Drug Development and Industrial Pharmacy, 31:577–588, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500214696

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A New HPLC Approach for the Determination of Hydrophilic and Hydrophobic Components: The Case of Pseudoephedrine Sulfate and Loratadine in Tablets

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ABSTRACT Effective isocratic separations of decongestants and antihistamines is a challenging analytical task due to wild differences in their lipohilicities (hydrophilic decongestants and hydrophobic antihistamines). In this paper a new approach for resolving such a problem is described taking pseudoephedrine sulfate and loratadine as an example. The chromatographic behavior of pseudoephedrine sulfate and loratadine on RP C18 and C8 columns were studied in presence and absence of sodium lauryl sulfate (SLS). The effect of combining two different types of stationary phases (cyano and C18 or C8) on the relative retention of the two compounds was investigated. In conclusion, it was found that the combination of a C18 column followed by a standard cyano column provides a stationary phase that separates both compounds effectively and within a reasonable time. This approach was compared to a literature method and demonstrated to have superior selectivity.

KEYWORDS Pseudoephedrine, Loratadine, HPLC, Combination stationary phase

INTRODUCTION

The use of medications containing a combination of antihistamine and a decongestant is quite common for alleviating symptoms of common cold and allergic rhinitis (Bronsky et al., 1995; Georgitis et al., 2000; Roman & Danzig, 1993; Serra et al., 1998; Weinstein, 2002). Perhaps the most common decongestant in such combinations is pseudoephedrine sulfate (Pseudo). Pseudo is one of the phenethylamine drugs that are characterized by a phenyl ring with an alkylamine chain. Other common decongestants used in cold preparations include phenylephrine, mephentiramine, and phenylpropanolamine (Weinstein, 2002).

Commercially available cold preparations usually contain an antihistamine such as triprolidine, chlorphineramine, or loratadine (Smith & Feldman, 1993).

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Loratadine (Lora) is a non-sedative second generation H1 receptor blocker (Kay & Harris, 1999). It is available commercially as mono component tablets (Claritin®) or in combination with the decongestant Pseudo (Clarinase®). Lora-Pseudo combinations are formulated to provide 12 or 24 h protection of allergic symptoms.

Several studies have reported the analysis of either compound separately. Pseudo has been analyzed in plasma and/or urine using gas chromatography (GC) (Brendel et al., 1988; Bye et al., 1975; Lai et al., 1979; Macek et al., 2002; Spyridaki et al., 2001; Till & Benet, 1979). HPLC methods have also been reported for the analysis of Pseudo in pharmaceutical preparations (Carnevale, 1983; Sagara et al., 1983). HPTLC method has also been developed for the determination of Pseudo in dosage forms (Makhija & Vavia, 2001). Many spectroscopic methods have been reported for the analysis of Pseudo either alone or in combination with different antihistamines including chlorpheniramine maleate and triprolidine (Davidson & El-Sheikh, 1982; Hoover et al., 1987). Lora has been determined using polarographic and spectroscopic methods (Ghoneim et al., 2001). Several chromatographic methods have been described for the determination of Lora in human plasma (Johnson et al., 1994; Kunicki, 2001; Sutherland et al., 2001). In pharmacokinetic studies, it is usually essential to determine the major metabolite of Lora (desethoxyloratadine, DEL), which is known to have significant pharmacological activity (Sutherland et al., 2001). It is also important to determine DEL in the quality control of raw material as it is a major breakdown product. Spectroscopic and chromatographic methods have been described for the simultaneous determination of Lora and DEL in raw material (Ruperez et al., 2002). Derivative spectroscopic methods have been described for the analysis of combination products that contain Pseudo, Lora, and ibuprofen (Ivanovic et al., 2000).

There has been no HPLC method for the determination of Lora-Pseudo combination (Clarinase®), but recently a study was published in which a reversed phase HPLC method for the simultaneous determination of Pseudo and Lora was described (Mabrouk et al., 2003). In the study, although the compounds have been shown to resolve within 4 min, Pseudo was eluted with the solvent front indicating insignificant retention. Significant retention is defined as $k>2$, where k is the capacity factor (Snyder et al., 1997). Consequently, it would be likely, and shown experimentally in this study, for Pseudo to interfere with other potential additives in the pharmaceutical preparations.

The problem of low affinity of Pseudo and phenethylamines in general to reversed phase (RP) stationary phases is well known (Gil-Agusti et al., 2001), and thus, if reasonable chromatography had to be achieved, some modifications would have to be made. Such modifications include the use of ion pairing agents to increase the retention of the hydrophilic Pseudo (Argekar et al., 1998; Chao et al., 1979; Ragonese et al., 2000). The low affinity of Pseudo to reversed phase packing material would be further complicated if it had to be separated from another hydrophobic substance such as loratadine or antihistaminic compounds in general (Carnevale, 1983; Chao et al., 1979). This is because at the retention time that would provide sufficient retention i.e., $k>2$ for Pseudo, Lora would elute at practically unacceptable time.

An obvious solution for such a problem would be to use a gradient HPLC technique. Gradient HPLC technique has been employed for the separation of the isomer of pseudoephedrine (ephedrine) from chlorphineramine (antihistamine) and codeine. However gradient elution suffers from long times that are required for equilibration between runs in addition to base line drifts [see for example Fig. 4 in Hood & Cheng (2003) and Snyder et al. (1997)]. Ion-pairing agents are known to help resolving such a problem in an isocratic mode of elution. This could be achieved by the differential interaction of the two compounds with the ion pairing agents, where the more hydrophilic compound might be influenced more relative to hydrophobic component and, consequently, the difference in the overall retention times between the two components decreases (Kord & Khaledi, 1992; Snyder et al., 1997).

Micellar liquid chromatography (MLC), where surface active agents are used at high concentrations in the mobile phase, has also been reported for the effective retention and potential separation of phenethylamine compounds on reversed phase (Gil-Agusti et al., 2001). Both ion pairing and MLC techniques are associated with long equilibration times e.g., 11 h, occasional appearance of extra peaks, and suboptimum reproducibility (Snyder et al., 1997).

The aim of this study was to find an HPLC method with significant retention ($k>2$) for such combination

of compounds (hydrophilic and hydrophobic) in a single isocratic run without the use of ion pairing agents or MLC. To achieve this goal, the potential of combination of two different stationary phases (C18 or C8 and Cyano) in achieving separation of such a mixture of hydrophilic and hydrophobic substances was investigated. Pseudo and Lora where chosen as a model of such combination. The hypothesis was that if the hydrophilic component (Pseudo) was not retained significantly on the C18/C8 column, then it would be retained on the second column (cyano). On the other hand, if the more hydrophobic component (Lora) was strongly retained on the C18/C8 column, then it would not be retained significantly on the second column (cyano).

MATERIALS AND METHODS

Materials

Working standards of Lora (99.8%) and Pseudo (98.7%) were kindly provided by Jordan-Sweddish Pharmaceutical Company (Jordan). Clarinase® tablets (5 mg Lora and 120 mg Pseudo) were purchased from local market (Batch No. 2D1044C). HPLC grade acetonitrile (actn), methanol, and tetrahydofuran were purchased from Merck (Dermstadt, Germany). All other reagents were purchased from Sigma (USA). Distilled water was filtered through 0.45 μm before use.

Equipment

Lachrom (Merck Hitachi) HPLC instrument equipped with L-7400 UV detector, L-7100 pump, and D-7500 integrator. Columns used were: Hypersil C18 (150 × 4.6 mm, 5 μm), Hypersil C8 (150 × 4.6 mm, 5 μm), Tracer Extrasil CN (150 × 4.6, 5 μm), and a guard column manually packed with CN material (4 cm). Injection loop of 20 μl (Rheodyne) was employed.

Preparation of Stock Solutions

Loratadine

A stock solution of loratadine (0.2 mg/ml) was prepared in 1% acetic acid solution. Aliquots of this solution were diluted with the mobile phase to obtain eight solutions having concentrations from 0.1 to 100 μg/ml.

Pseudoephedrine Sulfate

A stock solution of Pseudo (10 mg/ml) was prepared in 1% acetic acid solution. Aliquots of this solution were diluted with the mobile phase to obtain eight solutions having concentrations in the range 10 to 600 μg/ml. Calibration curves were constructed by plotting the peak area of the corresponding analyte against its concentration.

Preparation of Standard Mixture

A standard mixture of both compounds was prepared to evaluate each of the examined conditions. Lora (25 mg) and Pseudo (600 mg) were dissolved in 0.1 M HCL in a 50 ml volumetric flask then diluted (1:10) with 50% Actn/water.

Method Development

The effect of proportion of Actn and the inclusion of sodium lauryl sulfate (SLS) in mobile phase on the retention and separation of Lora and Pseudo using single columns (Standard C18 or C8) was investigated. The effect of combining two stationary phases and the order of the combination on the retention and separation of the two compounds was systematically addressed according to the obtained compound retention and resolution. Accordingly, the manually packed CN column was attached before the C18 or C8 columns, and after the C8 column, and then the standard packed cyano column after the C8 or C18 columns, were used for the effect of stationary phase combination. All mobile phases used in method development were prepared by mixing filtered ion pairing agent solution (when required) and/or buffer system with the organic solvent in the desired proportions. The apparent pH of the mixtures was adjusted to desired value using either acetic acid, 1 M KOH, or ammonium acetate solution. Mobile phases were then filtered through 0.45 μm membrane filters and sonicated before being used for chromatography.

Proposed Chromatographic Method

From the method development, the finally recommended conditions for the separation and

quantification of Lora and Pseudo utilized a combination of columns C18 (150×4.6 mm, 5 μm) followed by Tracer Extrasil CN (15×4.6, 5 μm). The mobile phase consisted of acetonitrile:10 mM ammonium acetate buffer (80:20 v/v), pH adjusted to 6.7. The mobile phase was prepared daily and delivered at a flow rate of 1.5 ml/min. The detector wavelength was set at 250 nm.

Validation and Application of the Proposed Chromatographic Method

The proposed method was validated in light of ICH Guidelines (1994, 1996) for linearity, precision, selectivity, sensitivity, and recovery. Selectivity was tested with respect to formulation additives and possible degradation products, while recovery was assessed by applying the proposed method for the assay of Pseudo and Lora in the commercial product Clarinase®. Consequently, the following were performed.

Preparation of Calibration Curves (Linearity)

Eight solutions of each of the two drugs were prepared as previously described. Calibration curves were constructed for each drug in the specified concentration range. Linearity was examined using the least square method within Excel program. Three calibration curves were constructed and the average values for intercepts, slopes, and correlation coefficients were reported.

Precision

Both repeatability (within a day precision) and intermediate precision (between days precision) were determined as follows: Solutions containing lowest, intermediate, and highest concentrations on the calibration curves for each compound i.e., 0.1, 50, and 100 μg/ml for Lora, and 10, 300, and 600 μg/ml for Pseudo were prepared. Nine injections of each compound at each of the specified concentration levels were obtained within the same day for repeatability, and over a period of 5 days (1–3 injections/day) for intermediate precision. Relative standard deviation was obtained and used to judge precision of the method.

Sensitivity (Quantification Limit)

Although it is not a must to determine the quantification limit according to the ICH Guidelines (1994, 1996) for the assay procedure (purity and potency), it was determined in this study. Quantification limit was determined by the two approaches recommended by ICH Guidelines which are 1) acceptable precision of the lowest concentration and 2) signal:noise ratio = 10.

Degradation Study (Selectivity)

Solutions of Lora (0.1 mg/ml) and Pseudo (2 mg/ml) were prepared in 0.1 M HCL and 0.1 M NaOH, separately. All of the four solutions were refluxed at 70°C for 5 h, filtered and neutralized before being injected into the chromatograph. For the purpose of identifying the major degradation product of lora, the residue of the degradation mixture, 0.5 g, was subjected to separation on silica gel column chromatography using 60 g of silica (mesh 70–230, GCC, UK). Mixture of ethyl acetate and methanol (80:20) was employed as a mobile phase. Fractions were collected from the column in volumes of 20 ml each. Total volume used was 460 ml and the degradation product appeared to elute within the first 60 ml of the mobile phase. The collected mobile phase containing the degradation product (first 60 ml) were evaporated to dryness and IR (KBr disks) were obtained. IR spectra for Lora sample subjected to similar treatment, i.e., dissolved in a mixture of ethylacetate and methanol (80:20) and then evaporated to dryness, were obtained and compared to that of the major degradation products. IR spectra were also obtained for DEL extracted (using ethyl acetate) from Aerius® tablets (Batch No. CA3STBC16E) in a similar manner. HPLC retention times of the extracted DEL from Aerius® tablets and that of the degradation product were also compared.

Assay of Lora and Pseudo in Clarinase® Tablet

Ten tablets were finely powdered and a portion equivalent to 25 mg of Lora and 600 mg Pseudo was suspended in 0.1 M HCL (50 ml volumetric flask). The suspension was sonicated for 15 min, completed to volume with 0.1 M HCL, filtered, and then diluted 1:10 ml with mobile phase. The concentration of Lora and Pseudo were calculated from the linear regression equations of calibration curves.

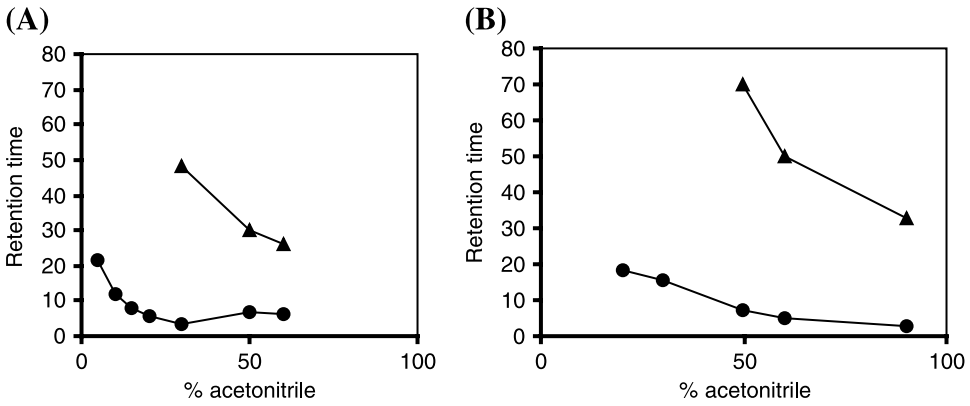

FIGURE 1 Plot of Retention Time of Pseudo (●) and Lora (▲) Against the Percentage of Actn in Mobile Phase (pH 3.2). In (A), C18 Column was Used and in (B), C8 Column was Used.

RESULTS AND DISCUSSION

Effect of Acn/Mobile Phase Proportion on Elution from Single Columns

The chromatographic behavior of both compounds (Pseudo and Lora) on C18 and C8 columns was studied at pH 3.2 and a flow rate of 0.8 ml/min. The ratio of organic component (acetonitrile, Actn) was varied in the range 5–90% and the observed retention times were plotted against the percentage of Actn. Figure 1A and 1B show plots of retention times of both compounds against the percentage of Actn using C18 and C8 columns, respectively. It is evident from both plots (C18 and C8) that a wild difference exists in the retention of the two compounds. At the lowest percentage where Lora can still be detected (30% with C18 and 50% with C8), there was a more than 45 min gap between the elution times of the compounds. In both cases, a gradient elution would be concluded necessary if the two compounds were to be retained effectively ($k>2$) and resolved within a reasonable time according to recommendations of Snyder.

Effect of Sodium Lauryl Sulfate (SLS) on Elusion from Single Columns

The effect of sodium lauryl sulfate on the relative retention of the two compounds was examined using C8 column. Using a flow rate of 2 ml/min and 90% Actn at pH 3.2, the effect of SLS was examined in the concentration range 0.03–0.25 mg/ml. Figure 2 represents a plot of the obtained retention times against the corresponding concentration of SLS in the mobile phase. The plot in Fig. 2 indicates that SLS had a more drastic effect on Lora than Pseudo. The retention time of Lora was decreased immediately from 33 min to 8 min when the lowest concentration of SLS was employed (0.03 mg/ml). The opposite effect (at the same concentration of 0.03 mg/ml) was observed on Pseudo, where the retention time was increased from about 3 min to about 9 min. This effect might be explained as a result of ion pair formation between either compound and SLS. Differences in hydrophilicity between the two compounds decreases as a result of ion pair formation. Thus, at that concentration of SLS, not only both compounds had close values of k,

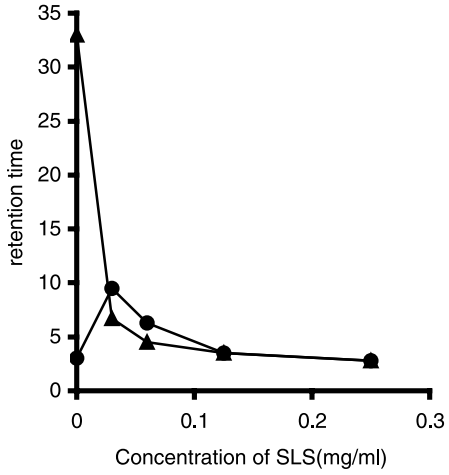

FIGURE 2 Plot of Retention Time of Pseudo (●) and Lora (▲) Against the Concentration of SLS in Mobile Phases Composed of 90% Actn (pH 3.2) Using C8 Column.

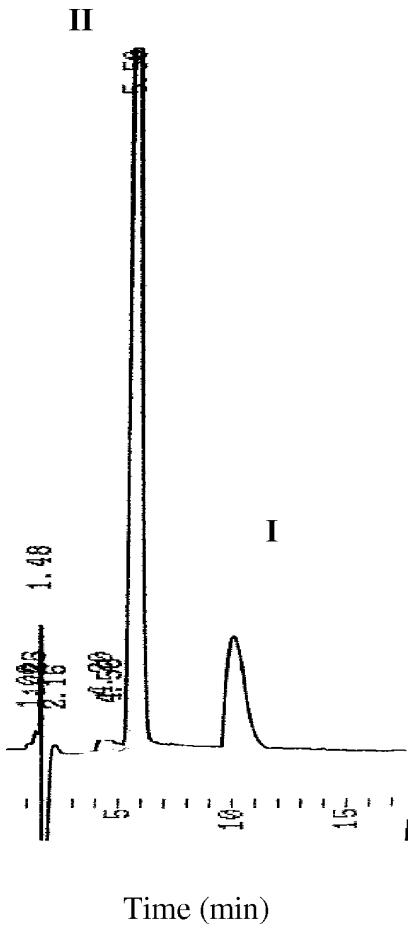

FIGURE 3 Representative Chromatogram of Pseudo (I) and Lora (II) Using a Mobile Phase Composed of 90% Actn and Having 0.03 mg/ml SLS (pH 3.2).

but also a reversal of the elution order of the two components could be achieved (Fig. 3). In spite of reasonable retention for both compounds, the condition was not satisfactory as the peak of Pseudo became significantly broad at useful concentrations of SLS. It is evident from Fig. 2 that the potentially useful range of SLS concentrations is limited to the range (0.03–0.125 mg/ml). At concentrations less than 0.03 mg/ml, Lora starts to significantly retain on the column, while at concentrations higher than 0.125 mg/ml, both compounds behave similarly and co-elute at very short times. Consequently, none of the concentrations within the specified range produced satisfactory resolution and retention of the two compounds.

Therefore, attempts to produce satisfactory retention and resolution of the two test compounds using single columns were concluded unsuccessful, and combination of two stationary phases was attempted next.

Attempts with Combination of Two Stationary Phases

C18 or C8 with Manually-Packed Guard Column with Cyano Backing Materials

The effect of guard column manually filled with cyano backing material and connected before a standard C18 column was investigated. For this purpose, chromatograms were obtained with and without the guard column (4 cm). A significant improvement in the retention of Pseudo was observed when cyano guard column was employed (Fig. 4). In spite of the

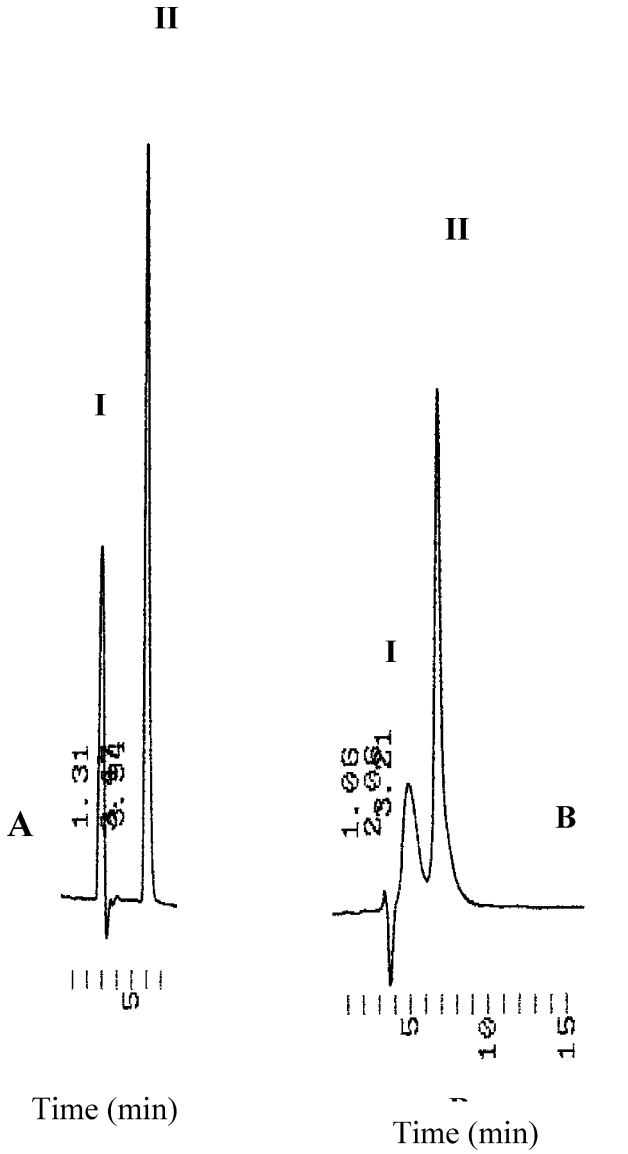

FIGURE 4 Representative Chromatograms of Mixtures of Pseudo (I) and Lora (II) Using a Mobile Phase Consisting of 90% Actn and C18 Column Without a Guard Column (A) and with Manually-Packed Cyano Guard Column (B).

significant improvement in the retention for Pseudo as a result of including a cyano column before the standard C18 column, the condition was still not optimum as the peaks became broad especially for Pseudo. However, these results were encouraging to further investigate the influence of combinations of columns on the chromatographic behavior of the two compounds. The C18 column was replaced with a C8 column and the effect of a cyano guard column was studied using different percentages of Actn in mobile phase. Generally, the retention time of Lora did not change significantly, while it was prolonged progressively for Pseudo with the increase in Actn. It was interesting to observe that the change of percentage organic from 90 to 95% led to reversal of the order of elution of the two compounds where Pseudo eluted after Lora. This can be explained as retention of Pseudo on cyano column appeared to be controlled by a normal phase mechanism (hydrophilic interaction). When the percentage of the relatively weak solvent according to normal phase principles (Actn) was increased from 90 to 95%, a chance was provided for the hydrophilic Pseudo to be retained more on the cyano column. As the hydrophobic Lora did not appear to have significant affinity towards the more hydrophilic cyano, it was not affected significantly by that change.

Effect of pH on the Elution Behavior (Cyano and Standard C8)

The pH effect was examined in the range 3–5.5 using a mobile phase consisting of 90% Actn with the combination of a guard cyano column followed by a standard C8 column. As basic compounds with expected pka values >8 (Ragonese et al., 2000), both compounds were expected to be fully ionized in the examined pH range (positively charged). However, the increase in pH from 3 to 3.5 was found to extend the overall retention time of both compounds with Pseudo being more seriously affected (Fig. 5). This effect might be explained as a result of ionization of the acidic silanol groups at higher values of pH. Thus a normal phase mechanism contributes to the retention of the test compounds (particularly Pseudo) as the pH is increased. This explanation accords with the peak broadening observed at higher pH values which is well known for the interaction of the basic

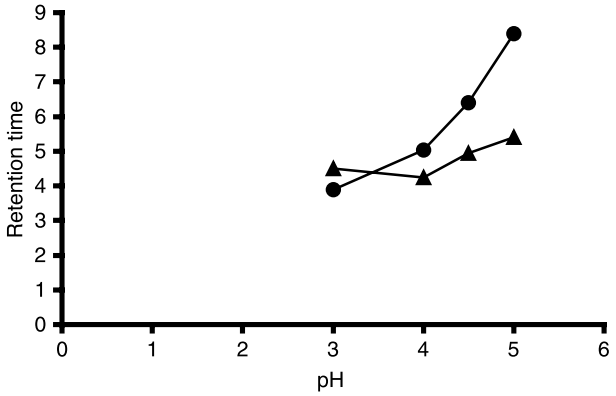

FIGURE 5 **Plot of Retention Times of Pseudo (●) and Lora (▲) Against pH Using Manually-Packed Cyano Column Attached Before a Standard C8 Column and a Mobile Phase Consisting of 90% Actn.**

compounds with the free silanol groups. Figure 5 indicates that better resolution could be obtained at higher values of pH. However, higher values of pH were associated with peak broadening that prohibited effective resolution.

Effect of the Order of Column Combination (Cyano and Standard C8)

The effect of the order of the columns was examined by placing the standard C8 column first followed by the manually-packed cyano column while keeping the mobile phase composition fixed (90% Actn, pH 6.7). Considerable improvement in peak shape was obtained as a result of changing the order of the columns and particularly for Pseudo (Fig. 6). Thus, when the cyano column was connected after the C8 column, higher efficiency was obtained (especially for Pseudo). This effect might be explained as follows: when the C8 column was connected after the cyano column, Pseudo was retained to some extent on the cyano column and then some degree of remixing occurred due to lack of retention on the late C8 column i.e., C8 worked as extra column length only. On the other hand, when cyano was connected after the cyano column and, hence, the effective retention of Pseudo occurred on the later cyano column, there was minimum of post packing volumes; thus the efficiency was maintained. Nevertheless, none of the examined conditions provided satisfactory retention and resolution of the test compounds using this assembly.

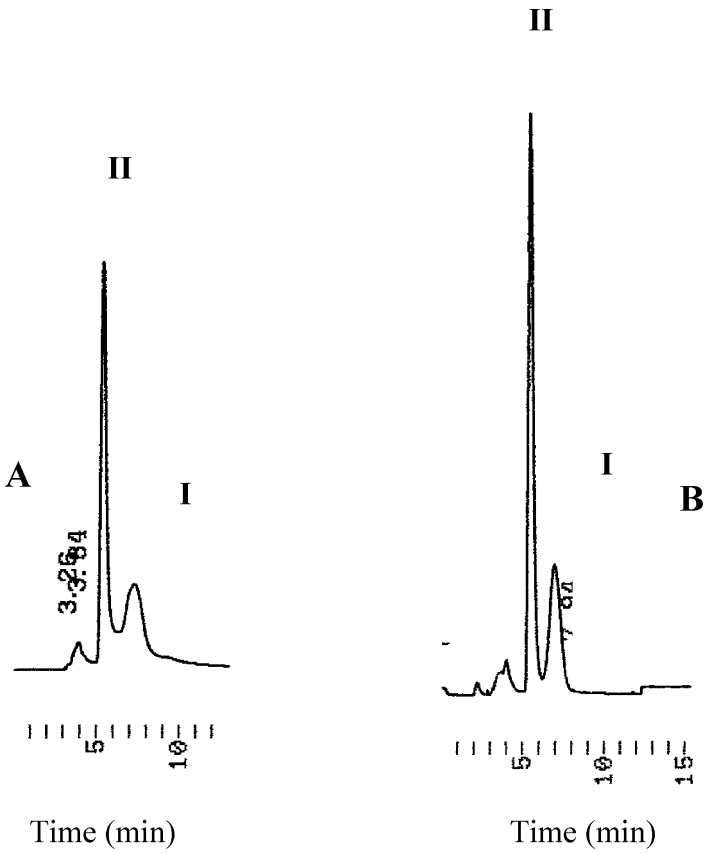

FIGURE 6 Effect of the Order of Column Combination on the Retention of Pseudo (I) and Lora (II). In (A), a Manually-Packed Cyano Column was Connected Before the Standard C8 Column. In (B), the Opposite Order of the Columns was Used.

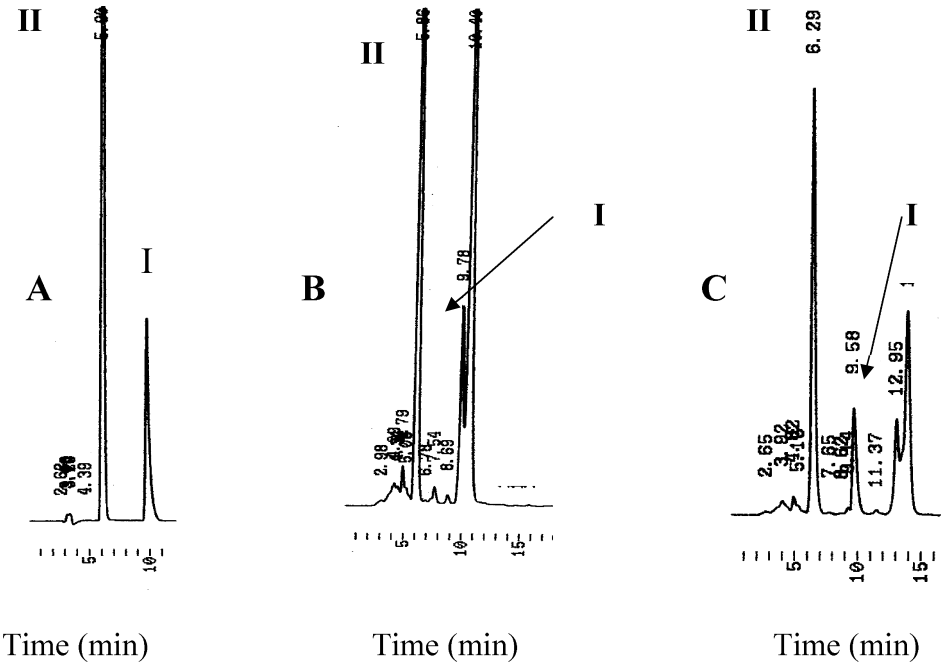

FIGURE 7 The Effect of Standard Cyano Column C8 or C18 Combination on the Retention of Pseudo (I) and Lora (II). In (A) and (B), Chromatograms were Obtained with a C8 Followed by a Standard Cyano Column with Mobile Phase Consisting of 80% actn (pH 6.7). In (C), a C18 Column was Followed by a Standard Cyano Column. Samples Injected were a Standard Mixture of Pseudo (I) and Lora (II) in (A); the Extract of Clarinase® Tablet in (B) and (C).

Effect of Ammonium Acetate Buffer

The effect of ammonium acetate concentration was examined in attempts to improve the retention and resolution of the test compounds. The pH was attempted to be controlled using ammonium acetate buffer instead of simple adjustment with acetic acid. Initial experiments showed significant effect of ammonium acetate (10 mM) on the chromatographic properties of test compounds. While the retention time of Lora remained almost constant (5–6 min), that of Pseudo decreased from about 11 min to less than 2 min. Significant improvement in the peak shape of Pseudo was achieved. However, the retention of Pseudo was still unsatisfactory ($k<1$) and the two compounds did not resolve sufficiently at that condition, but due to the improvement in the peak shape of Pseudo, ammonium acetate was used for pH adjustment in the next experiments.

Effect of Standard (Ready-Packed, Extrasil) Cyano Column

All previous attempts were not considered satisfactory in providing adequate resolution and retention of the test compounds. The manually-packed guard column has a length of only 4 cm and a particle size of the packed material of 10 μm, while the standard cyano column has a length of 15 cm and a packed material size of 5 μm. These differences, and the less efficient manual packing compared to standard packing, were the reasons for trying the standard column instead of the manually-packed column. Accordingly, a standard cyano column (15×4.6, 5 μm) was connected after the standard C8 column so that more efficient polar stage is involved in the system. Initial experiments with this setup showed promising results. A mobile phase composed of Actn (80%) was found satisfactory for separating the two compounds within a reasonable time (Fig. 7a). There was a clear dramatic increase in peak sharpness for both compounds and Pseudo in particular. The addition of SLS into the mobile phase was found not to be necessary for the effective separation of the two compounds. Thus, an isocratic condition was developed that was capable of separating the test compounds without the need for ion pairing agent and within a reasonable time (12 min). However, when the condition was applied to the commercially available Pseudo-Lora tablets (Clarinase®), an unknown peak was found to overlap the peak of Lora (Fig. 7b). Thus, the conditions were changed around the recommended one in order to resolve the observed unknown peak which appeared to correspond to some of the inactive additives in Clarinase® tablets. All attempts (so far) failed to obtain a reasonable separation of all components. When the C8 column was replaced with a standard C18 column, a complete resolution of all components was obtained (Fig. 7c). This clearly shows the potential of this approach to achieve the desired separation through manipulating various parameters. In comparison to a previously reported method (Mabrouk et al., 2003) for the analysis of Pseudo-Lora combination (Clarinase®), the proposed method was shown to be more reliable. The reason is that in the reported method only two peaks were observed in the chromatogram of the sample

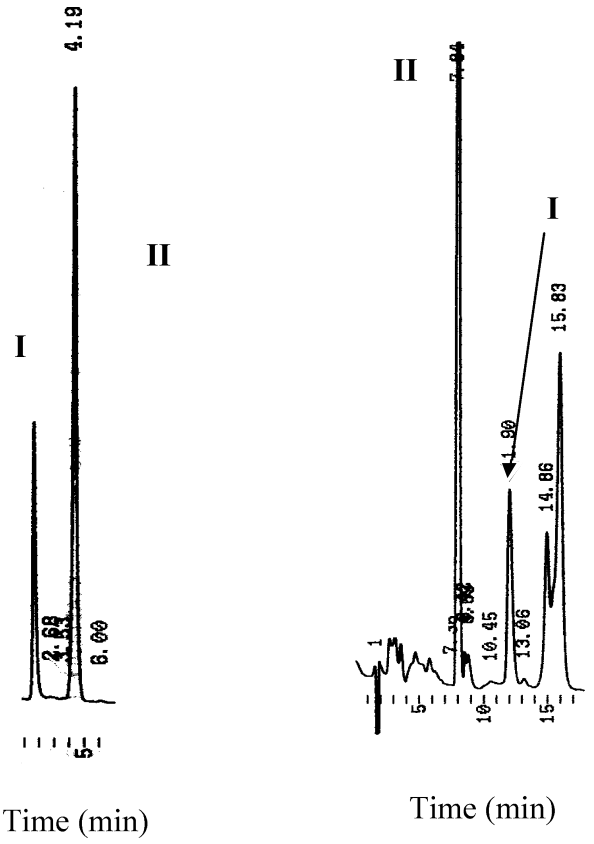

FIGURE 8 **Representative Chromatogram Samples of Pseudo and Lora Extracted from Clarinase® Tablet. In (A), the Sample was Prepared and Chromatographed as Recommended by (Snyder et al., 1997). In (B), the Same Sample of (A) but Chromatographed Using the Proposed Conditions.**

which tends to suggests that the reported method was either not selective enough i.e., an overlap between the peaks of components existed, or the reported method utilized sample preparation procedures so that only the test compounds were extracted from the tablet. In order to examine for these possibilities, samples from Clarinase® tablets were prepared according to the method reported in Mabrouk et al., 2003 and then chromatographed using both the reported method and the proposed method. Figure 8 shows that the same sample of Clarinase® (prepared as recommended by Mabrouk et al. 2003) produced two peaks when chromtographed using the conditions of the reported method and four peaks when chromatographed using the proposed conditions. These observations further stress the need to have adequate retention for the hydrophilic components when having such mixtures of hydrophilic and hydrophobic components. A relevant example was the separation of Lora from its major metabolite (DEL). DEL is significantly more hydrophilic than Lora making a gradient system necessary for their effective separation (Sutherland et al., 2001). However, at least one literature method could be found that describes (recommends) sub-optimum retention of DEL due to lack of retention (El-Ragehy et al., 2002).

Validation and Application of the Proposed Method

Linearity

The linearity of the method was examined using eight concentration points in the concentration range 10–600 μg/ml for Pseudo and 0.1–100 μg/ml for Lora. Linearity was established over the examined concentration ranges and the average linear regression equations of the two compounds were:

Lora	$Y = 352400X$	$(r^2 = 1)$
Pseudo	$Y = 4519.6X + 7140.1$	$(r^2 = 0.9997)$

Precision

Nine injections of each compound were obtained within the same day or over a period of five days. The summary of precision data is presented in Table 1. Overall, the data summarized in Table 1 enabled the conclusion that a satisfactory precision was obtained.

Sensitivity

Arbitrary examined lowest concentrations (0.1 mg/ml for Lora and 10 mg/ml for Pseudo) were significantly lower than required for analysis of both compounds in the dosage form (5 mg Lora and 120 mg Pseudo per tablet). However, the lowest limit of quantification was determined according to ICH Guidelines (1994, 1996). Accordingly, 0.1 μg/ml for Lora and 10 μg/ml for Pseudo were decided as the lowest limits of quantifications because it was shown that high precision can be achieved at these concentration levels. If a signal 10 times the noise level was taken as the limit of quantification (ICH Guidelines, 1994, 1996), then the limit of quantification would be 10 ng/ml for Lora and 150 ng/ml for Pseudo.

Selectivity

The selectivity of the method was ensured through two approaches. In the first approach (purposeful degradation), saturated solutions of each of the test

TABLE 1 Within Day and Between Days Precision (RSD) of Pseudo and Lora Using the Proposed Condition ($n = 9$)

	Precision (RSD)			
	Between days (intermediate precision)		Within a day (repeatability)	
Concentration* (μg/ml)	Pseudo	Lora	Pseudo	Lora
Lowest	1.46	1.73	1.30	1.65
Intermediate	1.41	1.66	1.31	1.26
Highest	1.21	1.11	1.20	1.03

*Lowest, intermediate, and highest concentrations examined were 10, 300, and 600 μg/ml for Pseudo and 0.1, 50, and 100 μg/ml for Lora, respectively.

compounds were refluxed in either 0.1 M NaOH or 0.1 M HCl. After filtration and neutralization, the reaction mixture was injected onto the proposed HPLC conditions and chromatograms examined for extra peaks. For Pseudo, no new peaks were observed and the peak area obtained remained within the expected range for a standard solution of Pseudo. For Lora, a new peak was observed (when refluxed in NaOH) which eluted at about 14 min and well-separated from either Pseudo or Lora. This new peak was attributed to the major degradation product of Lora (DEL). Identity of the degradation product was confirmed to be DEL through two approaches: 1) by injecting solutions of DEL from Aerius® tablets and comparing the obtained retention time with that of the degradation product (14 min) and 2) by comparing IR spectra of DEL extracted from Aerius® tablets to that for the obtained degradation products. In conclusion, IR spectra and retention times on HPLC were matching for DEL and the obtained degradation product which confirms the identity of the latter as DEL. Thus, the proposed method was also capable of separating Lora from its main degradation product (stability indicating assay). The ability of the proposed method to separate DEL which is known to be significantly more hydrophilic than Lora, and consequently difficult to separate in one chromatographic run (Sutherland et al., 2001) is another demonstration of the potential of this approach for separation of such mixtures of compounds. In the second approach, the potential interference of some expected additives with the chromatography of the two test compounds was examined. Some excipients (povidone iodine, starch, lactose, hydroxymethyl cellulose, titanium dioxide, stearic acid) were prepared at similar range that is expected to be found in the tablet and subjected to analysis according to proposed procedure. Results have indicated no potential interference (overlapping peaks) in the analysis.

Recovery and Application to Commercial Tablets (Clarinase®)

The method was applied for the determination of Pseudo and Lora in Clarinase® tablets. Average ($n=5$) percentages per label for Lora and Pseudo in commercial tablets were 97.7% (RSD=1.22) and 98.3% (RSD=1.36), respectively. Recovery of the proposed method was assessed by adding standard amounts (20% of the labeled content) of the two compounds to powdered Clarinase® tablets i.e., spiking in the solid form. Spiked samples were analyzed using the proposed method and the recovered amount (from the added standard) was calculated by subtracting the amount present in the tablets (practically found by direct application of the proposed method) from the practically found for the spiked tablets. Accordingly, average recovery ($n=5$) was estimated to be 99.8% (RSD=1.21) and 99.5% (RSD=1.39) for Lora and Pseudo, respectively.

CONCLUSION

In conclusion, the chromatographic behavior of Pseudo and Lora was studied in presence and absence of ion pairing agents. No satisfactory condition was found using either C18 or C8 alone for the separation of Pseudo and Lora. Using a combination of C18 followed by a cyano column enabled optimum separation and retention of both compounds. Comparison with previously published methods showed the importance of providing adequate retention of the hydrophilic components. The proposed method was validated in terms of selectivity, linearity, precision, sensitivity, and recovery with satisfactory results and applied for the analysis of commercial Clarinase® tablets.

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