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Rapid separation of desloratadine and related compounds in solid pharmaceutical formulation using gradient ion-pair chromatography

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ABSTRACT

We reported the development of an ion-pair chromatographic method to separate desloratadine and all known related compounds in Clarinex Tablets, which use desloratadine as active pharmaceutical ingredient (API). For the first time, baseline separation for desloratadine and all known related compounds was achieved by utilizing a YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size) and a gradient elution method. The mobile phase A contains 3 mM sodium dodecylsulfate (SDS), 15 mM sodium citrate buffer at pH 6.2, and 40 mM sodium sulfate, while the mobile phase B is acetonitrile. Chromsword®, an artificial intelligence method development tool, was used to optimize several key chromatographic parameters simultaneously including buffer pH/solvent strength, and temperature/gradient profile. The resolution of desloratadine and desloratadine 3,4-dehydropiperidine derivative, one of the critical pairs was improved by adding 40 mM sodium sulfate. Ultraviolet detection at 267 nm was used to achieve the detection for desloratadine and all compounds. This method has been successfully validated according to ICH guidelines in terms of linearity, accuracy, quantitation limit/detection limit, precision, specificity and robustness. It could be used as a stability indicating method for desloratadine drug substances or drug products that use desloratadine as active pharmaceutical ingredient.

1. Introduction

Desloratadine is a tricyclic antihistamine, which is an orally active nonsedating, peripheral histamine H1-receptor antagonist. It has been used as the active pharmaceutical ingredient (API) in several drugs to treat allergy symptoms such as Clarinex. There are six desloratadine related degradation products that have been identified by Schering-Plough Research Institute (Kenilworth, NJ, USA) including desloratadine despiperidyl keto derivative (DKD); N-formyl desloratadine (NFD); N-acetyl desloratadine (NAD), desloratadine N-oxide pyridine derivative (OPD); desloratadine N-oxide hydroxypyridine derivative (OHD); and desloratadine N-hydroxypiperidine derivative (DHD). In addition, there are three synthesis related impurities including desloratadine fluoro derivative (DFD), desloratadine 3,4-dehydropiperidine derivative (DDD), and N-methyl desloratadine (NMD). Loratadine, also an antihistamine compound, is often cited as a related compound in desloratadine, although in desloratadine drug substance or drug product, loratadine typically is not detected. The structures of these desloratadine related compounds are shown in Fig. 1. Identification and quantitation of these desloratadine related impurities and degradation products is critical to maintain high quality of the drug products.

Since desloratadine is an active metabolite of loratadine, the separation of desloratadine from loratadine is required for a loratadine stability indicating method. The separation of loratadine and desloratadine using high performance liquid chromatography (HPLC) methods has attracted interest and several HPLC methods have been developed [1–3]. For example, Qi reported the separation of loratadine and desloratadine on a Diamonsil BDS C_{18} column using heptanesulfonic acid sodium salt as ion pairing agent and methanol as eluent [1]. Ruperez reported the separation of loratadine and seven loratadine related compounds including desloratadine, DKD, NMD etc. on a SymmetryShield RP8 column using phosphoric acid/triethylamine buffer at pH 7.0 and methanol as eluent [2]. Krishna Reddy developed a HPLC/MS method to study the degradation chemistry of loratadine [3]. However, to the best of our knowledge, the separation of desloratadine and all known desloratadine related compounds in one single method has not been achieved yet.

As shown in Fig. 1, there are multiple critical pairs that have similar physicochemical characteristics such as desloratadine vs. DDD; OPD vs. OHD; NFD vs. NAD etc. From the molecular structure, it is also clear that several basic compounds such as desloratadine, DFD, DDD, NMD, and DHD have very weak retention on most reverse phase stationary phases such C_{18}, C_8, Cyano or Phenyl. This

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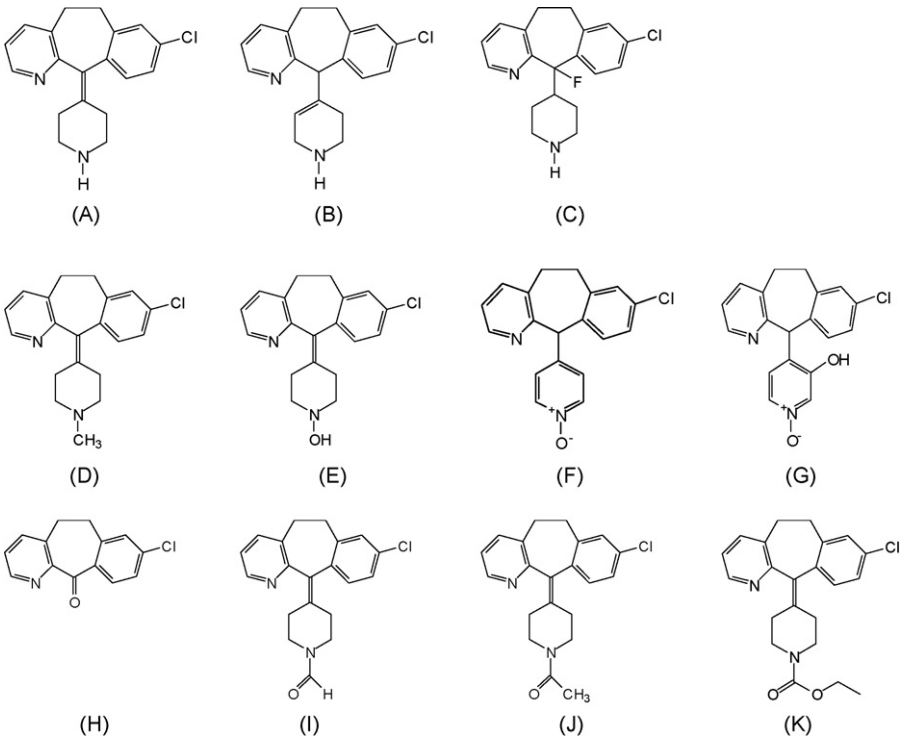

Fig. 1. Molecular structures of desloratadine and its related compounds. (A) Desloratadine; (B) desloratadine 3,4-dehydropiperidine derivative (DDD); (C) desloratadine fluoro derivative (DFD); (D) N-methyl desloratadine (NMD); (E) desloratadine N-hydroxypiperidine derivative (DHD); (F) desloratadine N-oxide pyridine derivative (OPD); (G) desloratadine N-oxide hydroxypyridine derivative (OHD); (H) desloratadine despiperidyl keto derivative (DKD); (I) N-formyl desloratadine (NFD); (J) N-acetyl desloratadine (NAD); and (K) loratadine.

is because the optimal operation pH for these stationary phases are typically around 2–8 while the pK_a values of desloratadine and several key related compounds including DFD, DDD, and NMD are around 10. At pH 2–8, these basic compounds will be protonated and positively charged, thus have very weak retention.

The retention of these basic compounds can be increased by using high pH mobile phases. There are several columns such as Waters XTerra column [4,5], Waters Xbridge column and Phenomenex Gemini column that can tolerate buffer pH up to 11 [6]. However, our preliminary experiment showed that no adequate separation can be achieved for one of the critical pairs, DDD vs. desloratadine, and severe peak tailing of several compounds (such as desloratadine and NMD) was observed.

An alternative approach to increase the retention of basic compounds and achieve adequate separation is to use ion-pair chromatography [7–18]. Ion-pair chromatography and reverse phase HPLC share several features. For example, most reverse phase HPLC columns can be used for ion-pair chromatography. One major difference is that in ion-pair chromatography, an amphiphilic ion-pair reagent is added to the mobile phase to increase the retention of small sample ions of opposite charge. Many theories have been proposed to interpret the ion-pair chromatography, and they can be divided into two categories: ion-pair model and dynamic ion-exchange model. Ion-pair model suggests that the analytes form ion-pair with the amphiphilic ion with opposite charge in the mobile phase, and the ion-pair complex was subsequently separated by following reverse phase mechanism. Dynamic ion-exchange model suggests that the stationary phase acts as a dynamically coated ion exchanger because of the adsorption of the amphiphilic ions, and analyte ions are retained and separated by ion-exchange mechanism. It appears that both theories can interpret ion-pair chromatography successfully in specific cases, and a mixed mode of mechanisms is possible in some cases. Depending on the chromatographic conditions such as stationary phase, hydrophobicity of ion-pair reagent, organic solvent type and concentration, one mechanism might dominate over the other. In this paper, we found that ion-pair theory gives reasonable interpretation on experimental results, and thus will be used. The mechanistic study of ion-pair chromatography is not the objective of this work, and more information with regard to the studies of ion-pair chromatography can be found in two recent reviews by Fritz [17] and Stahlberg [18].

To enhance the efficiency of HPLC method development, two advanced tools were used in this study: Chromsword® (Iris Technologies International, GA, USA) [19–21] and LC Spiderling™ automated column selector (Chiralizer Services, LLC, PA, USA). Chromsword® is an artificial intelligence method development tool. It analyzes the HPLC retention data, builds retention models, simulates and predicts the separation. It allows for optimization of two variables simultaneously, such as buffer pH and organic modifier concentration, or temperature and organic modifier concentration, etc. It can also be used to simulate the robustness of the HPLC method without actually running the separation. This software not only significantly reduces the method development time, but also increases the probability of developing a more efficient and robust method. Several other HPLC method development softwares such as ACD Method Development Suite and Dry Lab have similar functions, and might be used to enhance the efficiency of HPLC method development in a similar way. The LC Spiderling™ automated column selector is a high throughput column screening platform. It can be integrated with Agilent Chemstation, and is capable of switching up to nine columns automatically.

To develop an efficient HPLC method for complex samples such as aforementioned desloratadine and related compounds, the following three-step strategies were used. First, a preliminary HPLC method is developed using Chromsword®. Second, high throughput column screening is conducted to search for the most suitable stationary phases using LC Spiderling™ column selector. Finally, all

key HPLC parameters are optimized on the most suitable stationary phase using Chromsword®.

In this work, we developed a gradient high performance ion-pair chromatographic method that provides baseline separation of desloratadine and all known related compounds using a YMC-Pack Pro C_{18} column. Optimization of several key chromatographic parameters such as buffer pH, temperature and gradient profile using Chromsword® will be presented. The impact of ionic strength on the retention as well as the selectivity will be discussed. The validation results of this method will be summarized.

2. Experimental

2.1. Reagents and materials

Acetonitrile (HPLC grade), sodium dodecylsulfate (SDS), citric acid anhydrous, and sulfuric acid were obtained from Fisher Scientific (USA). Sodium citrate dehydrate, and sodium sulfate anhydrous were obtained from Sigma–Aldrich (USA). All reagents are ACS Reagent or have purity higher than 99%. Milli-Q water (resistivity = 18.2 Ω cm) was obtained using Millipore Gradient A10 Water Purification System (Millipore Corporation, USA).

Desloratadine and its related compounds were obtained from Schering-Plough Research Institute (Kenilworth, NJ, USA) including desloratadine Fluoro derivative (DFD); desloratadine 3,4-dehydropiperidine derivative (DDD); desloratadine despiperidyl keto derivative (DKD); N-formyl desloratadine (NFD); N-acetyl desloratadine (NAD), desloratadine N-oxide pyridine derivative (OPD); desloratadine N-oxide hydroxypyridine derivative (OHD); desloratadine N-hydroxypiperidine derivative (DHD); N-methyl desloratadine (NMD); and loratadine. The structure for each compound was confirmed using NMR and LC/MS, and the purity of each compound was determined using HPLC area normalization and correction for volatile contents.

2.2. Standard solutions and sample preparation

A mixture of 30% acetonitrile, 70% water, and 0.05% citric acid was used as sample diluent for all sample preparations. The addition of citric acid is to protonate desloratadine and related compounds and increase the solubility. Stock solution of desloratadine related compounds was prepared by dissolving approximately 10 mg of each desloratadine related compounds in 100 mL sample diluent at room temperature. The stock solution of desloratadine was prepared by dissolving approximately 50 mg desloratadine in 50 mL sample diluent. A mixture of desloratadine and desloratadine related compounds was prepared by adding 1.0 mL desloratadine stock solution and 2.0 mL stock solution of desloratadine related compounds into a 50-mL volumetric flask, and diluting to volume with sample diluent. This mixture contains approximately 0.2 mg/mL desloratadine and 0.004 mg/mL desloratadine related compounds.

2.3. Mobile phase preparation

- 0.1 M sodium citrate solution: in a 1000 mL volumetric flask, add 29.4 g sodium citrate dehydrate. Add about 500 mL Milli-Q water to dissolve. Dilute to volume with Milli-Q water.
- 0.1 M citric acid solution: in a 1000 mL volumetric flask, add 19.2 g citric acid anhydrous. Add about 500 mL Milli-Q water to dissolve. Dilute to volume with Milli-Q water.
- 0.1 M sodium citrate buffer: in a 500 mL beaker, add about 300 mL 0.1 M sodium citrate solution. Use 0.1 M citric acid solution to adjust the pH to 5.9.
- Mobile phase A (aqueous phase) solution: in a 2000 mL mobile phase bottle, add 1700 mL Milli-Q water and 300 mL 0.1 M sodium citrate buffer (pH 5.9). Add 1.73 g SDS. Stir to dissolve completely.
- To adjust ionic strength, add desired amount of sodium sulfate and stir to dissolve. The pH of this aqueous phase is about 6.2. Adjust the pH to 6.2 ± 0.1 with 1N sulfuric acid or 1N sodium hydroxide as needed.
- Mobile phase B: acetonitrile was selected as the organic modifier because of its low viscosity resulting in low column back-pressure. Also, acetonitrile has a low UV wavelength cut-off (190 nm) minimizing any UV interference and resulting in a stable baseline during the gradient run.

2.4. Instrumentation

For HPLC method development, an Agilent 1100 Series HPLC system equipped with a Diode Array Detector (DAD), a thermostated column compartment, and an autosampler was used (Agilent, CA, USA). Chemstation software was used for data acquisition, chromatogram integration and data processing.

A Waters Atlantis dC_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size, Waters Corporation, MA, USA) was used to develop a preliminary method. YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size, manufactured by YMC CO., LTD. And distributed by Waters Corporation, MA, USA) was used to develop and optimize chromatographic separations.

3. Results and discussion

3.1. Development of a preliminary method

Based on our understanding of chemical/physical characteristics of the analytes, we think that an ion-pair chromatographic method is most promising. For basic analytes, alkanesulfonate compounds or alkylsulfate compounds are typically used as ion-pair reagent. The sulfonate or sulfate group will form ion-pair with the protonated sample ions, which will increase the retention of the sample ions because of its hydrophobic alkyl chain. Usually the longer the alkyl chain on an ion-pair reagent, the more increase in retention. Several ion-pair agents have been tested including dodecanesulfonate, decanesulfonate, octanesulfonate, hexanesulfonate, and dodecylsulfate. It was found that dodecylsulfate sodium salt (SDS) provided the best selectivity for desloratadine and its related compounds, especially for DDD, which is an isomer of desloratadine and always elutes close to desloratadine. DDD is one of the major synthesis related impurities, and is typically about 0.1% or less of desloratadine in desloratadine drug substance. Therefore, the separation of DDD and desloratadine is most critical in this method. Since SDS provided the best selectivity between DDD and desloratadine, it was selected for further method development and optimization.

For HPLC column, a C_{18} column is preferred over C_8, phenyl or CN column because of its higher hydrophobicity. An Atlantis dC_{18} column is selected as the starting column because it is well known that Atlantis dC_{18} column provides good retention for polar compounds without over-retaining the hydrophobic compounds, which appears to be a good stationary phase for the analytes shown in Fig. 1 that contain both polar compounds and very hydrophobic compounds.

The pK_a values of desloratadine and its related compounds span a large range due to different functional groups on each compound. For example, the pK_a is estimated to be approximately 3.0 for desloratadine N-oxide hydroxypyridine derivative (OHD) while it is about 11.0 for N-methyl desloratadine (NMD). Therefore, selecting an appropriate buffer pH is critical to the separation of these compounds. Simultaneous optimization of buffer pH and organic

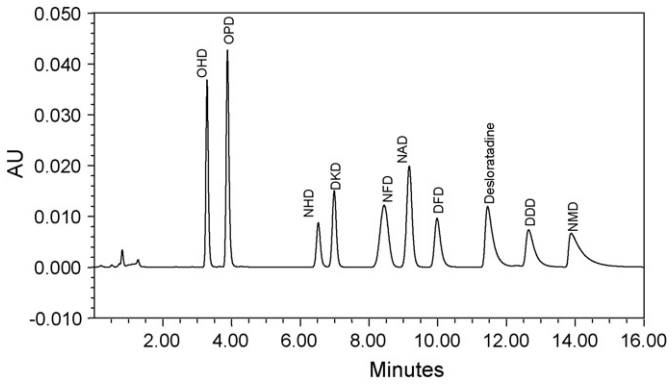

Fig. 2. Chromatogram obtained using conditions predicted by Chromsword®. HPLC column: Waters Atlantis dC_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size); temperature: 35 °C; flow rate: 1.5 mL/min. UV detection at 267 nm. Mobile phase: 39% acetonitrile: 61% aqueous (3 mM SDS + 15 mM sodium citrate at pH 6.2). Sample: 4 μg/mL desloratadine and desloratadine related compounds. Injection volume: 10 μL.

modifier (acetonitrile) concentration using ChromSword® software was performed.

First, three different aqueous mobile phases were prepared. Each solution contains 3 mM SDS and 10 mM sodium citrate buffer but has different pHs: 4.5, 5.5, and 6.5. The citrate buffer was selected because it provided continuous buffering capacity from pH 2.1 to pH 7.4, which allowed a wide pH range to be examined without changing the buffer salt. Mobile phase buffers at higher pH were not evaluated because of the potential for HPLC column deterioration. SDS was chosen as the ion-pair reagent because preliminary experiment showed that SDS provided the best resolution between the critical pair, DDD and desloratadine.

Second, two isocratic separations were performed using each aqueous phase at different pHs: 4.5, 5.5, and 6.5. The concentration of acetonitrile is 35% and 40% for each isocratic separation, respectively. The temperature was set at 35 °C because it allows for good control of temperature using thermostated column compartment. The impact of temperature on the separation and the optimization of temperature will be further discussed.

Using the retention data obtained from the above six injections, a pair resolution map was constructed using ChromSword® with respect to changes in pH and organic modifier concentration. Based on this pair resolution map, a Chromsword® simulation was performed, which predicted that at pH 6.2 and with 39% acetonitrile as the organic modifier, good separation for all compounds could be achieved.

The chromatogram using the predicted Chromsword® conditions was shown in Fig. 2. Baseline separation for desloratadine and all key related compounds was achieved. The experimental results match very well with the predicted results.

3.2. Column screening for most suitable stationary phases

After developing a preliminary method using Atlantis dC_{18} column, column screening was conducted using LC Spiderling™ automated column selector to search for most suitable stationary phases. About 40 HPLC columns were tested. Most stationary phases are C_{18}, and others include C_8, C_{12}, C_{16}, Cyano (CN), phenyl, hexyl-phenyl, fluorophase, and polar embedded stationary phases. It was found that the best separation was obtained using a YMC-Pack Pro C_{18} column as shown in Fig. 3. Several other columns also show promising separation such as ACE-3 C_{18}, Acclaim PA II C_{18}, Capcell Pak C_{18} UG, Develosil ODS-UG, Hydrosphere C_{18}, Hypersil Gold C_{18}, Sunfire C_{18}, and Synergi Max RP (C_{12}).

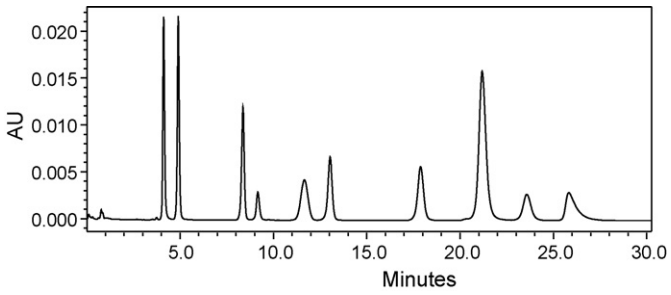

Fig. 3. Chromatogram obtained using most suitable stationary phase. HPLC column: YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size); temperature: 35 °C; flow rate: 1.5 mL/min. UV detection at 267 nm. Mobile phase: 36% acetonitrile: 64% aqueous (3 mM SDS + 15 mM sodium citrate at pH 6.2). Sample: 20 μg/mL desloratadine and 4 μg/mL desloratadine related compounds. Injection volume: 10 μL.

Although good separation was obtained as shown in Fig. 3, there are several problems such as the low sensitivity of late eluting peaks including desloratadine, DDD, and NMD, peak broadening of NFD, and retention time shift with regard to slight change in organic solvent concentration. Therefore, further optimization using YMC-Pack Pro C_{18} column is needed.

3.3. Effect of ionic strength on the separation

It is well known that ionic strength has a big impact on the retention of ionic samples in chromatographic separation, especially in ion-exchange chromatography or ion-pair chromatography. Usually, the increase in ionic strength reduces the retention of sample ions. For different sample ions, the change in ionic strength might result in different changes in the retention. Therefore, the peak spacing as well as the selectivity can be modified by adjusting the mobile phase ionic strength.

In this work, sodium sulfate was added to mobile phase A (aqueous phase) to change the ionic strength. Other sodium salt such as sodium chloride has also been tried. However, very noisy baseline was observed even at a very low concentration of sodium chloride (~5 mM). The overlay of chromatograms at different sodium sulfate concentrations (0 mM, 5 mM, 10 mM, 20 mM, 30 mM, and 40 mM) was shown in Fig. 4. For the first six peaks including desloratadine N-oxide pyridine derivative (OPD), desloratadine N-oxide hydroxypyridine derivative (OHD),

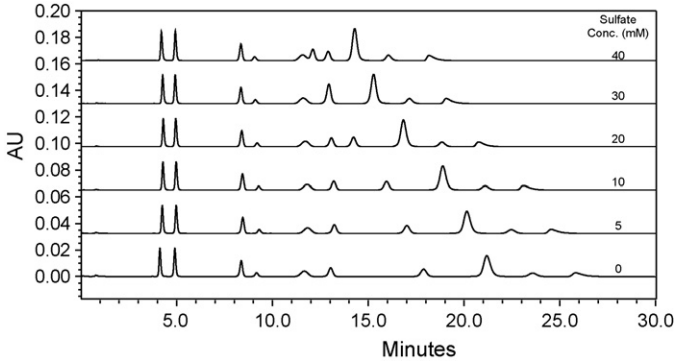

Fig. 4. Overlay of chromatograms with the addition of different concentrations of sodium sulfate. HPLC column: YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size); temperature: 35 °C; flow rate: 1.5 mL/min. UV detection at 267 nm. Mobile phase: 36% acetonitrile: 64% aqueous (3 mM SDS + 15 mM sodium citrate at pH 6.2 + sodium sulfate). Concentration of sodium sulfate is 0 mM, 5 mM, 10 mM, 20 mM, 30 mM, and 40 mM as specified in each chromatogram. Sample: 20 μg/mL desloratadine and 4 μg/mL desloratadine related compounds. Injection volume: 10 μL.

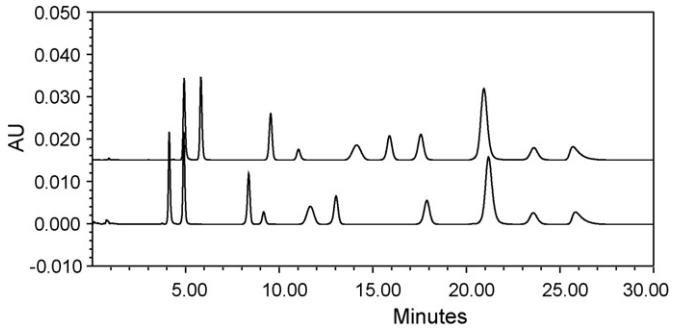

Fig. 5. Comparison of the resolution of DDD vs. desloratadine with and without sodium sulfate. HPLC column: YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size); temperature: 35 °C; flow rate: 1.5 mL/min. UV detection at 267 nm. Mobile phase for top chromatogram: 34.5% acetonitrile: 65.5% aqueous (3 mM SDS + 15 mM sodium citrate at pH 6.2 + 40 mM sodium sulfate). Mobile phase for bottom chromatogram: 36% acetonitrile: 64% aqueous (3 mM SDS + 15 mM sodium citrate at pH 6.2). Sample: 20 μg/mL desloratadine and 4 μg/mL desloratadine related compounds. Injection volume: 10 μL.

desloratadine despiperidyl keto derivative (DKD), desloratadine N-hydroxypiperidine derivative (DHD), N-formyl desloratadine (NFD), N-acetyl desloratadine (NAD), the retention time is almost unaffected by the addition of sodium sulfate. This is because these compounds are neutral compounds in this mobile phase. They were retained by hydrophobic interaction with the stationary phase (typical reverse phase retention), and their retention is less impacted by the change in ionic strength. The retention time of the last four peaks including desloratadine fluoro derivative (DFD), desloratadine, desloratadine 3,4-dehydropiperidine derivative (DDD), and N-methyl desloratadine (NMD) decreased with the increase of sodium sulfate concentration. This could be attributed to the competition of sulfate ion with SDS, which reduced the formation of analyte-SDS ion-pair and thus retention. If ion-exchange mechanism is used to interpret the separation, then it could be attributed to the elution of analytes by sodium ion.

From Fig. 4, it is noticed that the increase in ionic strength results in the decrease in retention time but surprisingly an increase in resolution of DDD vs. desloratadine. The resolution of DDD vs. desloratadine can also be increased by decreasing the organic solvent concentration. However, it will result in an increase in retention time. In this work, we took advantage of these two factors by increasing the ionic strength and decreasing the organic solvent concentration simultaneously. The capacity factors (retention) was kept constant, while the maximum resolution of critical pair, DDD vs. desloratadine was achieved. The results are shown in Fig. 5. The mobile phase ionic strength was increased by adding 40 mM sodium sulfate, and the acetonitrile concentration was decreased from 36% to 34.5%. The retention time of DDD in both chromatograms is about 23.6 min. However, the resolution of DDD vs. desloratadine increased from 3.2 to 3.8 with the addition of 40 mM sodium sulfate. As discussed above, the separation of DDD and desloratadine is most critical in this method because DDD elutes right after desloratadine, and the typical concentration of DDD is only about 0.1% or less of desloratadine. The average recovery of DDD at 0.05% level (10 μL injection of 0.2 μg/mL DDD in the presence of 0.4 mg/mL desloratadine) was improved from 84% to 93%, which is attributed to the higher resolution between DDD and desloratadine.

3.4. Isocratic separation vs. gradient separation

For an ion-pair chromatographic method, isocratic separation usually is preferred because it eliminates the time required for re-equilibration of stationary phase. However, for analytes that span a large range of capacity factors, the sensitivity of late eluting peak is always a big concern. It is well known that the peak width increases proportionally with the square root of retention time. That means that the peak height as well as the sensitivity decreases proportionally with the inverse of the square root of retention time. For a stability indicating method, a typical quantitation limit for API and related compounds has to be 0.05% or lower of the 100% assay concentration. At such a concentration, the signal to noise (S/N) ratio is only about 10 for desloratadine, 6 for DDD, and 6 for NMD. This does not meet a general requirement of S/N ratio larger or equal to 10 for quantitation limit. Increasing injection amount is limited by the loading capacity of the column, and thus does not seem to be a good solution.

Another problem with isocratic separation is the retention time shift of desloratadine peak. A one percent change in acetonitrile concentration would cause a 3-min shift in desloratadine retention time. The relative retention times (RRT) of desloratadine related compounds, which are calculated by comparing their retention times with that of desloratadine, would also change. This makes it difficult to identify desloratadine related compounds in a sample chromatogram.

To address these problems, gradient elution was performed with other HPLC parameters kept constant including temperature, flow rate, and detection. At least more than two folds increase in sensitivity for late eluting peaks was achieved and the retention time shift of late eluting peak was minimized. Therefore, gradient separation appears to be a better choice for this method.

3.5. Optimization of temperature and gradient separation

Preliminary studies indicated that the resolution of one of the critical pairs, DDD and desloratadine, increases with decreasing temperature. However, peak broadening of NFD was observed as the temperature decreases. To further study the effect of temperature on the NFD peak, a LC/MS compatible method was developed. The YMC-Pack Pro C_{18} column was used for this study, but the mobile phase was modified to be compatible with Mass Spectrometer. The mobile phase contains 38% acetonitrile: 62% aqueous phase (15 mM ammonium acetate buffer, pH adjusted to 6.2 using glacial acetic acid), and at flow rate 1.5 mL/min. Isocratic separations at different temperatures from 55 °C and 10 °C were performed, and the results were shown in Fig. 6. At 55 °C, one sharp symmetric peak was observed. As the temperature decreases, the peak becomes broader and broader. Eventually peak splitting was observed when the temperature was decreased to 15 °C and 10 °C. LC/MS analysis shows that these two peaks have identical MS spectra, indicating

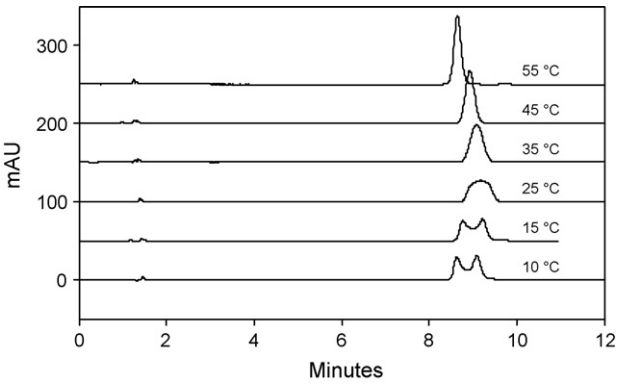

Fig. 6. The impact of column temperature on the peak shape of N-formyl desloratadine (NFD). HPLC column: YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size). Mobile phases: 38% acetonitrile: 62% aqueous phase (15 mM ammonium acetate, pH 6.2). UV detection at 267 nm. Temperatures: 55 °C, 45 °C, 35 °C, 25 °C, 15 °C, and 10 °C as indicated in each chromatogram.

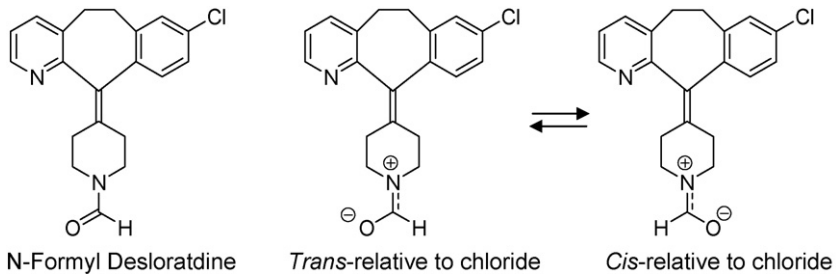

Fig. 7. Illustration of the equilibration of *trans*- and *cis*- isomers of N-formyl desloratadine.

that these two peaks are from two isomers. This ruled out the possibility of aldehyde hydrolysis, which would cause a difference of 18 (water) in molecular weight.

From the molecular structure that is shown in Fig. 1, it is noticed that the N-formyl desloratadine (NMD) has a unique *N–HC═O* bond. Resonance of the carbonyl group could give the C–N single bond some double bond character and slows the rotation as reported by Kim et al. [22–24]. Therefore, NMD could be considered as the mixture of *cis*- and *trans*- forms as shown in Fig. 7. The dynamic equilibrium of the *cis*- and *trans*- forms is quick at high temperature and cannot be separated chromatographically. However, at low temperature, the equilibrium of these two forms slows down, and the separation of these two forms can be observed. That is why peak splitting happened when the temperature was decreased to 10 °C. The plateau between two peaks indicates the dynamic equilibrium of these two isomers.

In order to obtain good separation between DDD and desloratadine as well as good peak shape for NMD, Chromsword® was used to optimize the temperature and gradient separation simultaneously. Three gradient injections were performed. The first two injections have the same gradient profile (30–50% acetonitrile in 40 min) but with different temperatures: 35 °C and 45 °C. The third gradient injection was performed at 35 °C with a different gradient profile (30–50% acetonitrile in 15 min). The retention data (retention time, peak area, and peak width) were used to construct a temperature-organic modifier concentration-resolution model. Using this model, simultaneous optimization of temperature and gradient profile was performed. The Chromsword® simulation results indicated that good separation as well as good sensitivity could be obtained at 40 °C and a linear gradient from 33% to 42% in 20 min. These predicted conditions were used to perform an actual injection on YMC-Pack Pro C_{18} column (150 mm × 4.6 mm, 3 μm), and the results confirmed this prediction as shown in Fig. 8. Good separation was achieved. The resolution is 3.6 for DDD and higher than 2.0 for all other peaks. This method also shows good sensitivity as the S/N ratio for quantitation limit solutions for all compounds (2 ng absolute amount assuming 10 μL injection volume of 0.2 μg/mL solution) is at least 15, which meet the requirement that the S/N ratio of quantitation limit solution is ≥10. The recovery of DDD at 0.05% level (10 μL injection of 0.2 μg/mL DDD in the presence of 0.4 mg/mL desloratadine) ranges from 90% to 99%, which is attributed to the improved resolution and sensitivity.

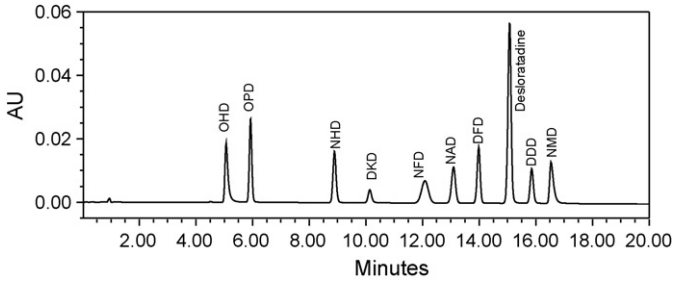

Fig. 8. Representative chromatogram using Chromsword® predicted HPLC conditions. HPLC column: YMC-Pack Pro C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size, 120 Å pore size); temperature: 40 °C; flow rate: 1.5 mL/min. UV detection at 267 nm. Mobile phases: A = 3 mM SDS + 15 mM sodium citrate at pH 6.2 + 40 mM sodium sulfate, B = 100% acetonitrile. Gradient separation from 33% B to 42% B in 20 min. Sample: 20 μg/mL desloratadine and 4 μg/mL desloratadine related compounds. Injection volume: 10 μL.

3.6. Summary of validation results

This method has been successfully validated according to ICH guidelines in terms of linearity, accuracy, specificity, precision, quantitation limit/detection limit, and robustness. Linear response was demonstrated between 0.2 mg/mL and 0.6 mg/mL for desloratadine, which has a correlation coefficient (*R*) of 1.000. For desloratadine related compounds, linear response was demonstrated between 0.2 μg/mL and 4 μg/mL, with a correlation coefficient (*R*) of 1.00 from two laboratories for all related compounds. The recovery ranged from 98.2% to 100.1% for desloratadine (API), and 80% to 105% for all related compounds. The precision ranged from 0.3% to 0.5% for desloratadine, and from 0.7% to 2.6% for desloratadine related compounds. The intermediate precision was 0.5% for desloratadine and ranged from 1.7% to 5.3% for desloratadine related compounds. The quantitation limit was 0.2 μg/mL, and the detection limit was 0.08 μg/mL for all compounds. The robustness of this method has been verified by deliberately changing HPLC parameters including temperature, flow rate, detection wavelength, buffer pH, ion-pair agent (SDS) concentration, mobile phase additive concentration, and gradient profile. The results indicated that baseline separation for all compounds was achieved with respect to the small but deliberate changes in the following critical HPLC parameters: buffer pH (6.2 ± 0.2), ion-pair agent concentration (3 ± 0.3 mM), temperature (40 ± 5 °C), organic modifier concentration (33 ± 2% to 42 ± 2% in 20 min), and gradient time (20 ± 2 min). The resolution of critical pair, DDD vs. desloratadine, was higher than 2.8 under all varied conditions. The solution stability was established as up to 3 days at room temperature and up to 7 days under refrigeration.

4. Conclusions

In summary, we successfully developed a gradient ion-pair chromatography method for the separation of desloratadine and its related compounds in solid pharmaceutical formulation. Baseline separation for all compounds is achieved, indicating that this method is specific. The quantitation limit for all desloratadine related compounds is equal to or lower than 0.2 μg/mL, which corresponds to 0.05% of desloratadine assay concentration (0.4 mg/mL). Therefore, this method is sensitive. The method robustness was simulated using Chromsword® software, and successfully validated in terms of buffer pH, ion-pair agent (SDS) concentration, temperature, acetonitrile concentration, and gradi-

ent time. This method has been successfully validated in terms of key method validation characteristics according to ICH guidelines including sensitivity, specificity, linearity, accuracy, precision, and robustness, and it can be used as a stability indicating method for desloratadine or drug products that use desloratadine as API.

The three-step method development strategies using Chromsword® method development software and high throughput column screening have proven to be very effective. These results indicated that the three-step strategies utilized in this study would significantly reduce the method development time and also increase the probability of developing a more efficient and robust method.

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