Ultron ES-OVM Column: A Bonded Ovomucoid Protein Column for Direct HPLC Separation of Chiral Compounds

Technical Overview

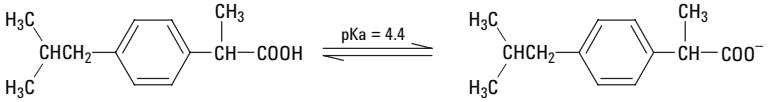
Many proteins demonstrate capability for chiral recognition and are used as the basis for chiral HPLC columns. The protein ovomucoid (molecular wight – 28,000) exhibits especially useful qualities for discriminating between enantiomers. Through the reaction with N, N^1–disuccinimidyl carbonate, this protein has been immobilized on 5-µm aminopropylsilane-derivatized silica particles with 120Å pores to form the packing in Ultron ES-OVM columns. This covalently bonded ovomucoid column is extremely useful for performing a wide variety of chiral separations with acidic, basic, and neutral compounds [1–10].

This technical overview describes some of the chromatographic properties of the Ultron ES-OVM column, and includes practical hints for optimizing the development of chiral separation methods. Included are illustrative applications that show the capability of this HPLC column for separating enantiomers of many different structures.

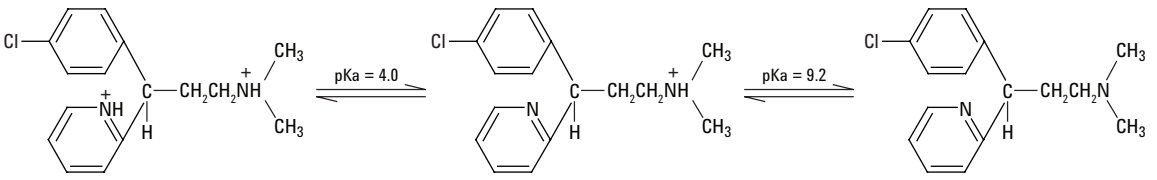
Effect of Mobile-Phase pH on Retention and Enantioselectivity

The isoelectric point, pI, of ovomucoid is 3.8 to 4.3. Therefore, Ultron ES-OVM packing assumes negative or positive charges when the pH of the mobile phase is above or below the pI of ovomucoid, respectively. The practical result is that the pH of the mobile phase used in the separation significantly affects the retention and selectivity of enantiomeric compounds. The effect of mobile-phase pH on the capacity factor (k'_1) of the first enantiomer peak and on enantioselectivity (α) was determined for an acidic compound, ibuprofen (pKa = 4.4); a neutral compound, hexobarbital (neutral in the range pH 3.0 to 6.0); and a basic compound, chlorpheniramine (pKa = 4.0, 9.2). Structures of these compounds are shown in Figure 1.

Ibuprofen



Chlorpheniramine



Hexobarbital

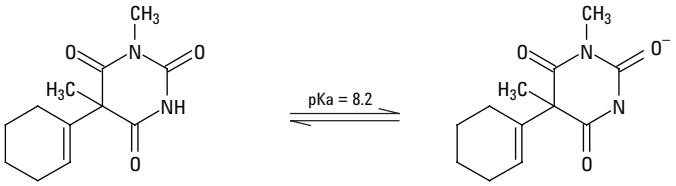


Figure 1. Acid-base equilibria of ibuprofen, chlorpheniramine, and hexobarbital.

For a mobile phase of 20 mM phosphate buffer/ethanol (100/5), the k'_1 of the first enantiomer peak and the separation factor ($\alpha = k'_2/k'_1$) of each test racemic compound for pH 3.0 to 6.0 are shown in Figures 2 and 3, respectively. Ovomucoid has a positive charge at pH 3.0, is neutral at pH 4.0 to 4.6, and is negatively charged at pH 5.5. Figure 2 shows that retention for uncharged hexobarbital is essentially constant throughout the pH range 3.0 to 6.0. The k'_1 value of the first-eluted enantiomer for this compound is slightly above zero, probably due to a weak hydrophobic interaction with immobilized ovomucoid.

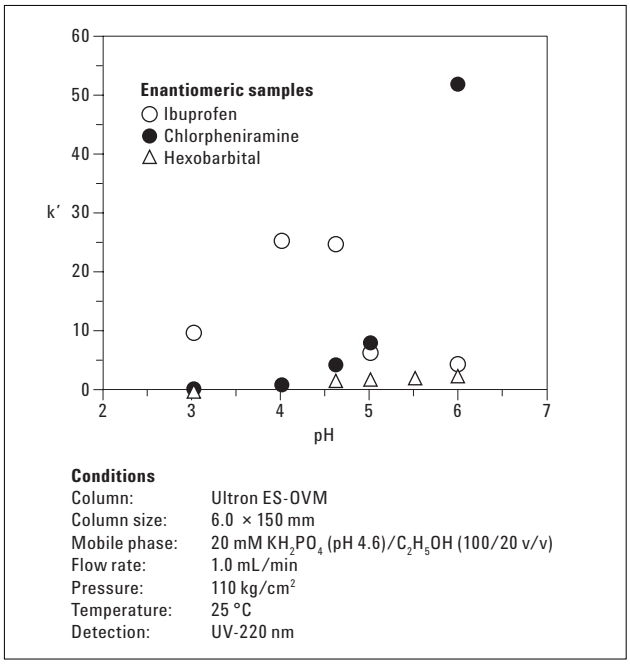


Figure 2. Change in k′ with change in pH.

For ibuprofen, maximum k'_1 values are in the range pH 4.0 to 4.6, as shown in Figure 2. Since the pKa of ibuprofen is 4.4, the compound is partially charged while ovomucoid is uncharged in this pH range; therefore, retention is likely due to strong hydrophobic interactions between the solute and the protein stationary phase. The decrease in k'_1 at higher pH (for example, 5.0 to 6.0) probably is the result of ion-repulsion of the negatively charged compound from the negatively charged protein stationary phase. The larger k'_1 values of ibuprofen compared to hexobarbital in the pH range 5.0 to 6.0 is considered to be due to a somewhat stronger hydrophobic interaction of ibuprofen. The k'_1 value of ibuprofen decreases at pH 3.0, presumably because of increased hydrophilicity of ovomucoid at this pH; however, hydrophobic interaction still dominates retention.

Figure 2 further shows that the k'_1 of chlorpheniramine increases at higher pH values. Both chlorpheniramine and ovomucoid are positively charged at pH 3.0, so electrostatic repulsion dominates and keeps retention low. This compound is more strongly retained at pH values higher than the pI of ovomucoid because of strong electrostatic forces plus the usual hydrophobic interaction.

Figure 3 shows that the enantioselectivity factors (α) for basic compounds can vary strongly with pH. The α value for chlorpheniramine increases sharply with pH increase; values for the other two compounds change only slightly.

These results indicate the importance of establishing the mobile phase pH that will produce the optimum chiral separation of ionic enantiomeric compounds. The effects just discussed imply that retention by the ovomucoid stationary phase is dominated by two mechanisms: electrostatic and hydrophobic interactions. Since electrostatic interactions are strongly influenced by the ionic strength of the mobile phase, reliable and reproducible separations are only obtained by careful control of both pH and salt concentration in the mobile phase.

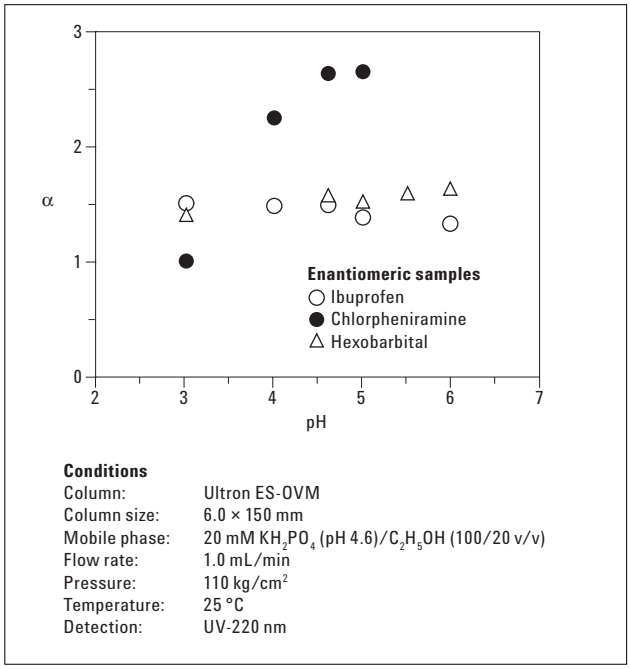
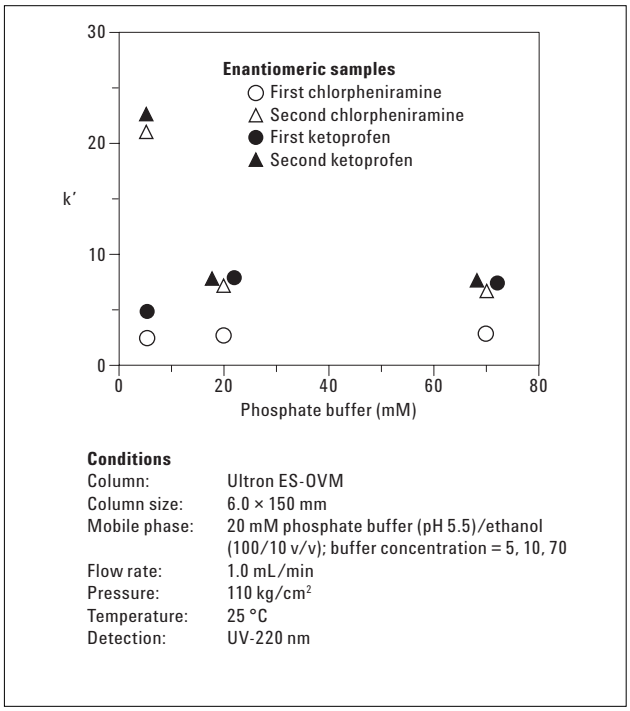


Figure 3. Change in α with change in pH.

Effect of Mobile Phase Ionic Strength on Retention

As discussed above, ionic strength partly influences retention on the ovomucoid stationary phase. This effect is demonstrated in Figure 4 for ketoprofen (pKa = 3.9) and chloropheniramine (pKa = 4.0, 9.2). Here, retention of these compounds is shown for three concentrations of pH 5.5 phosphate buffer (5, 20, 70 mM)/ethanol (100/10). About the same k' values occur for both compounds with 20 and 70 mM buffer, but larger k' values are shown for the negatively charged ketoprofen at 5 mM buffer. This effect may arise from the strong electrostatic interaction that occurs at low ionic strength, as is common in ion-exchange chromatography. Results such as those just described, combined with the tendency of buffers to precipitate with higher concentrations of organic mobile-phase modifier, suggest that 20 mM phosphate buffer my be appropriate for many separations. (Note that potassium salts are generally more soluble than sodium salts where solubility must be considered.)



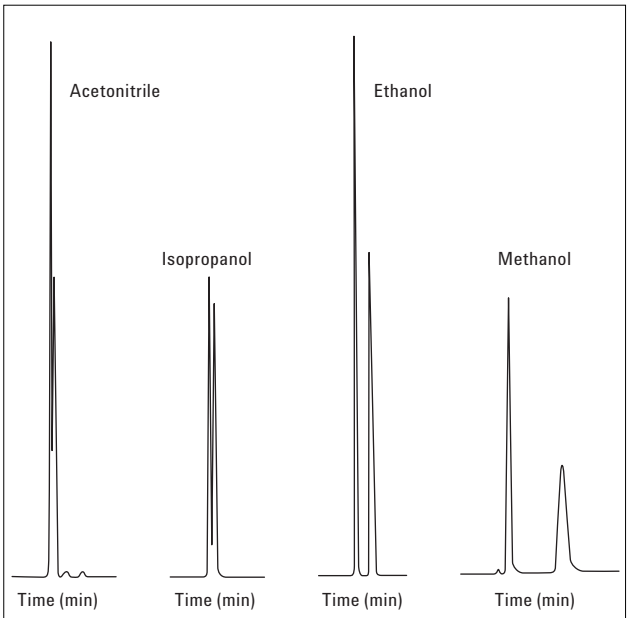
Conditions

Column:	Ultron ES-OVM
Column size:	6.0 × 150 mm
Mobile phase:	20 mM phosphate buffer (pH 5.5)/ethanol (100/10 v/v); buffer concentration = 5, 10, 70
Flow rate:	1.0 mL/min
Pressure:	110 kg/cm^2
Temperature:	25 °C
Detection:	UV-220 nm

Figure 4. Effect of ionic strength of phosphate buffer on retention.

Effect of Organic Modifiers on Retention and Enantioselectivity

The type of organic modifier in the mobile phase can have a strong effect on both retention (k') and enantiomer band spacing (α) values for chiral compounds with the Ultron ES-OVM column. Chiral separations of tolperison (pKa = 8.9) were performed with mixtures of phosphate buffer (pH 4.6) and four different modifiers: methanol, ethanol, isopropanol, and acetonitrile. Figure 5 shows that different separations were produced with the various modifiers. At the same organic concentration (v/v), the elution strength order of acetonitrile > isopropanol > ethanol > methanol was observed.



Conditions

Column:	Ultron ES-OVM
Column size:	6.0 × 150 mm
Mobile phase:	20 mM phosphate buffer (pH 4.6)/organic modifier (100/10 v/v)
Flow rate:	1.0 mL/min
Pressure:	110 kg/cm^2
Temperature:	25 °C
Detection:	UV-220 nm

Figure 5. Effect of type of organic modifiers on elution profile of enantiomeric tolperison.

The data in Table 1 show that changes (for example, 5 v/v) in organic modifier concentration can change retention profiles drastically. These results were obtained for chiral separations of acidic ibuprofen, neutral hexobarbital, and basic chlorpheniramine with mobile phase mixtures of 20 mM phosphate buffer (pH 4.6) and ethanol and acetonitrile in the ratio of 100/5 and 100/10 (v/v).

Compared to acetonitrile, ethanol as a modifier produced higher values for these test compounds when organic concentrations were used to obtain essentially the same k' values. (In Table 2, roughly equivalent k' values were obtained by mixing appropriate ratios of phosphate buffer and organic modifier.)

Differences in values could be due to the conformational change in ovomucoid, causing structural changes in the recognition site(s) for different compounds. In this regard, it was reported that the elution order of propranolol enantiomers was reversed by changing organic modifiers [5].

These and other studies indicate that retention characteristics of chiral compounds can be significantly influenced by the type and concentration of organic modifier used in the mobile phase. Different organic modifiers are favored for different compound structures. The optimum organic modifier should be determined for each system.

Table 1. Effect of Type and Contents of Organic Modifiers in the Mobile Phase on the Capacity Factors and Enantioselectivities on Ultron ES-OVM Column

		Ethanol content				Acetonitrile content			
		5		10		5		10	
Type of drug	**Compound**	**k'_1**	**α**	**k'_1**	**α**	**k'_1**	**α**	**k'_1**	**α**
Acidic[a]	Ibuprofen	24.76	1.45	12.38	1.40	24.29	1.25	3.10	1.06
Basic[a]	Chloropheniramine	4.10	2.60	1.60	1.93	3.25	2.00	2.00	1.00
Uncharged[a]	Hexobarbital	1.70	1.52	*		1.15	1.23	*	

[a]Mobile phase used was a mixture of 20 mM phosphate buffer (pH 4.6) and ethanol or acetonitrile (v/v).

*Hexobarbital did not retain.

Table 2. Change in the Enantioselectivity of Enantiomeric Compounds in Mobile Phases Containing Ethanol or Acetonitrile at Equivalent Solvent Strength

		Ethanol		Acetonitrile	
Type of drug	**Compound**	**k'_1**	**α**	**k'_1**	**α**
Acidic[a]	Ibuprofen	12.40	1.40	13.20	1.16
Basic[b]	Chloropheniramine	8.00	2.14	7.80	1.46
Uncharged[c]	Hexobarbital	2.19	1.59	1.85	1.42

[a]Mobile phase used was a mixture of 20 mM phosphate buffer (pH 4.6) and ethanol (100/10, v/v) or acetonitrile (100/7, v/v).

[b]Mobile phase used was a mixture of 20 mM phosphate buffer (pH 5.5) and ethanol (100/10, v/v) or acetonitrile (100/8, v/v).

[c]Mobile phase used was a mixture of 20 mM phosphate buffer (pH 6.0) and ethanol (100/5, v/v) or acetonitrile (100/4, v/v).

Effect of Column Temperature on Retention and Enantioselectivity

Temperature can also be used to optimize the retention and selectivity characteristics of the Ultron ES-OVM column. Table 3 shows the effect of temperature on the chromatographic characteristics of propranolol enantiomers separated with a mobile phase of 20 mM phosphate buffer (pH 4.6)/ethanol (100/10, v/v) at various temperatures between 5° and 40 °C. Table 3 summarizes the k' and α values; the plate number values, N; and the resolution values, Rs, found for the propranolol enantiomers in this study. With temperature increase, k' and α values decreased while N values increased, the highest resolution value occurring at 19 °C. Other studies have shown that certain compounds exhibit best resolution at higher temperatures, while others are better resolved at lower temperatures. Therefore, to optimize any chiral separation on ES-OVM, the effect of temperature should be investigated.

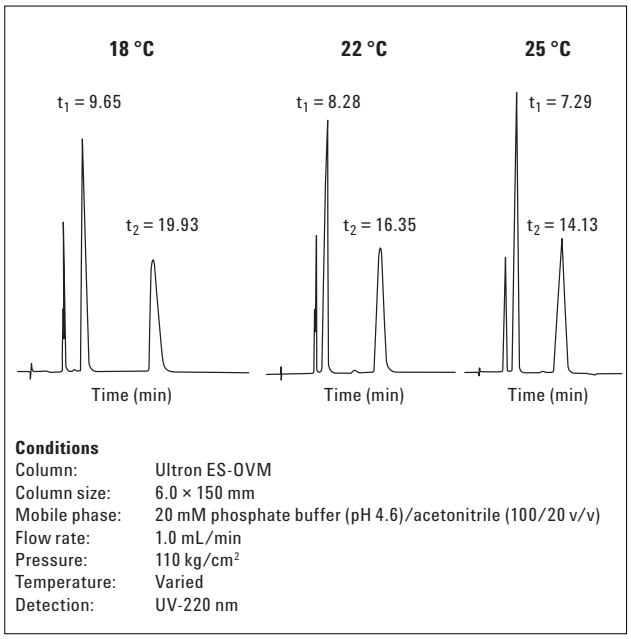


Figure 6. Effect of column temperature on the separation of enantiomeric trimipramine maleate.

Table 3. Effect of Column Temperature on the Chromatographic Parameters of Propranolol[a]

	5 °C	11 °C	19 °C	28 °C	40 °C
k'_1	18.67	11.49	7.79	4.97	2.59
k'_2	23.94	14.59	9.68	6.05	3.06
α	1.28	1.27	1.25	1.22	1.18
N_1	446	722	1059	1224	1899
N_2	380	563	798	890	1282
R_s	1.12	1.35	1.44	1.21	1.08

[a]Mobile phase used was a mixture of 20 mM phosphate buffer (pH 4.6) and ethanol (100/10, v/v).

Precise temperature control of the Ultron ES-OVM column is recommended to ensure good reproducibility of separations. This characteristic is illustrated by the data in Figure 6 for the chiral separation of trimipramine maleate. With a mobile phase of pH 4.6 phosphate buffer (20 mM)/acetonitrile (100/20, v/v), column temperatures in the narrow range of 18 to 25 °C gave significant differences in retention times, although α values were essentially constant in this case.

In some instances, racemization of the enantiomers can occur at higher temperatures, and lower operating temperatures are required for determining true enantiomer composition. This situation is illustrated in the separation of lorazepam with a mobile phase of pH 4.6 phospate buffer (20 MM)/isopropanol (100/10, v/v). Peak coalescence due to racemization of lorazepam on ovomucoid takes place at 15 °C or higher. Actual enantiomer composition is determined by operating the column at 7 °C [8].

Effect of Sample Loading

As with most HPLC columns, measured plate number is a function of the amount of sample injected on the column. The effect of sample mass is illustrated in Figure 7 for a 4.6 × 150 mm Ultron ES-OVM column. These chromatograms were obtained by increasing sample concentration at a fixed volume (0.5 µL) to increase sample loading. Studies have indicated that sample loadings of 1.5 to 3 nmol/g packing produced a decrease of 15% or less in enantiomer resolution (30% decrease in column plate number) with the 4.6 × 150 mm Ultron ES-OVM column [5]. Samples of about 2 nmol (1 µg) are typical for most analytical separations on this column. These results show that small sample loadings should be used to obtain the sharpest peaks and highest resolution.

The effect of sample volume on column efficiency and resolution is similar to that for other HPLC columns of equivalent efficiency. Sample volumes in the 1 to 50 µL range generally are permitted, with 10 or 25 µL volumes used in typical applications [4]. As in all HPLC separations, sample loading is most critical when peaks elute at low k' values. Sample mass and volume are less critical and can usually be increased when peaks are adjusted to elute at higher k' values.

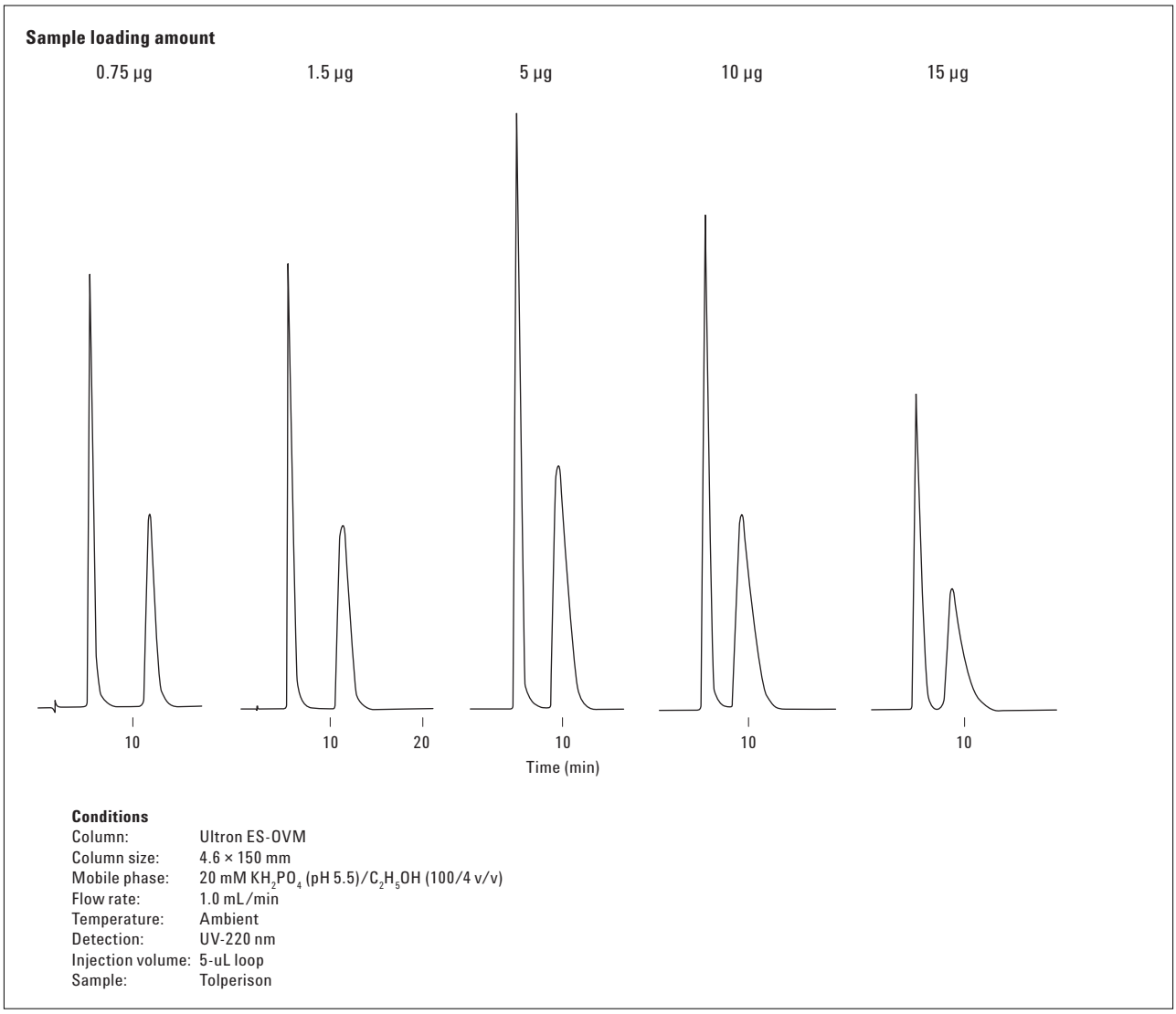


Figure 7. Effect of sample loading – tolperison.

Effect of Mobile Phase Flow Rate on Column Efficiency

As is typical of other HPLC columns, column efficiency is influenced by the mobile phase flow rate used in the separation. Figure 8 shows plate height versus flow rate plots for two Ultron ES-OVM columns of different internal diameters. These and other results suggest that a flow rate of about 1.0 mL/min often produces the highest column efficiency and peak resolution for these two columns. The flow rate for the highest column efficiency actually is dependent on the type and concentration of organic modifier and the temperature of operation. For best results, the optimum flow rate should be determined for each separation system.

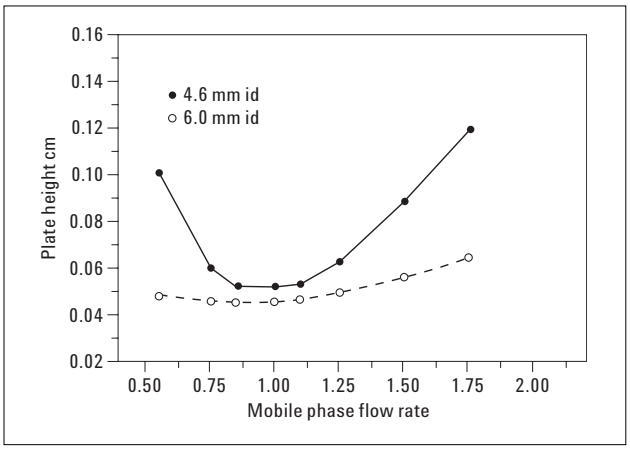
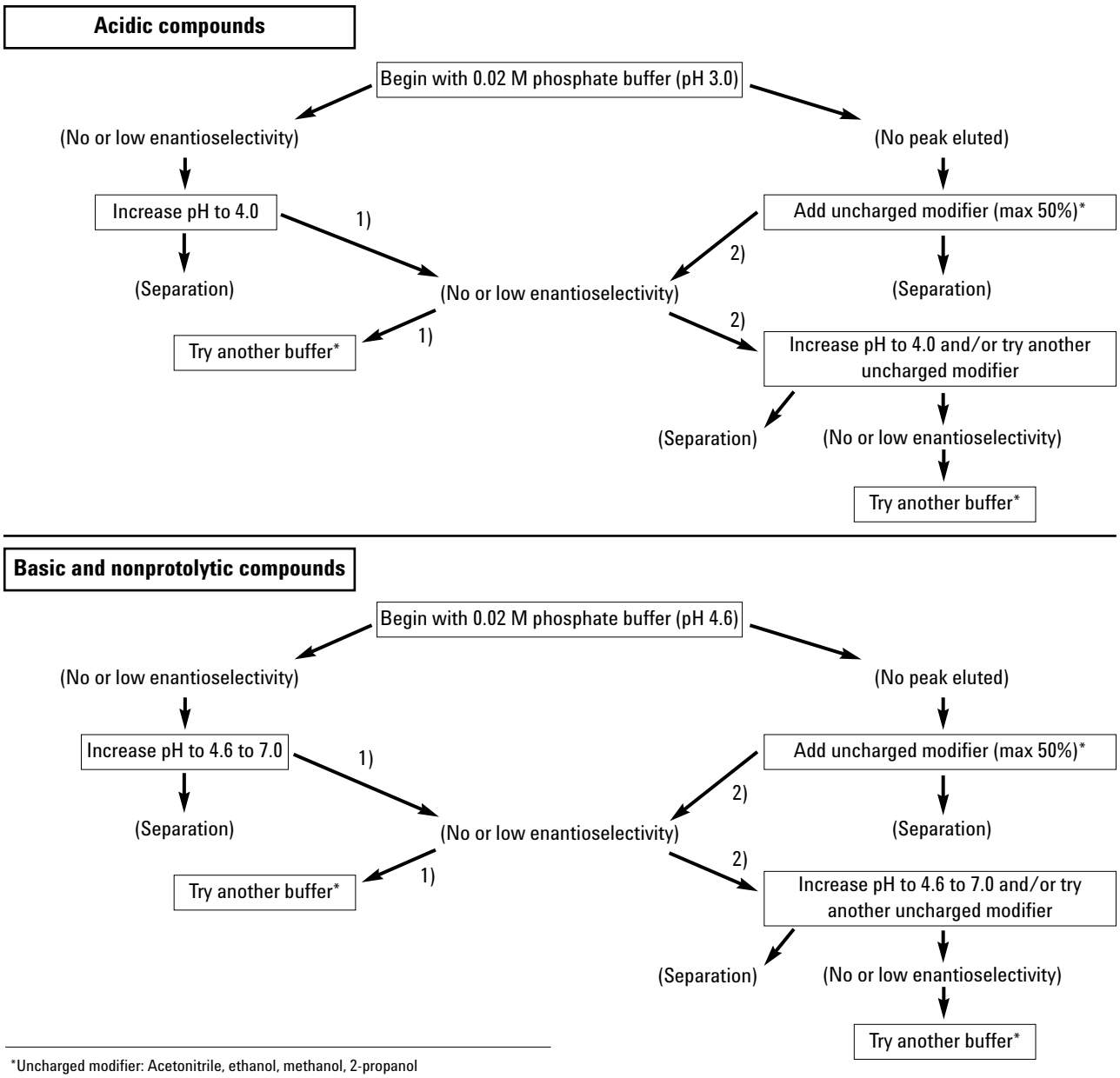


Figure 8. Influence of flow rate on plate height, Ultron ES-OVM columns, 150 mm

Optimizing Chromatographic Conditions for Ultron ES-OVM Column

Suggested steps for optimizing a separation of enantiomers are given in Figure 9. An initial temperature of 25 °C is recommended, since higher temperatures could racemize the chiral compounds, decrease resolution, and may also lead to denaturation of the ovomucoid stationary phase. An initial mobile phase of 20 mM phosphate with acetonitrile organic modifier is suggested [5]. For acidic enantiomeric compounds, a starting pH of 3.0 is recommended – α values generally are constant in the pH range 3.0 to 4.6, and faster separations usually are available at low pH. If the desired separation is not attained, increase the pH to 4.0, then, if needed, add additional organic modifier. If the desired separation still is not obtained, follow the same steps with a different modifier or with a different buffer (other than phosphate).

For basic or neutral enantiomers, an initial mobile phase of phosphate buffer pH 4.6/acetonitrile is recommended. If the desired separation is not obtained, raise the pH to 7.0 and increase the concentration of organic modifier to improve the separation. If the desired separation still is not obtained, follow the same steps with a different organic modifier or with a different buffer (other than phosphate) as described above for acidic samples.



*Uncharged modifier: Acetonitrile, ethanol, methanol, 2-propanol
Buffer solution (20 mM): KH_2PO_4/K_2HPO_4, $HCOOH/HCOONH_4$, CH_3COOH/CH_3COONa, $C_3H_4OH(COOH)_3KH_2PO_4$

Table 4 summarizes the resolution and optimized mobile phase that were obtained for 43 enantiomeric pairs, using the approach of Figure 9. Other useful optimization approaches for the Ultron ES-OVM column are given in References 4 and 5.

Table 4. Separation of Enantiomeric Pairs with Ultron ES-OVM

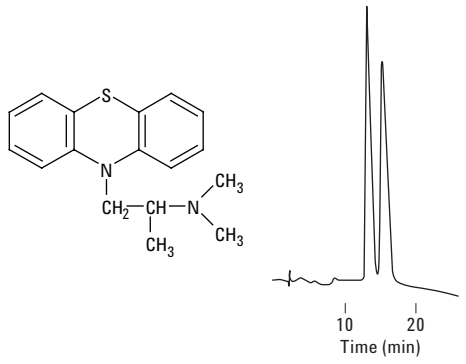
Substance	Mobile Phase	Rs
Acetylpheneturide	20 mM phosphate buffer (pH 4.6)-ethanol (100/7.5 v/v)	2.74
Alimemazine	20 mM phosphate buffer (pH 4.6)-ethanol (100/25 v/v)	6.06
Bay K 8644	20 mM phosphate buffer (pH 4.6)-ethanol (100/25 v/v)	5.92
Benproperine	20 mM phosphate buffer (pH 4.6)-ethanol (100/20 v/v)	3.27
Benzoin	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	8.41
Biperiden	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	3.17
Bunitrolol	20 mM phosphate buffer (pH 6.0)-ethanol (100/3 v/v)	3.08
Bupivacaine	20 mM phosphate buffer (pH 5.5)-acetonitrile (100/10 v/v)	1.26
Chlormezanone	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	6.48
Chlorphenesin	20 mM phosphate buffer (pH 5.5)	2.23
Chlorpheniramin	20 mM phosphate buffer (pH 5.0)-acetonitrile (100/5 v/v)	2.36
Chlorprenaline	20 mM phosphate buffer (pH 5.5)-ethanol (100/3 v/v)	2.34
Cloperastin	20 mM phosphate buffer (pH 4.6)-ethanol (100/15 v/v)	2.85
Dimetindene	20 mM phosphate buffer (pH 4.6)-ethanol (100/15 v/v)	4.33
1,2-Diphenylethylamine	20 mM phosphate buffer (pH 5.5)	1.74
Disopyramid	20 mM phosphate buffer (pH 5.5)-ethanol (100/10 v/v)	2.04
Eperisone	20 mM phosphate buffer (pH 5.5)-ethanol (100/5 v/v)	1.15
Ethiazide	20 mM phosphate buffer (pH 4.6)	1.42
Flurbiprofen	20 mM phosphate buffer (pH 3.0)-ethanol (100/30 v/v)	1.26
Glutethimide	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	1.36
Glycopyrronium	20 mM phosphate buffer (pH 4.6)	1.73
Hexobarbital	20 mM phosphate buffer (pH 5.5)-ethanol (100/5 v/v)	1.70
Homochlorcyclizine	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	3.04
Hydroxyzine	20 mM phosphate buffer (pH 4.6)-ethanol (100/15 v/v)	2.15
Ibuprofen	20 mM phosphate buffer (pH 3.0)-ethanol (100/10 v/v)	1.73
Ketoprofen	20 mM phosphate buffer (pH 3.0)-acetonitrile (100/10 v/v)	1.37
Meclizine	20 mM phosphate buffer (pH 4.6)-ethanol (100/35 v/v)	3.71
Mepenzolate	20 mM phosphate buffer (pH 4.6)	1.40
Mephobarbital	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	1.70
Methylphenidate	20 mM phosphate buffer (pH 5.7)	1.13
Oxprenolol	20 mM phosphate buffer (pH 5.5)-ethanol (100/10 v/v)	1.38
Pindolol	20 mM phosphate buffer (pH 5.5)-ethanol (100/3 v/v)	2.04
Pranoprofen	20 mM phosphate buffer (pH 3.0)-acetonitrile (100/8 v/v)	1.01
Prenylamine	20 mM phosphate buffer (pH 5.0)-acetonitrile (100/15 v/v)	1.02
Profenamine	20 mM phosphate buffer (pH 4.6)-ethanol (100/25 v/v)	3.31
Proglumide	20 mM phosphate buffer (pH 4.6)-ethanol (100/20 v/v)	1.32
Promethazine	20 mM phosphate buffer (pH 4.6)-ethanol (100/20 v/v)	0.98
Propranolol	20 mM phosphate buffer (pH 6.8)-acetonitrile (100/30 v/v)	1.24
Thioridazine	20 mM phosphate buffer (pH 5.5)-acetonitrile (100/30 v/v)	0.98
Tolperizone	20 mM phosphate buffer (pH 5.5)-ethanol (100/10 v/v)	1.50
Trihexyphenidyl	20 mM phosphate buffer (pH 4.6)-ethanol (100/10 v/v)	5.16
Trimipramine	20 mM phosphate buffer (pH 4.6)-ethanol (100/30 v/v)	3.69
Verapamil	20 mM phosphate buffer (pH 4.6)-ethanol (100/5 v/v)	1.49

Column Care

Buffered mobile phases with pH ranges of 2 to 7.5 (3 to 7 preferred) and common organic water-miscible solvents (for example, acetonitrile, methanol, ethanol, and propanol) can be used safely with the Ultron ES-OVM column. Mobile phases with > 50% organic solvent should be used sparingly. Guard columns are recommended for most applications, to protect the analytical column against deleterious contaminants. Samples containing extraneous materials that will be highly retained on the column should be carefully avoided. Contaminated columns exhibiting poor peak shapes sometimes are conveniently restored by flushing with 20 to 40 column volumes of 50% acetonitrile/distilled water. Columns are preferably stored in a mobile phase of 10 to 20% acetonitrile/distilled water (after flushing out any previously used buffer) when not in use for long periods.

Illustrative Applications

In the appendix are chromatograms of a wide range of enantiomeric compounds that have been successfully separated with the Ultron ES-OVM column. In each case, no attempt was made to optimize results, the main criterion being a separation that would have distinct utility in the characterization of racemic mixtures. Also included is a separation on a larger diameter column (6.0 × 150 mm), which can accept semipreparative samples (Figure 10).



Conditions

Column:	Ultron ES-OVM
Column size:	6.0 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH = 4.6)/C_2H_5OH = 100/20 v/v
Flow rate:	1.0 mL/min
Pressure:	110 kg/cm^2
Temperature:	25 °C
Detection:	UV-220 nm

Figure 10. Promethazine.

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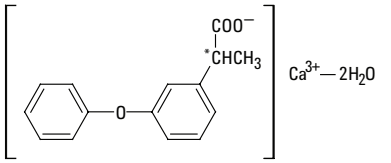
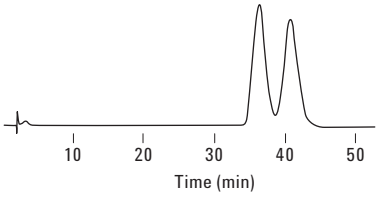
Appendix

Antiinflammatory
Fenoprofen calcium

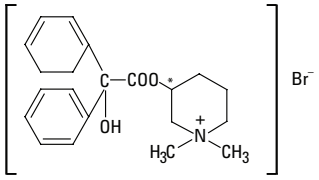


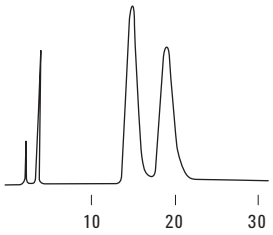


Conditions

Column:	Ultron ES-OVM
Column size:	6.0 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 5.6)/ C_2H_5OH (100/10 v/v)
Flow rate:	1.0 mL/min
Temperature:	27 °C
Detection:	UV-220 nm (0.04 AUFS)

Cholinergic blocking (parasympatholytic) drugs
Mepenzolate bromide

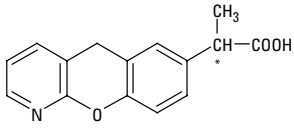


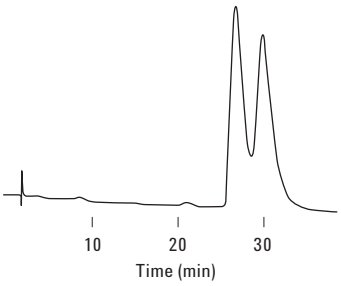


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH = 4.6)
Flow rate:	1.0 mL/min
Temperature:	27 °C
Detection:	UV-220 nm (0.04 AUFS)

Antiinflammatory
Pranoprofen

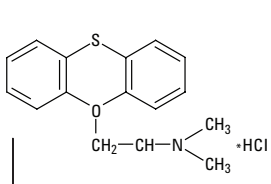


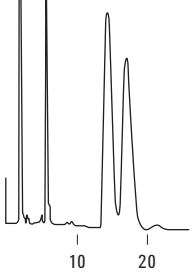


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 6.0)/ C_2H_5OH (100/5 v/v)
Flow rate:	1.0 mL/min
Temperature:	27 °C
Detection:	UV-220 nm (0.04 AUFS)

Antihistamines
Promethazine hydrochloride

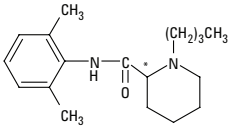


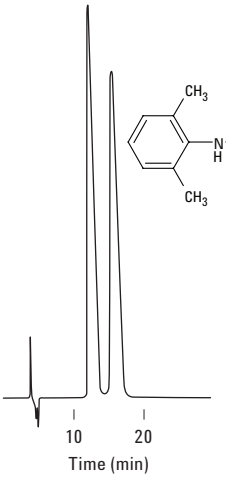


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)/ C_2H_5OH (100/20 v/v)
Flow rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV-220 nm (0.04 AUFS)

Local Anesthetic
Bupivacaine

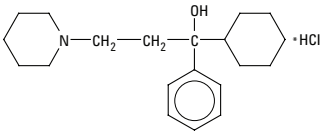


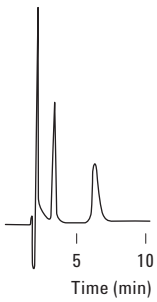


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 5.5)/ CH_3CN (100/10 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm (0.04 AUFS)

Antiparkinson drugs
Trihexyphenidyl



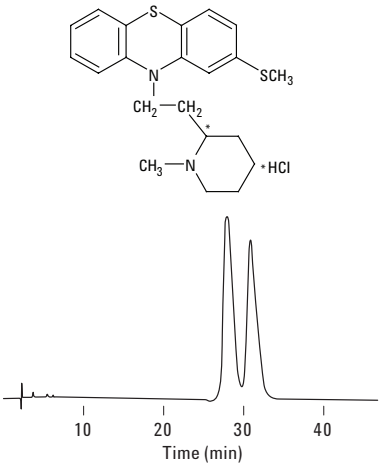


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)/ C_2H_5OH (100/10 v/v)
Flow rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV-220 nm (0.04 AUFS)

Antipsychotics

Thioridazine hydrochloride

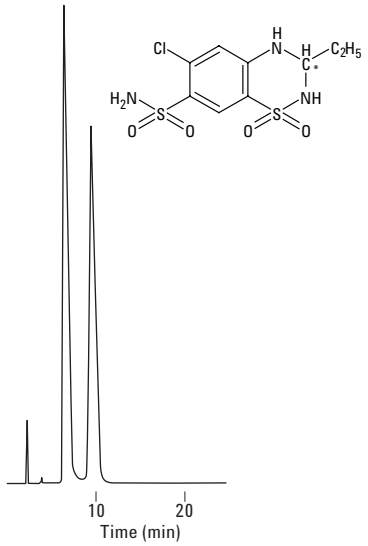


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 5.5)/ CH_3CN (100/30 v/v)
Flow rate:	1.0 mL/min
Temperature:	31 °C
Detection:	UV-254 nm (0.04 AUFS)

Diuretic drugs

Ethiazide

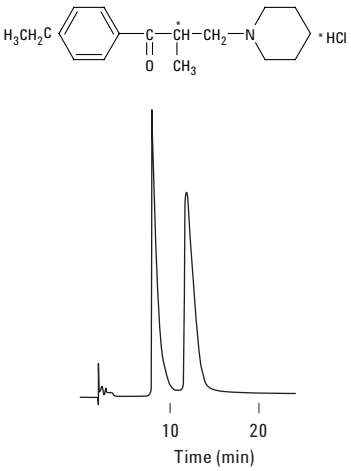


Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm (0.04 AUFS)

Skeletal muscle relaxants

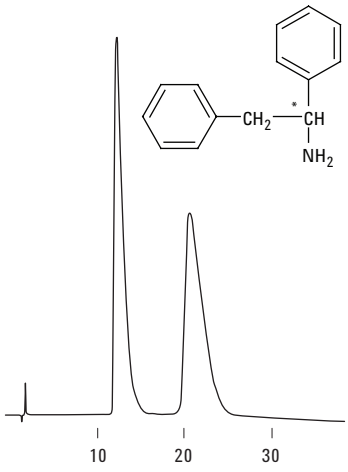
Eperisone hydrochloride



Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 5.5)/ C_2H_5OH (100/5 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm (0.04 AUFS)

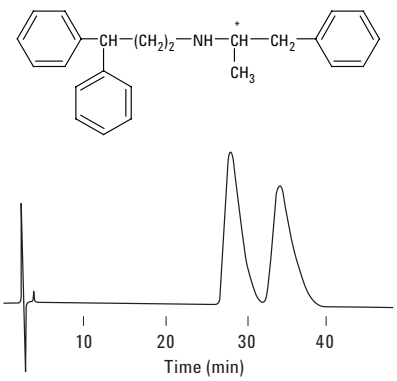
1,2-Diphenylethylamine



Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 5.5)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm (0.04 AUFS)

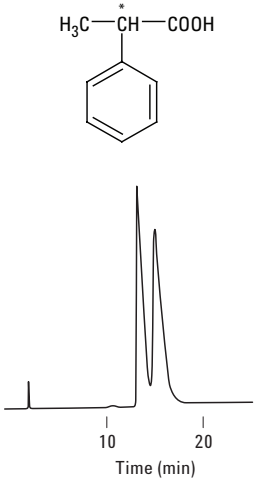
Prenylamine lactate



Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 5.0)/ CH_3CN (100/15 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm (0.04 AUFS)

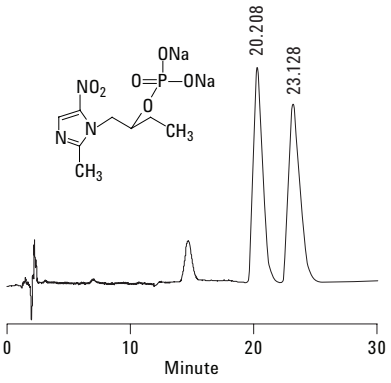
2-Phenyl propionic acid



Conditions

Column:	Ultron ES-OVM
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm (0.04 AUFS)

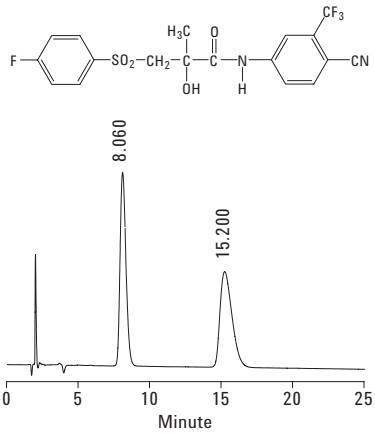
Ornidazol (Antibiotic)



Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 3.0)/ CH_3CN (88/12 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm
Sample:	Ornidazol disodium phosphate 0.2 mg/mL
Injection vol:	3 µL

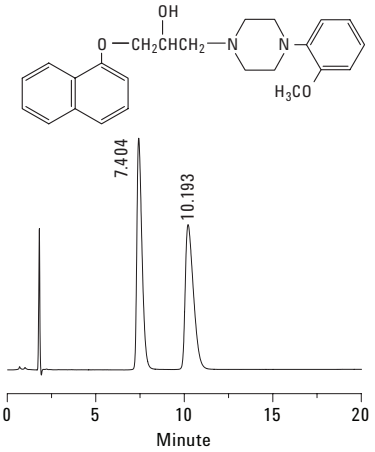
Bicalutamide (Antiandrogen)



Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)/ CH_3CN (88/12 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm
Sample:	Bicalutamide in CH_3CN 0.1 mg/mL
Injection vol:	3 µL

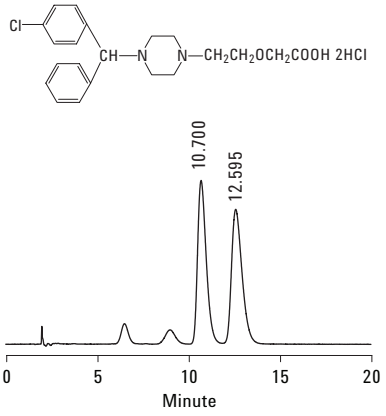
Naftopidil (Alpha1a-adrenergic receptor antagonist)



Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 7.0)/ CH_3CN (75/25 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm
Sample:	Naftopidil 0.5 mg/mL
Injection vol:	1 µL

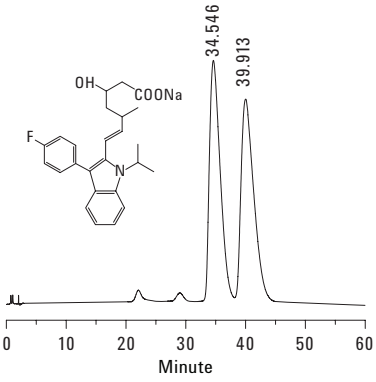
Cetirizine hydrochloride (Antihistamine)



Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)/ CH_3CN (90/10 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-235 nm
Sample:	Cetirizine hydrochloride 0.2 mg/mL
Injection vol:	2 µL

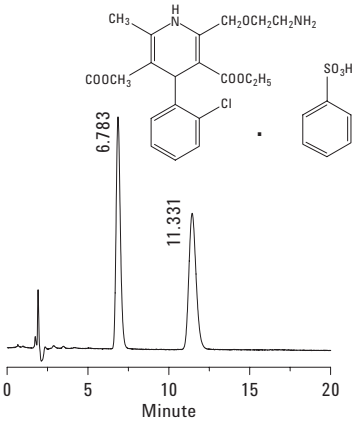
Fulvastatin sodium (HMG-CoA reductase inhibitor)



Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 3.0)/ CH_3CN (88/12 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-235 nm
Sample:	Fulvastatin sodium 0.5 mg/mL (Solvent: methanol)
Injection vol:	1 µL

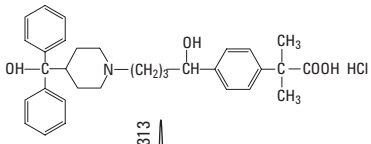
Amlodipine besilate (Calcium channel blocker)

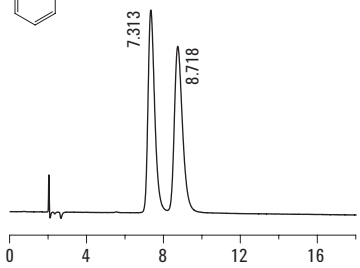


Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	10 mM Na_2HPO_4 (pH 7.0)/ CH_3CN (78/22 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-237 nm
Sample:	Amlodipine besilate 0.02 mg/mL (Solvent: Methanol)
Injection vol:	2 µL

Fexofenadine hydrochloride
(Antihistaminic)

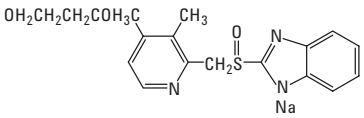


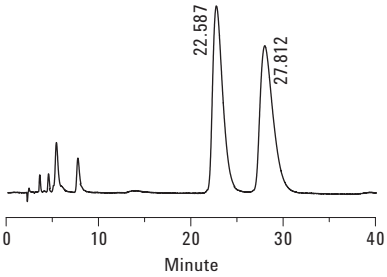


Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 4.6)/ CH_3CN (95/5 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-220 nm
Sample:	Fexofenadine hydrochloride 0.3 mg/mL
Injection vol:	1 µL

Sodium rabeprazole
(Proton pump inhibitor)





Conditions

Column:	Ultron ES-OVM (5 µm)
Column size:	4.6 × 150 mm
Mobile phase:	20 mM KH_2PO_4 (pH 6.0)/ CH_3CN (95/5 v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV-225 nm
Sample:	Sodium rabeprazole 0.2 mg/mL
Injection vol:	3 µL

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Printed in the USA
July 11, 2008
5989-8748EN

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