An Improved Ion Chromatographic Method for Fast and Sensitive Determination of *N*-Methylpyrrolidine in Cefepime Hydrochloride

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Abstract

An alternative ion chromatographic method to the existing USP method for the determination of *N*-methylpyrrolidine (NMP) in cefepime hydrochloride is developed. The cefepime in solution behaves as a strong cation and gets retained in the analytical column, leading to reduction in column capacity and irreproducible retention time. The retained drug has to be removed with a special rinsing solution, followed by re-equilibration with the mobile phase. This process takes at least 3 to 4 h time for sample analysis. We used a silica-based cation exchange column with poly-butadiene-maleic acid functional group attached with an optimized mobile phase composition. The characteristic feature of this method is the short analysis time with a clear separation of NMP and the cationic drug molecule within a run-time of 30 min. The developed method overcomes the limitations of the USP method. This method describes the matrix elimination by choosing appropriate column and eluent condition. The method is tested for selectivity, linearity, limits of detection and quantification, accuracy, and precision and is suitable for continuous sample analysis.

Introduction

Cefepime is a fourth generation, semisynthetic cephalosporin. Like other fourth generation cephalosporins, cefepime demonstrates good activity against gram-negative organisms such as *Pseudomonas aeruginosa*, and gram-positive organisms such as *Staphylococcus aureus*. It also exhibits increased stability against β-lactamase-overproducing bacteria.

Cefepime is unstable and degrades slowly even when it is stored at 4ºC. Degradation of the β-lactam ring can result in the release of *N*-methylpyrrolidine (NMP), an alicyclic tertiary amine. This was the expected breakdown of cefepime because two related compounds, ceftazidime and cefpirome, have shown similar breakdown. It also indicates that the ring opening occurs before the cleavage of NMP (1). Cefepime has been examined for stability, potential liberation of degradation products, and compatibility with other drugs under conditions mimicking its potential use by continuous infusion in cystic fibrosis and intensive care patients (5–12% w/v solutions; temperatures from 20 to 37°C; 1 h contact at 25°C with other drugs frequently co-administered by intravenous route to these types of patients). Cefepime releases so far unidentified degradation products if maintained at > 30°C for > 12 h as shown from a marked increase in pH and from the development of a strong red-purple color (2,3). Intravenously administered NMP, or NMP arising from the degradation of cefepime in vivo, is subject to rapid metabolic clearance and oxidation to *N*-oxide (4). Hence the toxicity of NMP warrants the determination of residual NMP in cefepime.

A gas chromatography method to determine NMP in cefepime and its preparation has been reported (5). However, it involves extraction of NMP from cefepime and uses pyridine, another known carcinogen, as the internal standard. A capillary electrophoresis method with indirect UV detection has been reported (6); it requires an application of small inlet pressure to get a stable base line, which would be difficult to implement in a pharmaceutical QC environment. It is preferable to use high-performance liquid chromatography, if possible, because these methods have routinely been proven to provide performance suitable for pharmaceutical assays.

Limitations of current USP method

The current USP31-NF26 method recommends using the L52 column with L17 guard column, with 0.01 N nitric acid and acetonitrile in the ratio 100:1 as the eluent. Cefepime behaves as a strong cation in aqueous solution and gets retained in the analytical column, and elutes as a broad peak at 55 min. The retained drug reduces column capacity, leading to a shift in the retention time of NMP in consecutive injections. The retention time variation of NMP from sample solution and standard solution is more than 10%. To remove the retained drug, a rinsing solution is used consisting of 50% of acetonitrile with nitric acid. This process of sample injection followed by column rinsing and column re-equilibration takes ~ 3 to

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4 h per test solution. Hence, the suggested method is not suitable for routine quality control testing. The aim of this work is to develop an alternative method which overcomes the limitations of the USP method.

Experimental

Instrument and accessories

An 861 Advanced Compact ion chromatography (IC) instrument from Metrohm (Herisau, Switzerland), together with 838 IC Filtration Sample Processor with built-in injector and peristaltic pump was used. Silica-based Metrosep cation C2–150 and polyvinyl alcohol-based Metrosep cation C3–250 columns were used for initial separation characterization. Both of these columns have identical poly-butadiene-maleic acid (PBDMA) functional group.

Chemicals

All solutions were prepared using deionized water (> 18MΩ) purified by a Milli-Q Gradient system (Millipore, Billerica, MA). Nitric acid puriss grade was from Fluka (Seelze, Germany). Acetone, acetonitrile, NMP from Merck (India) were used as received. Cefepime hydrochloride was received from a pharmaceutical company in Chennai, India.

Standard preparation

Approximately 0.32 mL of NMP, accurately weighed, was transferred to a 100-mL volumetric flask, dissolved, and made up to volume with water. Four milliliters of this solution was transferred to a 100-mL volumetric flask, diluted to volume with water, and mixed. This solution contains ~ 0.1 mg of NMP per mL. Lower concentrations of NMP were prepared from this stock solution by appropriate dilution with water.

Sample preparation

One hundred milligrams of cefepime hydrochloride, accurately weighed, was transferred to a 10-mL volumetric flask, dissolved, and made up to volume with water. This solution may be kept up to 6 h if maintained < 5°C; otherwise, this solution should be used within 30 min.

One container of cefepime was constituted for injection with the volume of water specified in the labeling. An accurately measured volume of this solution was diluted to obtain a solution with a concentration of ~ 10 mg of cefepime per mL.

Results and Discussion

Chromatography optimization for NMP and cationic drug elution

NMP separation in a matrix of strong cationic substance was investigated with columns having identical functional group but different base material, using a fixed nitric acid concentration of 5 mmol/L; the lipophilic effect was studied by changing the acetonitrile and acetone concentrations from 5% to 20%. The resulting retention time profile is given in Figure 1.

With both the columns, acetone as the solvent resulted in a further reduction in retention when compared with the same amount of acetonitrile. This was thought to be the result of hydrogen bonding between acetone and ammonium ion, resulting in a further decrease in interaction with the stationary phase and, therefore, lower retention time than with acetonitrile.

The column base materials had altogether different selectivity towards NMP and the cationic drug. With 5 mmol/L nitric acid as the eluent, NMP could be eluted with the polyvinyl-alcohol-based column, whereas it was retained in the silica-based column due to stronger interaction with polar silica base material. Hence, to elute NMP from the silica-based column, the addition of organic modifier was necessary.

Using 5 mmol/L nitric acid with 10% acetonitrile as the eluent, both NMP as well as the cefepime drug could be eluted well within 25 min run-time with a silica-based column. In this case, the addition of small amounts of an organic modifier resulted in reduced ionic interactions between strongly retained amines and the stationary phase. The additions of these organic modifiers have proven especially useful for strongly retained amines and cationic drugs.

With the same eluent composition, the cefepime drug was eluting as a big broad peak at 45 min with a polyvinyl alcohol-based column. This was mainly due to the strong interaction of the drug molecule with the non-polar base material. This is one of the major problems with the polymer-based cation exchange columns. To achieve a good separation of weakly retained amine from strongly retained drug, it is required to use an elution of either a linear gradient or a step gradient. This makes the system more complicated and difficult to maintain. Another problem with polymer-based column and gradient separation is the extended re-equilibration time needed, which defeats the purpose of developing simple and rapid analytical method.

Similarly, arginine, which is used as a buffering agent in cefepime for injection, had different selectivity with these two

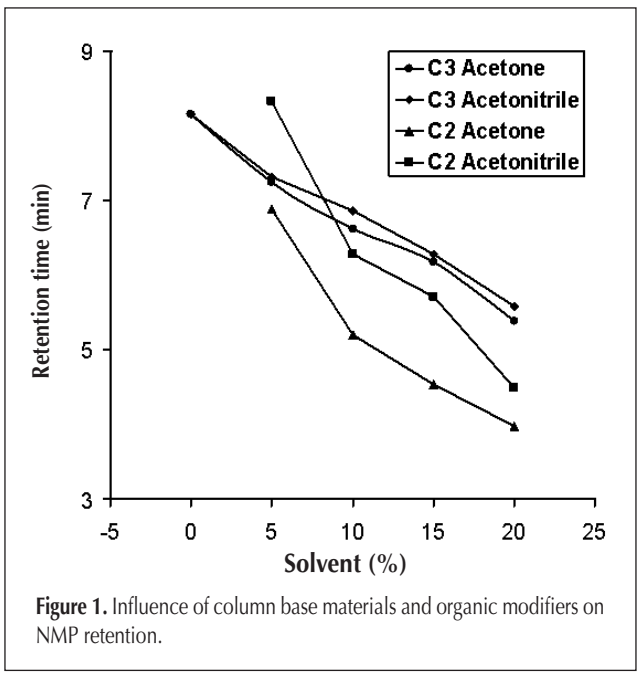

Figure 1. Influence of column base materials and organic modifiers on NMP retention.

columns. Arginine was retained with the polyvinyl alcohol-based column and eluted close to the drug, where as it eluted well before NMP with a silica-based column.

Considering all these limitations with a polymer-based column and gradient separation, it was decided to use the silica-based column for further method optimization and validation. To further reduce the run-time, an eluent composed of 6 mmol/L nitric acid with 10% acetonitrile was used. This eluent at 1 mL/min flow-rate produces background conductivity of ~1725 µS/cm with a noise level of 0.005 to 0.006 µS/cm.

Specificity

The method selectivity was checked by injecting blank water, mobile phase, NMP standard, mixed cation standard consisting of lithium, sodium, ammonium, potassium, calcium and magnesium, mixed aliphatic amines standard consisting of monomethylamine, dimethylamine, trimethylamine, ethanolamine, diethanolamine, triethanolamine, the drug, and drug spiked with NMP. Clear separation of NMP from the standard cations and mixed amine was achieved. A merged chromatogram of the blank, mixed cations, mixed amines, and 50 µg/mL NMP is shown in Figure 2. The clear separation of NMP from the main drug component, common cations, and aliphatic amines proves that this chromatographic condition was suitable for the separation and quantification of NMP in this drug sample.

System precision

System precision was checked by performing six replicate injections of 50 µg/mL NMP standard, and the resulting % relative standard deviation (RSD) was calculated for area and retention time. The %RSD for six replicate injections was 1.01% and 0.3%, respectively, for area and retention time. These values are well within the acceptable limit of 5% set by the U.S. Food and Drug Administration.

Linearity

Linearity was checked by injecting 5, 10, 15, 20, 30, 40, and 50 µg/mL of NMP prepared from NMP stock solution. Each standard was injected six times to check the precision. The calculated slope value was 2.601 ± 0.01801 and *Y*-intercept value was –2.142 ± 0.5163. The correlation coefficient was r^2 = 0.99904. The calculated residual standard deviation was $S_{y/x}$ = 1.777. The RSD of the response factor was 2.81%.

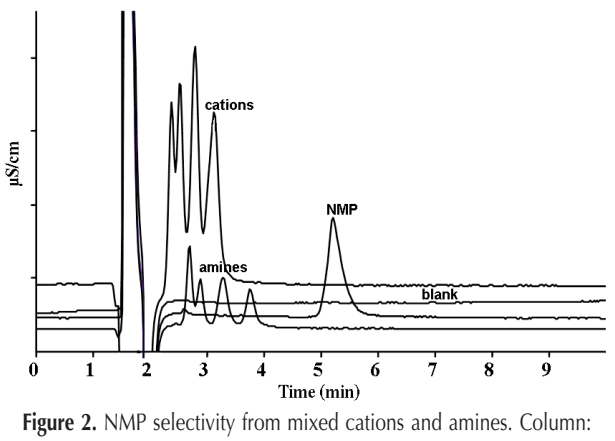

Figure 2. NMP selectivity from mixed cations and amines. Column: Metrosep Cation C2–150; eluent: 6 mmol/L nitric acid + 10% acetonitrile; flow: 1.0 mL/min.

Sample analysis and method precision

Three samples were prepared from the same cefepime batch, and 20 µL of the sample was injected to the IC system. From the NMP content, the method precision was calculated as 1.8%. A sample chromatogram is shown in Figure 3.

Limits of detection and quantification

Based on the linearity data, limit of quantification (LOQ) and limit of detection (LOD) were predicted using the following formula:

LOD (µg/mL) = noise × 3.0
LOQ (µg/mL) = noise × 10

The LOD and LOQ were found to be 0.15 µg/mL and 0.4 µg/mL of NMP, respectively, which would correspond to 0.001% and 0.005% of NMP with respect to the sample weight. The LOQ achieved by this method was well within the USP requirement of 0.3%.

Accuracy and precision

Spiking study at LOQ level could not be carried out, as the sample analyzed had an NMP concentration of 0.0963%, which was higher than the LOQ. However, to check the accuracy, a known quantity of NMP was spiked to the accurately weighed cefepime hydrochloride sample to get a final NMP concentration of 0.3% with respect to the drug weight. Three samples were prepared to check the precision. Recovery ranging from 99% to 101% was achieved with an RSD of 1.15%. This indicates that the proposed method is suitable for the determination of NMP from the LOQ level to tolerance level.

Cefepime for injection

Arginine and NMP were co-eluting under the chromatographic condition used for cefepime. Hence, the eluent composition was modified to 5 mmol/L nitric acid with 5% acetonitrile to get clear separation of NMP from arginine and common cations. Under this chromatographic condition, the drug elutes well within a 35 min run-time. The tolerance level for NMP in cefepime for injection is less than 1% and hence,

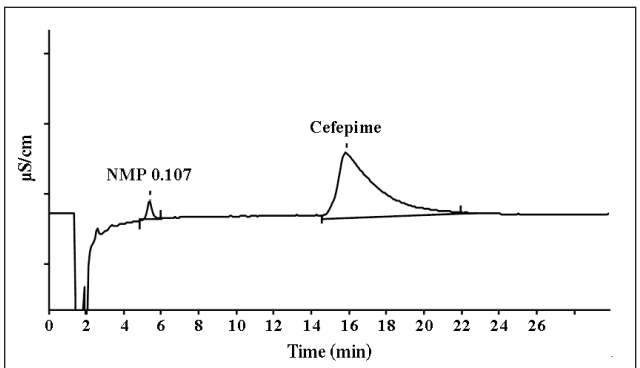

Figure 3. Cefepime sample.

if needed, the injection volume can further be reduced to 5 µL. A sample chromatogram is shown in Figure 4.

Summary

This method describes the matrix elimination by choosing appropriate column and eluent condition. The cationic drug molecule elutes within 25 min in the case of cefepime drug and within 35 min run-time in the case of cefepime for injection. It is very easy to swap between the two eluent compositions, as they differ only slightly in their composition. The equilibration time needed for this column was less than 30 min. Overall, this method has improved sensitivity for NMP, short analysis time, and improved reproducibility compared to the current USP method is suitable for the continuous sample injections. Important method validation parameters are summarized in Table I.

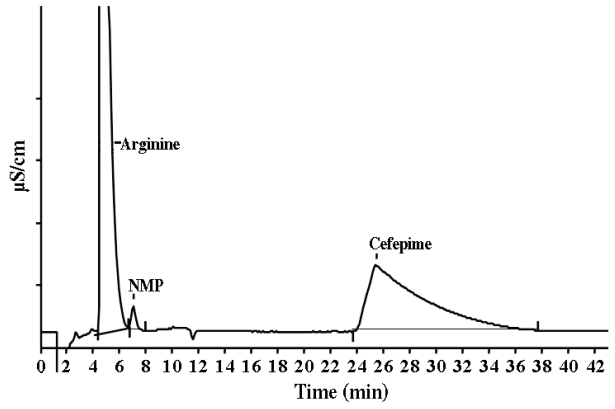

Figure 4. Cefepime for injection. Clean separation of NMP from Arginine. Eluent 5 mmol/L nitric acid with 5% acetonitrile.

Table I. Validation Summary

Validation parameter	Acceptance criteria	Validation result	Result
Selectivity	Baseline separation of NMP from cations and amines	RRT < 0.8 for cations and amine	+
LOD	Noise × 3.0	0.15 µg/mL; 0.001%	+
LOQ	Noise × 10	0.4 µg/mL; 0.005%	+
System precision	RSD of peak area, retention time < 5%	< 1.01%	+
Linearity	$r^2 \geq 0.9980$ for six-point calibration	$r^2 = 0.99904$; RSD = 2.81%	+
Accuracy	Spike recovery: 80…120%	99…101.0%	+
Precision	RSD ≤ 15%	RSD < 1.15%	+

Acknowledgment

The authors N. Harihara Subramanianan and S. Thyagarajan, and P. Manigandan greatly acknowledge the support provided by Metrohm India Limited, Chennai.

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Manuscript received December 31, 2008;
Revision received March 4, 2009.