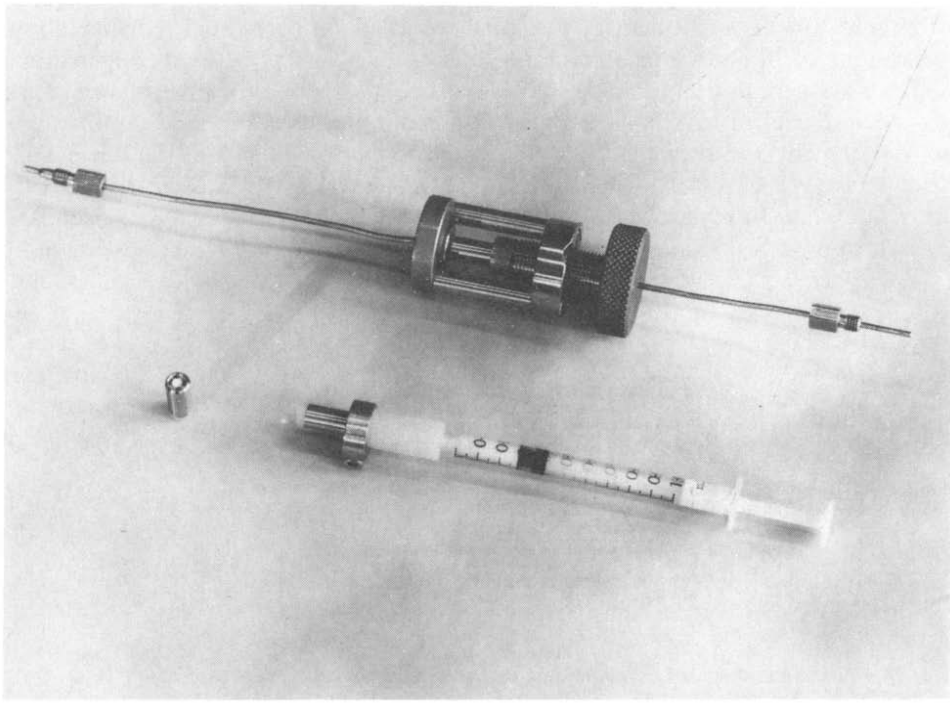

Fig. 2. Precolumn holder and cartridge (*cf.*, Fig. 1) with syringe and adapter used for slurry packing of the precolumn. The syringe can also be used for loading of samples.

TABLE IV

SUMMARY OF PROJECTS IN WHICH TYPE VIII AND IX PRECOLUMNS HAVE BEEN USED

Stationary phase	*Analytes*	*Remarks*	*Ref.*
C_{19}; metal-loaded C_{18}	Phenylurea herbicides	River-water analysis	13
C_{18}	Carbaryl	Surface water	14
PRP_1	Anti-cancer drugs	Serum and plasma; automated analysis	15
Ion exchangers and PRP_1	Phenylenediamines and related compounds	River water	16
C_{18}	Synthetic organic colourants	Cosmetics	*
C_{18}, PRP_1 and cation exchanger	Selected pollutants	Industrial effluents	*

* Current research project.

on type VIII and IX precolumns ($n = 5$) are included in Table III; here a new analytical column, although of the same type and with the same dimensions, was used having $N = 9700$ and $A_{0.1} = 1.0$. It is evident that the newly designed precolumns perform equally well or somewhat better than the other columns tested. The fully satisfactory precision of 4 9%, recorded in repeatability tests on series of five precolumns, has been referred to above. The main advantage of the new precolumn design is, however, in the ease of handling. In recent times they have, therefore, repeatedly and successfully been applied in research projects in our laboratory, using widely divergent chromatographic and flow-rate conditions, and employing manually operated as well as automated systems. A summary of such work is presented in Table IV.

CONCLUSIONS

Various papers report on the use of on-line trace-enrichment techniques in LC. These studies usually deal with the trace enrichment of very hydrophobic solutes onto non-polar stationary phases. In such cases the solutes of interest display high retention and are, consequently, retained in a very small zone at the top of the precolumn, *i.e.*, the separation process starts with a favourable profile of the solute zone. Under such conditions, the on-line coupling of a precolumn and an analytical column generally has a negligible effect on band broadening (typically less than 3%; *cf.*, ref. 11) and system performance is hardly dependent on the precolumn design used.

If, on the other hand, rather polar and, therefore, more weakly retained compounds such as, *e.g.*, low-chlorinated phenols and anilines have to be concentrated, the analytes are distributed over a larger part of the precolumn and precolumn geometry may, in principle, be more critical. The data presented in the present paper demonstrate, however, that even in such cases the several commercially available and home-made precolumns show rather similar performance. Still, short precolumns seem to be generally preferable to long ones. The latter, which must be packed under high pressure, initially may well have a high efficiency. This efficiency, however, deteriorates rather rapidly during trace enrichment of solutes from purely aqueous

sample solutions, and good performance is only obtained in the backflush mode. Even so, one should realize that they display a rather high back-pressure during loading, possess a relatively large dead volume and are rather expensive.

During the present research project a new type of precolumn has been designed which allows easy slurry packing with a micro-spatula or via syringe loading. Since experience has shown that 20 mg of packing materials are ample in most trace-enrichment work, all precolumn cartridges were constructed with a geometric volume of 30–40 μl. Today, 2.0 × 4.6, 5.0 × 3.0 and 11 × 2.0 mm I.D. precolumns are used indiscriminately in our laboratory, and are equally successful. Highly different stationary phases, *e.g.*, non-polar chemically bonded phases, metal-loaded bonded phases, styrene divinylbenzene copolymers and ion exchangers, and flow-rates ranging from 0.5 to 10 ml min^{-1} have been used without any real problem. The precolumns are installed in both manually operated and (semi-)automated systems. Precolumn repacking has turned out to be particularly simple and in our laboratory several novices to the art of precolumn technology have mastered the technique within a few days.

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