Validation of Capillary Electrophoresis Method for Determination of *N*-Methylpyrrolidine in Cefepime for Injection

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Abstract

The present study relates to a new capillary electrophoresis method for the determination of *N*-methylpyrrolidine, an impurity considered to be toxic and also potential degradation impurity in cefepime hydrochloride drug substance. The newly developed capillary electrophoresis method for determining the content of *N*-methylpyrrolidine in cefepime for injection has been validated as per International Conference on Harmonization (ICH) guidelines to prove the selectivity, sensitivity, suitability, robustness, and ruggedness of the method. This simple, efficient, and rapid methodology may be used by pharmaceutical industry for routine analysis as well as during stability studies. The newly developed capillary electrophoresis method to determine the content of *N*-methylpyrrolidine in cefepime for injection requires 10 min for data acquisition, and uses an indirect UV photometry method to detect the analyte signal at 240 nm against the reference signal at 210 nm. The electrophoretic system is optimized to get stable base line, higher signal to noise ratio and peaks with narrow peak width. The method employs bare fused silica capillary with extended light path, effective length of capillary is 56 cm and inner diameter of capillary is 50 µm, 5 mmole of imidazole buffer adjusted to pH 5.1 with 3 molar acetic acid solution is used as background electrolyte. The sample is introduced in hydrodynamic mode employing pressure of 50 mbar for 5 s, and the desired separation is achieved with constant applied voltage of 25 kV at ambient temperature (~25°C).

Introduction

Cefepime hydrochloride (as an injectable composition), an improved cephalosporin salt is used in the treatment of broader spectrum of antibiotic activity (1,2). Cefepime hydrochloride (as cefepime for injection) is available in the market under the brand name of Maxipime. Cefepime for injection is a dry mixture of cefepime dihydrochloride and *L*-arginine in the ratio of approximately 62:38.

Cefepime for injection is unstable at room temperature and loses 30% or more of its activity on storage at elevated temperature leading to formation of degradation impurities (i.e., other than active moiety). *N*-Methylpyrrolidine is one of the potential known degradants of cefepime hydrochloride, and it is also a residual process impurity from synthesis. *N*-Methylpyrrolidine is reported to be an inert of unknown toxicity by US Environmental protection agency (3). It is also observed that the increase in *N*-methylpyrrolidine content is proportional to the decrease in potency of cefepime. Consequently, monitoring and control of *N*-methylpyrrolidine in cefepime for injection is essential for preserving the desired quality of active moiety during release as well as throughout its shelf life.

The cefepime for injection monograph is available in United States Pharmacopoeia (USP), wherein the content of *N*-methylpyrrolidine in cefepime hydrochloride is controlled to "not more than 1.0% w/w" by using ion chromatography method (4). However, the ion chromatography method described in USP for the determination of *N*-methylpyrrolidine in cefepime for injection has many disadvantages, and so does the ion chromatography method described in Dionex Application note 199 (5). Therefore, to overcome the challenges posed by ion chromatography methods, a relatively simple, efficient, selective, reliable, and rapid capillary electrophoresis method has been developed.

N-Methylpyrrolidine accepts a proton to form the *n*-methylpyrrolidinium ion in acidic solution and is inactive to UV absorbance. Due to its low UV activity and high polarity, it cannot be determined by conventional analytical techniques like high-performance liquid chromatography (HPLC) and gas chromatography (GC). However, a validated GC method for determination of *N*-methylpyrrolidine in Cefepime and its preparations was reported and *N*-methylpyrrolidine was extracted into chloroform from Cefepime (6). The UV-inactive cations are generally determined by using cationic probes such as histidine, creatinine, histamine, ephedrine, and imidazole in capillary electrophoresis (7). Many capillary electrophoresis (CE) methods have been reported for the separation and subsequent determination of inorganic ions, low molecular weight organic acids and bases by means of indirect UV-photometric detection.

In the present method, a CE method was developed to achieve suitable selectivity with desired sensitivity by means of opti-

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mizing the conditions of electrophoretic system. The peak due to *N*-methylpyrrolidine was well resolved from other component of sample matrix, and the limit of detection of *N*-methylpyrrolidine in cefepime for injection is found to be 5.4 µg/mL (0.05% w/w) w.r.t cefepime, suggesting that the method is sensitive.

The determination of *N*-methylpyrrolidine in cefepime for injection by CE has many significant advantages over the ion chromatography (IC) method reported by Dionex. The advantages include low cost per analysis. The cost as well as quantity of reagents and columns used by CE method are much less and are significantly higher in the case IC method by Dionex (5). CE also has a shorter analysis time (total acquisition time is 10 min by CE method, whereas the acquisition time is 35 min in the case of IC method by Dionex). Importantly, ambient temperature is being used for achieving the separation in CE method. In the case of the IC method by Dionex, the column compartment temperature is maintained at 40°C. This is of serious concern, as cefepime is known to degrade at elevated temperature. Therefore, the content of *N*-methylpyrrolidine is likely to be overestimated in the IC method by Dionex. Further, no sample plug is possible during the separation by CE method, as the solute in the sample matrix migrates depending upon its electrophoretic mobility. In the case of the IC method, the exchange of ions influences the separation, thereby causing sample plug and reduced column life time.

Subsequently, considering its selectivity, sensitivity, and efficiency, this optimized CE method was validated to verify its performance characteristics according to ICH guidelines (8), and it is found that the CE method to determine the content of *N*-methylpyrrolidine in cefepime for injection is selective, sensitive, linear, precise, accurate, robust, and rugged.

Experimental

Chemicals and reagents

N-Methylpyrrolidine and imidazole were purchased from Fluka (Sigma-Aldrich, St. Louis, MO). Nitric acid and acetic acid were supplied by E. Merck (Mumbai, India) and water was from Milli–Q purification system (Millipore, Billerica, MA). Cefepime for injection drug product and cefepime related impurities were obtained from Aurobindo Pharma Ltd., (Hyderabad, India).

Instrumentation

An Agilent instrument CE system equipped with a diode array detector along with chemstation software for data acquisition and processing was used. Separation was carried out in fused silica capillary with extended light path length (Agilent, Böblingen, Germany) of effective length of 56 cm and i.d. of 50 µm.

Standard solution preparation

Standard solution was prepared by dissolving accurately weighed 600 mg of *N*-methylpyrrolidine in 100 mL of dilute nitric acid (1 in 2000). Further, 2 mL of this solution was diluted to 200 mL with dilute nitric acid, and filtered through 0.22-µm or finer porosity membrane filter.

Sample solution preparation

Sample solution was prepared by dissolving accurately weighed 500 mg of drug substance in 25 mL of dilute nitric acid (1 in 2000), and filtered through 0.22-µm or finer porosity membrane filter.

Procedure

The electrophoretic system consisted of a bare fused silica capillary with extended light path length, effective length to detector was 56 cm and inner diameter of capillary was 50 µm. The background electrolyte used was 5 mmole imidazole buffer adjusted to pH 5.1 with 3 molar acetic acid solution. The sample and standard solutions were introduced by hydrodynamic pressure of 50 mbar for 5 s, and the separation was carried out with constant applied voltage of 25 kV at ambient temperature (~25°C). Before introducing the sample, the capillary was conditioned with background electrolyte for 3 min at the inlet pressure of 5 bars. The analyte signal was detected by indirect UV photometric method, the wavelength was set at 240 nm against reference signal at 210 nm. New capillaries were rinsed with water for 5 min and followed by background electrolyte for 15 min.

Method development

It is important to note that a CE method to determine the content of *N*-methylpyrrolidine in cefepime for injection has already been reported in literature (9), wherein creatinine is used as the background electrolyte. However, the method posed many disadvantages like peak split and peak broadening (if the injection time was increased), more base line noise (due to that the reference signal was set at off), and was less efficient (i.e., sample plug formation). The reference signal is employed to achieve positive peak in the electopherogram in indirect UV detection method; hence, it is more appropriate to set the reference signal on. These problems posed by earlier reported CE method articulate the incomplete optimization of the electrophoretic system.

To overcome these drawbacks, a robust and rugged method was developed by optimizing the electrophoretic system, wherein 5 mmole imidazole buffer adjusted to pH 5.1 with acetic acid was used as background electrolyte. The selection of suitable background electrolyte, its optimum concentration, and its pH are the critical parameters of an electrophoretic system to make the separation efficient, selective, and sensitive. Cefepime is reported to be stable in the pH range between 4.0 and 6.0 (10). The pH of the buffer was carefully optimized in this pH range. The pKa of imidazole and acetic acid are 7.0 and 4.85, respectively, the pKa of the acetic acid lies in between 4.0 and 6.0; therefore, it was selected for adjusting the pH of the electrolyte to 5.1. A background electrolyte concentration of 5 mmole was selected, which leads to a very good base line, less noise, and excellent buffering capacity.

This optimized method did not allow any sample plug formation even while applying the pressure of 50 mbar for 5 s and baseline drift was minimal. There was no need to apply any hydrodynamic pressure in order to make the baseline flat during the analysis while employing imidazole buffer as background electrolyte instead of creatinine. However, an increase in absorbance was observed at the beginning of the separation (0–0.5 min), and thereafter baseline was flat.

Results and Discussion

N-Methylpyrrolidine is an organic base and inactive to UV absorption. Therefore, it is difficult to determine this impurity in cefepime for injection by using conventional HPLC method. In addition, cefepime is very sensitive to thermal exposure, and thus was not able to be analyzed by direct GC analysis either.

N-Methylpyrrolidine accepts a proton and exists as positively charged ion in aqueous acidic solution, therefore, a capillary zone electrophoresis method was developed to quantify the content of *N*-methylpyrrolidine in cefepime for injection at low levels. The analyte signal was set at 240 nm, in-line with the detection of cationic probe, and reference signal was set at 210 nm, which helps to convert a negative peak into positive peak in the electropherogram. The optimized electrophoresis conditions yielded a stable baseline. The selectivity between *L*-arginine and *N*-methylpyrrolidine peaks, stable base-line, narrow peak shape, and no sample plug have indicated that the sample concentration, and pH of imidazole buffer, as well as its concentration were suitably optimized. The method is simple, efficient, selective, and has desired level of sensitivity.

The optimized method was validated according to ICH guidelines (8) to prove its performance characteristics, thereby verifying its suitability and reliability for monitoring the *N*-methylpyrrolidine in cefepime for injection during routine analysis as well as stability studies. The validation parameters studied in this study are selectivity, sensitivity, linearity, precision (system precision, method precision, and intermediate precision), accuracy, stability of sample solution, and robustness. The results obtained from the experiments are summarized in the next paragraphs.

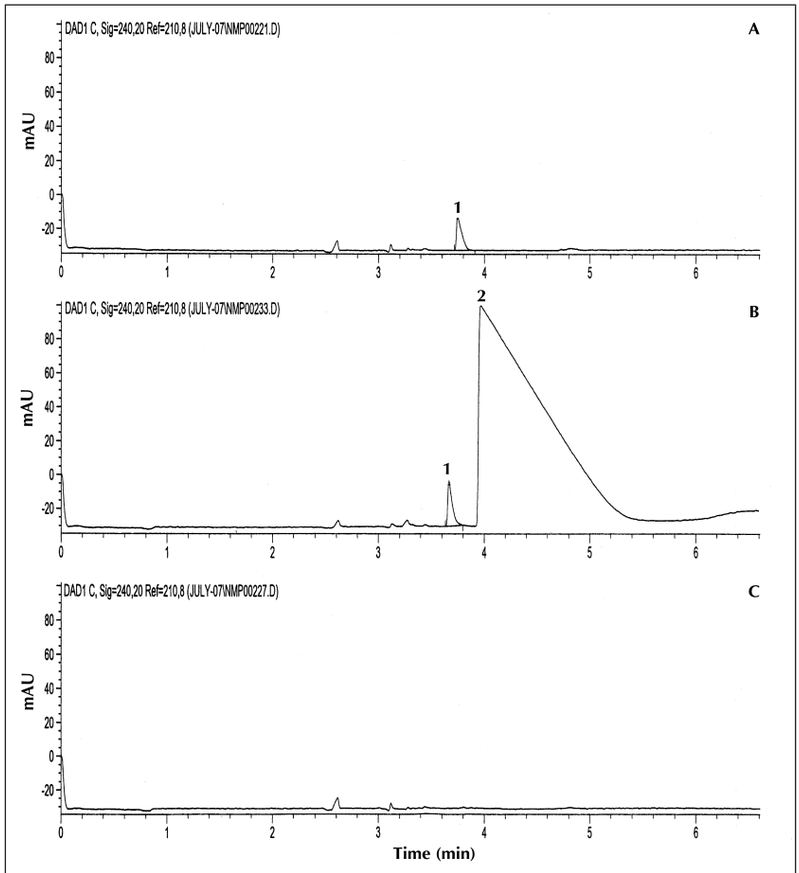

Figure 1. Overlay electropherogram of *N*-methylpyrrolidine standard solution (A), sample solution spiked with known impurities of cefepime (B), and blank solution (C). Peak numbers are as follows: peak 1, *N*-methylpyrrolidine and peak 2, *L*-Arginine.

Selectivity

The solutions of blank, *N*-methylpyrrolidine, *L*-arginine, cefepime hydrochloride, and cefepime for injection were introduced into the capillary electrophoresis system to identify the migration time. The migration time of *N*-methylpyrrolidine and *L*-arginine was found to be 3.7 min and 4.4 min. However, due to high molecular weight of cefepime, it does not elute through the capillary column and was flushed by rinsing the system for 3 min. The sample was found to contain *N*-methylpyrrolidine at a very low level, and therefore, the sample (cefepime for injection) was spiked with *N*-methylpyrrolidine at a level of 0.5% w/w w.r.t cefepime, along with other known impurities, of cefepime hydrochloride. The peak due to *N*-methylpyrrolidine is well resolved from the peaks due to blank, other known impurities and *L*-arginine, thereby indicating that the method is selective for determining the content of *N*-methylpyrrolidine in cefepime for injection. In conjunction, Figure 1 depicts an overlay electropherogram of blank solution, *N*-methylpyrrolidine standard solution, sample spiked with *N*-methylpyrrolidine along with other known impurities of cefepime.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were predicted using slope (S) and residual standard deviation (SD) obtained from a linear regression line performed at lower concentration levels.

The predicted limit of detection and quantification was found to be 5.4 µg/mL and 16.4 µg/mL respectively, and each predicted level was verified for precision by analyzing six replicate measurements. The percentage relative standard deviation for six replicate measurements at predicted LOD and LOQ concentration levels was found to be 10.9 and 5.2 respectively, verifying the predicted values.

Linearity

The linearity of the detector response for the determination of *N*-methylpyrrolidine in cefepime for injection was verified by analyzing series of different levels of solution containing *N*-methylpyrrolidine in the concentration range of approximately 15 µg/mL to 150 µg/mL [i.e., LOQ level to 150% of specification level (1%)]. The regression coefficient of the linear regression line was found to be 0.9994 by plotting the peak area against the concentration of *N*-methylpyrrolidine. The statistical analysis for linearity data is tabulated in Table I.

Precision

The system precision was demonstrated by performing six replicate introduction of *N*-methylpyrrolidine standard solution (60 µg/mL) into capillary electrophoresis system, and the percentage relative standard deviation of response for six replicate measurements was found to be less than 2.0.

Repeatability of the method (method precision) was demonstrated by preparing six replicate sample preparations by spiking known concentration of *N*-methylpyrrolidine in a single lot of cefepime for injection. These were analyzed as per the method, and the content of *N*-methylpyrrolidine was determined. The percentage relative standard deviation for six replicate measurements was found to be 3.7.

The results of two batches of Maxipime samples (Lot. No. 6K10579 and 6L14517) were analyzed as per the method and found to be 0.303% and 0.330%, respectively. The results of same two batches of samples using USP method were 0.308% and 0.335%, respectively.

Intermediate precision of the method (ruggedness) was performed in the same way as described in method precision, however, by employing different analyst on another day using another lot of capillary. The content of *N*-methylpyrrolidine was determined in each preparation, and the percentage relative standard deviation for six replicate measurements was found to be 2.8.

Stability of sample solution

The sample solution prepared by spiking known concentration of *N*-methylpyrrolidine was stored at 4 ± 2°C temperature conditions, and was introduced into capillary electrophoresis system at different time intervals. The content of *N*-methylpyrrolidine was determined at each interval, the sample solution was found to be stable over a period of 8 h. A cumulative percent RSD was found to be 7.0%. However, the sample solution was found to be stable for an hour at room temperature (~25°C).

Accuracy

The accuracy of the method was verified by preparing sample solution spiked with known amount of *N*-methylpyrrolidine at different concentration levels ranging between 0.5% and 1.5% with respect to cefepime concentration. Each concentration of sample solution was prepared in triplicate and analyzed as per the method. The percent recovery of *N*-methylpyrrolidine was found to be in the range of 98.1 to 103.2, mean percent recovery was 100.4, when calculated against the known added amount, indicating that the method is accurate. The results are tabulated in Table II.

Table I. The Statistical Evaluation of Linearity Data and Determine the Limit of Quantitation and Limit of Detection

Statistical parameter	Results
Correlation coefficient (r)	0.9994
Concentration range (µg/mL)	15.0–150.0
Intercept (a)	0.0681
Slope (b)	0.0499
Standard deviation (intercept)	0.0291
Standard deviation (slope)	0.0019
Standard error estimate (Residual standard deviation)	0.0820
Limit of detection (µg/mL)	5.4
With respect to cefepime (%w/w)	0.052
Limit of quantification (µg/mL)	16.4
With respect to cefepime (%w/w)	0.156

Robustness

Robustness of the method was verified by deliberately altering the critical method parameters from that of actual conditions. The altered conditions include change in temperature, buffer pH, and applied voltage. The results obtained from robustness experiments indicated that, the method parameters were suitably optimized to tolerate minor variations.

Conclusion

This optimized capillary electrophoresis method to determine the content of *N*-methylpyrrolidine in cefepime for injection is simple, efficient, rapid, selective, and sensitive. The low cost per analysis, shorter analysis time, ambient temperature during the analysis, no sample plug formation, and stable baseline are noted to be specific advantages of this optimized capillary electrophoresis method over the ion chromatography methods reported so far. The selection of background electrolyte as imidazole buffer, and its optimum concentration and pH, in addition to sample concentration are the critical electrophoretic parameters that have been suitably optimized in this method to achieve narrow peak shape, stable baseline and higher signal to noise ratio.

The results obtained from validation experiments proved that this optimized capillary electrophoresis method is selective, sensitive, linear, precise, accurate, and robust. Therefore, this optimized capillary electrophoresis method is suitable for the intended purpose and can be used during routine as well as

Table II. Recovery Results from Spiking of Sample with *N*-Methylpyrrolidine

% Conc.* spiked in the sample	% Amount found*	% Amount Recovered	% Recovery	Statistical analysis	
0.00	0.110	–	–	–	–
0.476	0.601	0.491	103.2	Mean	101.4
0.479	0.584	0.474	99.0	SD	2.15
0.474	0.593	0.483	101.9	%RSD	2.1
0.947	1.039	0.929	98.1	Mean	98.8
0.948	1.042	0.932	98.3	SD	1.04
0.955	1.065	0.955	100.0	%RSD	1.1
1.427	1.538	1.428	100.1	Mean	100.9
1.423	1.540	1.430	100.5	SD	1.12
1.422	1.563	1.453	102.2	%RSD	1.1
		Mean	**100.4**		
		SD	**1.78**		
		%RSD	**1.8**		

* Concentration level was relative to Cefepime concentration

during stability storage analysis of samples of cefepime for injection for *N*-methylpyrrolidine content.

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