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Analysis of carbamazepine in serum by liquid chromatography with direct sample injection and surfactant-containing eluents

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ABSTRACT

Surfactant-containing eluents are evaluated for the analysis of carbamazepine in serum with conventional reversed-phase columns. Bovine serum was quantitatively eluted at the column void volume using surfactant concentrations in conventional reversed-phase eluents. The effect of pH, guard columns and column switching was evaluated with respect to separating and detecting clinical levels of the drug and its primary metabolite. Column lifetime was also investigated.

INTRODUCTION

Because the requirement for sample preparation adds to the labor and cost associated with liquid chromatographic (LC) methods for drug analysis, a number of alternatives for minimizing sample preparation have been investigated [1,2]. Some of these approaches allow the direct injection of serum into the LC system. One such approach is the use of specially designed column packings which do not promote the adsorption or precipitation of proteins. The internal-surface reversed-phase (ISRP) [3] and shielded hydrophobic phase [4] packings are the best known commercial implementations of this strategy. A related strategy is the coating of conventional column packings with surfactants [5] or serum proteins [6,7], in order to permit their use with direct serum injection. One disadvantage of all special packings is the relatively small number of available phases. For example, Haginaka *et al.* [8] could not adequately retain hydrophilic drugs on the commercial ISRP phase and found it necessary to synthesize a new ISRP phase with a more hydrophobic ligand. Another consideration in the use of coated columns is the need to restrict the use of certain eluent components (*e.g.*, organic solvents) capable of removing or deactivating the column coating [5], possibly leading to a change in retentivity toward drug solutes.

Another approach to direct serum injection is the use of conventional column packings in combination with eluents capable of solubilizing serum components. In micellar LC, eluents are used which contain surfactants at concentrations above the critical micelle concentration (CMC), and these eluents have been shown to permit

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direct serum injection [9–11]. Properly designed eluents bring about the elution of serum proteins at or near the column void volume.

We have recently demonstrated [12] that the constraints which micellar LC imposes upon eluent composition can be relaxed considerably without compromising the ability to perform direct serum injection. Specifically, much lower surfactant concentrations (*i.e.* sub-CMC) and much higher organic solvent compositions (*e.g.*, 40% methanol) can be used in eluents for direct serum injection. The broader allowable ranges for these eluents permit direct serum injection methods to be developed similarly to paired-ion chromatographic methods [13], with the use of columns, eluents and rules of thumb familiar to chromatographers.

In the previous study [12], a sample consisting of 50 mg/ml bovine serum albumin (BSA) was used in the testing and development of eluents. This paper extends those efforts, substituting a more realistic sample matrix for the model serum and focusing on practical issues. Column lifetime was studied both with and without a guard column, and the stability of retention time and peak area were evaluated. The conditions used for the analysis of carbamazepine and its 10,11-epoxide metabolite have permitted hundreds of analyses via direct injection of spiked serum samples.

EXPERIMENTAL

Instrumentation

The modular liquid chromatograph from Waters Chromatography Division of Millipore (Milford, MA, U.S.A.) consisted of a Model 510 solvent delivery module(s), a Model 712 WISP autoinjector with cooling unit, and a Model 441Z absorbance detector or a Model 440 absorbance detector, equipped with an extended-wavelength module. A detection wavelength of 214 nm was used for all of the work reported here. Instrument control was provided by a Model 840 chromatography control station, analog data being monitored with a Model SE120 dual-pen recorder. Digital data were auto-archived to a Model 860 networking chromatography station for processing and storage. An eluent stabilization system (Waters) was pressurized with helium and used in the blanket mode to provide a steady supply of eluent to the solvent delivery modules. A flow-rate of 2.0 ml/min was used, unless otherwise noted.

For the column switching methods, a column switching valve (Waters) was controlled by the 840 chromatography control station. Connections to the six valve ports (see Fig. 1) were: (1) guard column inlet, (2) injector outlet, (3) waste, (4) guard column outlet, (5) analysis pump outlet and (6) analysis column inlet. The column switching method required two pumps, a loading pump for the aqueous 5 m*M* sodium dodecyl sulfate (SDS) loading eluent and an analysis pump for the methanol–water –SDS analysis eluent. The injector was positioned between the loading pump and port 2 of the column switching valve.

Columns, eluents and reagents

A fresh μBondapak Phenyl steel column (15 × 0.39 cm I.D.) (Waters) was used for each of the lifetime studies or examples discussed. When used, the guard column was either a μBondapak Phenyl Guard Pak cartridge, housed in a Guard Pak holder (Waters), or a Guard Column kit, dry-packed with Corasil Phenyl bulk packing (Waters). The particle size of the pellicular Corasil Phenyl packing is 37–53 μm. An

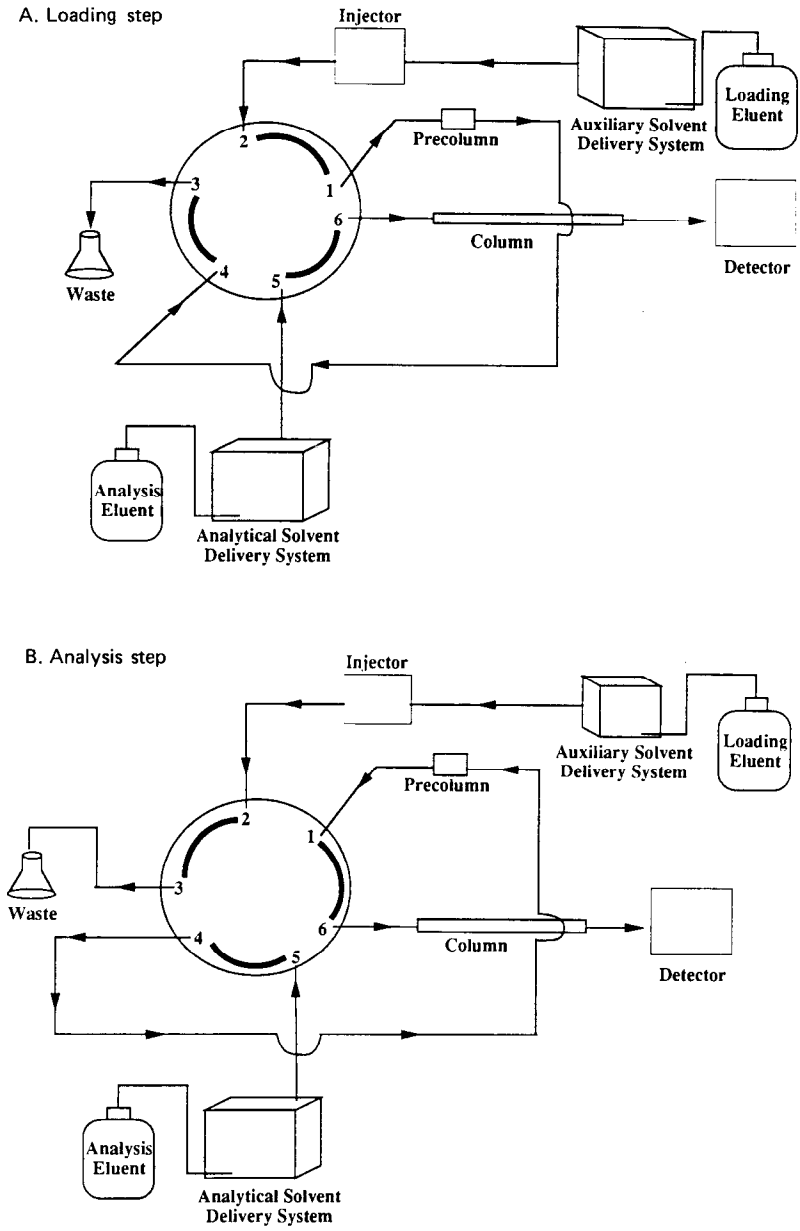

Fig. 1. Valve port and system diagram used with the column switching option.

in-line precolumn filter kit, containing a 2-μm filter assembly, was used throughout this work, with the filter assembly changed regularly as noted in the text. For the separation in Fig. 2, a Pinkerton ISRP GFF column (15 × 0.46 cm I.D.) was obtained from Regis (Morton Grove, IL, U.S.A.) and used according to the manufacturer's recommendations.

Purified, filtered water was obtained with a Milli-Q water purification system (Millipore Corporation, Bedford, MA, U.S.A.). HPLC-grade methanol was purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.), and SDS was obtained in puriss. quality from Fluka (Ronkonkoma, NY, U.S.A.). The SDS-containing eluents were prepared by dissolving the required quantity of SDS in a water–methanol blend of the desired composition. When foaming was not severe, eluents were degassed by filtration under vacuum through a 0.45-μm cellulose acetate filter, housed in a solvent clarification kit (Waters) or equivalent. Otherwise, ultrasonication under vacuum was used for degassing.

The sample matrix was adult bovine serum (triple filtered through a 0.1-μm filter by the manufacturer) from HyClone Labs. (Logan, UT, U.S.A.). When received, the adult bovine serum was divided into 10-ml aliquots and stored frozen until needed. Spiked bovine serum samples were prepared by adding small volumes of 1 mg/ml methanolic solutions of carbamazepine (Sigma, St. Louis, MO, U.S.A.) and carbamazepine-10,11-epoxide (Alltech-Applied Science, State College, PA, U.S.A.) to aliquots of adult bovine serum. Unless otherwise specified in the text, all spiked serum samples contained 2.0 μg carbamazepine-10,11-epoxide (CBE) and 4.8 μg carbamazepine (CBZ) per ml of bovine serum.

RESULTS AND DISCUSSION

CBZ analysis was selected as the example of a drug analysis problem for this work. Serum levels of this anticonvulsant must be monitored regularly in patients taking the drug, due to its narrow therapeutic range (4–10 μg/ml serum) as well as the poor correlation of dosage to serum concentration. *In vivo*, CBZ is metabolized to the pharmacologically active CBE, which has little ultraviolet absorbance at 254 nm. LC is frequently used for CBZ analysis, especially when the epoxide metabolite must be monitored, since currently available immunoassay approaches do not respond selectively to the metabolite [14].

Our previous report [12] suggested that both methanol and surfactant concentration could be varied over a broader-than-expected range for direct serum injection methods with complete elution of the protein. However, the "model serum" used in that work, a 50 mg/ml aqueous solution of BSA, provided a relatively simple matrix. Adult bovine serum was used as a more realistic sample matrix in the studies described here. Additional testing and confirmation with human serum samples [15] indicated that the results obtained for bovine serum will also be obtainable for human serum samples.

The choice of CBZ analysis as an analytical problem imposes a detection challenge not usually discussed in reports on direct serum injection methods, since the analysis of CBE requires UV monitoring at a low wavelength (*e.g.*, 214 nm). Serum proteins respond strongly at this detection wavelength, and excessive tailing of the early-eluted serum peak can potentially interfere with quantitation of the less-retained analytes.

Effect of eluent variables on serum-related peaks

Serum peak tailing is a problem in all direct serum injection methods and is not specific to surfactantcontaining eluents. For example, Fig. 2 shows the separation of

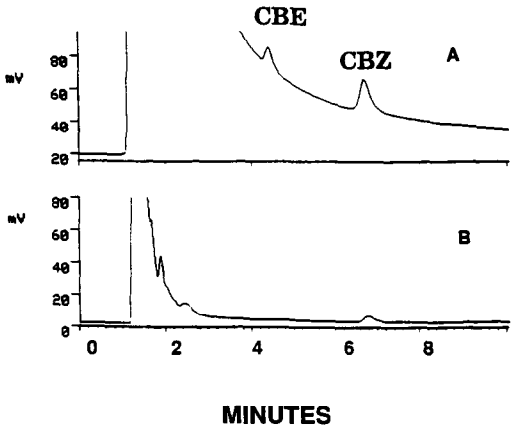

Fig. 2. Effect of detection wavelength on chromatograms of CBE and CBZ in spiked bovine serum with UV detection at (A) 214 nm and (B) 254 nm. Column, Regis 5-μm ISRP GFF (15 × 0.46 cm); eluent, 0.1 *M* KH_2PO_4 (pH 6.8)–acetonitrile (80:20, v/v), flow-rate, 1.0 ml/min. Ordinate scale: 0.08 a.u.f.s.

clinical levels of CBZ and CBE added to bovine serum on a Regis ISRP column with the eluent for carbamazepine analysis suggested by the manufacturer. At 254 nm (Fig. 2B), the serum peak is relatively small and the peak of CBZ is eluted on a flat baseline. However, clinical levels of CBE cannot be detected at 254 nm. Detection at 214 nm (Fig. 2A) reveals a peak for CBE, but the serum peak tail is increased so dramatically that even CBZ occurs as a rider peak. Thus, the control of serum peak tailing becomes a part of the methods development process for carbamazepine analysis because of the requirement for monitoring at low UV wavelengths.

As a transition between our previous work [12] with model serum (50 mg BSA/ml) and the use of bovine serum in the work reported here, we considered the effect of methanol and surfactant concentration on the elution of serum proteins from these matrices. Fig. 3 compares the peak profiles obtained with eluents containing 1 or 50 m*M* sodium dodecylsulfate (SDS) for 10-μl injections of the model serum and bovine serum. Confirming our previous findings, there is little discernible difference between the model serum chromatograms with either an aqueous eluent (Fig. 3A) or in the presence of 30% methanol (Fig. 3B). However, bovine serum presents a substantially more complex peak profile. Tailing is significant at either surfactant concentration, and actually appears much worse in the 100% aqueous eluent (Fig. 3C) than in the methanol-containing eluent (Fig. 3D). In addition, a number of small peaks follow the main serum peak, suggesting the possibility of interference problems with early-eluted sample components.

It was not clear initially whether the small peaks obtained in Fig. 3C and D were due to the elution of distinct proteins present in serum or whether they represent an artifact related to column overload. To better understand this, we collected two fractions of the serum peak tail for subsequent reinjection. (Fraction 1 was the volume collected between 0.6 and 2.0 min and fraction 2 was between 2.0 and 3.1 min.) When portions of the collected fractions were reinjected, sharp peaks were observed at the column void volume. Apparently, the severe tailing and multiple peak production

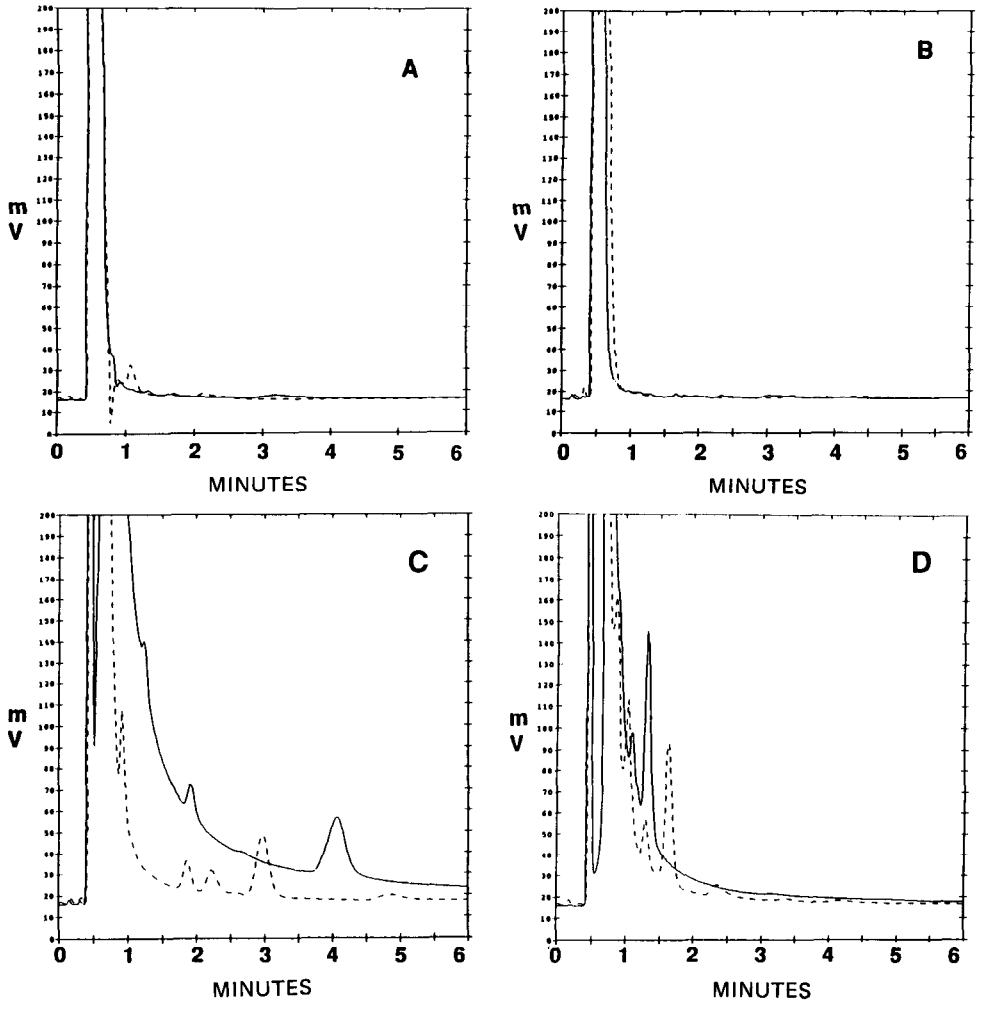

Fig. 3. Effect of methanol and SDS concentration on peaks observed for 10-μl injections of model serum (A, B), and bovine serum (C,D) at two levels of SDS concentration (solid line is 1 m*M* and dashed line is 50 m*M*). Methanol concentration of 0% (v/v) in A and C, or 30% in B and D. Column μBondapak Phenyl (15 × 0.39 cm); flow-rate 2 ml/min; 214 nm. Ordinate scale: 0.20 a.u.f.s.

associated with the injection of bovine serum is the result of overload. Fortunately, most of the eluents used in this study contain enough methanol and SDS to produce the relatively early elution of the serum peak seen in Fig. 3D.

Although pH is not a key variable for controlling the elution of CBZ and CBE, we were interested in the influence of this variable on the serum peak profile. A series of 10 m*M* phosphate buffers at pH 2.8, 5.0 and 7.5 were prepared. Eluents containing 50 m*M* SDS, 30% methanol and 70% of these buffers were compared with an unbuffered but otherwise identical eluent for the elution of 10-μl injections of bovine serum. Fig. 4 summarizes the results of the pH study. The greatest amount of tailing is observed for

the pH 5.0 eluent, possibly reflecting the reduced solubility of serum albumin near its pI value. The pH 2.8 eluent produces the greatest number of small peaks following the main serum peak, with the last of these eluted at *ca.* 7 min. The possibility of interference with analyte peaks is significant for this low pH eluent.

The best results in Fig. 4 are obtained with the pH 7.5 eluent, which produces a serum peak profile nearly identical with that of the unbuffered eluent. While unbuffered eluents are used in all of the work reported here, it appears that a buffered eluent at a neutral or slightly basic pH would also be appropriate. Interestingly, Fig. 4 shows that the solubilization of serum proteins by SDS is not sufficient to completely eliminate the influence of eluent pH on the elution of serum components, at least in eluents containing 30% methanol and 10 m*M* buffer strength. However, the situation is greatly improved compared to the use of eluents containing no surfactant. When ISRP columns are applied to direct serum injection, Pinkerton *et al.* [3] recommend the use of eluents at pH 6.8 as the best compromise in order to avoid problems of protein precipitation and to maintain column life.

While methanol-containing eluents are mentioned throughout this report, we briefly considered the influence of other organic modifiers upon serum-related peaks. As Fig. 5 shows, eluents containing acetonitrile or 2-propanol produce a serum chromatogram similar to that with a methanol-containing eluent when all three are adjusted to give a comparable retention of carbamazepine (7–8 min). Based on our past experience with methanol, we continued working with this modifier. However, recent efforts in our laboratory have produced excellent results for direct serum injection methods with 2-propanol-containing eluents.

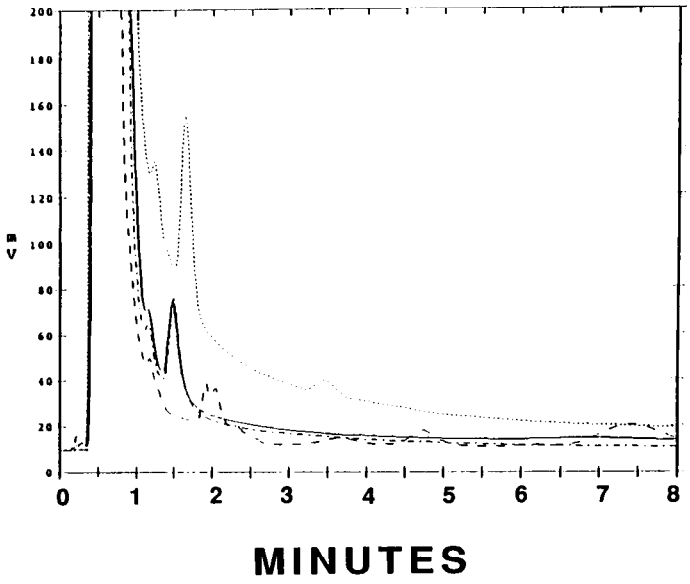

Fig. 4. Effect of pH on the chromatogram observed for 10-μl bovine serum. Eluent, 50 m*M* SDS in methanol–aqueous 10 m*M* sodium phosphate (30:70, v/v) at pH 2.8 (– – –), pH 5.0 (·····) or pH 7.5 (– · – · –). For comparison, the solid line chromatogram is methanol–water (30:70) using no buffer. Other conditions as in Fig 3.

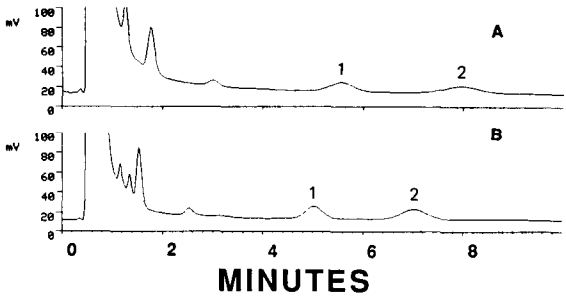

Fig. 5. Effect of organic modifier on the serum chromatogram under conditions which produce a retention time of 7 to 8 min for CBZ. Eluent, 50 m*M* SDS in water and (A) 10% 2-propanol or (B) 15% acetonitrile. Other conditions as in Fig. 3. Injection volume, 10-μl containing 2.0 μg/ml of CBE (peak 1) and 4.8 μg/ml of CBZ (peak 2).

Effect of eluent variables on retention of CBE and CBZ

The selection of a suitable eluent for direct serum injection involves a trade-off between analysis time, column efficiency and serum peak tailing. The most significant variables for retention adjustment are methanol and SDS composition in the present system. An increase in either of these variables will result in earlier elution of CBE and CBZ, as Table I shows. However, increasing the SDS concentration from 10 to 100 m*M* causes at least a two-fold reduction in plate count. An increase in methanol, on the other hand, alters the serum peak profile significantly (Fig. 3). As a compromise, we selected an eluent consisting of methanol–water (30:70) with an overall SDS concentration of 50 m*M*. This eluent provides an analysis time under 10 min without excessive loss of column efficiency, while eluting the majority of the serum-related peaks in less than 4 min.

Column lifetime studies

Having selected a suitable eluent (30% methanol, 50 m*M* SDS), it was possible

TABLE I

EFFECT OF SDS CONCENTRATION ON RETENTION AND EFFICIENCY

t_R = Retention time, min; N = plate number.

Methanol (%, v/v)	Parameter	[SDS] (m*M*)					
		10		50		100	
		CBE	CBZ	CBE	CBZ	CBE	CBZ
15	t_R	39.79	74.59	8.63	11.53	4.70	5.98
	N	1417	2081	838	840	580	580
22	t_R	20.98	40.97	8.06	10.70	4.23	5.31
	N	1333	1989	810	789	721	708
30	t_R	14.55	19.19	4.98	7.15	3.48	4.44
	N	1381	1687	1008	1121	807	635

to consider the effect of a large number of serum samples on chromatographic resolution, system pressure and the stability of retention time, peak height and peak area for CBZ and CBE. A series of three lifetime studies were conducted, with the lessons learned in each applied to improve performance in the next.

Experiments conducted prior to the first lifetime study showed that spiked bovine serum samples could not be left in the autoinjector at room temperature for more than a few hours without the formation of precipitates and consequent pressure problems. This was avoided either by using a refrigerated autoinjector or by keeping serum samples at room temperature for less than 4 h. As an additional precaution, the analytical column was protected by an in-line filter, containing a 2-μm stainless-steel frit. A new filter frit was installed daily (after each 50–100 injections), although a much less frequent change would probably have provided acceptable results.

Using these precautions, a 15-cm μBondapak Phenyl steel column was able to provide stable retention times (relative standard deviation, R.S.D. = 3.0–3.5%) for CBZ and CBE over the course of 738 injections (10-μl) of spiked bovine serum. In the course of these injections, two observed trends were of some concern. First, the serum peak increased in width throughout the study (see Fig. 6). Second, the CBZ and CBE peaks broadened steadily, despite the stability of their retention times. While neither of these phenomena prevented quantitation of the drugs, we were interested in minimizing both types of peak broadening in subsequent lifetime studies.

Use of guard columns

The changes in the size of the serum peak which were observed during the first lifetime study might arise from the accumulation of serum components at the head of the analytical column. While SDS is known to be an effective solubilizing agent for

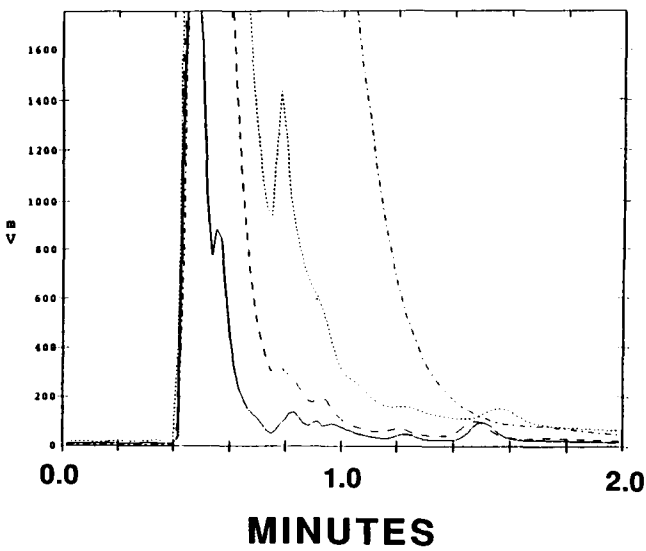

Fig. 6. Change in the serum chromatogram of 10-μl of spiked bovine serum during the first lifetime study (no guard column). Injection number 1 (———), 30 (– – – –), 200 (·····) and 490 (– · – · – ·) are shown. Eluent, 50 m*M* SDS in methanol–water (30:70). Ordinate scale: 1.75 a.u.f.s. Other conditions as in Fig. 3.

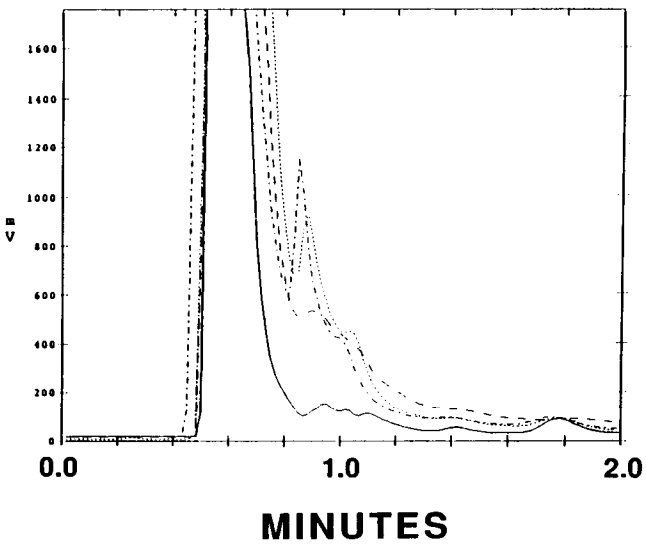

Fig. 7. Change in serum chromatogram of 10 μl of spiked bovine serum during the second lifetime study (with μBondapak Phenyl Guard Pak cartridge). Injections number 1 (———), 30 (– – – –), 200 (·····) and 863 (– · – · – ·) are shown. Conditions as in Fig. 6.

serum albumin, other serum components (*e.g.* lipids or proteins other than serum albumin) might be strongly retained. In other direct serum injection studies, backpressure problems observed with the use of aqueous–organic eluents have been attributed to buildup of serum lipids and related compounds [16], and guard columns have been used successfully to improve performance. Thus, Pinkerton *et al.* [3] were able to substantially extend the lifetime of the analytical column in their direct serum injection studies when a guard column was included in the system.

The system used in the first lifetime study was modified to include a μBondapak Phenyl Guard Pak cartridge between the in-line filter and analytical column. The serum samples were handled identically, and a second lifetime study was begun with a fresh μBondapak Phenyl analytical column. The Guard Pak cartridge was replaced with a fresh cartridge after each 50–100 injections, although the cartridges could perhaps have been used much longer.

The effect of the Guard Pak cartridge in controlling the width of the serum peak is clear in the chromatograms presented in Fig. 7. Some broadening was observed during the first few injections, but beyond about the 30th injection the serum peak profile changed relatively little until the termination of the study at 880 injections. Retention time precision improved to 2.1–2.4% R.S.D. for the drug peaks, and the width of those peaks was considerably more stable than in the first lifetime study. After about 830 injections, system backpressure problems began to appear, and efforts to prolong the study were ineffective. Changes of the column inlet and outlet frits and reversal of the direction of flow provided only short-term improvements. Nonetheless, we were encouraged by the ability to perform over 800 injections of serum without significant loss in chromatographic performance for CBZ and CBE.

In the first two lifetime studies, CBZ and CBE were present in the spiked bovine

serum at relatively high levels to simplify detection and to maintain a focus on column longevity. Building upon the results of these two studies, a lifetime study was then conducted using bovine serum spiked with clinical levels of CBZ and CBE. All other conditions were as in the second study. As was noted above, the use of 214 nm as a detection wavelength is required for the quantitation of CBE but leads to a chromatogram which is dominated by the serum peak tail. Nonetheless, peak areas can be easily obtained with the integration software, which permits tangent skimming on a sloped linear baseline. We were able to perform 900 injections (10-μl) of spiked bovine serum before it was necessary to terminate the study due to backpressure problems and excessive analyte peak broadening.

For the retention time, relative standard deviations of 2.0% (CBE) and 2.9% (CBZ) were obtained. This is comparable with the results of the second lifetime study. More importantly, peak area precisions were disappointing at 10.0% (CBE) and 8.0% (CBZ), although there was no overall trend in either the retention or peak area data. While the reason for the relatively high R.S.D. values for peak area is unknown, one possibility is difficulty of reproducible integration due to the large serum peak tail. To allow the analysis of CBZ and CBE peaks on a flat baseline, we investigated column switching strategies which would permit the majority of the serum peak to be sent to waste prior to the analysis.

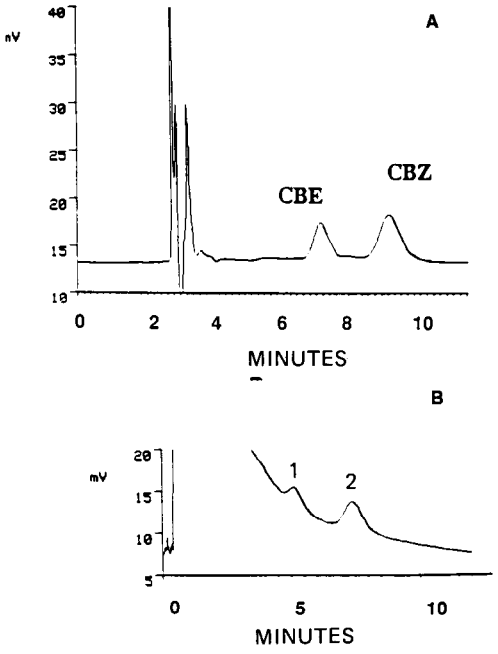

Fig. 8. Comparison of column switching method to non-column switching method. Chromatogram A was of a 10-μl sample of spiked bovine serum obtained with the column switching method. Valve position for the six-port valve was changed at 2.0 min. Loading eluent: 5 m*M* SDS in water. Guard column: μBondapak Phenyl Guard Pak cartridge. Analysis eluent and column as in Fig. 6. Flow-rate: 2 ml/min. Ordinate scale: 0.03 a.u.f.s. Chromatogram B represents injection 379 of the third lifetime study with direct sample injection and no column switching. Ordinate scale: 0.015 a.u.f.s. Other conditions as in Fig. 6. Injection volume, 10 μl containing 2.0 μg/ml of CBE (peak 1) and 4.8 μg/ml of CBZ (peak 2).

Column switching

Because the serum peak is eluted near the column void volume with the SDS-containing eluents discussed here, it is a relatively simple matter to design a sample cleanup strategy which will remove the majority of the serum peak from the chromatogram. The column switching cleanup strategy consists of two steps (see Fig. 1). In the loading step, the sample is injected into a μBondapak Phenyl Guard Pak cartridge, using an aqueous SDS-containing loading eluent. Most of the serum peak is eluted to waste in this step, while the drugs are strongly bound. In the analysis step, the Guard Pak cartridge is backflushed with the analysis eluent (in this case, the same eluent was used in the lifetime studies). The methanol-containing analysis eluent elutes the drugs from the Guard Pak cartridge into the analysis column, leading to the chromatogram shown in Fig. 8A. This is contrasted to the typical chromatogram obtained with direct injection using no column switching shown in Fig. 8B.

As desired, the baseline in Fig. 8A is flat in the region where the drug peaks are eluted, and the serum peak appears to be almost completely removed. Note that the 5 m*M* SDS loading eluent for this analysis contains surfactant at a concentration below the CMC, in keeping with our previous findings that micelles are not a requirement for direct serum injection. Given the absence of serum-related peaks in the analytical chromatogram (Fig. 8A), it may be possible to reduce or even eliminate the SDS component of the analysis eluent as well. This would improve column efficiency, according to Table I. The use of other organic modifiers to modify selectivity should also be possible.

In a preliminary column switching lifetime study with 200 injections, the same chromatographic performance as shown in Fig. 8A was maintained. Work is currently underway in our laboratory to select the best combination of guard column and analysis column, and to determine the column lifetime and stability of retention time and peak area that can be expected for an optimized column-switching strategy.

REFERENCES

1 D. Westerlund, *Chromatographia*, 24 (1987) 155.
2 Z. K. Shihabi, *J. Liq. Chromatogr.*, 11 (1988) 1579.
3 T. C. Pinkerton, T. D. Miller, S. E. Cook, J. A. Perry, J. D. Rateike and T. J. Szczerba, *Biochromatogr.*, 1 (1986) 96.
4 D. J. Gisch, B. T. Hunter and B. Feibush, *J. Chromatogr.*, 433 (1988) 264.
5 C. Desilets and F. E. Regnier, presented at the *12th International Symposium on Column Liquid Chromatography, Washington, DC, June 19–24, 1988*, poster WP 326.
6 H. Yoshida, K. Takano, I. Morita, T. Masujima and H. Imai, *Jpn. J. Clin. Chem.*, 12 (1983) 312.
7 J. A. Adamovics, *J. Pharm. Biomed. Anal.*, 5 (1987) 267.
8 J. Haginaka, N. Yasuda, J. Wakai, H. Matsunaga, H. Yasuda and Y. Kimura, *Anal. Chem.*, 61 (1989) .2445.
9 L. J. Cline Love, S. Zibas, J. Noroski and M. Arunyanart, *J. Pharm. Biomed. Anal.*, 3 (1985) 511.
10 L. J. Cline Love and M. Arunyanart, *J. Chromatogr.*, 342 (1985) 293.
11 K. B. Sentell, J. F. Clos and J. G. Dorsey, *Biochromatogr.*, 4 (1989) 35.
12 R. A. Grohs, F. V. Warren, Jr. and B. A. Bidlingmeyer, *Anal. Chem.*, in press.
13 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
14 R. Hartley, M. Lucock, W. I. Forsythe and R. W. Smithells, *J. Liq. Chromatogr.*, 10 (1987) 2393.
15 L. Bowers, University of Minnesota at Minneapolis, personal communication.
16 T. Arvidsson, K. G. Wahlund and N. Daoud, *J. Chromatogr.*, 317 (1984) 213.