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PRECOLUMN-VENTING PLUG TECHNIQUE FOR DIRECT INJECTION OF UNTREATED BLOOD PLASMA SAMPLES INTO REVERSED-PHASE LIQUID CHROMATOGRAPHY SYSTEMS

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SUMMARY

A simple column switching technique for the direct injection of untreated blood plasma samples is presented for the determination of drugs and related compounds. The system consists of injector valves, a precolumn, a three port valve and a separation column. The precolumn is used for trace enrichment and sample clean-up. Aqueous plugs are introduced on both sides of the plasma sample before it enters the precolumn. This results in a stable system, because contact between plasma proteins and the organic solvent usually present in the mobile phase is prevented. The proteins are eluted to waste with the aqueous plug fluid.

The stability of the chromatographic system was studied with respect to efficiency and column back-pressure. The influence of the concentration of organic solvent in the eluent, type and particle size of the packing material, precolumn filters, pH, and ionic strength was investigated for large sample volumes. Optimal stability was obtained at low concentrations of organic solvent in the eluent, with plugs of phosphate buffer (μ = 0.1) and with a spreader at the inlet and a screen (2 μm) at the outlet of the precolumn. The precolumn was packed with 10-μm particles for trace enrichment. Under favourable conditions 40–50 large-volume (0.5-ml) plasma injections can be made into a single precolumn.

INTRODUCTION

For many years there has been great interest in the development of analytical methods for the determination of drugs and other compounds in body fluids by applying the biological sample directly to a reversed phase liquid chromatographic column[1]. Such a procedure would make time-consuming steps like solvent extractions unnecessary leading to simpler methods, which can easily be automated and to methods of higher accuracy. The main problem in injecting an untreated plasma sample into the aqueous mobile phase is the incompatibility of the plasma proteins

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with the column system. Plasma proteins can be adsorbed on the solid phase and denatured or precipitated by components of the eluent.

One way to solve this problem may be to use solid phases and eluents that do not cause adsorption or denaturation–precipitation. In one study[2], we used eluents containing only water with added salts, thus avoiding the usual water-miscible modifiers (*e.g.* methanol, acetonitrile) that are known to cause protein precipitation. Direct injection of untreated plasma was possible in these systems, but column stability was somewhat limited as was the possibility of regulating the retention when no organic solvents were allowed in the eluent.

To prolong the life-time of the separation column, we used the precolumn venting technique[3], and we were able to inject about 0.5 ml of plasma before the column performance declined. In this technique, the main fraction of the plasma proteins comes in contact with only the precolumn but does not enter the separation column. The separation column was completely stable, but the precolumn had to be changed after about 0.5–1.0 ml plasma, and thus, this system is practical only if small sample volumes (≈ 10 μl) are applied. Several factors which affect column stability were studied, and it was found that the kinetics of protein denaturation must be taken into account in order to increase precolumn stability. We also found that moderately high organic solvent concentrations in the eluent can be tolerated with acceptable precolumn stability. The methodology was applied to the determination of theophylline and antiepileptics in small plasma samples[4,5].

Other groups have successfully applied 10–20 ml of serum to a column system where the drug is enriched on a precolumn and the proteins are washed away with water. Then the drug is eluted from the precolumn in a back-flush mode into the separation column with a suitable eluent[6,7]. After back-flushing, the precolumn is washed with water in a rather time-consuming procedure. Often two precolumns and two pumps are used in order to increase sample through-put. Detailed discussions of the stability in these systems are scarce, but some guidelines have been reported[8]. Recently, this technique has been applied by using elution from the precolumn in a forward-flush mode[9], which seemed to increase the life-time of the system.

In this paper, we introduce a precolumn-venting plug technique. In the regular precolumn-venting technique[3–5], the plug of plasma sample comes in direct contact with the eluent as it is injected into the short precolumn (10 mm). This may decrease precolumn stability if the eluent contains protein-denaturing agents, such as methanol or acetonitrile. By introducing "plugs" of a liquid, *e.g.* water, that will surround the plasma plug during its passage through the precolumn, protein denaturation will be minimized and precolumn stability increased. This is done very conveniently by using valve injectors in series. This makes the use of the precolumn-venting technique available for large sample volumes and increases enormously the number of small-volume plasma injections that can be made into a single precolumn. Several plugs of different composition may be introduced so that one can, *e.g.*, influence the retention of proteins and that of the drug selectively. The composition of the surrounding plug must allow efficient enrichment of the drug in the precolumn, especially if the plasma sample is large (100 μl–1.0 ml).

The present study is aimed at the performance and the stability of the precolumn-venting plug technique. The stability has been studied with respect to peak efficiency and column back-pressure under different conditions, for example with

different concentrations of organic solvent in the eluent, different compositions of plug fluid, different types and particle sizes of the solid phase, and different types of filters in the precolumn.

EXPERIMENTAL

Chemicals

Naproxen, primidone, and theophylline were of pharmacopoeial grade. Methanol (obtained from E. Merck, Darmstadt, F.R.G.) was of p.a. quality. Solvents and other chemicals were of analytical-reagent grade.

LiChrosorb RP-2 (10 μm particle size), LiChrosorb RP-8 (5 and 10 μm), LiChrosorb RP-18 (10 μm), LiChrosorb CN (10 μm), LiChrosorb DIOL (10 μm), and LiChroprep RP-8 (25–40 μm) were obtained from E. Merck. μBondapak Phenyl (10 μm) was obtained from Waters Assoc. (Milford, MA, U.S.A.).

Equipment

The pump was an LDC solvent delivery system 711-47 (Milton Roy minipump with pulse dampener; LDC, Riviera Beach, FL, U.S.A.). The sample injector was a Rheodyne syringe loading injector 7120 (Rheodyne, Berkeley, CA, U.S.A.) equipped with loops of 20–500 μl. A three-port valve Valco CV-3-HPax (Valco, Houston, TX, U.S.A.) was used as a venting valve and a Valco-6-HPax injector equipped with loops of 0.5–5.0 ml was used for injection of the plug liquids. The detector was an LDC UV III monitor (1203), operating at 254 nm.

The separation columns, 100 × 4.6 mm I.D., were Lichroma tubes (316 stainless steel, Handy & Harman Tube Co., Norristown, PA, U.S.A.) and Altex (Altex, Berkeley, CA, U.S.A.) 2-μm stainless-steel fritted disks were placed in both ends. The precolumn, 10 × 3.2 mm I.D., was constructed similar to the separation column after modification of the fitting. The bed supports were equipped with either Altex stainless-steel frits or a spreader (HPLC-Teknik, Robertsfors, Sweden) at the inlet and a 2-μm stainless-steel screen (Skandinaviska GeneTec, Kungsbacka, Sweden) at the outlet. The spreader and the screen were fitted with circular PTFE gaskets on both sides for sealing. The gaskets were cut from thin PTFE sheets. The width of the sealing edge was equal to the thickness of the precolumn wall. A pressure regulator, constructed in our laboratory[10] was used to regulate the back-pressure in the venting line when the precolumn-venting plug technique was used.

Procedures

Blood plasma samples were prepared by adding 100 μl of a stock solution of the drugs to 1000 μl of completely homogenized pooled blank plasma. Prior to injection of the blood plasma, it was completely homogenized and centrifuged at about 5000 *g*. All experiments were performed at 25°C.

The buffers were prepared by mixing 1.0 *M* sodium dihydrogen phosphate, 0.5 *M* disodium hydrogen phosphate or 1.0 *M* phosphoric acid, and diluted with deionized water, to give solutions of pH 2.0, 6.0, and 7.0 and the specified ionic strength. The mobile phases were prepared by mixing volumes of buffer and organic solvent in the specified ratios.

The separation columns were packed with a slurry of the packing material in

chloroform at 300 bar with a high-pressure pump. The precolumns were packed by means of an ordinary LC pump with a slurry of the packing material in a mixture of methanol–dichloromethane (1:1, v/v).

The void volume was determined by injection of sodium nitrate. For determining the column efficiency as a test for the stability of the system, the plate number (N) was calculated from the retention time and the base-width. The efficiency can be expressed either by the plate number, the theoretical plate height ($H = L/N$) or the reduced plate height ($h = H/d_p$) even if the chromatographic system consists of a precolumn combined with a separation column containing particles of different size. In these cases, the column length (L) and particle diameter (d_p) used in the calculation were those of the separation column.

The column resistance factor (φ') is expressed by

$$\varphi' = 100 \frac{P\, t_m\, d_p^2}{\eta\, L^2}$$

were P is the column back-pressure in bar, t_m is the retention time of an unretained solute in seconds, d_p is the particle diameter of the packing material in μm, η is the viscosity in cP and L is the column length in mm.

The precolumn stability was expressed by two methods: either as the total volume of plasma samples that can be injected until the peak efficiency (h) and the column resistance factor (φ') have changed by 10%, or as the precolumn life-time, which is defined as the total volume of plasma applied until the back-pressure reaches a level close to the maximal pressure capacity of the pump (*ca.* 300 bar). During the stability measurements, blank plasma was injected and the efficiency was measured by injection of solutes, dissolved in buffer (non-venting).

Plug liquid was introduced by filling the 2-ml loop of the plug liquid valve with it. The plug liquid was injected into the precolumn (10 × 3.2 mm I.D.) while the three-port valve was maintained in the "waste" position. After passage of 1.0 ml, the plasma sample was injected. When the total volume from both injectors had passed the precolumn, the three-port valve was switched to direct the eluent into the separation column (100 × 4.6 mm I.D., packed with 5-μm LiChrosorb RP-8).

RESULTS AND DISCUSSION

System design

The regular precolumn-venting system[3] was designed to provide very high stability for the separation column through the on-line coupling of a short precolumn (10 mm) to the separation column via a three-way valve. The plasma sample was injected directly into the precolumn. Separation in the precolumn of the high-molecular-weight plasma proteins from low-molecular-weight drugs enabled the elution of proteins from the precolumn through the three-port valve to "waste". This required that the proteins be unretained (or excluded from the pores) in the precolumn and that the drug be retained.

The precolumns had a limited stability; at best, 140 plasma samples of small volume (10 μl) could be injected[4], before column back-pressure and efficiency started to change by 10%. This was the case when the eluent contained an organic solvent.

The limited stability makes the application of this technique to large sample volumes difficult because of the need for frequent replacement of the precolumn. It should be possible to increase the stability by preventing the plasma proteins from contacting the organic solvent usually present in the eluent. This can be done by introducing a "plug" of a suitable liquid, *e.g.* water, on both sides of the plasma plug before it enters the precolumn. This is conveniently done with regular sample valve injectors, one for the plasma sample and the other for the plug liquid. The loop of the plug liquid valve, situated upstream from the sample valve, is filled with the plug liquid. After the plug liquid is introduced into the eluent stream, the sample valve is switched while the plug liquid is flowing through this valve, *i.e.*, the sample will be injected into the stream of the chosen plug liquid instead of into the eluent and will thus be preceeded and followed by certain volumes of plug liquid. These volumes can be regulated by the total volume of plug liquid introduced and by the time difference between the switching of the two valves.

Pre-column design

The precolumns were constructed to allow for a variable length and inner diameter[3]. In this study, they were 10 mm long with 3.2 mm I.D. Other research groups have found that the use of screens instead of fritted disks for retaining the packing in the precolumn gave much less problems with pressure increase upon injection of plasma samples[6,8,11,12]. Therefore, our precolumn design features a 2 μm screen at the outlet (Fig. 1) and only a spreader at the inlet. The spreader serves to distribute the plasma sample over the entire cross-sectional area of the precolumn.

Stability of the precolumn

As described before[3–5], the precolumn is operated in such a way that the stability of the separation column is not affected by the plasma samples. However, the samples can limit the stability of the precolumn, primarily its flow resistance and efficiency. The precolumn stability can be affected by various factors, such as the concentration of organic solvents in the eluent, type and particle size of the packing material, whether frits or screens are used, pH, and ionic strength. These factors will be discussed in the following. Most of the stability studies were performed in an accelerated mode[3], *i.e.* a large series of blank plasma samples were injected at short time intervals. A few experiments indicated that the same stability values are obtained in routine use of the sample injection procedure where the time intervals between plasma injections are longer.

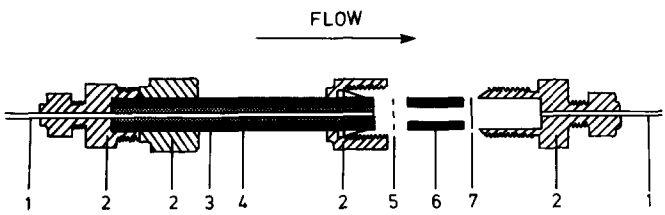

Fig. 1. Pre-column design. 1 = Capillary tube, 0.25 mm I.D.; 2 = swagelok connectors; 3 = stainless-steel tube, 50 × 3.2 mm I.D.; 4 = PTFE tube; 5 = stainless-steel spreader fitted with thin PTFE gaskets on both sides; 6 = precolumn, 10 × 3.2 mm I.D.; 7 = stainless-steel screen filter (2 μm) fitted with thin PTFE gaskets on both sides.

The advantage of the plug technique, as compared to the previous technique[3], is best demonstrated by injection of plasma samples and by the use of aqueous eluents with a high concentration of methanol, which is known to cause protein denaturation[3]. High methanol concentrations severely limit the life-time of the pre-column. Fig. 2 demonstrates that the stability increased two- to five-fold when the plug technique, with water in the plugs, was used. Obviously, the water plugs partly protect the plasma from the eluent. Although the presence of the plugs vastly improves the stability, the plasma proteins will obviously still damage the precolumn packings. The composition of the eluent affects the stability of the precolumn in a similar way in the plug technique and in the precolumn-venting technique (Fig. 2), *i.e.* an increase of the methanol concentration decreases the stability of the precolumn.

Screens and frits

The use of the spreader and screen in the precolumn (Fig. 1) instead of fritted disks increased the stability about three- to four-fold as demonstrated in Fig. 2. Perhaps the high surface area of the fritted stainless-steel disk that comes into contact with the plasma sample induces some denaturation or precipitation of plasma proteins, thus limiting stability more than the screen, for which the contact area is much smaller. Thus, the previously published stability levels for applications of the precolumn-venting technique[4,5] can be raised by using this new precolumn design.

Composition of the plug fluid

The composition of the plug fluid has a pronounced influence on precolumn stability. The role of the organic solvent, which has a denaturing effect on plasma proteins, has been demonstrated earlier[3]. We have found that the presence of a buffer, its pH and ionic strength also affect the stability of the precolumn considerably. Fig.

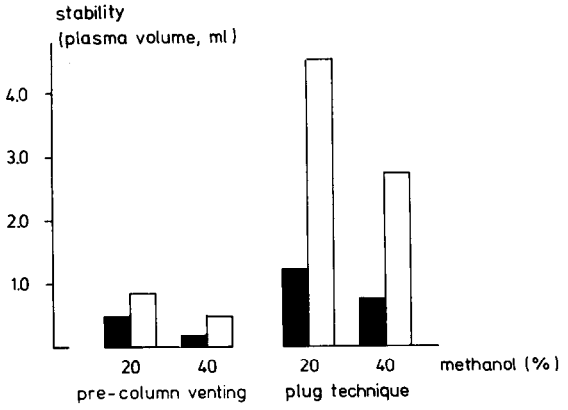

Fig. 2. Influence of the type of filters and of eluent composition on stability. Eluent: phosphate buffer (pH 6.0)–methanol; separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8 (5 μm); precolumn: 10 × 3.2 mm I.D., packed with LiChrosorb RP-8 (10 μm); flow-rate: 0.9 ml/min; plasma sample size: 20 μl; plug fluid, water; solutes: theophylline in 20% and naproxen in 40% methanol; the stability measured at 10% change of the starting values of peak efficiency and column back-pressure. (■) Precolumn with 2-μm stainless-steel fritted disk; (□) precolumn with as spreader at the inlet and a 2-μm screen at the outlet.

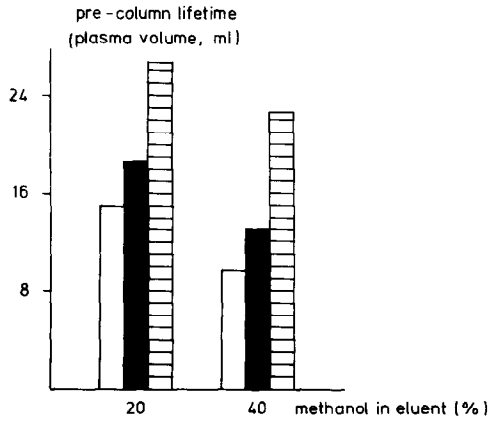

Fig. 3. Effect of plug fluid on stability. Eluent: phosphate buffer (pH 6.0)–methanol; separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8 (5 μm); precolumn: 10 × 3.2 mm I.D., packed with LiChrosorb RP-8 (10 μm); plasma sample size: 0.5 ml; flow-rate: 0.9 ml/min; solutes, theophylline in 20% and naproxen in 40%. (□) Water, (■) phosphate buffer (pH 7.0, μ = 0.1), (⊟) phosphate buffer (pH 2.0, μ = 0.1).

3 shows some improvement of the stability when phosphate buffer (pH 2.0) is used instead of pure water. However, the actual pH is also important, pH 2.0 giving a higher stability than pH 7.0. At present we do not have any explanation for these effects. The extremely high stability, 26 ml of plasma, corresponds to thousands of injections of small (10 μl) plasma samples and shows that the plug technique has great potential as a valuable tool. Fig. 4 illustrates the very small decline in column efficiency obtained after injecting up to 50 samples of plasma, each of 500 μl volume, with phosphate buffer in the plugs. Pure water drastically decreases the efficiency after only 10 samples.

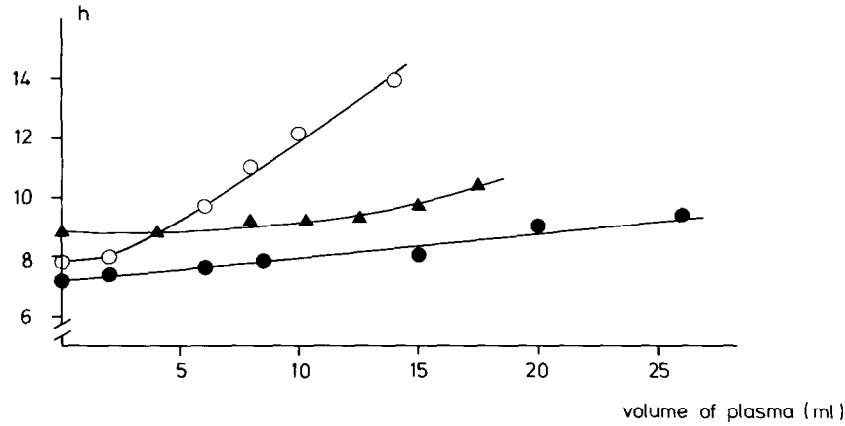

Fig. 4. Effect on peak efficiency of plasma injections using different plug fluids. Eluent: phosphate buffer (pH 6.0)–methanol (4:1); separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8 (5 μm); precolumn; 10 × 3.2 mm I.D., packed with LiChrosorb RP-8 (10 μm); flow-rate: 0.9 ml/min; plasma sample size: 0.5 ml; solute: theophylline. (○) Water, (▲) phosphate buffer (pH 7.0, μ = 0.1), (●) phosphate buffer (pH 2.0, μ = 0.1).

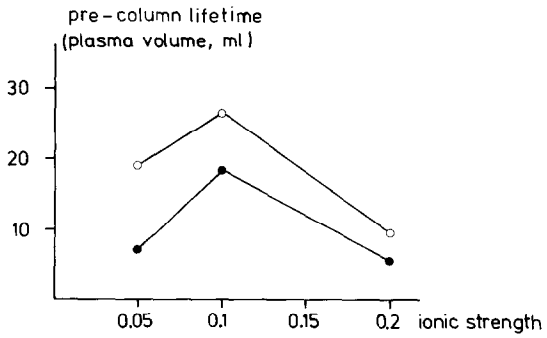

Fig. 5. Effect of ionic strength of plug fluid on stability. Eluent: phosphate buffer (pH 6.0)–methanol (4:1); separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8 (5 μm); precolumn: 10 × 3.2 mm I.D., packed with LiChrosorb RP-8 (10 μm); plasma sample size: 0.5 ml; flow-rate: 0.9 ml/min; solute: theophylline; plug fluid: (○) phosphate buffer (pH 2.0), (●) phosphate buffer (pH 7.0).

The ionic strength of the buffer in the plugs is also important. Fig. 5 shows that maximum stability was obtained at an ionic strength of 0.1, rather than at higher or lower values both at pH 7.0 and 2.0. Of course, care must be taken not to include components like ion-pair reagents in the plug as these may be retained on the pre-column and thereby cause extra peaks in the chromatograms.

Size of the plugs

The protecting effect of the plugs must depend to a certain degree on their volume and the requirements may even be different for the volume of the front and

TABLE I

EFFECT OF THE SIZE OF THE FRONT AND BACK FLUID PLUG ON STABILITY

Chromatographic conditions: eluent, phosphate buffer (pH 6.0)–methanol (3:2); separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8, 5 μm; precolumn: 10 × 3.2 mm I.D., packed with LiChrosorb RP-8, 10 μm; flow-rate: 0.9 ml/min; injected volume: 0.5 ml; solute: naproxen; plug fluid: phosphate buffer (pH 7.0, ionic strength = 0.1).

Volume of plug fluid (ml)		*h**		*φ'***		*Precolumn lifetime (total plasma volume, ml)*
Front	*Back*	*Start value*	*Stability*** (total plasma volume, ml)*	*Start value (× 10^{-3})*	*Stability*** (total plasma volume, ml)*	
0.0	2.0	9.0	4.5	1.8	6.5	7.5
0.5	1.5	9.4	6.0	1.8	8.0	1.0
1.0	1.0	8.3	7.5	1.9	1.0	13.5
1.5	0.5	8.7	3.0	1.9	6.0	7.0
2.0	0.0	9.6	1.0	1.8	2.5	4.0
0.25	0.25	9.7	2.0	1.8	3.5	4.5
1.0	4.0	9.6	6.5	1.9	9.5	11.5

* h = reduced plate height.
** φ' = column resistance factor.
*** 10% change of the measured parameter from the starting value.

of the back plug. Some experiments involving different volumes of the front and back plug are summarized in Table I. They indicate that the presence of the back plug is more important, because even when there was no front plug the stability was reasonably good. The back plug, obviously, is needed to wash out the proteins. More evidence for the importance of the back plug is given by the fact that the stability decreased drastically when the back plug was absent.

Plasma injection on the precolumn alone

Despite the use of plugs, plasma samples cause a deterioration of the precolumn. Direct information on this effect was obtained by using the precolumn alone as a chromatographic column, with phosphate buffer (pH 7.0) as eluent. The use of this buffer as a plug fluid for large plasma samples will be discussed below. The results are summarized in Fig. 6. Injections of large volumes of plasma caused a continuous decrease in the retention of a test compound and in the plate count. However, the decrease seems to be so low that it does not affect the use of the precolumn for trace enrichment, which will be discussed later. The slight decrease in retention and plate count, even when no plasma was injected, may be due to some physical or chemical breakdown of the packing material.

Packing material

The stability of the precolumn depends significantly on the type of packing material and its particle size. Other authors have recommended the use of large particles (*ca.* 30 μm) in the precolumn[6,8,11], because with smaller particles a high pressure drop over the precolumn arose after a limited number of plasma injections. We have found that in the spreader-equipped precolumn 25–40-μm particles provide a better stability than smaller particles (Fig. 7). However, even 10-μm particles show a very good performance, *i.e.* about 60% of the stability obtained with the larger particles; at optimal conditions (Fig. 3) more than 20 ml of plasma can be applied.

In our opinion, 10-μm particles are the best choice, for two reasons: (1) smaller particles give a better efficiency, which helps to avoid insufficient enrichment, *e.g.*, in

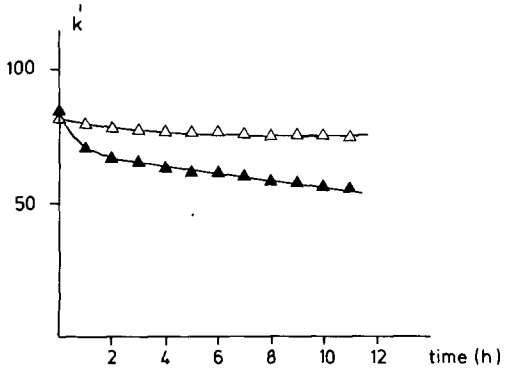

Fig. 6. Solute retention in the precolumn after plasma injections with buffer as eluent. Eluent: phosphate buffer (pH 7.0, μ = 0.1); precolumn: 10 × 3.2 mm I.D., packed with LiChrosorb RP-8 (10 μm); flow-rate: 1.0 ml/min; solute: caffeine; injected volume: 0.5 ml for plasma sample (total volume = 6.0 ml) and 10 μl for standard solution. (△) Standard solution, (▲) blank plasma.

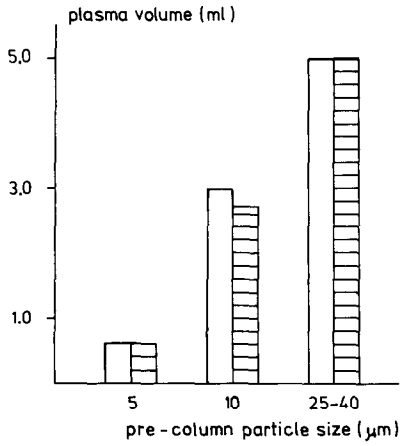

Fig. 7. Effect of pre-column particle size on stability. Eluent: phosphate buffer (pH 6.0)–methanol (3:2); separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8 (5 μm); precolumn: 10 × 3.2 mm I.D., packed with LiChrosorb RP-8 (5 and 10 μm) and LiChroprep RP-8 (25–40 μm); plasma sample size: 100 μl; plug fluid: water; flow-rate: 0.9 ml/min; solute: naproxen; the stability measured at 10% change of the starting values with respect to column back-pressure (□) and peak efficiency (⊟).

cases when the solute has a low capacity ratio[13,14] and (2) large particles in the precolumn very drastically increase the band-broadening of the combined precolumn–separation column system[3].

Table II summarizes the effect of different packing materials. More hydropho-

TABLE II

STABILITY OF PRECOLUMN PACKED WITH DIFFERENT STATIONARY PHASES

Chromatographic conditions: eluent, phosphate buffer (pH 6.0)–methanol (4:1); separation column: 100 × 4.6 mm I.D., packed with 5-μm LiChrosorb RP-8; precolumn: 10 × 3.2 mm I.D., packed with 10-μm stationary phase; solute: theophylline; injected volume: 0.5 ml; plug fluid: phosphate buffer (pH 7.0, ionic strength = 0.1).

Solid phase in precolumn	*h**		*φ'***		*Precolumn lifetime (total plasma volume, ml)*	*Enrichment**** *efficiency (%)*
	Start value	*Stability*§ *(total plasma volume, ml)*	*Start value* ($\times 10^{-3}$)	*Stability*§ *(total plasma volume, ml)*		
LiChrosorb SI 1	7.3	1.0	1.7	0.5	1.5	0.0
LiChrosorb CN	7.0	3.5	1.7	3.5	4.5	0.0
μBondapak Phenyl	8.8	2.5	1.9	4.5	5.5	40
LiChrosorb RP-2	9.5	4.0	1.7	4.0	5.5	100
LiChrosorb DIOL	6.6	8.5	1.9	8.5	11.0	0.0
LiChrosorb RP-18	7.1	9.5	1.7	1.5	12.0	100
LiChrosorb RP-8	7.4	14.5	1.7	14.5	18.5	100

* h = reduced plate-height.

** φ' = column resistance factor.

*** Enrichment efficiency = absolute recovery of the solute during the venting procedure (solute dissolved in buffer pH 6.0).

§ 10% change of the measured parameter from the starting value.

bic column materials, LiChrosorb RP-8 and RP-18, as well as the polar material, LiChrosorb DIOL, tend to have a high stability, whereas pure silica, LiChrosorb Si 100, has a low stability. These results may reflect differences in the protein denaturing properties of these materials. Materials like LiChrosorb DIOL, containing weakly polar alcohol groups, have been chosen as packings for size-exclusion chromatography of proteins, because they have a small denaturing effect on the proteins[15].

Plasma sample size and enrichment

The plug technique was developed with the aim of increasing precolumn stability so that large plasma samples could be analyzed without causing rapid precolumn deterioration. The number of possible plasma injections can be estimated from the stability data in terms of total plasma volume. Experience has shown that the total plasma volume that can be applied is independent of the volume of each sample injected.

For large plasma samples, *e.g.* >0.5 ml, analytical conditions must permit an enrichment of the analyte in order to yield good separation efficiency and allow precolumn venting. The enrichment on the precolumn requires that the analyte has a very high retention during the passage of the plasma sample. The composition of the plasma itself may contribute to the enrichment, owing to its high water content, but on the other hand, it can also have a negative effect, if, *e.g.*, strong solute–protein binding decreases retention during enrichment and thus may cause low recoveries[16].

In the plug technique presented here the plugs also serve to free the precolumn from plasma proteins. The aqueous buffers used as plug thus serve two purposes, *i.e.* to clean the precolumn and to keep the analyte enriched. Chromatograms illustrating enrichment and separation of primidone from a 500-μl plasma sample are shown in Fig. 8.

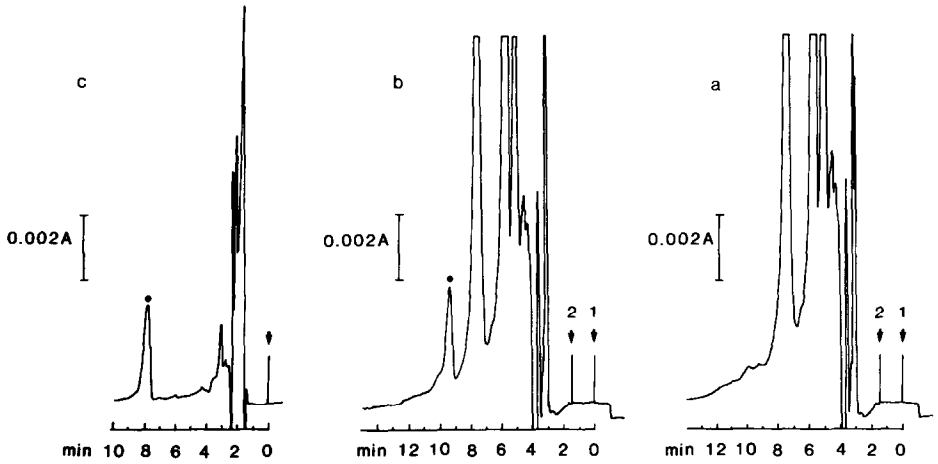

Fig. 8. Chromatograms for separation of primidone. (a) Plug technique, blank plasma; (b) plug technique, primidone (●) 4.13 mg/l in plasma; (c) non-plug, standard solution of primidone (●) 4.13 mg/l; eluent: phosphate buffer (ph 6.0)–methanol (7:3); flow-rate: 0.9 ml/min; separation column: 100 × 4.6 mm I.D., packed with LiChrosorb RP-8 (5 μm); precolumn: 10 × 3.2 mm I.D., packed with Lichrosorb RP-8 (10 μm); sample size: 0.5 ml; plug fluid: phosphate buffer (pH 7.0, μ = 0.1). 1 = Sample injected after 1 ml of the plug fluid pass the precolumn to waste, 2 = venting valve is switched to direct the eluent into the separation column.

Enrichment occurs only if the solute exhibits very high retention on the packing material in the precolumn while the latter is filled with the plug fluid. When an aqueous buffer (pH 7.0) is used as plug fluid, the precolumn packing material ought to be rather hydrophobic in order to retain many types of organic compounds. Data expressing the enrichment efficiency of theophylline, chosen as a polar model compound, in Table II confirm this, as complete enrichment was obtained only with alkyl silicas, whereas pure silica derivatized with phenyl-, cyano- and diolgroups yielded incomplete or zero enrichment.

CONCLUSIONS

The advantages of the precolumn plug technique are: (1) trace enrichment; (2) clean-up of the sample; and (3) protection of the separation column. With the plug technique it is possible to inject large sample volumes (0.5 ml), which is necessary for the analysis of samples with low drug levels, needed for pharmacokinetic studies.

The system described can easily be adapted to the determination of different types of compounds in untreated blood plasma. It can be assumed that quantitation will have acceptable accuracy and precision, as determined by external standardization, even if the peak efficiency changes by 10%. This means that, under favourable conditions, at least 40–50 plasma injections (0.5-ml sample volume) can be made. Quantitation will show recoveries close to 100% with low relative standard deviation.

Work is in progress on the application of the precolumn-venting plug technique to the determination of drugs in large plasma samples[17].

REFERENCES

1 K.-G. Wahlund and U. Lund, *J. Chromatogr.*, 122 (1976) 269.
2 K.-G. Wahlund, *J. Chromatogr.*, 218 (1981) 671.
3 T. Arvidsson, K.-G. Wahlund and N. Daoud, *J. Chromatogr.*, 317 (1984) 213.
4 N. Daoud, T. Arvidsson and K.-G. Wahlund, *J. Pharm. Biomed. Anal.*, 4 (1986) 253.
5 N. Daoud, T. Arvidsson and K.-G. Wahlund, *Acta Pharm. Suec.*, 23 (1986) 65.
6 W. Roth, K. Beschke, R. Jauch, A. Zimmer and F. W. Koss, *J. Chromatogr.*, 222 (1981) 13.
7 W. Voelter, T. Kronbach, K. Zech and R. Huber, *J. Chromatogr.*, 239 (1982) 475.
8 A. Nazareth, L. Jaramillo, B. L. Karger, R. W. Giese and L. R. Snyder, *J. Chromatogr.*, 309 (1984) 357.
9 U. Juergens, *J. Chromatogr.*, 310 (1984) 97.
10 W. Lindner and H. Ruckendorfer, *Intern. J. Environ. Anal. Chem.*, 16 (1983) 205.
11 K. Zech, *Symposium on Handling of Environmental and Biological Samples in Chromatography*, Lausanne, 1983.
12 W. Roth, *J. Chromatogr.*, 278 (1983) 347.
13 H. P. M. van Vliet, Th. C. Bootsman, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 185 (1979) 483.
14 C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 53 (1981) 2072.
15 D. E. Schmidt, R. W. Giese, D. Conron and B. L. Karger, *Anal. Chem.*, 52 (1980) 177.
16 T. Arvidsson, *2nd Symposium on Handling of Environmental and Biological Samples in Chromatography, Freiburg, 1985.*
17 N. Daoud, T. Arvidsson and K.-G. Wahlund, *J. Pharm. Biomed. Anal.*, submitted for publication.