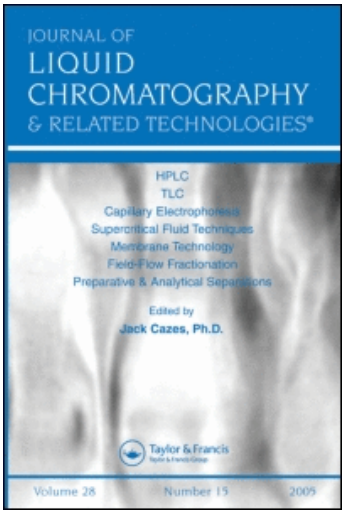

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PRACTICAL OPTIMIZATION OF AN ION-PAIR HPLC ASSAY FOR 1,3-DICHLORO-6,7,8,9,10,12-HEXAHYDROAZEPINO[2,1-B] QUINAZOLINE MONOHYDROCHLORIDE BULK PHARMACEUTICAL CHEMICAL

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ABSTRACT

An ion-pair HPLC method was optimized for the assay of the experimental Alzheimer's bulk pharmaceutical chemical CI-1002 Hydrochloride and ten potential impurities. The process of selecting the most robust chromatographic system (i.e., mobile phase composition, pH and column) was completed. A mobile phase consisting of aqueous sodium dodecyl sulphate (SDS) and methanol was utilized. Several commercially available base-deactivated C_{18} columns were evaluated and a Waters Symmetry 150 x 4.6 mm, 5 μm particle size column was selected. The Waters Symmetry column was used in subsequent experiments to optimize the mobile phase pH, the percent methanol, and the SDS content. The ruggedness of the HPLC method was evaluated by documenting slight modifications under controlled conditions. The ion-pairing method enables the assay and impurity profile for ten potential impurities to be carried out simultaneously. The method also provides baseline resolution for all components and a run time of approximately 30 minutes.

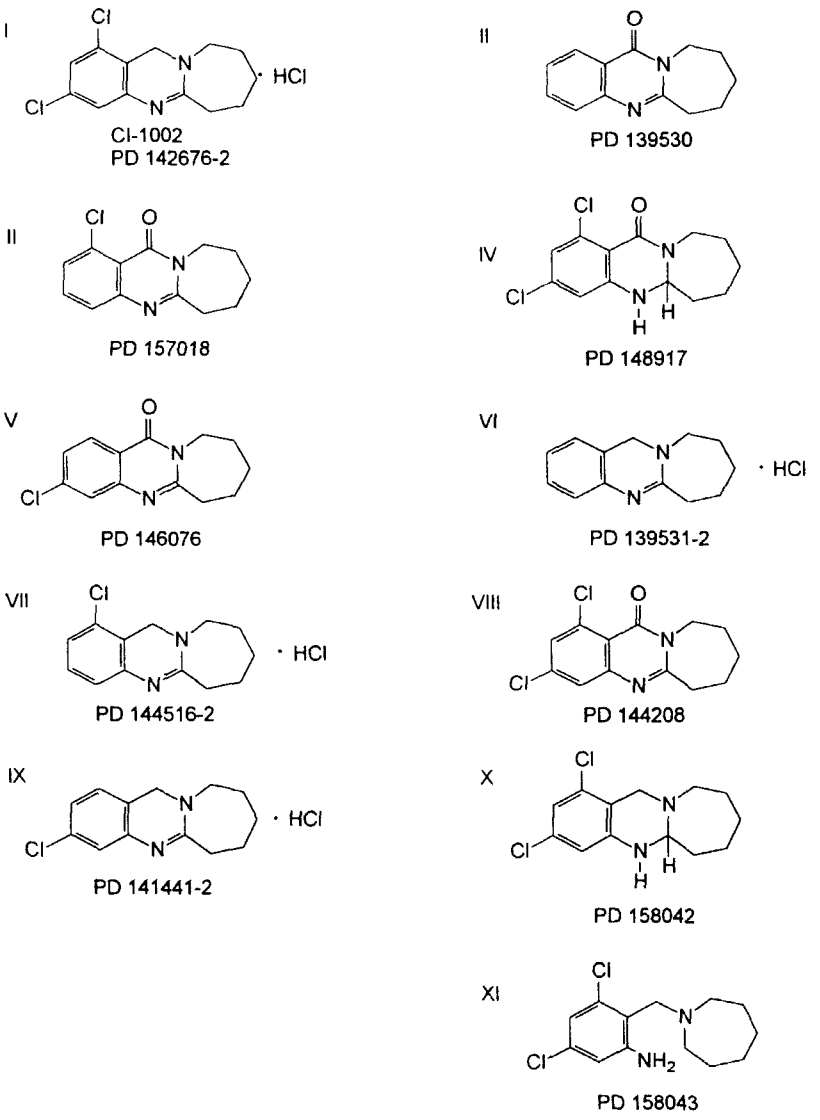


Figure 1. Chemical Structures for CI-1002 and Ten Potential BPC Impurities

INTRODUCTION

CI-1002 hydrochloride bulk pharmaceutical chemical (BPC) (PD 142676-2, compound I in Figure 1), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-b] quinazoline monohydrochloride, is being developed as a cognition activator for the treatment of Alzheimer's. The original HPLC assay for the BPC was a reverse phase method which utilized a C_8 column. A 60-minute run time was required for this method, and it was not able to resolve several potential impurities from CI-1002 .

The chemical structures of CI-1002 and the ten potential impurities are shown in Figure 1.

A shorter run time, baseline resolution, and ruggedness were key factors considered in the development and optimization of a replacement HPLC method. Ion-pair theory[1-2] and investigative efforts suggested the use of the ion-pairing agent sodium dodecyl sulfate (SDS) as the mobile phase modifier and methanol as the organic solvent.

An ion-pair reverse phase HPLC method was optimized to reduce the run time to approximately 30 minutes, while maintaining baseline resolution of CI-1002 and the ten potential impurities. Specifically, several commercially available base-deactivated C_{18} columns were evaluated. Also, experiments to optimize the percent methanol, the SDS concentration, and the mobile phase pH were conducted to enable all of the potential BPC components to be baseline resolved.

EXPERIMENTAL

Reagents

Methanol (HPLC grade) and ammonium hydroxide (reagent grade) were purchased from EM Science, Gibbstown, NJ. Phosphoric acid (85%, reagent grade) and sodium dodecyl sulphate (electrophoresis grade) were obtained from Fisher Scientific, Fair Lawn, NJ. Water (HPLC grade) was obtained from a Waters, Milford, MA Milli-Q water purification system. The ten potential impurities and CI-1002 shown in Figure 1 were prepared at Parke-Davis Pharmaceutical Research Division, Holland, MI.

Chromatographic Columns

The following base-deactivated C_{18}, 5 μm particle size, 4.6 mm ID x 150 mm length columns were evaluated: a Waters Symmetry, (Waters, Milford, MA); a Mac Mod Zorbax RX-C18, (Mac Mod, Chadds Ford, PA); and an Alltech Alltima, (Alltech, Deerfield, IL).

Chromatographic and Other Equipment

Initially, chromatographic analysis was performed using a Hitachi L-6200A Intelligent Pump, a Micromeritics 728 autosampler, a Rheodyne 7010 injector with a 20 μL sampling loop, a Hitachi 655A variable wavelength UV detector, a Hitachi D-2500 Chromato-Integrator, and the three base-deactivated columns selected. The final chromatograms used the three columns and a chromatographic system from Waters (i.e., a 600E pump, a 700 WISP, and a 996 photo diode array detector). The system was controlled, and the data were collected and processed with Waters Millennium (version 2.1) software. The mobile phase pH was determined using an Orion Model 701A digital IonAnalyzer with a Orion 8103 ROSS combination pH electrode.

USP Plate Count, Tailing Factor, and Resolution Test Solution

Aliquots (1.0 mL) of individually prepared stock solutions of PD 158042 (compound X in Figure 1) and CI-1002 were added to a 25 mL volumetric flask and diluted to volume with mobile phase. Each stock solution concentration was 1.0 mg/mL in methanol.

Elution Optimization Test Mix Solution

Aliquots (0.5 mL) of individually prepared stock solutions of each compound were added to a 25 mL volumetric flask and diluted to volume with mobile phase (see Figure 1). Each impurity stock solution (*II* through *XI*) concentration was 1.0 mg/mL in methanol. The concentration of the BPC stock solution was 5.0 mg/mL in methanol.

RESULTS AND DISCUSSION

The original reverse phase HPLC method, which utilized an aqueous triethylamine buffer at pH 3.0 mixed with acetonitrile and methanol (3:1:1,

v/v), and a 4.6 x 250 mm C_8 column, eluted CI-1002 at approximately 10 minutes, and the penultimate (PD 144208, compound VIII in Figure 1) at about 51 minutes. Based on the basicity (tertiary vs. secondary vs. primary amine content) of CI-1002 and the potential impurities, it was decided that ion-pairing might be useful as an alternative HPLC approach. According to ion-pair theory, the relatively basic amine analytes would be retained on the column by acidic ion-pair reagents such as octane sulfonic acid or SDS.[1-2] SDS was used initially and found to provide a promising separation, with (in accordance with the basicity of the analytes) a resulting reversal in elution order of the penultimate (VIII) and CI-1002 (in comparison to the original reverse phase method). This reversal in elution order was significant since it gave a much sharper peak for the penultimate (VIII) and resulted in a lower limit of detection and quantitation for this BPC impurity. Next, experiments were run to compare the three base-deactivated C_{18} columns. The resolution and USP tailing factor was superior with the Waters column, and this column was selected for further optimization experiments.

The ion-pairing of the compounds with SDS was structurally dependent on the basicity of the various amines. The greater the ion-pairing, the longer the retention time of the compounds.[1-2] A number of parameters play a significant role in ion-pairing chromatography: mobile phase organic modifier and ion-pair reagent concentration, as well as the pH of the mobile phase.[1-2] These parameters were therefore systematically varied to optimize the final HPLC method.

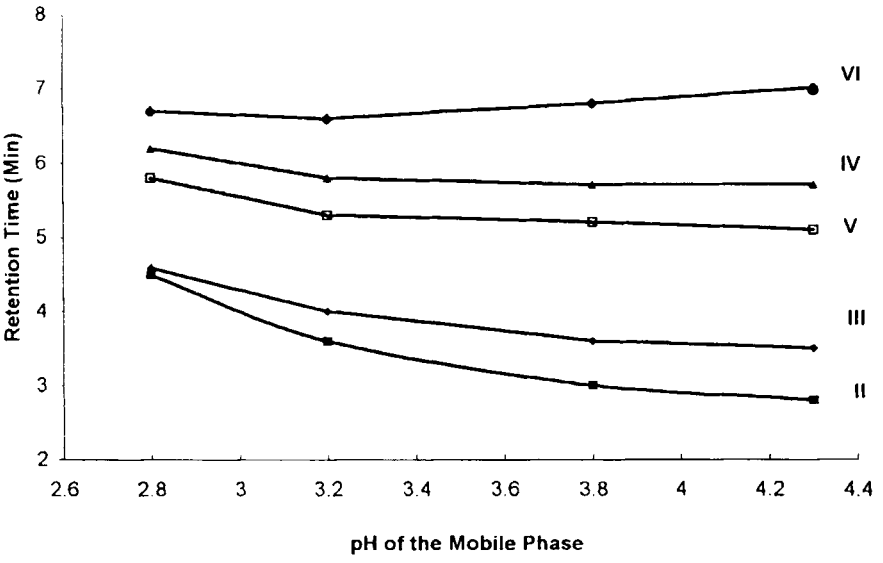
Optimization of Methanol Content

The elution time for the ion-pair HPLC method was greatly effected by the content of methanol in the mobile phase. For example, with all other parameters being equal, the increase of methanol content in the mobile phase from 67% to 69% (with the balance of the mobile phase being 0.03 M SDS, with a final apparent pH of 4.0) changed the retention time of the last eluting component (XI) by 9.3 minutes. However, the system suitability results remained essentially unchanged. A 68% methanol content (v/v) in the eluent was selected to achieve the desired 30 minute run time.

Optimization of SDS Concentration

A range of SDS concentrations from 0.006 M to 0.02 M (concentration per liter of mixed mobile phase) were evaluated. The concentration of the counterion altered analyte retention times, and subsequent resolution. The

Part A



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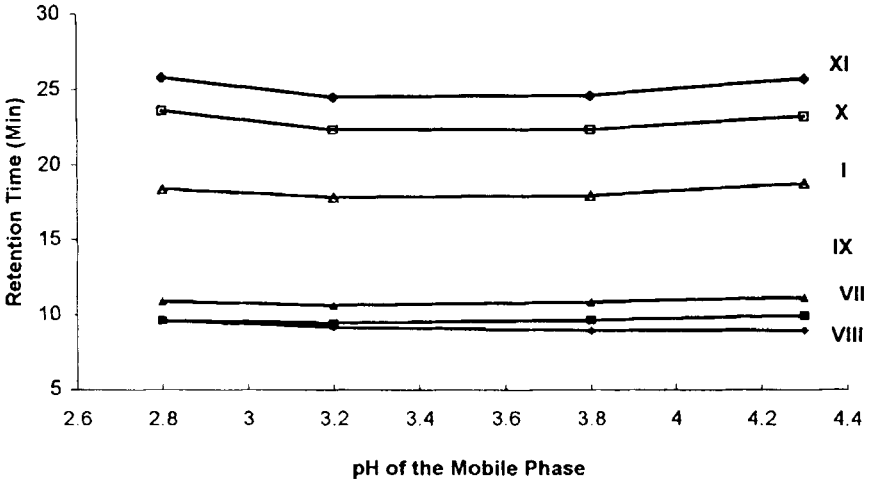

alterations were predictable based on the basicity of the analytes (refer to Figure 1). The 0.006 M counterion concentration reversed the elution order of VII and the penultimate (VIII), creating potential quantitation problems due to co-elution of the two compounds.

No significant advantages were found in the separation above 0.013 M SDS. The 0.01 M SDS concentration enabled the penultimate (VIII) to be quantitated without interference, at a relative retention time of approximately 0.46 vs 1.00 for CI-1002.

Based on these results, and in order to reduce the potential for poor system performance and improve ruggedness,[2] the optimum SDS concentration was determined to be 0.01 M. This SDS concentration was therefore used for subsequent experiments.

Optimization of pH

The final (apparent) pH of the mobile phase was evaluated and the results are shown in Figure 2A and 2B. The resolution of the impurities increased with increasing mobile phase pH. The resolution of VII, the penultimate (VIII) and IX showed the greatest increase. At pH 2.8, VII and the penultimate (VIII) co-eluted, but were resolved at pH 3.8. These effects were critical since the penultimate (VIII) in the CI-1002 Hydrochloride BPC synthetic scheme needed to be resolved and quantitated.

The system suitability resolution parameters were unchanged over the pH range of 2.8 to 4.3. As the pH was increased to 4.3, the retention time decreased for impurities II, III, and V. Similarly, as the pH was increased to 3.2 the retention time consistently decreased for all components, and then at pH values greater than 3.2, retention times increased or remained unchanged for I, IV, VI, VII, IX, X, and XI (see Figure 2A and 2B).

The effects of pH on resolution and retention time resulted in pH 4.0 being selected as the most desirable in terms of ruggedness.

Figure 2. (left) Effect of Mobile Phase pH on the Retention of Compounds I through XI as Shown in Figure 1. Part A shows Compounds II through VI, while Part B shows Compounds VII through XI and Compound I.

Table 1

System Suitability Measurements (Resolution, USP Plate Count and Tailing Factor) for the Three Columns Tested

Column	Resolution	Plate Count	Tailing Factor
Waters Symmetry	5.3	9184	1.03
Mac Mod Zorbax	4.2	8155	1.12
Alltech Altima	2.4	9885	1.25

Table 2

Waters Millennium Resolution Values for Each Compound Identified in Figure 1

Compound	Waters CD-2522	Waters IMP-MIX	Zorbax IMP-MIX	Alltima IMP-MIX
II	NA*	NA*	NA*	NA*
III	5.29	3.19	2.38	4.62
V	6.63	6.51	1.12	1.71
IV	NA*	2.20	4.61	5.60
VI	NA*	3.67	3.18	1.76
VIII	10.91	5.38	6.30	<1.0
VII	2.39	2.23	1.11	7.54
IX	3.00	2.78	1.78	>1.0
I	12.34	12.01	12.32	12.16
X	5.70	5.31	4.16	2.44
XI	NA*	2.45	3.61	3.07

*See the Results and Discussion Section for an explanation of the not-applicable (NA) values reported.
IMP-MIX refers to the Elution Optimization Test Mix Solution.

Column Comparison with Optimized Conditions

Column selection involved testing and evaluating the following C_{18} columns: a Waters Symmetry, a Mac Mod Zorbax RX-C18, and an Alltech Alltima. The columns were compared by running the elution optimization

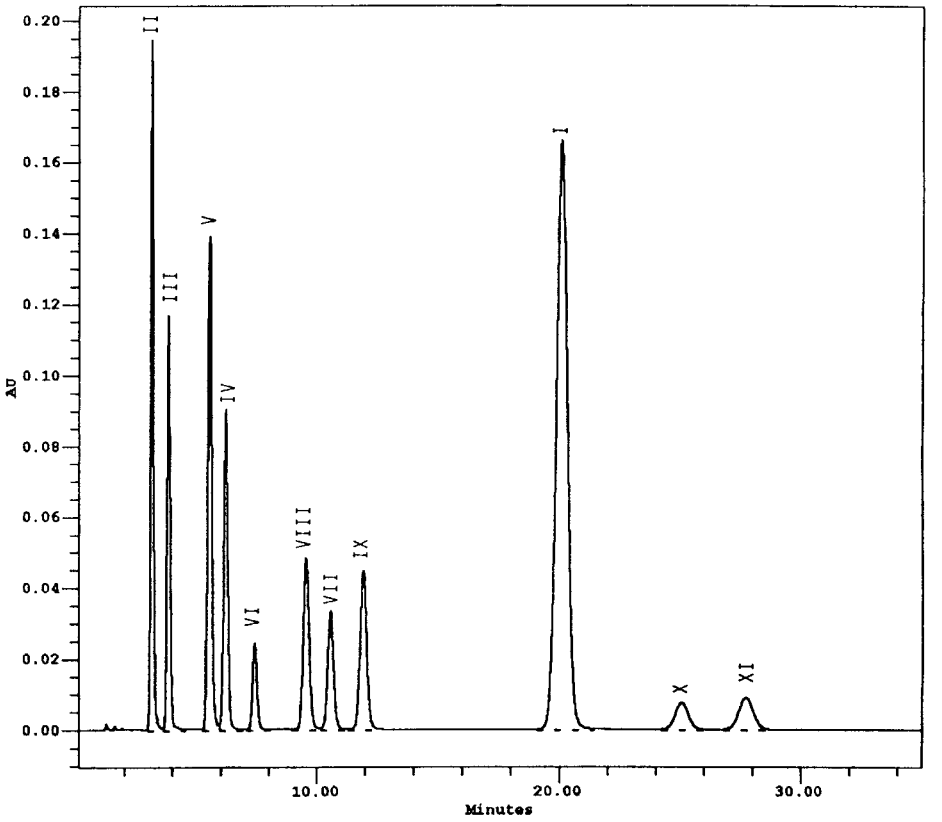

Figure 3. Waters Symmetry Chromatogram of the IMP-MIX. Experimental Conditions: Mobile Phase of 68 parts MeOH and 32 parts 0.03 M SDS with a final pH of 4.0, Flow Rate of 1.0 mL/min, Column Temperature of 27°C, Detection Wavelength of 228 nm, Injection Volume of 20 µL. Figure 1 shows the Structures of Compounds I through XI.

test mix at a concentration of 0.02 mg/mL for (I through XI), and 0.10 mg/mL for CI-1002. The run conditions for each system tested varied slightly in percent mobile phase composition in order to keep the total run time equal to approximately 30 minutes. System suitability results shown in Table 1 were used to evaluate overall column performance. The system suitability results showed: the Waters column with a higher plate count and resolution than the Zorbax column; the Alltech column with the highest plate count and lowest resolution.

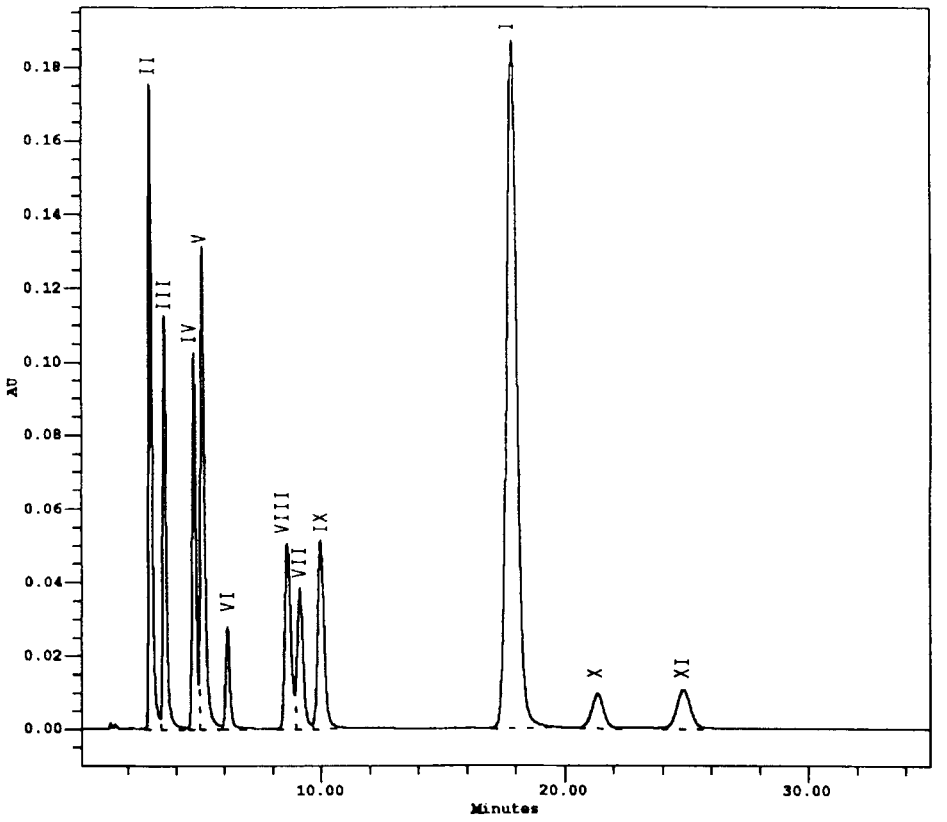

Figure 4. Zorbax Rx-C18 Chromatogram of the IMP-MIX. Experimental Conditions: Mobile Phase of 67 parts MeOH and 33 parts 0.03 M SDS with a final pH of 4.0, Flow Rate of 1.0 mL/min, Column Temperature of 27/C, Detection Wavelength of 228 nm, Injection Volume of 20 µL. Figure 1 shows the Structures of Compounds I through XI.

The tailing factors were all found to be below 1.5, with the highest being found for the Alltech column at 1.3. In summary, the Waters column was selected for routine use based on superior resolution and tailing factor results.

Tabulated resolution results using the Waters Millennium Software for CI-1002 and the ten impurities on each column tested can be found in Table 2. The resolution in Table 2 refers to the separation from the previous eluting impurity. Not applicable (NA) applied when the previous compound was not detected.

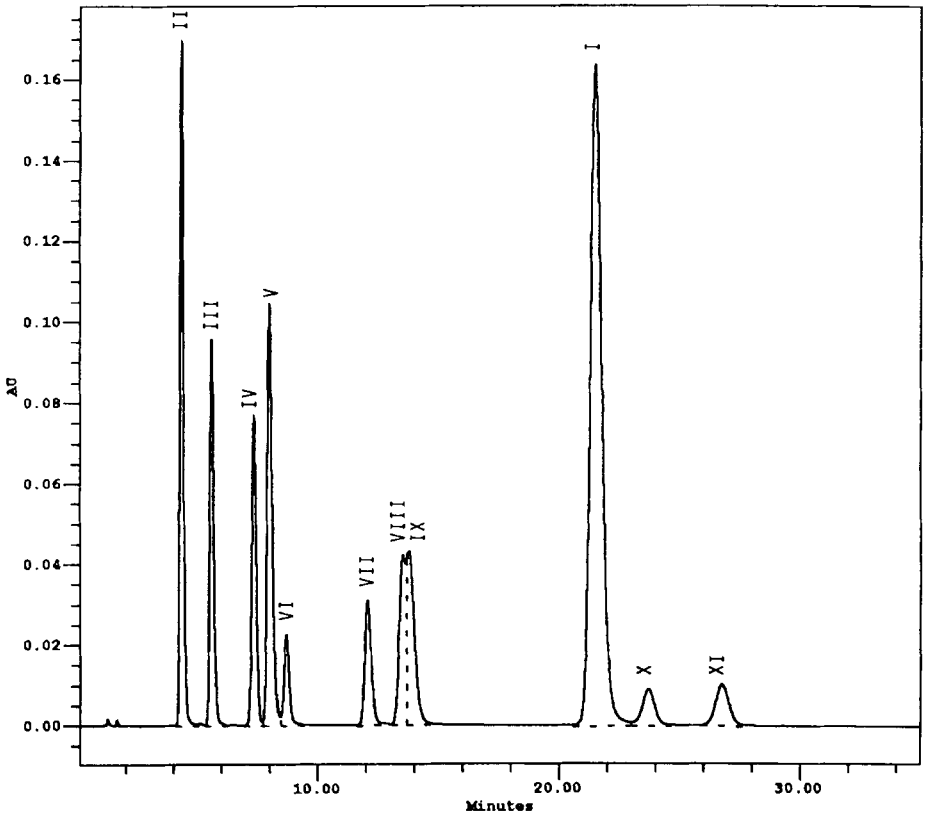

Figure 5. Alltech Alltima Chromatogram of the IMP-MIX. Experimental Conditions: Mobile Phase of 69 parts MeOH and 31 parts 0.03 M SDS with a final pH of 4.0, Flow Rate of 1.0 mL/min, Column Temperature of 27°C, Detection Wavelength of 228 nm, Injection Volume of 20 µL. Figure 1 shows the Structures of Compounds I through XI.

The Waters column did not exhibit a resolution factor less than 2.0 for any single compound. This resolution of at least 2.0 met recent FDA recommendations.[3] The Zorbax and Alltech columns exhibited some resolution factors less than 2.0 with the Alltech column unable to separate the penultimate (VIII) and IX. Recall that the penultimate compound in the process needed to be resolved for quantitation. It was interesting to note, however, that the Alltech column provided the best resolution for the early eluting potential

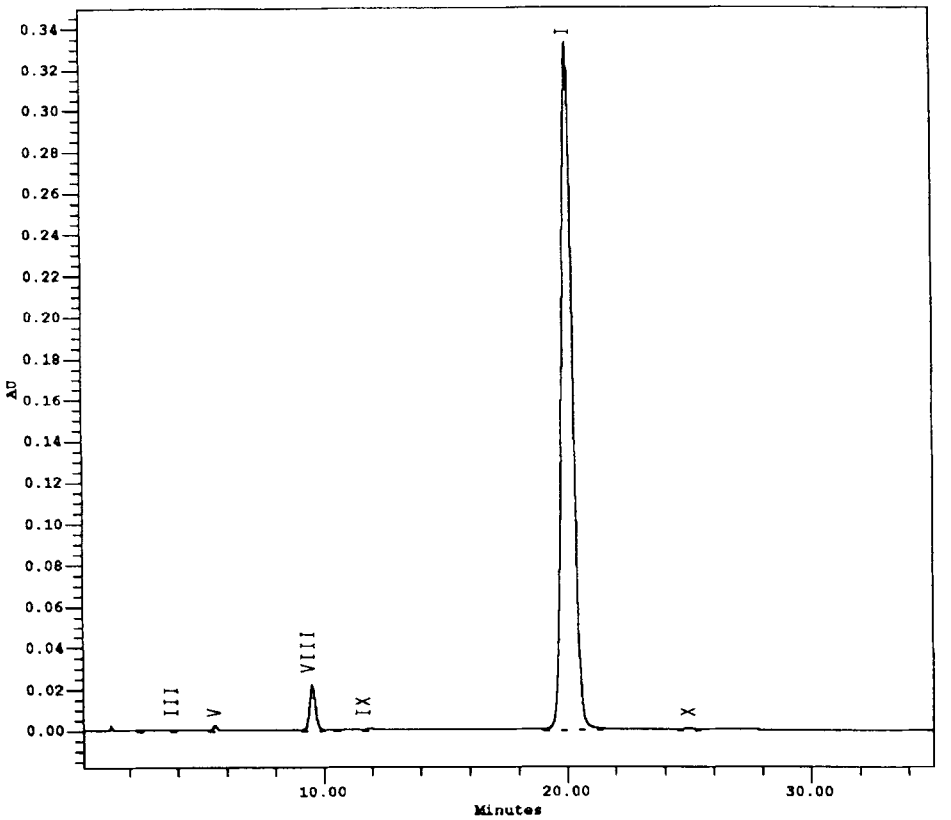

Figure 6. Chromatogram of a Typical Lot of CI-1002 Hydrochloride BPC. Experimental Conditions: Mobile Phase of 68 parts MeOH and 32 parts 0.03 M SDS with a final pH of 4.0, Flow Rate of 1.0 mL/min, Column Temperature of 27°C, Detection Wavelength of 228 nm, Injection Volume of 20 µL. Figure 1 shows the Structures of Compounds I through X.

impurities II and III. The HPLC chromatograms of the impurity mix for each column tested are shown in Figures 3-5. A typical production lot of BPC assayed using the Waters column showed excellent resolution of all the compounds identified, and the penultimate (VIII) was resolved from the nearest pre-eluting compound by a resolution factor of 10.9 (see Figure 6).

CONCLUSIONS

These experiments demonstrated that by careful optimization of mobile phase characteristics and column selection, it was possible to develop a rapid isocratic method for the BPC and numerous potential impurities. The selection of SDS as the counterion proved beneficial (i.e., the twelve carbon length) by providing the versatility necessary to achieve baseline resolution for the ten potential impurities and CI-1002, within approximately 30 minutes. The Waters Symmetry column selected has approximately 20 percent carbon loading, this feature helps explain the superior resolution and low tailing factor observed with this column. The hydroxyl groups of the silanol backbone are well protected and this protection benefits column robustness and column longevity in this application. The well protected silica surface of the Waters Symmetry column does not require silanol blockers in the mobile phase (e.g., triethylamine) to help control tailing and improve resolution.

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