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# Analytical and preparative resolution of enantiomers of verapamil and norverapamil using a cellulose-based chiral stationary phase in the reversed-phase mode

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ABSTRACT

Analytical HPLC methods were developed for the chiral resolution of verapamil and norverapamil using a cellulose-based chiral stationary phase (Chiralcel OD) in the reversed-phase mode. The effect of pH, buffer concentration, and temperature on the analytical chiral separation were investigated. It was shown that retention decreased slightly with increasing pH, while $\alpha$ and resolution were unaffected by pH. Increasing buffer concentration from 0.01 to 0.125 *M* resulted in increased retention, while concentrations above 0.125 *M* resulted in decreased retention. Separation was unaffected by buffer concentration, while resolution increased with increasing buffer concentration. Maximum analytical resolution was obtained at subambient temperatures. Separation was unaffected by temperature, while an increase in temperature resulted in decreased retention. In addition, the preparative resolution of the enantiomers of verapamil and norverapamil was investigated. The analytical methods were scaled up to preparative loadings and the chromatographic parameters varied to determine their effect on the preparative separations.

## INTRODUCTION

Verapamil (Fig. 1) is a calcium antagonist which is used in the management of hypertension. Norverapamil is the primary metabolite of verapamil. Both verapamil and norverapamil contain a chiral carbon and exist as enantiomers. There are two approaches to obtaining enantiomerically pure chemicals. These are asymmetric synthesis of the desired isomer and resolution of a racemic mixture into individual isomers. Various synthetic methods to produce the individual enantiomers of verapamil have been developed [1]. Methods for the resolution of a racemic mixture include recrystallization of diastereomeric salts. formation of diastereomeric derivatives followed by chromatographic resolution on an achiral stationary phase, and direct chromatographic resolution of enantiomers using a chiral stationary phase or a chiral mobile phase additive. The resolution of verapamil by the re-

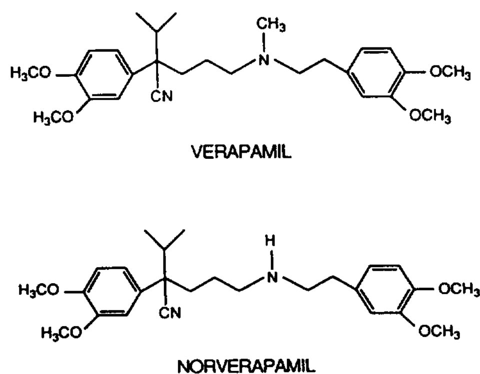


Fig. 1. Structure of verapamil and norverapamil.

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crystallization of diastereomeric salts has been reported [2]. The enantiomers of verapamil have also been separated by liquid chromatography on various chiral stationary phases [3–6]. Ikeda et *al.* have reported on the analytical chiral separation of verapamil using a cellulose-based phase in the reversed-phase mode [6]. Liquid chromatographic resolution of the enantiomers of norverapamil has also been reported [3]. The preparative separation of the enantiomers of verapamil or norverapamil has not been reported.

This paper reports on the use of liquid chromatography for the direct enantiomeric resolution of verapamil and norverapamil at both analytical and preparative loadings using a cellulose-based chiral stationary phase in the reversed-phase mode. The effect of pH, buffer concentration and temperature on the analytical separation of the enantiomers of verapamil and norverapamil will also be discussed.

## EXPERIMENTAL

### *Materials*

The chiral stationary phases used for these studies were obtained from Daicel (Tokyo, Japan) through Regis Chemical (Morton Grove, IL, USA) as prepacked analytical (250 × 4.6 mm I.D.) and preparative columns (500 × 10 mm I.D.). Samples of verapamil were received from Searle Technical Operations. Norverapamil was synthesized in the Chemical Development laboratories of Searle (Skokie, IL, USA). The solvents and other chemicals used were reagent grade or better and were obtained from a variety of sources.

### *Equipment*

The analytical chromatograph consisted of a Spectra-Physics (San Jose, CA, USA) SP8700 pump or a Waters Assoc. (Milford, MA, USA) Model 590 solvent delivery system, a Waters Intelligent Sample Processor Model 712, a Kratos (Ramsey, NJ, USA) Model 757 variable wavelength UV detector, a Kipp and Zonen (Delft, Netherlands) Model BD41 two-channel recorder and a Digital Equipment Corporation (Maynard, MA, USA) VAX 11/785 computer with a Searle chromatography data system. Subambient and elevated temperatures were achieved with a Kariba (Cardiff, South Wales, UK) Research Series advanced air oven.

The preparative chromatograph consisted of two Beckman (Berkeley, CA, USA) Model 101 pumps with preparative heads, a Model 165 variable wavelength detector with a 5 mm semi-preparative flow cell, a Model 450 data system/controller and a Kipp and Zonen Model BD41 two channel recorder. A Rheodyne (Cotati, CA, USA) Model 7125 syringe loading sample injector equipped with a 10 ml loop (Valco, Houston, TX, USA) was used. The column effluent was fractionated using a Gilson (Middleton, WI, USA) Model FC80 or Model FC220 fraction collector.

### *Recovery of chemical from mobile phase*

The purified chemical was recovered from the mobile phase using the following procedure. Fractions containing pure chemical were combined and evaporated under vacuum at 50°C to remove acetonitrile. Sodium chloride (approximately 50 g per 100 ml of mobile phase) was added and the pH adjusted to 11-12 with ammonium hydroxide. The basic aqueous solution was then extracted three times with one equivalent volume of toluene. The toluene phases were combined and the solvent removed using a rotary evaporator. Verapamil and norverapamil were recovered as the free base. The yields for these extractions varied between 60 and 90%.

## RESULTS AND DISCUSSION

### *Analytical HPLC*

When developing an analytical chiral HPLC separation that will be scaled up to preparative loadings, it is desirable to achieve maximum resolution of the two enantiomers. It is also desirable to develop the separation on a stationary phase that is available in larger column sizes or as bulk packing and to use a volatile mobile phase, allowing for easy recovery of the separated enantiomers. The separation of the enantiomers of verapamil has been previously reported using an AGP chiral stationary phase (CSP) [3], a cyclodextrin CSP [4], an ovomucoid

CSP [5] and a cellulose-based CSP in the reversed-phase mode [6]. Due to the low loadability of AGP and cyclodextrin and ovomucoid columns, as well as the unavailability of large columns ( > 10 mm I.D.), they are not desirable for preparative work. The cellulose-based column used nonvolatile mobile phase additives and was not ideally suited for our work. Pirkle CSP- and cellulose-based phases using normal-phase solvents were investigated and did not separate the enantiomers of verapamil or norverapamil. Since separation was not achieved on any of the other columns attempted or with a mobile phase that contained volatile buffers, the cellulose-based CSP in the reversed-phase mode [6] was chosen for our work.

The reported analytical separation of the enantiomers of verapamil used a Chiralcel OD CSP (See Fig. 2) and a mobile phase of acetonitrile–0.05 *M* sodium perchlorate (pH 3.0 with perchloric acid) (33:67, v/v). While this method only reported on the chiral separation of verapamil, it was shown in our laboratories that the method also separated the enantiomers of norverapamil. The analytical HPLC separations for verapamil and norverapamil using these conditions are shown in Fig. 3. Table I summarizes the capacity factors (k'), separation factor ($\alpha$), and resolution ($R_s$) for the enantiomeric separation of these compounds.

Prior to preparative work, further analytical HPLC method development using cellulose-based CSP in the reversed-phase mode was done using verapamil and norverapamil in an attempt to maximize the separation and to determine if buffers were required. The results of this work are summarized in Table II. When a neutral,

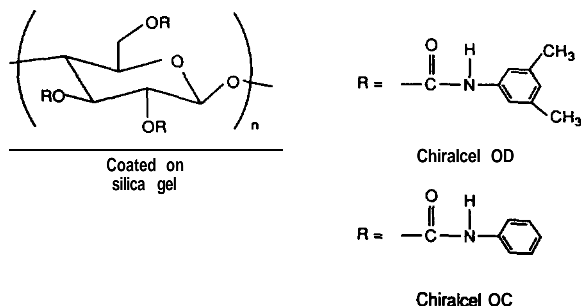


Fig. 2. Structure of Chiralcel OC and OD packings.

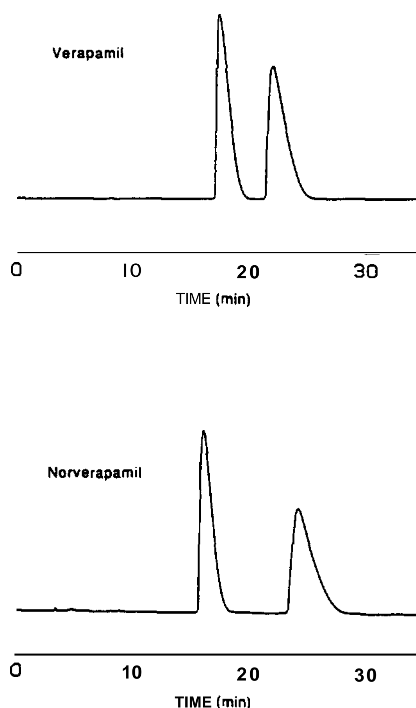


Fig. 3. Analytical HPLC separation of verapamil and norverapamil. Analysis conducted on Chiralcel OD column (250 mm x 4.6 mm I.D.), detection at 205 nm, with a mobile phase of acetonitrile-0.05 M $NaClO_4$ (pH 3.0 with $HClO_4$) (33:67) and a flow rate of 1.0 ml/mm. 15 $\mu$l of a 1 mg/ml solution were injected.

unbuffered mobile phase was attempted, poor peak shape was obtained. Replacing the buffers with volatile acids lead to adequate separations but broad peaks. Further method development using different buffer systems (sodium formate–formic acid, sodium acetate-acetic acid) was attempted. The separations were not as good as

TABLE I

VALUES FOR ANALYTICAL SEPARATION OF ENANTIOMERS OF VERAPAMIL AND NORVERAPAMIL

See Fig. 3 for HPLC conditions.

| Compound | $k'_1$ [a] | $k'_2$ [b] | a | $R_s$ |
|---|---|---|---|---|
| Verapamil | 4.92 | 6.40 | 1.30 | 2.12 |
| Norverapamil | 4.41 | 7.06 | 1.60 | 3.76 |

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.

TABLE II

CHROMATOGRAPHIC RESULTS

HPLC conditions: flow rate, 1.0 ml/min; detection, 205 nm.

| Mobile phase | Column | Verapamil | | | | Norverapamil | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $k'_1$ [a] | $k'_2$ [b] | $\alpha$ | $R_s$ | $k'_1$ [a] | $k'_2$ [b] | a | $R_s$ |
| Acetonitrile-water (4060) | OD | 4.38 | 4.87 | 1.11 | 0.33 | 1.76 | 2.33 | 1.32 | 0.74 |
| Acetonitrile-aqueous acetic acid (pH 3.0) (15:85) | OD | – [d] | – | – | – | 2.91 | 5.55 | 1.90 | 1.79 |
| Acetonitrile-aqueous formic acid (pH 3.0) (15:85) | OD | 5.70 | 9.89 | 1.74 | 1.13 | 8.33 | 14.74 | 1.77 | 2.02 |
| Acetonitrile–0.05 *M* sodium formate (pH 3.6 with formic acid) (20: 80) | OD | 4.81 | 6.50 | 1.35 | 1.30 | 4.18 | 6.86 | 1.64 | 2.57 |
| Acetonitrile–0.05 M sodium acetate (pH 4.5 with acetic acid) (20:80) | OD | 4.41 | 5.96 | 1.35 | 1.40 | 3.94 | 6.57 | 1.67 | 2.60 |
| Methanol-O.05 *M* sodium perchlorate (pH 3.0 with perchloric acid) (50:50) | OD | 8.32 | 12.09 | 1.45 | 0.72 | – [e] | – | – | – |
| Acetonitrile-0.05 *M* sodium perchlorate (pH 3.0 with perchloric acid) (50:50) | *o c* | 5.85 | 6.40 | 1.09 | 0.41 | 4.29 | 4.73 | 1.10 | 0.39 |

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.
[c] Detection at 220 nm.
[d] No analysis of verapamii was attempted with these conditions.
[e] No analysis of norverapamil was attempted with these conditions.

that obtained with sodium perchlorate–perchloric acid buffer. Replacing methanol for acetonitrile resulted in reduced separation and broad peaks. We then attempted the separation using a different cellulose-based phase (Chiralcel OC, Fig. 2). Reduced separation was obtained using the Chiralcel OC column. A separation which did not contain non-volatile buffers could not be developed. Therefore, it was decided that the original HPLC conditions reported by Ikeda et al. [6] would be used for the preparative work.

### *Preparative HPLC*

To determine the feasibility of isolating the individual enantiomers of verapamil. and norverapamil, the analytical methods reported in Fig. 3 were scaled up to preparative loadings. The percent acetonitrile in the preparative mobile phase was increased to 35% to achieve a $k'$ close to that of the analytical column. A 500 x 10 mm I.D. column containing approximately 25 g of stationary phase and a flow rate of 4.2 ml/min was used for preparative method development. The linear velocity for the preparative separation was lower than the analytical separation due to pressure limitations of the preparative column. In order to maximize the throughput of the preparative method, experiments were performed to determine the effect of increasing sample load on the separation. Column loadings of 1, 2 and 4 mg of sample per g of packing were investigated. Preparative loadings greater than 4 mg of sample per g of packing. were not investigated due to the small degree of separation seen analytically and the need to keep the isolated yields as high as possible. Loadings less than 1 mg sample per g of packing were not investigated since they would be inefficient to produce the desired quantities of pure enantiomers. Due to greater solubility in the preparative mobile

phase, the hydrochloride salts of verapamil and norverapamil were used for all preparative work. The presence of non-volatile buffers in the mobile phase required an extraction procedure to isolate the enantiomers. This recovery procedure is detailed in the Experimental section.

From these preparative loading experiments it was determined that the first eluting enantiomer could be isolated pure for both verapamil and norverapamil: The results of these experiments are summarized in Table III. These data show that as loading increases, the amount of enantiomer produced per injection increases even though the percentage of enantiomer isolated decreases.

For the preparative separation of verapamil we found that the second eluting enantiomer could not be isolated pure at any of the column loadings attempted; however, the second eluting enantiomer of norverapamil could be isolated at all column loadings attempted. This was expected because of the increased analytical separation seen for the enantiomers of norverapamil compared to verapamil. This also results in greater isolated yields for the first eluting enantiomer of norverapamil.

### *Effect of pH, buffer concentration and temperature*

After completion of the preparative work, further analytical HPLC method optimization was performed. The use of cellulose-based CSP in the reversed-phase mode had not been used previously in our laboratories. In addition, little had been reported in the literature on this subject. The original reference by Ikeda *et al.* [6] only reported on the separation of verapamil; no investigation of the separation parameters of pH, buffer concentration or temperature was reported. Experiments were therefore designed to determine how varying the mobile phase composition affected the separation of the enantiomers of verapamil and norverapamil. The first area investigated was the effect of pH on the separation. A pH range of 2.5 to 5.7 was explored. A $NaClO_4$ concentration of 0.050 *M* was used for all experiments. Plots of pH vs. $k'$, $\alpha$, and $R_s$ for verapamil and norverapamil are shown in Fig. 4. These results show that pH has little effect on retention and $\alpha$. Increased mobile phase pH resulted in a slight reduction in resolution. Maximum resolution was obtained at pH 3.0. Due to the buffer system being used, the pH of the mobile phase could not be adjusted to the pk of verapamil or norverapamil (*ca.* 8). If a pH closer to 8 could have been obtained it is possible that more drastic effects could have been realized.

The effect of buffer concentration for the chiral separation of verapamil and norverapamil was also investigated. Sodium perchlorate con-

TABLE III

RESULTS OF PREPARATIVE EXPERIMENTS FOR VERAPAMIL AND NORVERAPAMIL

HPLC conditions: Chiralcel OD (500 × 10 mm I.D.) containing approximately 25 g of packing; mobile phase, acetonitrile-0.05 *M* sodium perchlorate (35:65, pH 3.0 with perchloric acid); flow rate, 4.2 ml/min.

| Compound | Loading (mg/g) | First eluting enantiomer | | Second eluting enantiomer | |
|---|---|---|---|---|---|
| | | Percent isolated[a] | mg per injection | Percent isolated[a] | mg per injection |
| Verapamil | 1 | 83 | 10.4 | 0 | – |
| | 2 | 66 | 16.5 | 0 | – |
| | 4 | 26 | 13.0 | 0 | – |
| Norverapamil | 1 | 90 | 11.3 | 66 | 8.3 |
| | 2 | 83 | 20.8 | 38 | 9.5 |
| | 4 | 45 | 22.5 | 16 | 8.0 |

[a] Isolated yields determined by peak area integration of preparative chromatogram.

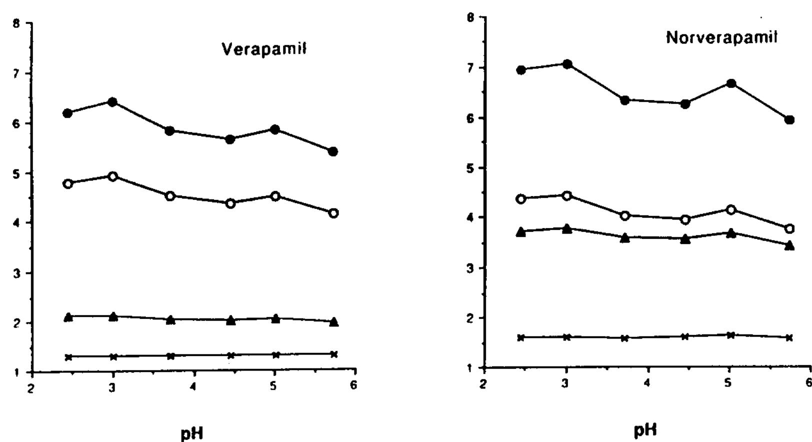


Fig. 4. Effect of pH on analytical HPLC separation of verapamil and norverapamil. See Fig. 3 for chromatographic conditions. ● = $k'$ for second eluting enantiomer, ○ = $k'$ for first eluting enantiomer, ▲ = resolution ($R_s$), × = alpha ($\alpha$).

centrations between 0.01 $M$ and 0.175 $M$ were investigated. A pH of 3.0 was used for all experiments. The results of these studies are shown in Fig. 5. These data show that increasing sodium perchlorate concentration from 0.01 to 0.125 $M$ results in an increase in retention for both verapamil and norverapamil. Retention is relatively unchanged above 0.125 $M$. Separation remained constant at all buffer concentrations studied. Increased buffer concentration resulted in an increase in resolution. Maximum resolution for both verapamil and norverapamil was obtained at 0.125 $M$ sodium perchlorate. Resolution decreased slightly above 0.125 $M$.

The effect of temperature on the chiral separation of verapamil and norverapamil was also studied. Column temperatures between 10 and 55°C were investigated. The results of these experiments are summarized in Fig. 6. As expected, retention decreased with increasing temperature. Good linear correlations were observed between ln $k'$ and $1/T$ for both verapamil and norverapamil, as shown in Fig. 7. Separation and resolution were maximized at subambient

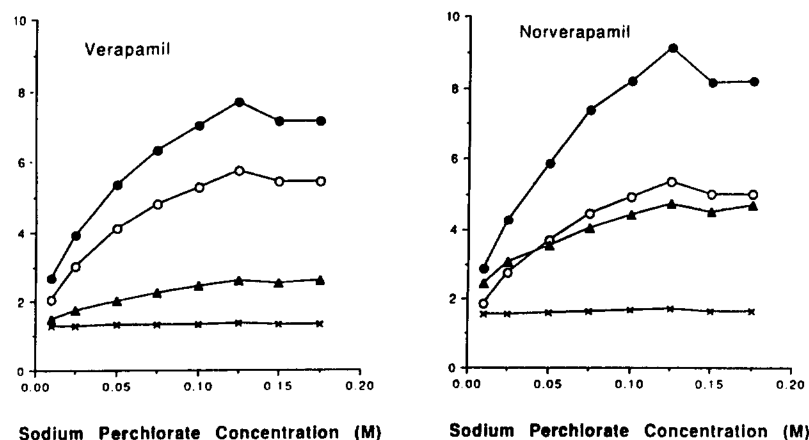


Fig. 5. Effect of buffer concentration on analytical HPLC separation of verapamil and norverapamil. See Fig. 3 for chromatographic conditions. Symbols as in Fig. 4.

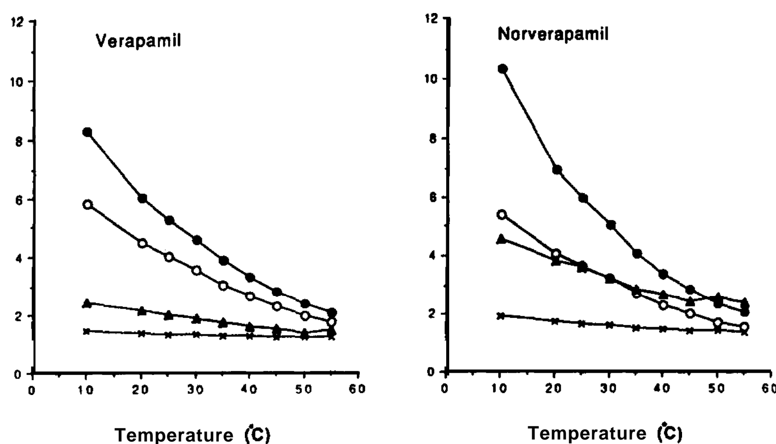


Fig. 6. Effect of temperature on analytical HPLC separation of verapamil and norverapamil. See Fig. 3 for chromatographic conditions. Symbols as in Fig. 4.

temperatures. This is probably due to an increase in the association times for the transient diastereomeric complexes formed between the chiral stationary phase and the solute.

## CONCLUSION

Analytical and preparative HPLC can be used for the direct resolution of the enantiomers of verapamil and norverapamil. For the analytical resolution of verapamil and norverapamil using cellulose-based phases in the reversed-phase mode, pH has little effect on $\alpha, k'$ and resolution. Buffer concentration has little effect on a, while increased buffer concentration increases $k'$ and resolution. Maximum analytical resolution of the enantiomers of verapamil and norverapamil was obtained at subambient temperatures, due to an increase in the association time between the chiral stationary phase and the solute. The resolution obtained at preparative loadings is directly related to the analytical separation.

The Chiralcel OD chiral stationary phase appears to be quite stable under the conditions used for this work. Mobile phase was continu-

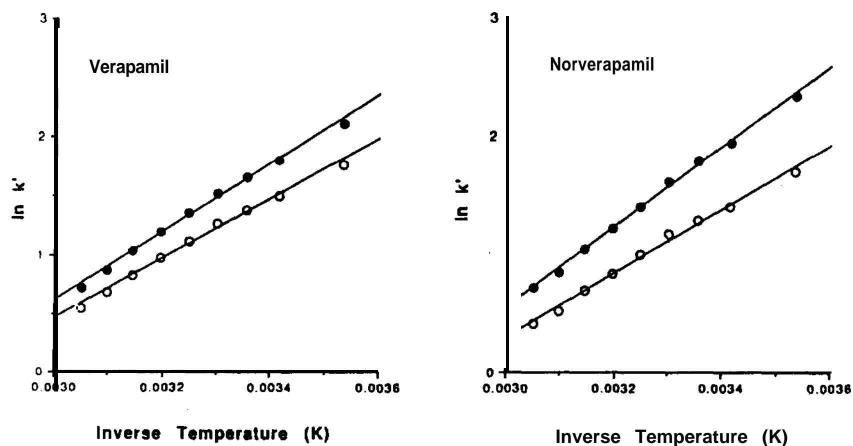


Fig. 7. Plot of ln $k'$ *vs.* inverse temperature (K) for verapamil. Symbols as in Fig. 4.

ously flowing through the analytical column and injections made regularly over a six-month period before a reduction in resolution was observed.

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