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Comparison of a new ovomucoid and a second-generation α_1-acid glycoprotein-based chiral column for the direct high-performance liquid chromatography resolution of drug enantiomers

K. M. KIRKLAND*, K. L. NEILSON and D. A. McCOMBS
ICI Pharmaceuticals Group, Concord Pike and Murphy Rd., Wilmington, DE 19897 (USA)
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ABSTRACT

A new commercially-available glycoprotein chiral stationary phase (CSP) based on immobilized ovomucoid protein has been evaluated. This column has been quantitatively compared to the second-generation α_1-acid glycoprotein CSP for the direct resolution of enantiomers of sixteen commercially-available racemic drugs and eight proprietary drug candidates. The experimental protocol utilized simple aqueous–organic mobile phases with optimization schemes varying only in phosphate buffer concentration, pH and organic solvent modifier type and concentration. Cationic and anionic modifiers were not used to achieve separations due to concerns for consistent column performance. Column stability was monitored by comparing separation factors, resolution, and column efficiency following 200 sample injections during method development. The ovomucoid column showed generally higher resolution, greater flexibility in operating parameters, and better long-term stability than the acid glycoprotein column.

INTRODUCTION

At present there are several choices of chiral stationary phase (CSP) high-performance liquid chromatographic (HPLC) columns that can directly separate enantiomers. These phases include the Pirkle type, inclusion complex formation such as with the cyclodextrin and cellulosic materials, ligand exchange and immobilized protein supports. A number of publications have classified the various chromatographic approaches and defined the bases of CSP–solute interactions [1–4]. Increased regulatory pressures on the pharmaceutical/agrichemical industries, coupled with interest in stereoselective bioprocesses [5,6], have resulted in chiral separations of racemic drugs and metabolites being an important issue.

Based on our interest in the chiral recognition of drugs and metabolites in biological systems, we have focused our attention on the protein-based columns, primarily because of the stereoselective network of enzymes, receptors, and cell membranes that exist *in vivo*. Protein-based chiral stationary phases have become popular for the direct separation of drug enantiomers because of their broad applicability, and the use of aqueous buffered mobile phases that are compatible with many biological

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samples [3]. The initial immobilized protein HPLC columns for chiral recognition were based on bovine serum albumin (BSA) and α-acid glycoprotein (orosomucoid), as a result of the pioneering work of Allenmark [7] and Hermansson and Eriksson [8], respectively. Recently, columns based on immobilized human serum albumin [9] and the glycoprotein, fungal cellulase [10], have been used to resolve enantiomers.

We have extensively evaluated another column with a different immobilized glycoprotein: ovomucoid. This stationary phase was reported by Miwa and co-workers [11,12] to have excellent chiral recognition properties. The advantages of this ovomucoid protein for chiral HPLC separations include good stability to changes in temperature, pH, organic solvent composition and antitrypsin activity [13]. To prepare a HPLC column, ovomucoid is isolated and purified from chicken eggwhite, then chemically bound to aminopropyl silica [11]. A column with this ovomucoid protein (OVM) has been recently commercialized and preliminary studies reported [14–16].

Distinguishing features of the various chiral columns with immobilized protein stationary phases are shown in Table I. The second generation α_1-acid glycoprotein (AGP) column primarily has been used to resolve basic or cationic enantiomers [17,18], however, applications for some acidic drugs also have been reported [8]. Most compounds resolved on the BSA column have been acids[17,18]. The major differences between the ovomucoid and orosomucoid proteins, aside from molecular weight, is in their isoelectric points. This is a direct consequence of the higher sialic acid residue content of orosomucoid. In addition, the larger number of disulfide bridges imparts a more rigid structure to the ovomucoid, which may account for the exceptional stability of the ovomucoid protein [13]. Table I shows that the physical characteristics of ovomucoid are intermediate between orosomucoid and BSA. This suggests that the ovomucoid protein might permit the separation of both acidic and basic enantiomers.

EXPERIMENTAL

Apparatus

Ovomucoid-based analytical and guard HPLC columns (Ultron ES-OVM, 15

TABLE I

CHARACTERISTICS OF SOME PROTEINS USED AS STATIONARY PHASES FOR CHIRAL HPLC COLUMNS

Property	Orosomucoid [19]	Ovomucoid [13]	Bovine serum albumin [7]
Molecular weight	41 000	28 800	66 000
Isoelectric point	2.7	3.9–4.5	4.7
Sialic acid residues	14	0.3	
Disulfide bridges	2	8	17
% Carbohydrate	45	30	

× 0.46 cm I.D., and 1.0 × 0.4 cm I.D., respectively) and the achiral column (Zorbax Rx-C_8, 25 × 0.46 cm I.D.) were obtained from Mac-Mod Analytical (Chadds Ford, PA, USA). Chiral AGP columns (10 × 0.4 cm I.D.) were acquired from ChromTech AB (Uppsala, Sweden). The HPLC system consisted of two pumps (Model 400, ABI Analytical, Ramsey, NJ, USA), a Model 7125 loop injector (Rheodyne, Cotati, CA, USA), and a variable-wavelength UV detector (ABI, Model 783G). The chromatographic data was acquired and analyzed using the Multichrom data system (Version 1.8, VG Laboratory Systems, Manchester, UK). The detector output was also monitored using a strip-chart recorder (Model BD-41, Kipp and Zonen, Delft, the Netherlands).

Chemicals

The seven proprietary racemic drug candidates (ICI-1–7), a proprietary mixture of diastereomers (ICI-8), lorglumide, and Trögers base all were synthesized by ICI Pharmaceuticals (Wilmington, DE, USA). Halofantrine hydrochloride was kindly provided by Dr. I. W. Wainer of McGill University (Montreal, Canada). The remaining compounds were obtained from either Research Biochemicals (Natick, MA, USA) or Sigma (St. Louis, MO, USA): 8-OH-DPAT, SKF-38393, SCH-83566, (±)-vesamicol, SCH-23390, SCH-23388, normethyl SCH-23390, normethyl SCH-23388, 2,3-dichloro-α-methylbenzylamine, verapamil, warfarin, lorazepam, atenolol, propranolol and pindolol. Structures for the commercially-available compounds are given in Fig. 1. Reagent-grade potassium dihydrogen phosphate, potassium hydroxide, HPLC-grade water, methanol and acetonitrile were obtained from J. T. Baker (Phillipsburg, NJ, USA). Absolute ethanol was from Quantum Chemicals (Cincinnati, OH, USA).

Chromatographic conditions

Phosphate buffers of pH 5–7 were 10 m*M*, or as described in the text or in figure captions. Organic modifiers were acetonitrile and ethanol of various concentrations. All separations were performed at ambient temperature (20–25°C). Mobile phase flow-rates were 1.0 ml/min, or as shown. Separation times were within 15 min with capacity factors: $k'_{OVM} < 10$, $k'_{AGP} < 15$.

Buffers were prepared from 1.25, 2.5, 5.0, 10, 20 or 25 n*M* solutions adjusted to the desired pH with 1.0 *M* potassium hydroxide. Stock solutions of 1 mg/ml in methanol were prepared for all test compounds. SCH-23390 and SCH-23388 and the normethyl derivatives of these compounds were obtained as single enantiomers. Chiral separations were performed using solutions of the racemic drugs prepared by mixing equal volumes of each enantiomer.

Chromatography procedures

Separations of sixteen commercially-available racemates and eight ICI proprietary racemic compounds were attempted on both the AGP and OVM protein columns. The commercial test compounds include β-blockers, dopamine agonists and antagonists, and various other pharmacological agents. Both sets of the commercial and ICI compounds contained basic or acidic (carboxylic) functionalities.

Our experiments used only the OVM and the AGP columns for comparison, because previous studies suggested that these two columns might have the desired

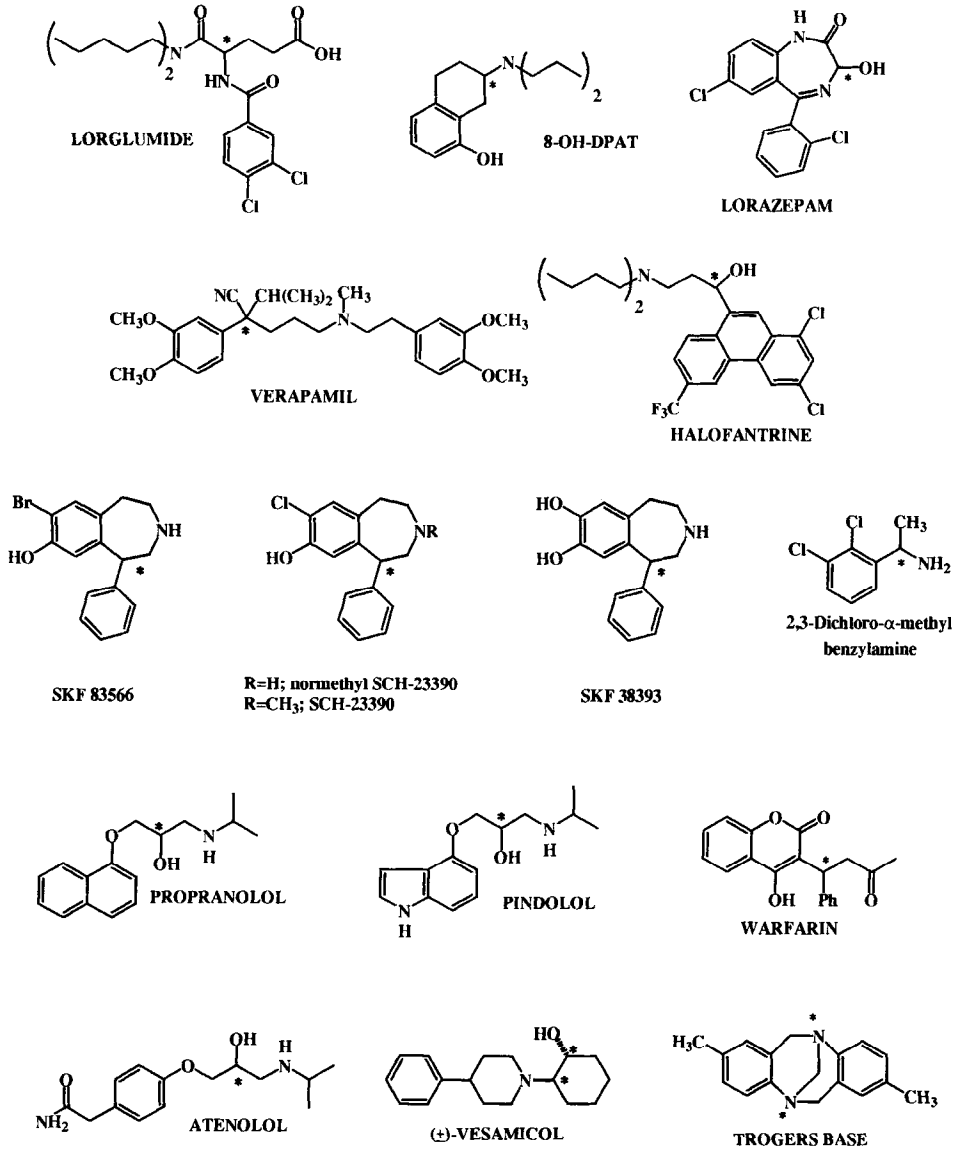

Fig. 1. Structures of commercially-available racemic compounds studied.

range of applicability [8,11,19]. HPLC separations were performed at ambient temperature using a mobile phase flow-rate of 1.0 ml/min. The UV detector was adjusted to the maximum-absorption wavelength for each drug. Injection volumes were 20 μl, corresponding to 0.5–1 μg of racemate. Separations were achieved with simple mobile phases consisting of only phosphate buffers and organic solvents. Dimethyloctylamine has been reported [19] to be a useful charged additive for separations with the

AGP column. However, in our work with the OVM column, this modifier was not required.

Buffers used were in the pH 5–7 range; initial buffer concentration was 10 m*M*. The effect of buffer concentration on resolution was studied using verapamil, halofantrine and lorglumide over the 1.25–25 m*M* range. (The chromatographic behavior of verapamil on the OVM column also has previously been reported by Miwa *et al.* [12].) Similarly, the effect of pH on resolution and selectivity was investigated for the same three compounds. The influence of organic modifier type and concentration was determined in detail only for verapamil.

Resolution of enantiomers for the 24 racemic drugs was optimized by varying pH and the organic modifier, usually ethanol or acetonitrile. The primary objective was to obtain baseline separations in less than 15 min. This corresponds to k' values of about 10 and 15 on the OVM and AGP columns, respectively.

Stability of both the OVM and AGP columns was studied by evaluating column performance after initial column installation and after 200 injections made during various method development studies. Separations of the test racemic drug probes, SKF-83566 and ICI-1 were performed at various times during these method development studies.

Column-to-column performance of the OVM column was examined using halofantrine as the probe. Separation of halofantrine enantiomers was established on two independent HPLC systems equipped with different OVM columns using identical mobile phases. One OVM column had a history of 90 injections before this test. The other OVM column had separated 400 method development samples prior to the test injection.

The effect of structure on retention and separation resolution for different nitrogen substitution was studied for both the OVM and AGP column using SKF-38393, the racemate prepared from SCH-23390 and SCH-23388 and its normethyl analogues.

Column loading capacity was determined for the OVM column by injecting increasing amounts of the basic drug, halofantrine, and the acidic compound, lorglumide. A 15% decrease in resolution was the arbitrary criterion used to establish column capacity.

Two applications of the OVM column were explored. First, column suitability for diastereomer separations was examined using the experimental protocol for ICI-8. Second, Sequential coupling of an achiral separation of Zorbax Rx-C_8, followed by a chiral separation on the OVM column, was performed with ICI-2 isolated from human plasma. For the chiral study, standard calibration curves were prepared in 0.5 ml of control human plasma, corresponding to the concentration range of 10–1000 ng/ml of each enantiomer. Plasma samples were adjusted with ammonia to pH 9 and extracted with five equal volumes of ethyl acetate. Following phase separation by centrifugation, the organic layer was removed and evaporated to dryness under nitrogen. The residue was reconstituted in 100 μl of the achiral mobile phase [methanol–water (50:50)] and 90 μl of this solution was injected into the HPLC system. Achiral reversed-phase separations were performed on a Zorbax Rx-C_8 column at 1.3 ml/min with the UV detector set at 270 nm. The HPLC peak at 5.5 min corresponding to ICI-2 elution was collected in polypropylene tubes, taken to dryness under nitrogen, and reconstituted in 50 μl of chiral mobile phase [acetonitrile–0.01 *M* KH_2PO_4

(15:85), pH 6.0]. Approximately 45 μl of this solution was injected on the OVM column. Peak areas for both enantiomers were determined with the VG Multichrom data system.

Chromatographic calculations

Capacity factor (k') values, selectivity factors (α), plate numbers (N), plate heights (H) and resolution values (R_S) were measured using the relationships given in ref. 20.

RESULTS AND DISCUSSION

Table II is a summary of data obtained on the 23 test compounds with the OVM and AGP columns. Optimum α values determined for 23 compounds are depicted for the OVM and AGP columns in Fig. 2 (ICI-8 was not included since it was a diastereomer). To determine the conditions for the best separations for all of these compounds, optimum pH and organic modifier type and composition were deter-

TABLE II

SUMMARY OF CHIRAL WORK ON AGP AND OVM COLUMNS

Compound	k'^a		N^a		HETP ($\cdot 10^{-3}$)^a		α		Resolution	
	AGP	OVM	AGP	OVM	AGP	OVM	AGP	OVM	AGP	OVM
8-OH-DPAT	6.3	0.9	1813	4169	5.5	3.6	1.2	1.4	1.7	2.8
SKF-38393	–	1.6	–	3191	–	4.7	1.0	1.3	–	1.9
SKF-83566	9.3	3.5	1484	2270	6.7	6.6	1.2	1.3	1.4	2.1
Vesamicol–HCl	10.2	1.9	–	2548	–	5.9	1.1	1.3	0.8	1.8
Methyl-SCH	8.7	2.6	1956	2242	5.4	6.7	1.2	1.2	1.6	1.3
Normethyl-SCH	–	1.6	–	2458	–	6.1	1.0	1.3	–	1.8
2,3-Dichloro-α-methylbenzylamide	6.8	2.7	2067	2720	4.8	5.5	1.3	1.2	2.4	1.2
Verapamil–HCl	6.8	4.0	1216	1625	8.2	9.3	1.3	1.4	1.7	2.5
Trögers Base	6.6	3.9	361	1648	27.7	9.1	1.6	1.2	2.3	1.2
Warfarin	4.6	2.2	–	1338	–	11.2	1.3	1.3	0.6	1.7
Lorazepam	5.9	2.7	114	859	87.5	17.5	1.4	1.3	1.0	1.8
Atenolol	6.9	–	550	–	18.2	–	1.2	1.0	1.2	–
Propranolol	29.2	3.9	1639	–	6.1	–	1.1	1.1	1.1	0.8
Pindolol	6.1	2.5	3005	992	3.3	15.1	1.2	1.6	1.9	2.3
Halofantrine	–	0.8	–	913	–	16.4	1.0	2.6	–	4.2
Lorglumide	–	1.5	–	1510	–	9.9	1.0	2.6	–	5.2
ICI-1	5.9	8.7	1734	2221	5.8	6.8	1.4	1.2	2.8	1.6
ICI-2	3.5	0.8	333	4928	30.0	3.0	1.6	1.4	1.7	2.6
ICI-3	7.6	3.7	369	2935	27.1	5.1	1.1	1.4	1.0	3.3
ICI-4	10.6	1.2	–	2325	–	6.5	1.1	1.2	0.7	1.3
ICI-5	4.6	1.7	152	2202	65.8	6.8	1.4	3.3	1.3	7.3
ICI-6	–	1.2	–	1415	–	10.6	1.0	1.3	–	1.8
ICI-7	–	4.2	–	1727	–	8.7	1.0	1.4	–	3.4

[a] Calculations for first-eluting enantiomer.

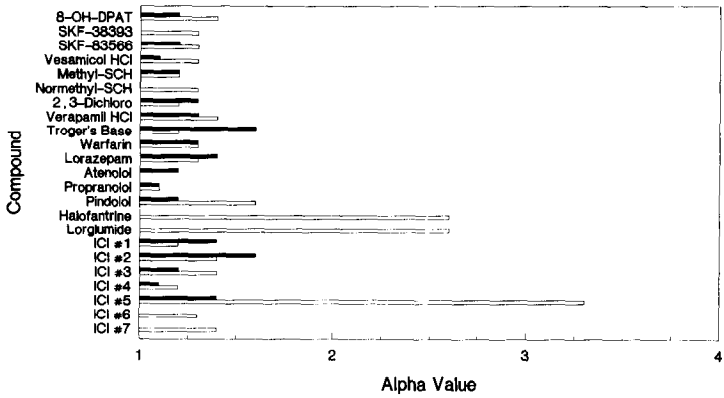

Fig. 2. Separation selectivity factors (α values) for the chiral separation of racemates. Columns: ■ = Chiral AGP, 10 × 0.40 cm I.D.; □ = Ultron ES-OVM, 15 × 0.46 cm I.D.; mobile phase: optimized as discussed in text; flow-rate: 1.0 ml/min; temperature: ambient; sample: 20 μl of 0.025 mg/ml; UV detector: 270 nm.

mined by experimentation. No separation of enantiomers was obtained for four of the commercial and two of the proprietary compounds on the AGP column. Atenolol was the only compound not separated on the OVM column. Note the very large α values (*ca.* 3) for halofantrine, lorglumide and drug ICI-5 on the OVM column. α Values were similar for the remaining compounds on both columns.

Resolution of most enantiomers was higher on the OVM column, as shown in Fig. 3. Atenolol and propranolol were better resolved on the AGP column. However, a very long retention time ($k' > 20$) was required to produce a resolution of 1.1 with an α value of 1.1 for propranolol. The acidic compounds, lorglumide and ICI-3, are better resolved on the OVM column. Typically, k' values (and retention times) required to produce the resolution values shown in Fig. 3 were 3 to 8 times larger on the AGP column compared to the OVM column.

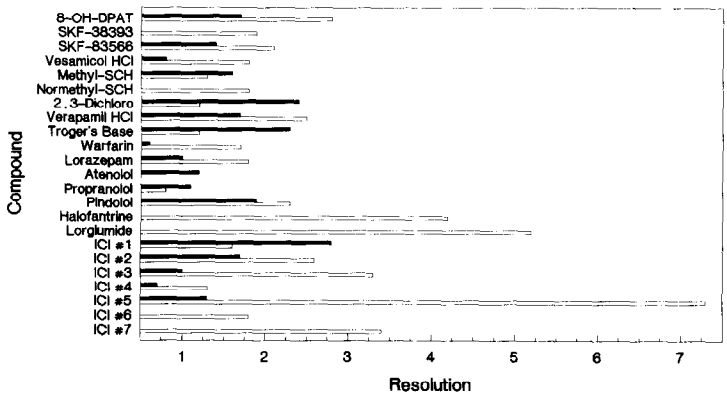

Fig. 3. Resolution values (R_S) for the chiral separation of racemates. Conditions as in Fig. 2.

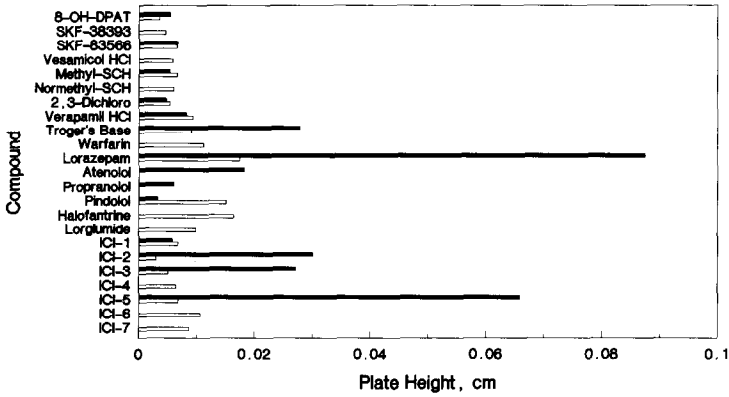

Fig. 4. Plate heights of columns used for the chiral separation of racemates. Conditions as in Fig. 2.

The greater resolution observed for the OVM column relative to the AGP column is a direct result of better column efficiency, as expressed by the plate height values summarized in Fig. 4. Plate heights could not be calculated for nine of the racemates on the AGP column and two racemates on the OVM column, because of poor resolution of the enantiomers.

The direct enantiomeric separation of halofantrine, verapamil and lorglumide on the OVM column was studied as a function of pH and buffer concentration. The effect of organic modifier concentration on chiral selectivity was investigated for verapamil. These particular compounds were selected as models since halofantrine and verapamil are basic compounds, while lorglumide is a carboxylic acid. We found that the α values for these particular compounds were relatively insensitive to pH changes in the mobile phase buffer, as shown in Fig. 5.

In contrast, as shown in Fig. 6, the enantiomeric resolution of these three

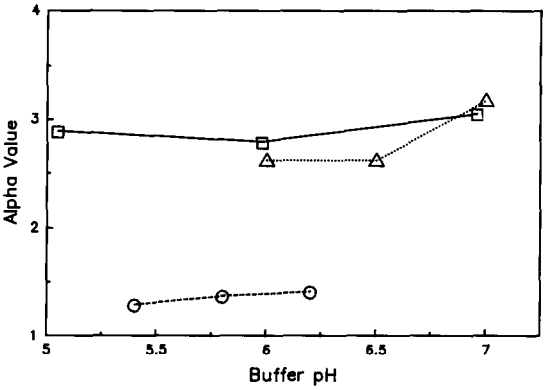

Fig. 5. Effect of buffer pH on enantiomer separation factors for OVM column. Column: Ultron ES-OVM. 15 × 0.46 cm I.D.; mobile phase: organic modifier as follows with 0.01 *M* phosphate buffer; compounds: ○ = verapamil, 17.5% ethanol; □ = halofantrine, 45% acetonitrile; △ = lorglumide, 26% acetonitrile; rest of conditions as in Fig. 2.

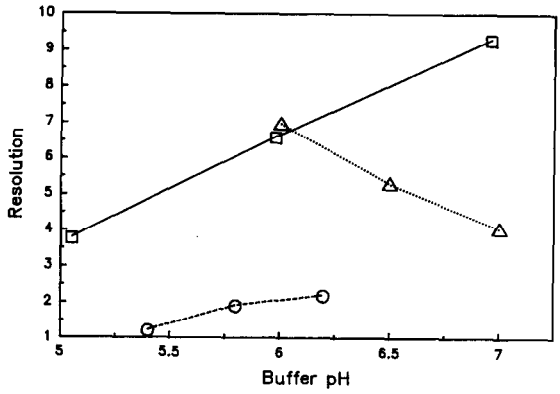

Fig. 6. Effect of buffer pH on enantiomer resolution values for OVM Column. Conditions and symbols as in Fig. 5.

compounds was significantly affected by buffer pH. This trend is the same as reported by Hermansson [19] for acidic and basic solutes on the AGP column. The resolution for the basic drugs increased with increasing pH. The acidic drug, lorglumide, showed increasing resolution with decreasing pH. These effects may be attributed to changes in the surface charge of the immobilized protein which affects coulombic interactions with cationic of anionic drugs. Since the α values for these enantiomers are not significantly altered by mobile phase pH, the change in resolution is a function of column efficiency. The large variation in column efficiency may result from mass transfer effects occurring from the pH alteration of the charge density on the protein substrate. Dependence of resolution on mobile phase pH may be related to the pK_a of the drug.

The effect of mobile phase velocity on the plate heights of the three test drugs is shown in Fig. 7. These data were determined using conditions that produced opti-

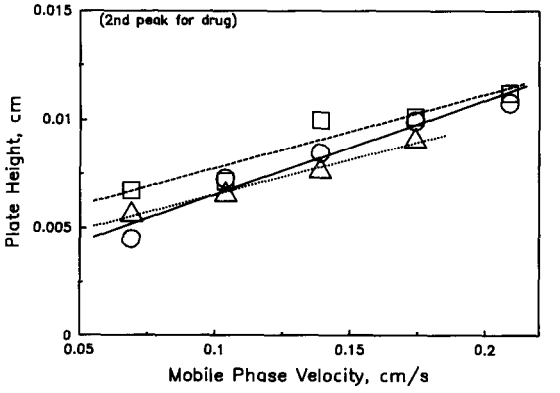

Fig. 7. Mobile phase velocity *vs.* plate heights for drug enantiomers on OVM column. Compounds and mobile phases (in 13 m*M* phosphate buffer): ○ = halofantrine, 45% acetonitrile, pH 6; □ = lorglumide, 26% acetonitrile, pH 6.3; △ = verapamil, 18% ethanol, pH 6.2. Conditions as in Fig. 5.

mum resolution of the enantiomeric pairs. For these systems, plate heights were similar for the three test compounds. The slightly lower plate heights for halofantrine at lower mobile phase velocities may be due to the lower viscosity associated with the mobile phase containing a high concentration of acetonitrile.

The influence of organic mobile phase modifiers was investigated with verapamil as an arbitrary test compound. Similar results were obtained on other drug compounds. Large decreases in α (and k') values were observed with increasing amounts of acetonitrile, ethanol, and methanol, as also reported by Miwa *et al.* [11,12]. The separation of verapamil enantiomers was not improved by using methanol as the organic modifier.

The α values for the three drugs, halofantrine, verapamil and lorglumide, were unaffected by buffer concentration in the range of 1.25 to 25 m*M*. Fig. 8 shows that resolution also is essentially independent of buffer concentration within the range studied. The exception was halofantrine which showed a slight decrease in resolution at the highest buffer concentration tested. The optimized separations that were achieved using the OVM column for lorglumide, halofantrine and verapamil are shown in Fig. 9. Most noteworthy is the mobile phase concentration of acetonitrile (45%) that was found best for the OVM column in separating the enantiomers of halofantrine. No significant changes in retention and resolution were seen for the OVM column during the method development period (*ca.* one week) with this 45%-acetonitrile mobile phase. This observation compares with the 15–20% limitation in organic solvent composition experienced in this laboratory and by others [21] for the second-generation AGP column.

The ability of a chiral column to resolve particular enantiomers is important. However, equally important is the question of column stability or robustness. The stability of both the OVM and AGP columns was studied using SKF-83566 and ICI-1 as probes. Approximately 200 injections were made during similar method development schemes, and the separations found for the probes was examined during the stability test. During these tests, the initial AGP column had to be replaced after demonstrating significant loss in resolution after only 80 injections. The results ob-

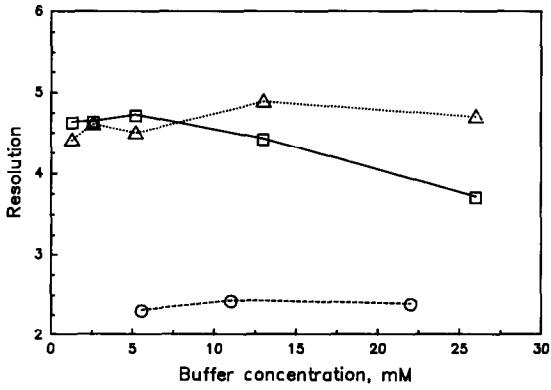

Fig. 8. Effect of buffer concentration on the resolution of enantiomers with OVM column. Compounds and organic modifier: ○ = verapamil, 17.5% ethanol; pH 6.2; □ = halofantrine, 45% acetonitrile, pH 5.5; △ = lorglumide, 26% acetonitrile, pH 6.5. Conditions as in Fig. 5.

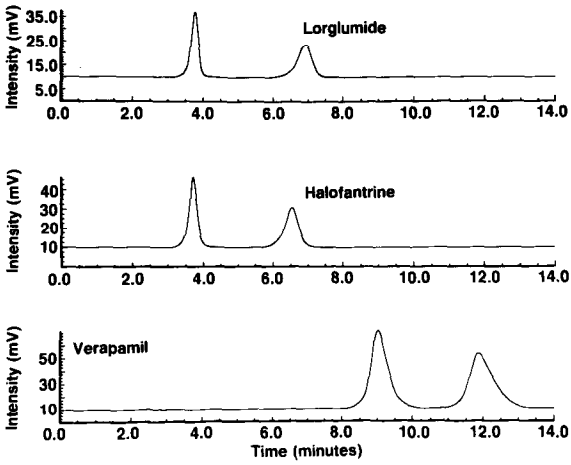

Fig. 9. Optimized separation of enantiomers with OVM column. Conditions as in Fig. 5, except mobile phases: lorglumide, 26% acetonitrile–74% 0.01 *M* phosphate buffer, pH 6.5; halofantrine, 45% acetonitrile–55% 0.01 *M* phosphate, pH 5.5; verapamil, 18% ethanol–82% 0.01 *M* phosphate buffer, pH 6.2.

tained for SKF-83566 on a fresh OVM column and the same column after 200 method development samples is shown in Fig. 10. Fig. 11 shows the same probe compound on an AGP column using a similar stability test protocol. In this test the OVM column exhibited significantly greater stability than the AGP column. This result was found to be typical in several tests. For example, similar results were obtained for the proprietary ICI-1 probe compound. Note that the separations in Fig. 10 with the OVM column used a guard column, whereas a guard column was not available for the AGP column. Proper use of the recently commercially-available guard column for the AGP column should increase its lifetime. Some investigators have reported that the AGP column has acceptable stability when the column is dedicated to a specific analysis without the organic mobile phase composition and pH changes that are often required in method development studies [19,21].

The data in Fig. 10 indicate excellent stability for the OVM column when used properly. Preliminary data on column-to-column reproducibility was also obtained, as illustrated in Fig. 12. We found that α values, resolution and column efficiency were unchanged on two different OVM column with up to 400 sample injections. For this test, halofantrine was used as the test compound with 45% acetonitrile in the mobile phase.

It has been reported that steric bulk around the basic nitrogen of some drugs can affect enantioselectivity on the AGP column [19,22]. As shown in Fig. 13, a selective dopamine antagonist containing a methyl substituent is well resolved on both the OVM and AGP columns. Removal of the methyl substituent results in a total loss of resolution of the AGP column, but not on the OVM column, as illustrated in Fig. 14. Similarly, we found that the desmethylated benzazepin, SKF-38393, was baseline resolved on the OVM column in less than 7 min, while only a single tailing peak was observed on an AGP column. It is possible that certain mobile phase additives (*e.g.*, dimethyloctylamine [19]) may improve the AGP column performance for these drugs; however, our experimental protocol did not provide for the use of

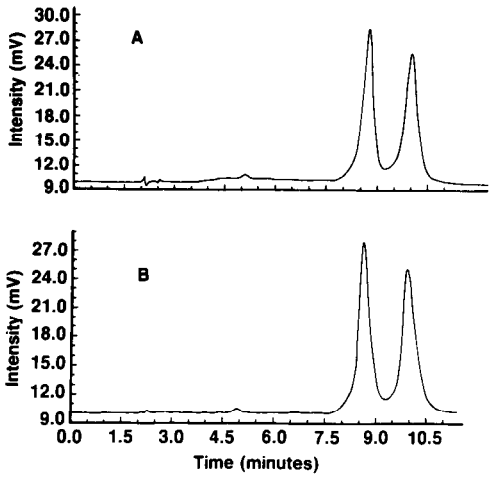

Fig. 10. Stability of OVM column for enantiomeric separation of SKF-83566. (A) Fresh column; (B) same column after 200 sample injections. Conditions: same as for Fig. 5, except mobile phase, 18% acetonitrile–82% 0.01 *M* phosphate buffer, pH 6.0.

such additives. These data suggest that the OVM column may be especially useful for stereoselective metabolism studies involving N-dealkylated products.

The effect of sample loading on the resolution of drug enantiomers of halofantrine and lorglumide for the OVM column is shown in Fig. 15. α Values (solid points) are independent of sample loading for both of these acidic and basic drugs throughout the limited range studied. However, compared to the basic halofantrine, about

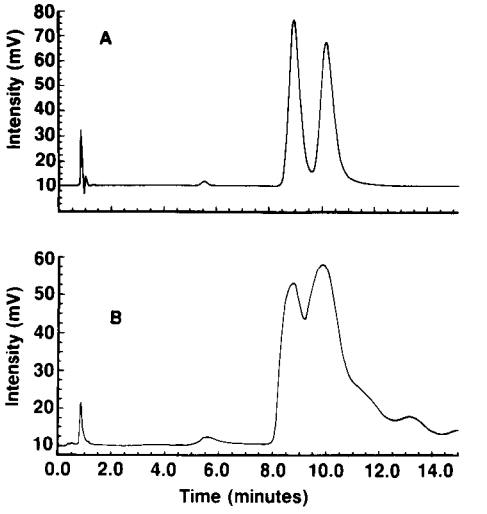

Fig. 11. Stability of AGP column for enantiomeric separation of SKF-83566. (A) Fresh column; (B) same column after 200 sample injections. Column: Chiral AGP, 10 × 0.40 cm I.D.; mobile phase: 12% acetonitrile–88% 0.01 *M* phosphate buffer, pH 6.0; flow-rate: 1.0 ml/min; other conditions as in Fig. 2.

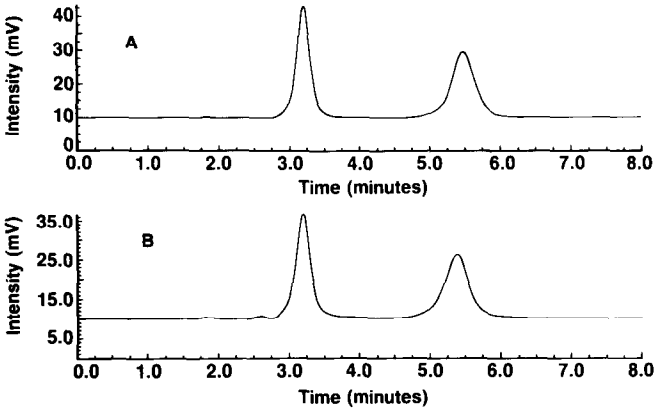

Fig. 12. Column-to-column stability of OVM with high concentration of organic modifier. (A) Column after 90 sample injections; (B) another column of the same type after 400 sample injections. Column: Ultron ES-OVM, 15 × 0.46 cm I.D.; conditions as in Fig. 9 for halofantrine.

twice the amount of the acidic lorglumide can be injected before a 15% decrease in resolution is observed. The low sample loading limit of 1.5–3 nmol/g (about 2.5–5 μg of these two drugs) for a 15% decrease in resolution confirms the relatively poor loading capacity of protein-based CSPs [19].

Preliminary experiments on applying the OVM column to enantiomers and diastereomers in biological systems have been quite encouraging [14–16]. For example, sequential coupling of an achiral reversed-phase separation with a chiral separation using the OVM column was performed to analyze for ICI-2 in human plasma. Fig. 16 indicates the fraction collected during the achiral separation of a plasma extract, and the resulting chiral separation of the enantiomers at a concentration of 10 ng/ml of each enantiomer. With appropriate validation, a method with this sensi-

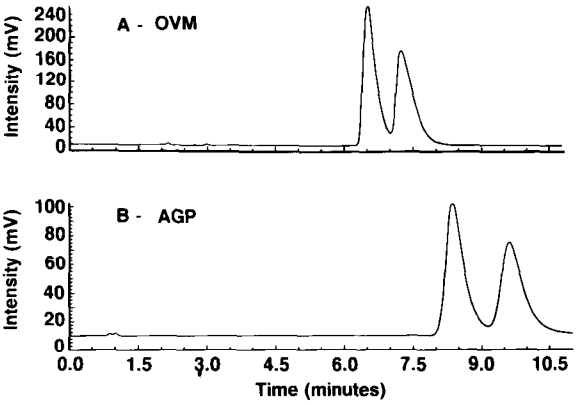

Fig. 13. Enantiomeric separation of SCH-23390 with methylated nitrogen SCH-23390 enantiomers. (A) OVM column; mobile phase, 15% acetonitrile–85% 0.01 *M* phosphate buffer, pH 6.0. (B) AGP column; mobile phase, 10% acetonitrile–90% 0.01 *M* phosphate buffer, pH 6.0. Rest of conditions as in Fig. 2.

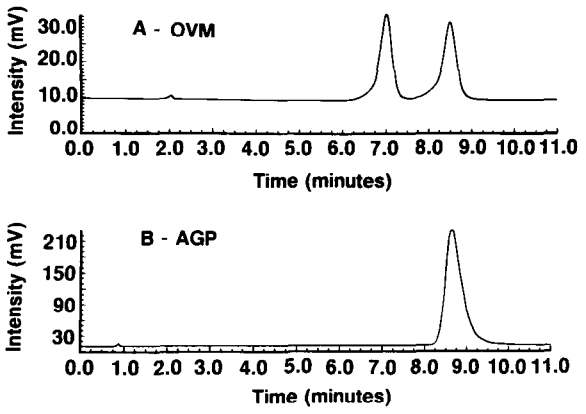

Fig. 14. Enantiomeric separation of SCH-23390 normethyl analogue. Conditions as in Fig. 13, except mobile phase for OVM column was 20% acetonitrile–80% phosphate buffer, pH 6.0.

tivity appears suitable for the pharmacokinetic monitoring of each enantiomer.

The OVM column also has demonstrated utility in resolving four diastereomers from drugs containing two chiral centers, as shown for ICI-8 in Fig. 17A. Fig. 17B shows that two of the diastereomers coeluted on an AGP column.

CONCLUSIONS

The new immobilized-ovomucoid OVM column represents a valuable addition to the family of protein-based columns for enantiomer separations by HPLC. The OVM column exhibits excellent stability during method development where pH and mobile phase composition must be frequently manipulated. Compared to the AGP column, the OVM column has demonstrated superior resolution and efficiency in our laboratory. These advantages permit rapid and simple method development. The

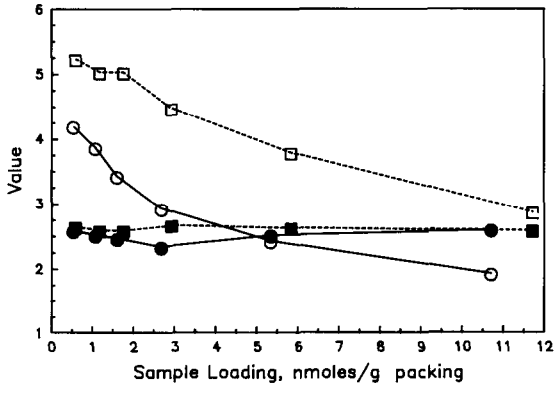

Fig. 15. Effect of sample loading on separation of enantiomers with OVM column. ○ = R_S and ● = α values of halofantrine; □ = R_S and ■ = α values of lorglumide. Conditions same as in Fig. 9.

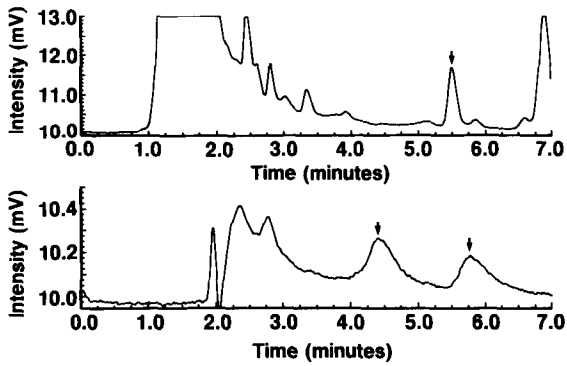

Fig. 16. Achiral/chiral trace analysis in spiked human plasma. Top: isolation of 20 ng of ICI-2 with achiral Zorbax Rx-C_8 column, 25 × 0.46 cm I.D.; mobile phase, 50% acetonitrile–50% water; flow-rate, 1.3 ml/min. Bottom: chiral separation of isolate with OVM column; mobile phase, 15% acetonitrile–85% 0.01 *M* phosphate buffer pH 6.0; flow-rate, 1.0 ml/min; enantiomers shown by arrows.

OVM column appears to be broadly applicable to both acidic and basic drugs without requiring special mobile-phase modifiers. The column is suited for trace analysis, with particular applicability to studies involving stereoselective pharmacokinetics and metabolism. The OVM column also appears useful for monitoring process intermediates during bulk synthesis, and for formulation studies to measure the rate of possible drug racemization.

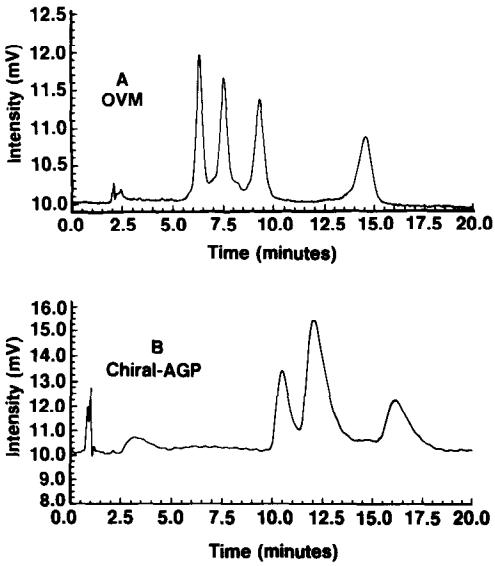

Fig. 17. Enantiomeric separation of diastereomers: ICI-8. (A) OVM column; mobile phase, 22% acetonitrile–78% 0.01 *M* phosphate buffer, pH 6.0. (B) AGP column; mobile phase, 15% acetonitrile–85% 0.01 *M* phosphate buffer, pH 6.5.

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