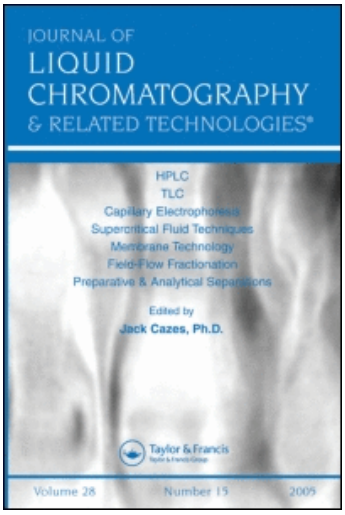


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# Separation of Fexofenadine, Pseudoephedrine, Potential Impurities, and Degradation Products Using Ion Interaction Chromatography

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# Separation of Fexofenadine, Pseudoephedrine, Potential Impurities, and Degradation Products Using Ion Interaction Chromatography

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**Abstract:** Ion interaction chromatography has been demonstrated to be a viable separation scheme for a wide variety of small molecules. This separation mechanism has been shown to be effective for a complex mixture of analytes that range from uncharged to positively and/or negatively charged. The analytes present in fexofenadine-D tablets are either uncharged or cationic. Therefore, if a single HPLC method is to be used for this difficult separation, a dual mechanistic system must be present so that the charged and neutral components are retained and separated. An ion interaction liquid chromatographic system that contains an anionic surfactant, such as sodium dodecyl sulfate (SDS), meets both of these requirements: a reversed stationary phase for a hydrophobic analyte and a fixed anionic charge site for a positively charged analyte. The effect that each mobile phase parameter may have on this complex separation was found to be crucial. These variables were studied and include: concentration of SDS, ionic strength, pH, concentration of phosphoric acid, concentration of organic modifier, column temperature, and different gradients. The results obtained in this study and the optimized separation is discussed.

**Keywords:** Fexofenadine, High performance liquid chromatography, Chromatography, Ion interaction, Ion pair, Pseudoephedrine, Ephedrine, Sodium dodecyl sulfate

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## INTRODUCTION

Fexofenadine-D is composed of fexofenadine (the acid metabolite of terfenadine) and pseudoephedrine hydrochloride, and is indicated for people who suffer with allergic rhinitis. A stability-indicating chromatographic method is required for the active components of the formulation, as well as any potential degradation products and impurities.

Pseudoephedrine and potential impurities/degradation products have chemical and physical properties such that an ion exchange column or a reversed-phase column alone will not adequately separate all of the analytes in a single run. Pseudoephedrine has little retention on a reversed-phase column, but is retained on a cation exchange column. Ephedrone and ephedrine, potential degradation products of pseudoephedrine, have some retention on both a cation exchange column and a reversed-phase column. Ephedrone and ephedrine co-elute on a reversed-phase column but are separated on a cation exchange column. Pseudoephedrine and ephedrine were also found to co-elute on a cation exchange column. Fexofenadine and its degradation products/impurities are separated on a reversed-phase column but are not resolved on a cation exchange column. Therefore, a mixed-mode system should provide an adequate separation for all of the analytes of interest that may be present.

A study was done to determine if the chromatographic separation of fexofenadine, pseudoephedrine, and all potential degradation products/impurities could be accomplished using IIC (ion interaction chromatography). In IIC, a molecule that contains both a fixed charge site and a hydrophobic tail is added to the mobile phase.[1–8] The hydrophobic portion of the molecule adsorbs onto the stationary phase and the fixed charged portion provides a site with which an analyte ion may interact. This allows a charged analyte ion that has little or no retention on a reversed-phase packing to be retained. Analytes that are retained on the reversed-phase packing will still be retained, but not necessarily with the same retention time if the IIR was not present.

An IIC separation was developed that uses sodium dodecyl sulfate in the mobile phase. The separation does require a gradient of increasing organic modifier in order to elute the most hydrophobic analytes in a reasonable amount of time. The separation and column re-equilibration can be accomplished in approximately 65 min with all of the analytes of interest baseline resolved. This report will discuss the ion interaction separation that was developed and the mobile phase parameters that were found to influence the separation.

## EXPERIMENTAL

### Reagents and Instrumentation

Fexofenadine, pseudoephedrine hydrochloride, and potential impurities and degradation products used in this study were supplied by Aventis (Kansas

City, MO, USA). Acetonitrile and methanol (HPLC-grade) were obtained from Burdick and Jackson (Muskegon, MI, USA). Phosphoric acid, sodium monobasic phosphate monohydrate, and sodium hydroxide were obtained from Mallinckrodt (Paris, KY, USA). Sodium dodecyl sulfate was purchased from J.T. Baker (Phillipsburg, NJ, USA). HPLC-grade water was obtained by passing de-ionized water through a Nanopure™ II water purification system (Barnstead, Dubuque, IA, USA). The instrumentation consisted of a ThermoSeparations P4000 quaternary pump, AS3000 autosampler, UV1000 variable wavelength UV detector (Fremont, CA, USA), and the PeakPro data acquisition system (Beckman, Fullerton, CA, USA). The YMC basic column (5 μm, 4.6 × 150 mm) was purchased from YMC, Inc. (Wilmington, NC, USA).

## Procedures

Fexofenadine and pseudoephedrine HCl samples were prepared at a concentration of 1 mg/g. The potential impurities were prepared at a level of 0.1% (wt/wt) of fexofenadine. A flow rate of 1.0 mL/min was used for all separations, along with UV detection at 220 nm, an injection volume of 10 μL, and a column temperature of 30°C, except for the temperature study. The pH of the phosphate buffer was adjusted using 1 N NaOH.



## RESULTS AND DISCUSSION

Enhanced retention of charged analyte ions on reversed stationary phases from a mobile phase containing an ion interaction reagent (a hydrophobic ion of opposite charge) is determined by two major equilibria.[1,3,4,6,9] The first equilibrium (equation (1)) accounts for the adsorption of the hydrophobic ion onto the stationary phase and the second equilibrium (equation (2)) describes the ion exchange selectivity between the charged analyte ion and the counterion associated with the retained hydrophobic ion. These equilibria are shown by equations (1) and (2), respectively.

$$A + RSO_3^-C^+ \rightleftharpoons A\cdots RSO_3^-C^+ \quad (1)$$

$$A\cdots RSO_3^-C^+ + X^+ \rightleftharpoons A\cdots RSO_3^-X^+ + C^+ \quad (2)$$

In these equations, A represents the stationary phase, $RSO_3^-$ represents the ion interaction reagent, $C^+$ represents the counterion provided by the IIR (ion interaction reagent), the buffer and/or ionic strength salt, and $X^+$ is the analyte ion. The variables that have been found to influence the separation of charged analytes by ion interaction chromatography include: the reversed stationary phase, the type and concentration of the IIR, the type and concentration of organic modifier, the type and concentration of counterion provided

by the buffer and/or ionic strength salt present in the mobile phase, mobile phase pH, mobile phase ionic strength, and column temperature.[4,8–11]

The IIR used in this study was SDS (sodium dodecyl sulfate). In most chromatographic systems that employ SDS, a micellar retention mechanism is observed rather than an ion interaction mechanism. However, studies that were performed in our laboratory have shown that micelles are not formed under the chromatographic conditions used throughout the separation. Therefore, the ion interaction retention mechanism was found to best describe the separation mode for charged analytes where SDS was employed.

Since the IIR offers an anionic charge site on the stationary phase as well as reversed phase sites, the diverse analytes present in a fexofenadine-D mixture should be retained and separated using this chromatographic system. The cationic analytes will be retained by ionic interactions with the fixed anionic charge sites of the IIR and hydrophobic interactions with the stationary phase, while the un-charged analytes will be retained by reversed-phase interactions with the stationary phase as well as with the hydrophobic center of the IIR. Therefore, an IIR system was studied as a possible separation scheme for fexofenadine, pseudoephedrine, and all potential degradation products/impurities that may be present in the fexofenadine-D formulations. The structures of fexofenadine, pseudoephedrine, ephedrine, and ephedrone are presented in Figure 1.

The various ion interaction chromatographic mobile phase parameters that may have an effect on the retention and separation of the analytes present in the fexofenadine-D formulations were studied. The results that were obtained for each chromatographic variable are discussed and the optimized separation is presented. This separation scheme was found to be extremely useful and reproducible for fexofenadine, pseudoephedrine, and all potential degradation products/impurities.



## Effect of Mobile Phase pH

The pH of the mobile phase will have a dramatic effect on the retention of weak organic acids and bases, while uncharged analytes that are not ionizable will not be affected by pH. Interactions between the IIR and analyte are different for the associated and unassociated forms of the analyte. Several studies have shown that small changes in the mobile phase pH will have an effect on retention, especially when the pH is close to the analyte's $pK_a$ value.[12,13]

The effect that mobile phase pH would have on the retention of fexofenadine, pseudoephedrine, and potential impurities/degradation products was studied. The results indicate that retention is dependent on the analyte's $pK_a$ and the pH of the mobile phase. Figure 2 shows the effect of the mobile phase pH on four analytes: fexofenadine, pseudoephedrine, ephedrone, and MDL 46,016 (most highly retained analyte studied). It is interesting to note

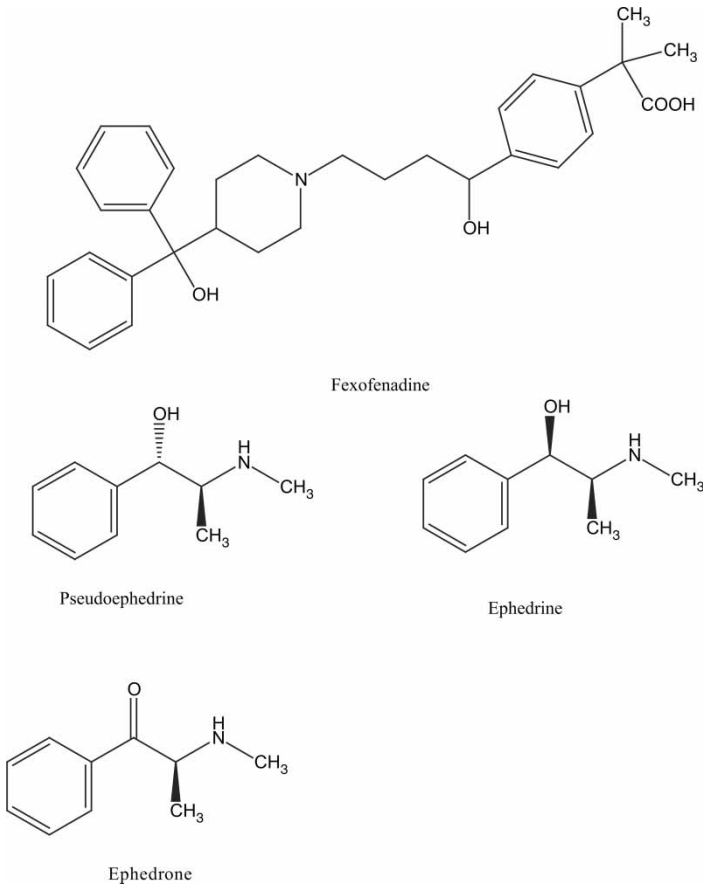


***Figure 1.*** Chemical structure of fexofenadine, pseudoephedrine, ephedrine, and ephedrone.

that three of the four analytes did not show a change in retention with increasing pH, however, fexofenadine showed a significant decrease. As fexofenadine converts from being cationic in nature to a zwitterion, retention was found to decrease. Typically retention of an analyte increases on a reversed-phase packing when changing from a charged form to a zwitterionic or uncharged form. However, the retention mechanism in ion interaction chromatography is different; in the charged form, fexofenadine is retained by both reversed-phase interactions and by cation interactions with the diffuse secondary layer of the SDS (equation (2)), while the uncharged form of fexofenadine is retained only by reversed-phase interactions.

It was also noted that several other analytes decreased in retention with increasing mobile phase pH. Typically, the analyte's retention was found to decrease as the mobile phase pH was increased above the analyte's $pK_a$ of

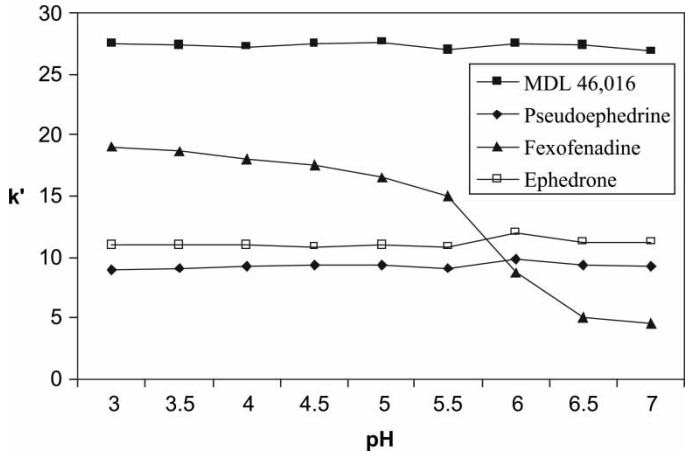


***Figure 2.*** The effect of mobile phase pH on analyte retention. Mobile phase: A)50 mM SDS, 25 mM $H_3PO_4$, 30% $CH_3CN$: B) 50 mM SDS, 25 mM $H_3PO_4$, 50% $CH_3CN$. Gradient: 1) 0–20 min 100% A, 2) 20–30 min 100% A to 10% A, 3) 30–60 min 10% A, 4) 60–70 min 100% A.



the carboxylate side chain. The analytes that had a $pK_a$, within the pH range that was studied, were found to decrease in retention during the study. Figure 3 shows the separation at a mobile phase pH of 2.5. Only nine of the ten analytes present in the mixture could be separated at this mobile phase pH (pseudoephedrine and ephedrine co-eluted). Therefore, a more in-depth study was performed at a higher mobile phase pH to determine if all ten analytes could be separated in a single run.

Figure 4 shows the separation of the same ten analytes studied at pH 2.5, plus two additional analytes at a mobile phase pH of 6.9. A critical parameter of the separation was the resolution between three different groups of analytes: fexofenadine/MDL 102,038, pseudoephedrine/ephedrone, and ephedrine/MDL 46,814. As expected, the elution order was different at the higher pH mobile phase when compared to the separation at pH 2.5 (Figure 3). The critical analytes were resolved at pH 2.5, but not baseline resolved, which was a requirement. The separation was found to be better at a mobile phase of pH 6.9 than at a pH of 2.5.

## Effect of Organic Modifier Concentration

The concentration of organic modifier in the mobile phase will play a major role on analyte retention for both the charged analyte cations and the uncharged hydrophobic analytes.[1,3,4,9,10] The retention of cations will depend on the number of ion exchange sites that are present from the adsorbed IIR. It has been shown, that as the concentration of organic

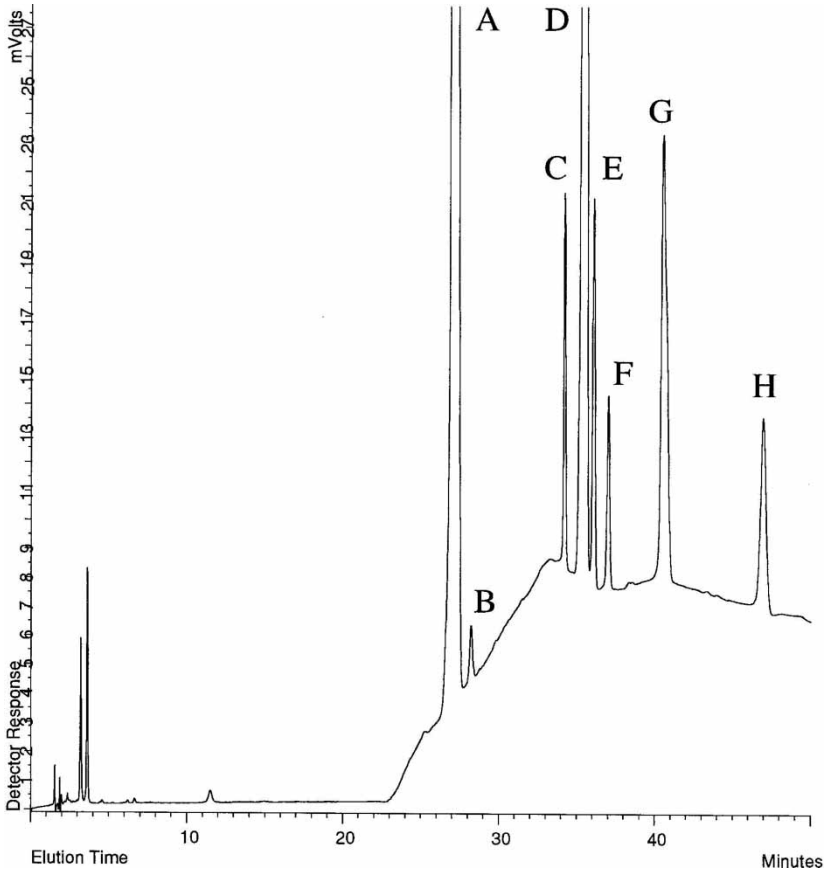


***Figure 3.*** The separation of fexofenadine, pseudoephedrine, and potential impurities by ion interaction chromatography at a mobile phase pH of 2.5. Mobile phase: A) 50 mM SDS, 25 mM $H_3PO_4$, pH 2.5, 30% $CH_3CN$; B) 50 mM SDS, 25 mM $H_3PO_4$, pH 2.5, 50% $CH_3CN$. Analytes: A) pseudoephedrine, ephedrine, B) ephedrone, C) MDL 4,829, D) fexofenadine, E) MDL 17,523, F) MDL 102,038, G) MDL 46,619, H) MDL 46,016. Gradient: 1) 0–20 min 100% A, 2) 20–30 min 100% A to 10% A, 3) 30–60 minutes 10% A, 4) 60–70 min 100% A.

modifier in the mobile phase is increased, the number of apparent ion exchange sites decreases.[3,9,10] This leads to a corresponding decrease in the retention of cations. The retention of the uncharged hydrophobic analytes will also decrease in retention as the organic modifier concentration is increased and is attributed to the increase in the mobile phase hydrophobicity. Therefore, retention of the analytes can be controlled by the amount of organic modifier present in the mobile phase.

The concentration of organic modifier was found to influence both the charged analytes (pseudoephedrine, ephedrine, and ephedrone) and the

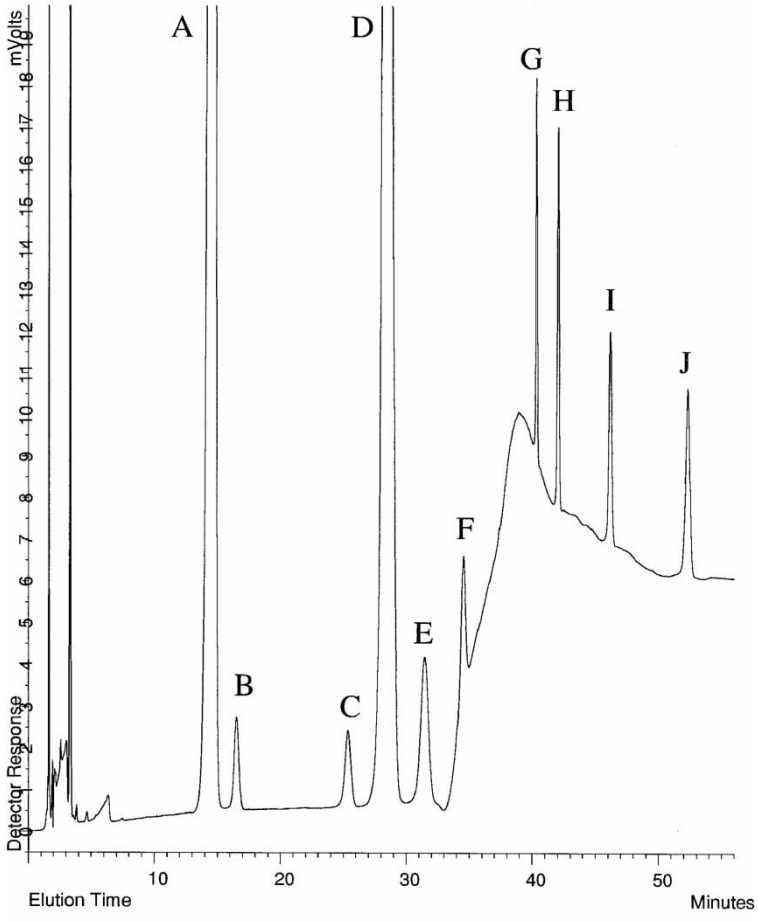


***Figure 4.*** The separation of fexofenadine, pseudoephedrine, and potential impurities by ion interaction chromatography at a mobile phase pH of 6.9 and acetonitrile. Mobile Phase: A) MDL 16,455, B) MDL 102,038, C) ephedrone, D) pseudoephedrine, E) ephedrine, F) MDL 45,814, G) MDL 4,829, H) MDL 17,523, I) MDL 46,619, J) MDL 46,016. Gradient: 1) 0–30 min 100% A, 2) 30–35 min 100% A to 0% A, 3) 35–55 min 0% A, 4) 55–65 min 100% A.

neutral, zwitterionic, or uncharged analytes (fexofenadine and related compounds). Table 1 shows the retention data obtained from several analytes where the concentration of acetonitrile was varied between 25 and 30% (mobile phase pH 6.9). Table 2 shows the selectivity between key analytes that were baseline resolved or nearly baseline resolved over this same acetonitrile concentration range. It is interesting to note that the pseudoephedrine/ephedrone selectivity was not affected by the change in the

*Table 1.* Retention of key analytes as a function of acetonitrile concentration (mobile phase pH 6.9)

| | Percent acetonitrile used (k′) (%) | | | |
|---|---|---|---|---|
| Analyte | 25 | 27 | 28 | 30 |
| Fexofenadine | 6.92 | 6.09 | 5.78 | 4.91 |
| MDL 102,038 | 8.28 | 7.16 | 6.75 | 5.65 |
| Pseudoephedrine | 12.6 | 11.1 | 10.4 | 8.74 |
| Ephedrone | 13.7 | 12.5 | 11.7 | 9.87 |
| MDL 46,814 | 14.5 | 13.9 | 13.6 | 12.4 |
| MDL 46,016 | 28.6 | 28.0 | 28.1 | 27.4 |



acetonitrile concentration, however, retention for both analytes was found to decrease when the concentration of acetonitrile was increased. This indicates that both of these analytes were retained by ionic interactions and not by reversed-phase interactions. Fexofenadine, MDL 102,038 and MDL 46,814 were affected by the changes in the mobile phase concentration of acetonitrile and this indicates that these analytes are retained predominantly by reversed-phase interactions. MDL 102,038 and ephedrone were not baseline resolved at lower concentrations of acetonitrile due to the higher retention of MDL 102,038, whereas fexofenadine and MDL 102,038 showed better resolution at lower concentrations of acetonitrile. Based on this information, the mobile phase concentration of acetonitrile will need to be carefully chosen and controlled so that the separation between MDL 102,038/fexofenadine and MDL 102,038/ephedrone are adequate.

Because of the great diversity with which the analytes present in the study were retained on the stationary phase, gradient elution was required for the separation. When the initial mobile phase contained a concentration of acetonitrile greater than 30%, pseudoephedrine/ephedrone were not baseline resolved. If the gradient produced an increase in the organic modifier that was steep, the more highly retained analytes decreased in retention with a corresponding decrease in resolution. Therefore, the

*Table 2.* Selectivity between key analytes as a function of acetonitrile concentration (mobile phase pH 6.9)

| % $CH_3CN$ | MDL 102,038/ fexofenadine | Ephedrone/ pseudoephedrine | MDL 102,038/ ephedrone |
|---|---|---|---|
| 25 | 1.20 | 1.09 | 1.06 |
| 27 | 1.18 | 1.13 | 1.11 |
| 28 | 1.17 | 1.12 | 1.16 |
| 30 | 1.15 | 1.13 | 1.26 |

initial organic modifier concentration of 30% acetonitrile was found to be the optimum amount, while the final organic modifier content was found to be acceptable at 60% acetonitrile at a mobile phase pH of 6.9 (Figure 4).

## Effect of Methanol on the Separation

The effect that different organic modifiers have on a separation may be significant due to differences in solvolysis. The separation using acetonitrile was acceptable, although not all of the analytes were baseline resolved. Therefore, methanol was used for the separation to determine if it would provide better resolution between all analytes.

The concentration of methanol in mobile phase A that was required for the separation of all ten analytes was determined and found to be 45% (see Figure 5 I). This compares to 30% acetonitrile that provided a similar separation (Figure 4). All of the analytes were baseline resolved using the methanol mobile phase. It appears as if methanol has a more favorable effect on the separation due to differences in solvolysis. Small changes in the amount of methanol in mobile phase A (45% versus 47%) were found



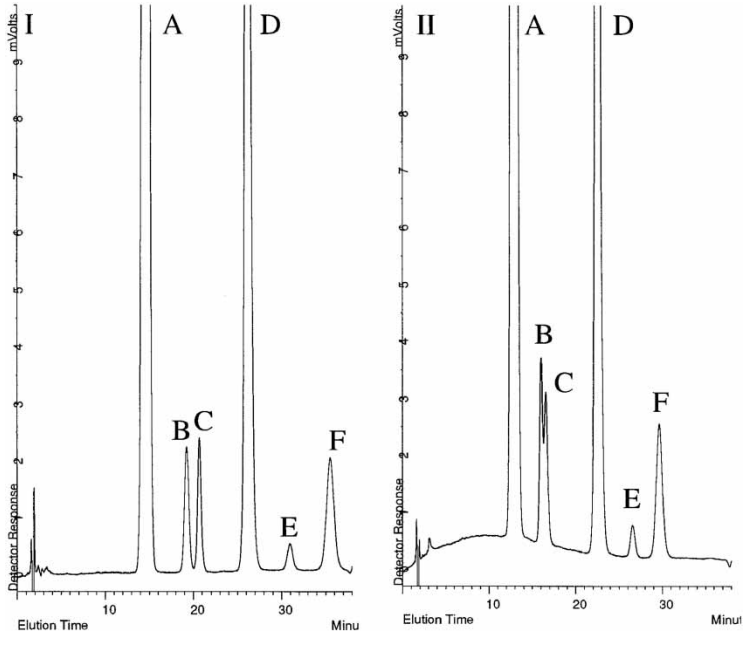


***Figure 5.*** The effect of methanol concentration on the fexofenadine separation. Mobile Phase: A) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, I) 45% $CH_3OH$, II) 47% $CH_3OH$. Analytes: A) fexofenadine, B) MDL 102,038, C) ephedrone, D) pseudoephedrine, E) ephedrine, F) MDL 46,814.

to have a significant effect on the separation as shown in Figure 5. The impact that a slight change in methanol concentration had on the separation required that each mobile phase be carefully prepared.

A major issue with the use of a methanol mobile phase is that at concentrations above 70%, which was required to elute all of the analytes in a reasonable amount of time, the phosphate buffer was found to precipitate out of solution. Therefore, the run time was longer when compared to the separations using acetonitrile, since mobile phase B contained 70% methanol, which is not equivalent to 60% acetonitrile. Since the phosphate buffer precipitated out at higher concentrations of methanol, a study was done to determine if a mixed methanol/acetonitrile mobile phase would produce similar results to the mobile phase that contained just methanol as the organic modifier. Several different ratios of methanol/acetonitrile were studied. It was determined that for mobile phase B, acetonitrile alone at a level of 55% would adequately separate the analytes in a reasonable amount of time. The separation was also optimized with respect to mobile phase A. The two mobile phases consisted



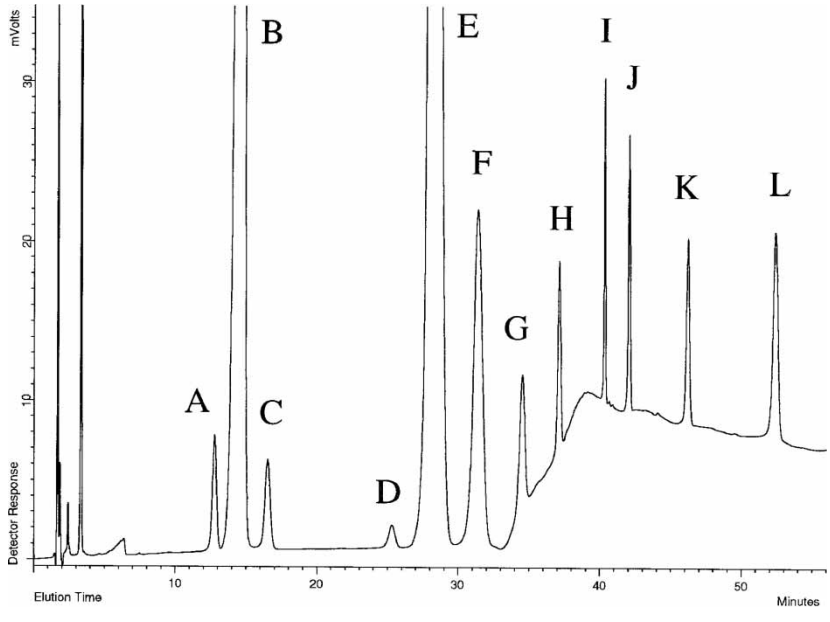


***Figure 6.*** The separation of fexofenadine, pseudoephedrine, and potential impurities by ion interaction chromatography using an initial mobile phase of acetonitrile and methanol. Mobile phase: A) 50 mM SDS, 15 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$, 28% $CH_3OH$; B) 50 mM SDS, 15 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. Analytes: A) MDL 47,397, B) fexofenadine, C) MDL 102,038, D) ephedrone, E) pseudoephedrine, F) ephedrine, G) MDL 46,814, H) MDL 47,794, I) MDL 4,829, J) MDL 17,523, K) MDL 46,619, L) MDL 46,016. Gradient: 1) 0–30 min 100% A, 2) 30–35 min 100% A to 0% A, 3) 35–55 min 0% A, 4) 55–65 min 100% A.

of: A) 50 mM SDS, 15 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$/28% $CH_3OH$; B) 50 mM SDS, 15 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. The gradient that was used was: 0 to 30 min 100% A, 30 to 40 min 100% A to 0% A, 40 to 55 min 0% A, 55 to 65 min 100% A. The separation employing these chromatographic conditions is presented in Figure 6.

## Effect of the Concentration of Ion Interaction Reagent

The amount of IIR that adsorbs onto a stationary phase has been shown to increase when the mobile phase concentration of the IIR is increased.[1,3,4,9–11] The increased adsorption of the IIR leads to an increase in the number of apparent cation exchange sites on the stationary phase and should lead to higher analyte cation retention. However, the concentration of countercation in the mobile phase is also increasing and results in greater competition for the cation exchange sites. Analyte retention will continue to increase until a certain concentration of IIR is reached, and the beneficial effect of more cation exchange sites will be overcome by the much higher concentration of countercation that competes for these sites (see equation (2)).

Table 3 lists the retention of several analytes obtained at different concentrations of SDS, while Table 4 shows the selectivity between three different critical pairs of analytes that must be resolved (MDL 102,038/fexofenadine; ephedrone/pseudoephedrine; MDL 45,814/ephedrine). The resolution between ephedrone and pseudoephedrine was not acceptable until a concentration of 30 mM SDS was reached and resolution continued to

***Table 3.*** Effect of the mobile phase concentration of SDS on analyte retention

| | Analyte (k′) | | | | | |
|---|---|---|---|---|---|---|
| mM SDS | Fexofenadine | MDL 102,038 | Pseudo[a] | Ephedrine | MDL 45,814 | MDL 46,016 |
| 0 | 13.3 | 22.9 | 1.4 | 5.7 | 29.6 | 33.8 |
| 5 | 13.4 | 18.6 | 12.2 | 17.2 | 29.4 | 34.4 |
| 10 | 14.0 | 18.7 | 13.0 | 22.1 | 29.3 | 35.7 |
| 15 | 14.5 | 19.2 | 15.4 | 25.8 | 29.5 | 36.4 |
| 20 | 14.9 | 19.0 | 18.5 | 27.4 | 29.6 | 36.4 |
| 30 | 13.4 | 17.3 | 19.7 | 27.2 | 29.3 | 36.9 |
| 40 | 11.6 | 14.7 | 17.3 | 25.3 | 28.2 | 37.2 |
| 50 | 10.3 | 12.9 | 16.8 | 22.3 | 27.2 | 37.1 |
| 60 | 9.4 | 11.7 | 16.4 | 20.1 | 24.9 | 36.9 |
| 70 | 9.0 | 9.8 | 13.6 | 17.3 | 19.5 | 36.7 |
| 100 | 5.9 | 7.1 | 10.3 | 12.2 | 12.9 | 32.3 |

[a]Pseudoephedrine.

*Table 4.* Selectivity between key analytes as a function of SDS concentration

| mM SDS | MDL 102,038/ fexofenadine | Ephedrone/ pseudo[a] | MDL 45,814/ ephedrine |
|---|---|---|---|
| 0 | 1.72 | 1.0 | 5.22 |
| 5 | 1.39 | 1.82 | 1.71 |
| 10 | 1.34 | 1.0 | 1.33 |
| 15 | 1.32 | 1.07 | 1.14 |
| 20 | 1.28 | 1.27 | 1.08 |
| 30 | 1.29 | 1.47 | 1.08 |
| 40 | 1.27 | 1.49 | 1.11 |
| 50 | 1.25 | 1.63 | 1.22 |
| 60 | 1.24 | 1.74 | 1.24 |
| 70 | 1.23 | 1.70 | 1.13 |
| 100 | 1.20 | 1.75 | 1.06 |

[a]Pseudoephedrine.



increase throughout the study. The best resolution for ephedrine and MDL 45,814 occurred at a SDS concentration of 50 mM, whereas resolution between MDL 102,038 and fexofenadine decreased until the SDS concentration reached 15 mM and then leveled off. The SDS concentration was found not to significantly affect the highly retained analytes throughout the study.

In the case of the critical pairs of analytes that must be resolved, a concentration of 50 mM SDS appears to provide the best compromise between retention, selectivity, and efficiency. Thus, this is the amount of SDS used throughout the rest of the study.

*Table 5.* Effect of mobile phase ionic strength on analyte retention (addition of NaCl)

| | Analyte (k′) | | | | |
|---|---|---|---|---|---|
| Ionic strength | Fexofenadine | MDL 102,038 | Pseudo[a] | Ephedrine | MDL 46,016 |
| 141 | 6.43 | 8.04 | 13.6 | 15.7 | 27.5 |
| 145 | 6.25 | 7.82 | 12.9 | 14.9 | 27.6 |
| 150 | 6.09 | 7.61 | 12.3 | 14.2 | 27.6 |
| 155 | 6.19 | 7.77 | 12.1 | 14.1 | 27.4 |
| 160 | 5.99 | 7.50 | 11.5 | 13.3 | 27.4 |
| 170 | 5.75 | 7.19 | 10.6 | 12.3 | 27.1 |
| 190 | 5.44 | 6.82 | 9.4 | 10.9 | 26.8 |
| 215 | 5.00 | 5.86 | 8.1 | 9.4 | 26.6 |

[a]Pseudoephedrine.

***Table 6.*** Effect of the mobile phase concentration of $H_3PO_4$ on analyte retention

| | Analyte (k′) | | | | |
|---|---|---|---|---|---|
| mM $H_3PO_4$ | Fexofenadine | MDL 102,038 | Pseudo[a] | Ephedrine | MDL 46,016 |
| 5 | 6.3 | 7.8 | 15.3 | 16.2 | 27.8 |
| 10 | 6.3 | 7.9 | 14.5 | 16.5 | 27.9 |
| 15 | 6.0 | 7.5 | 13.3 | 15.1 | 27.5 |
| 20 | 6.2 | 7.7 | 12.4 | 14.3 | 27.5 |
| 25 | 6.8 | 8.6 | 13.1 | 15.1 | 27.7 |
| 30 | 6.0 | 7.5 | 11.1 | 12.8 | 27.2 |
| 40 | 5.6 | 7.0 | 9.7 | 11.2 | 27.3 |
| 50 | 5.5 | 6.9 | 8.9 | 10.3 | 26.9 |
| 60 | 5.2 | 6.5 | 7.8 | 9.2 | 26.5 |
| 75 | 5.1 | 6.4 | 7.3 | 8.4 | 26.5 |

[a]Pseudoephedrine.



## Effect of Mobile Phase Ionic Strength

As the ionic strength of the mobile phase is increased, competition for the fixed cation exchange sites increases (equation (2)). This should lead to a reduction in retention of analytes that are retained predominantly by an ion exchange mechanism.[1,5–7,9–11]

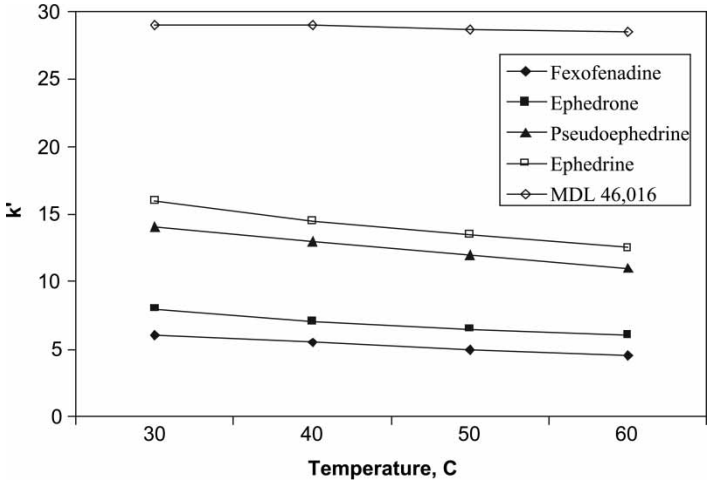


***Figure 7.*** The effect of column temperature on analyte retention. Mobile phase: A) 50 mM SDS, 15 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$, 28% $CH_3OH$; B) 50 mM SDS, 15 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. Gradient: 1) 0–30 min 100% A, 2) 30–35 min 100% A to 0% A, 3) 35–55 min 0% A, 4) 55–65 min 100% A.

In general, as the ionic strength of the mobile phase was increased (addition of NaCl), retention of the analytes decreased, especially for ephedrine/ephedrone/pseudoephedrine. The data in Table 5 shows the effect that the addition of NaCl to the mobile phase had on analyte retention. The analytes that were not highly retained were affected more by the mobile phase ionic strength than the highly retained hydrophobic analytes.

A second study was done where the concentration of the $H_3PO_4$ buffer was varied and this data is presented in Table 6. Analyte retention was found to decrease moderately as the concentration of $H_3PO_4$ was increased. The analytes that are predominantly retained by an ion exchange

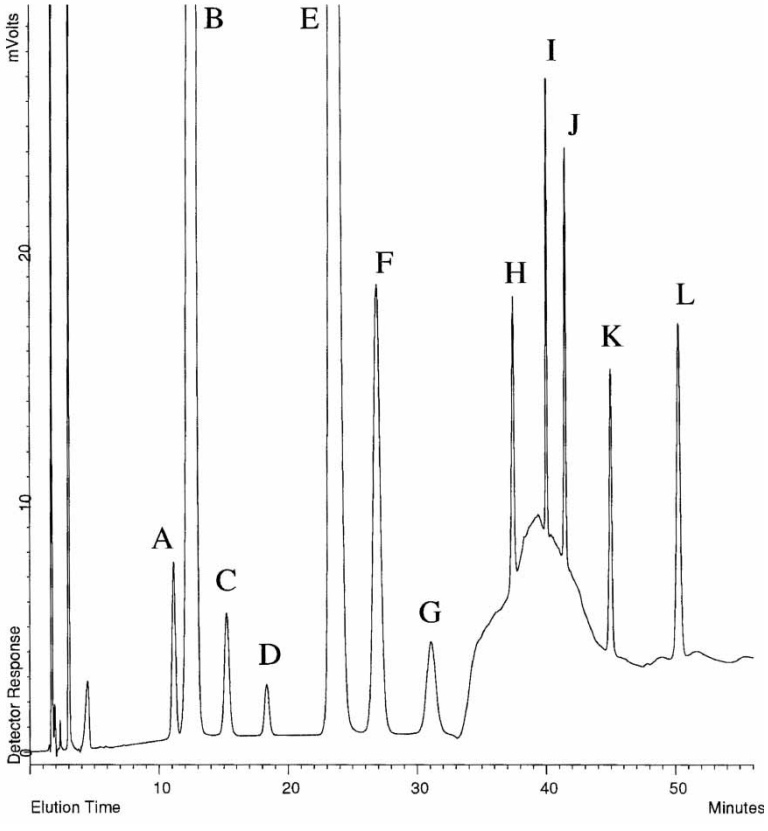


***Figure 8.*** The optimized separation of fexofenadine, pseudoephedrine, and potential impurities by ion interaction chromatography. A) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$, 28% $CH_3OH$; B) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. Analytes: A) MDL 47,397, B) fexofenadine, C) MDL 102,038, D) ephedrone, E) pseudoephedrine, F) ephedrine, G) MDL 46,814, H) MDL 47,794, I) MDL 4,829, J) MDL 17,523, K) MDL 46,619, L) MDL 46,016. Gradient: 1) 0–30 min 100% A, 2) 30–35 min 100% A to 0% A, 3) 35–55 min 0% A, 4) 55–65 min 100% A.

mechanism (pseudoephedrine, ephedrone, and ephedrine) were affected the most by the increased $H_3PO_4$ concentration, whereas the analytes that were retained by reversed-phase interactions showed only a small decrease in retention. It was determined from this data that the best separation for all of the analytes was a mobile phase composed of 50 mM SDS and 20 mM $H_3PO_4$.

## Effect of Column Temperature

The column temperature has been shown to affect chromatographic separations by changing the kinetics that take place between the analytes, the stationary phase, the mobile phase pH, and the IIR.[14,15] Typically, when

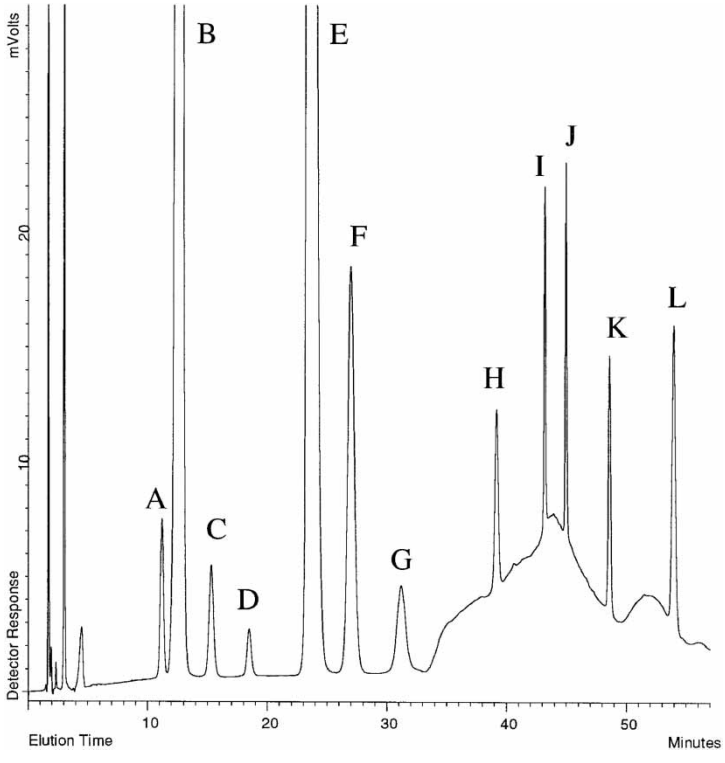


***Figure 9.*** The separation of fexofenadine, pseudoephedrine, and potential impurities by ion interaction chromatography using a longer gradient ramp time. A) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$, 28% $CH_3OH$; B) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. Analytes: A) MDL 47,397, B) fexofenadine, C) MDL 102,038, D) ephedrone, E) pseudoephedrine, F) ephedrine, G) MDL 46,814, H) MDL 47,794, I) MDL 4,829, J) MDL 17,523, K) MDL 46,619, L) MDL 46,016. Gradient: 1) 0–30 min 100% A, 2) 30–40 min 100% A to 0% A, 3) 40–55 min 0% A, 4) 55–65 min 100% A.

the column temperature is increased, analyte retention decreases. In most cases peak shape also improves.

The data obtained for the temperature study is presented in Figure 7 where the column temperature over a range of 30 to 60°C was studied. From this data, it was determined that a column temperature of 30°C was the optimal temperature when resolution, selectivity, and peak shape were all taken into account.

## Optimized Separation

The data from each study was used to determine the optimal separation conditions for fexofenadine, pseudoephedrine, and potential degradation products/impurities. The optimal mobile phase conditions consisted of: A)

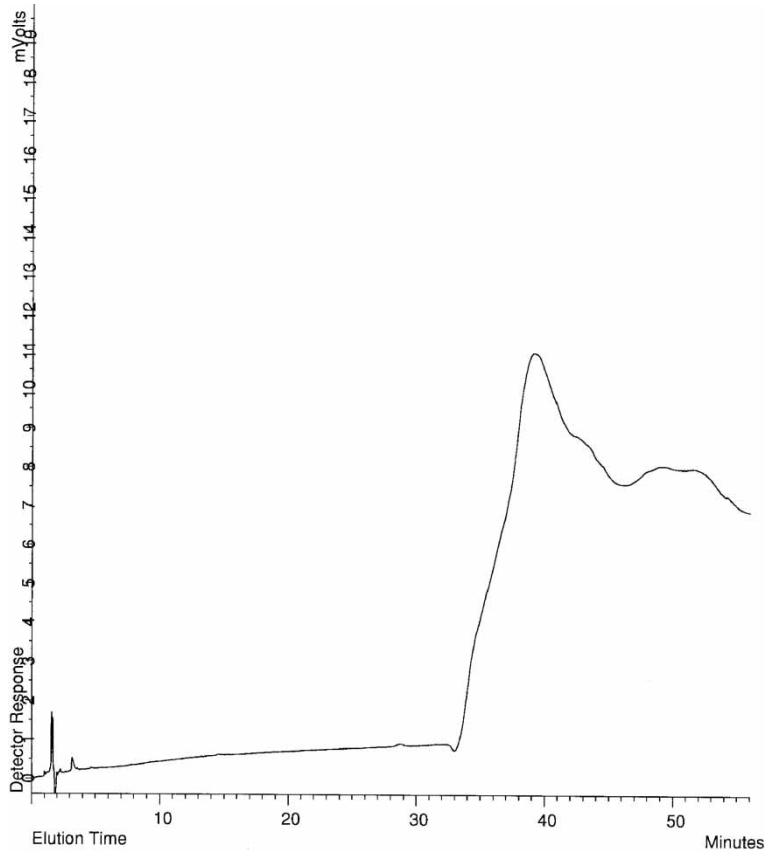


***Figure 10.*** A water blank injection. A) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$, 28% $CH_3OH$; B) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. Gradient: 1) 0–30 min 100% A, 2) 30–35 min 100% A to 0% A, 3) 35–55 min 0% A, 4) 55–65 min 100% A.

50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 15% $CH_3CN$, 28% $CH_3OH$; B) 50 mM SDS, 20 mM $H_3PO_4$, pH 6.9, 55% $CH_3CN$. A flow rate of 1.0 mL/min, a column temperature of 30°C, and an injection volume of 10 μL were all used. The gradient consisted of: 0–30 min 100% A, 30–35 min 100% A to 0% A, 35–55 min 0% A, 55–65 min 100% A. The optimized separation for a mixture of twelve analytes is presented in Figure 8. A longer gradient (30–40 min 100% A to 0% A) did not improve the separation significantly or cause an increase in the run time (Figure 9).

A water blank was injected into the chromatographic system in order to determine if the gradient would produce any extraneous peaks (Figure 10). No extraneous peaks were observed, only a shift in the baseline, which is due to differences in the refractive index of the organic modifiers during the gradient run.

## CONCLUSIONS

The use of mobile phases that contain an ion interaction reagent for the separation of fexofenadine, pseudoephedrine, and their potential impurities/degradation products was studied. The retention of the different analytes was found to be affected by the different mobile phase parameters. The various parameters were identified and studied. The separation, which was optimized with respect to the mobile phase components and the gradient profile, provided baseline resolution for all of the analytes of interest studied with a run time of 65 min.



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