Determination of *N*-Methylpyrrolidine in Cefepime with Nonsuppressed Conductivity Detection

INTRODUCTION

Cefepime (Figure 1A) is a semi-synthetic, fourth-generation cephalosporin with low toxicity and a broad antimicrobial activity range against Gram-negative and Gram-positive bacteria.[1, 2] However, this antibiotic is thermally unstable and will rapidly degrade at temperatures ≥ 25 °C and slowly degrade at lower temperatures (e.g., 4 °C).[3] One of the degradation products is *N*-methylpyrrolidine (NMP, Figure 1B).[3] The primary concerns with NMP formation are loss of cefepime potency and potential toxicity to patients. Although NMP is metabolized to the N-oxide and cleared rapidly,[4] the potential for side effects is still a health concern. Therefore, the determination of NMP in cefepime is critical to assess the purity of the pharmaceutical product due to potential toxicity of NMP to patients.

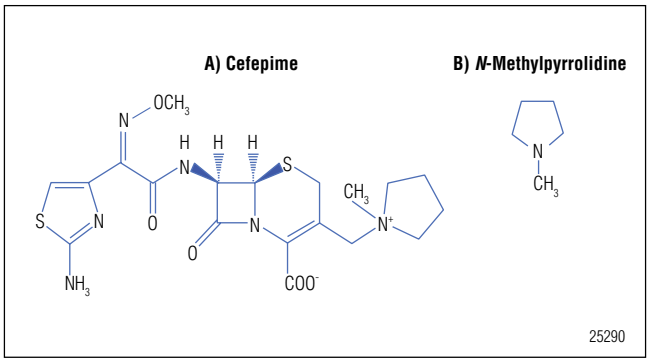

Figure 1. Chemical structures of A) cefepime and B) N*-methylpyrrolidine.*

Cefepime and its related compounds have been characterized by HPLC with UV detection,[5] whereas the NMP degradation product in cefepime has been determined by cation-exchange chromatography with suppressed conductivity detection using a Reagent-Free™ Ion Chromatography (RFIC™) system.[6] The U.S. Pharmacopeia (USP) has proposed improving compendial methods for determining the limit of NMP in cefepime hydrochloride[7] and Cefepime for Injection[8] by eliminating column rinse and re-equilibration steps, thereby increasing sample throughput from 3–4 h to 60 min per sample.[9, 10]

This work describes the determination of NMP by cation-exchange chromatography with nonsuppressed conductivity detection.[11] The results meet the USP criteria for Organic Impurities, Procedure 1 (Limit of *N*-Methylpyrrolidine) in the proposed methods for cefepime hydrochloride[9] and Cefepime for Injection.[10]

EQUIPMENT

Dionex ICS-2100 system* including:

- Single isocratic pump
- Vacuum degasser
- High pressure, 6-port injector
- Column heater enclosure
- Conductivity cell detector
- EO Eluent Organizer, including pressure regulator, and 2 L plastic bottle

AS Autosampler with sample tray temperature control and 2 mL vial tray

Chromeleon® 6.8 or greater Chromatography Data System (CDS) software

Helium or nitrogen, 4.5-grade (99.995%) or better, <5 ppm oxygen (Praxair)

Filter unit, 0.2 µm nylon (Nalgene 90 mm Media-Plus, Nalge Nunc International P/N 164-0020) or equivalent nylon filter

Vacuum pump (Gast Manufacturing Corp. P/N DOA-P104-AA) or equivalent, for degassing eluents

Glass injection vials (1.5 mL) with caps (Vial Kit, Dionex P/N 055427)

*This application can also be run using an ICS-1100, -1600, -3000, or -5000 system.

CONSUMABLES

IonPac® SCG1 column, 4 × 50 mm (Dionex P/N 061523)

IonPac SCS1 column, 4 × 250 mm (Dionex P/N 061521)

IonPac Mixer (Dionex P/N 063443)

REAGENTS AND STANDARDS

Deionized water, 18 MΩ-cm resistance or higher, filtered and degassed

Nitric acid, Ultrex® II ultrapure reagent (VWR P/N JT6901-5)

Acetonitrile, UV (VWR P/N BJ015-4)

Cefepime Hydrochloride Reference Standard (USP P/N 1097636), Lot H0G278 was used in this study.

DL-Arginine (Sigma-Aldrich P/N A4881)

N-Methylpyrrolidine, 97% (Sigma-Aldrich P/N M79204)

CONDITIONS

Columns:	IonPac SCG1 4 × 50 mm (Dionex P/N 061523) IonPac SCS1 4 × 250 mm (Dionex P/N 061521)
Eluent:	10 mM nitric acid/5% acetonitrile
Flow Rate:	1.00 mL/min
Inj. Volume:	10 µL (full loop)
Column Temp.:	30 °C
Detector Temp.:	40 °C
Detection:	Nonsuppressed conductivity
Background:	~3300 µS
Noise:	Typically <20 nS/min
Backpressure:	~2300 psi

ELUENT AND STANDARDS PREPARATION

Eluent Solution

Fill a 2 L glass volumetric flask to the mark with filtered and degassed deionized (DI) water. Remove 101.2 mL of DI water, add 1.2 mL of concentrated nitric acid (70%, 15.8 N), and mix thoroughly. Add acetonitrile with mixing to the mark to produce 2 L of eluent.

Standard and Sample Solvent Solution

The NMP stock, calibration standards, and cefepime surrogate sample solutions use 2 mM nitric acid as the diluent. To prepare 2 mM nitric acid, add 500 g of filtered and degassed DI water to a tared glass bottle, add 0.127 mL of concentrated nitric acid to the flask, mix well, and add enough filtered and degassed DI water to make 1.0 kg of solution.

Standard NMP Solution

To prepare a 1000 µg/mL NMP solution, add 19 mL of 2 mM nitric acid solvent to a tared glass scintillation vial, add 0.0200 g NMP solution to the vial beneath a well-ventilated fume hood, and add enough 2 mM nitric acid solvent to prepare 20 g of solution. Store the stock solution at 4 ºC when not in use. Make all subsequent dilutions of NMP stock solution gravimetrically with 2 mM nitric acid for generating the calibration curve and system precision. Store at 4 ºC.

SAMPLE PREPARATION

Cefepime Hydrochloride Samples

Prepare 5 mg/mL cefepime matrix solution based on the cefepime assay result listed on the USP Reference Standard label (0.865 mg cefepime/mg solid in Lot H0G278). For this study, weigh 0.0289 g cefepime hydrochloride into a tared, 20 mL glass scintillation vial and dissolve in 5 g of 2 mM nitric acid to produce a 5 mg/mL cefepime solution. Mix to dissolve the solid, then immediately withdraw 1.0 mL of the matrix solution and place the aliquot in a 1.5 mL glass autosampler vial. This vial then should be placed into the AS sample tray cooled to 4–6 ºC and analyzed immediately to minimize sample degradation. To determine the method's accuracy and precision, reweigh the scintillation vial, add an appropriate weight of 1000 µg/mL NMP solution to produce a working sample of 15 µg/mL NMP in 5 mg/mL cefepime, and immediately analyze the solution.

Cefepime for Injection Simulated Samples

Retention time and peak area precision of NMP in the simulated Cefepime for Injection samples were evaluated. In Cefepime for Injection, arginine is added at an approximate concentration of 725 mg/g of cefepime to maintain the pH of the constituted solution between 4 and 6. Prepare 5.0 g of 5 mg/mL cefepime solution in 2 mM nitric acid as described above. Based on the expected 0.827 mg arginine/mg arginine hydrochloride, add 0.0219 mg arginine hydrochloride to the cefepime solution. Mix, immediately withdraw 1.0 mL of this matrix solution, place the aliquot in a 1.5 mL glass autosampler vial, and place the vial into the AS sample tray cooled to 4–6 ºC. Start the sequence soon after placing the vials in the autosampler.

RESULTS AND DISCUSSION

Chromatography

The IonPac SCS1 column is a low-capacity, silica-based, weak cation-exchange column functionalized with carboxylic acid groups.[12] Typical recommended eluent conditions for this column are 3 mM MSA at 1 mL/min to elute six common cations in about 35 min (guard and analytical columns). In the proposed USP method, the described eluent composition consists of 10 mM nitric acid with 5% acetonitrile. The organic solvent reduces the hydrophobic interactions of cefepime with the stationary phase to improve sample throughput, whereas higher acid concentrations decrease the retention of alkali and alkaline earth metals on the column. However, an increase in the hydronium ions in the eluent produces a proportional increase in the background conductivity and, therefore, baseline noise, which reduces the sensitivity of the method. Despite the reduction in sensitivity, the method retains the ability to quantify at or below the USP specification for NMP in cefepime hydrochloride. Figure 2A demonstrates the separation of common cations on the IonPac SCS1 column using the eluent conditions described in the USP method. As shown, the retention time of these cations is significantly reduced relative to the standard conditions with 3 mM MSA. Figure 2B shows the separation of NMP eluting at 10 min as a sharp peak with an asymmetry of 1.1 and a plate count > 15000.

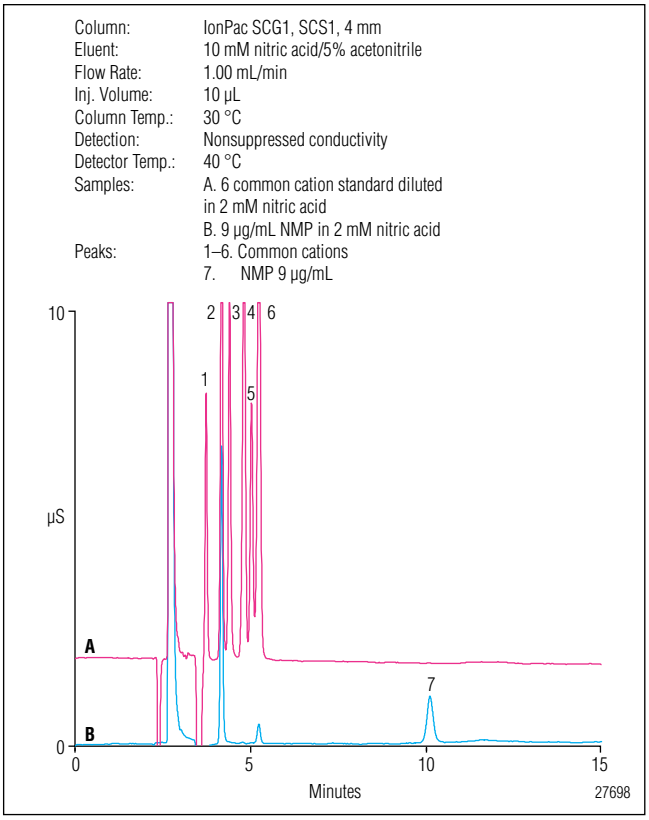

Figure 2. Chromatography of A) six common cation standard and B) 9 µg/mL NMP standard dissolved in 2 mM nitric acid.

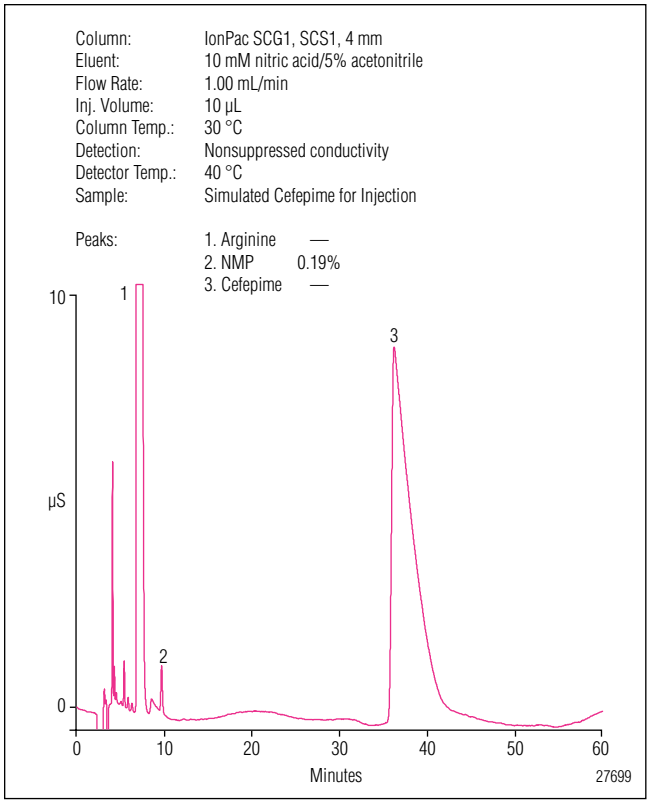

Figure 3. Determination of NMP in simulated Cefepime for Injection sample.

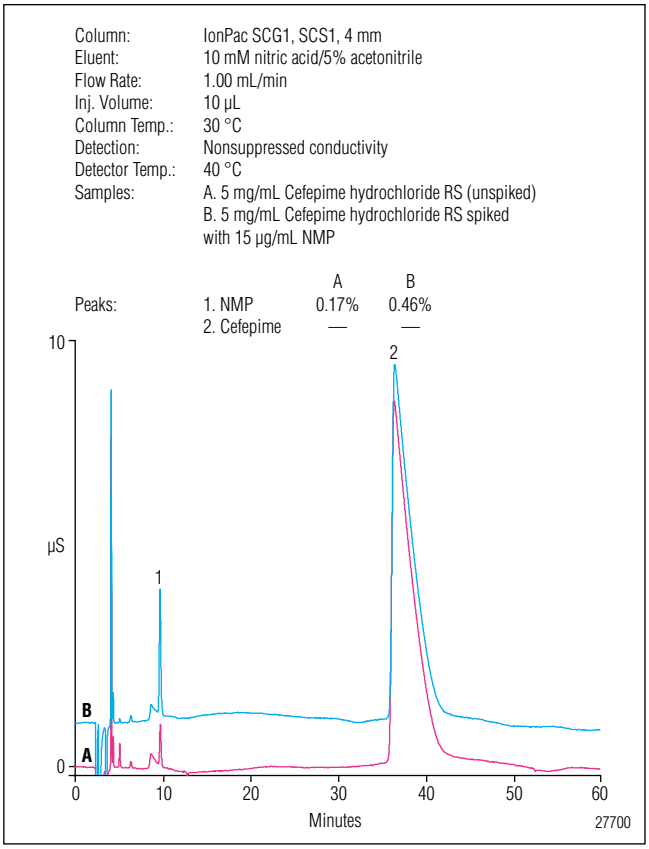

Figure 4. Comparison of A) unspiked and B) 15 µg/mL (0.3%) NMP-spiked solutions of 5 mg/mL cefepime in 2 mM nitric acid.

Figure 3 shows the separation of NMP in a simulated Cefepime for Injection sample, which also contains relatively high concentrations of arginine. As shown, arginine and NMP are well resolved (R_s = 8.2) on the IonPac SCS1 column using the conditions specified in the USP monograph. This chromatogram also demonstrates that cefepime elutes completely within 6× the NMP retention time limit as specified in the USP proposed monograph, thus avoiding cefepime carryover.[9, 10] However, the total analysis time is twice as long as the IC method described in Dionex AN 199.[6]

Limit of Detection, Limit of Quantification, and Linearity

The USP General Chapter on validation of compendial methods <1225> specifies a signal-to-noise (S/N) ratio of three for the limit of detection (LOD) and 10 for the limit of quantification (LOQ).[13] Baseline noise was determined to be 18 nS by averaging the peak-to-peak noise of seven system (no injection) blanks over two 1 min windows centered on the NMP retention time. Peak heights from triplicate injections of standards in 2 mM nitric acid were plotted versus NMP concentration. The LOD and LOQ estimates for NMP were 0.5 and 1.6 µg/mL, respectively, corresponding to 0.01 and 0.032% in 5 mg/mL cefepime. The LOQ estimate was 10-fold lower than the 0.3% acceptance criterion cutoff level for cefepime hydrochloride and more than 30-fold lower than the 1.0% acceptance criterion cutoff level for Cefepime for Injection.

To determine method linearity, calibration standards were prepared at nine concentration levels in the range of 5–100 µg/mL NMP in 2 mM nitric acid, corresponding to 0.1–2.0% in 5 mg/mL cefepime. A plot of peak area versus concentration produced a correlation coefficient (r^2) value of 0.9996 using a linear least squares regression fit. The relative standard deviation of the measured peak areas based on the calibration curve was < 1.8%.

Accuracy and Precision

Method accuracy was evaluated by spiking 15 µg/mL of NMP into a 5 mg/mL cefepime sample solution (Figure 4). The unspiked cefepime sample contained 8.41 µg/mL (0.17%) NMP, which was below the acceptance criterion cutoff value. After correcting for the amount of NMP in the cefepime sample, the average recovery for three replicates was 99 ± 1.0%.

Table 1. Retention Time and Peak Area Precisions for *N*-Methylpyrrolidine in Cefepime Samples						
Sample[a]	**N**	**NMP Conc.**	**Average Retention Time (min)**	**Retention Time RSD**	**Average Peak Area (µS*min)**	**Peak Area RSD**
15 µg/mL NMP Standard	6	15.0 µg/mL	9.742	0.02	0.3686	0.4
USP Cefepime Hydrochloride RS[b]	6	0.18%	9.715	0.06	0.2033	1.1
Simulated Cefepime for Injection[c]	6	0.19%	9.704	0.13	0.2248	1.6

[a] - 2 mM nitric acid solvent

[b] - 5 mg/mL cefepime

[c] - 5 mg/mL cefepime + 3.6 mg/mL arginine

Table 1 summarizes the results of three sets of repeatability experiments. System precision refers to repeatability for a standard without cefepime or cefepime/arginine mixture present. Excellent system precisions for NMP retention time (0.02%) and peak area (0.4%) were measured for an NMP concentration of 15 µg/mL. Precision of NMP retention time and peak area was also determined for cefepime HCl and Cefepime for Injection samples. Retention time and peak area repeatabilities were 0.06% and 1.1%, respectively, for NMP in 5 mg/mL cefepime and 0.13% and 1.6% for NMP in 5 mg/mL Cefepime for Injection containing 3.6 mg/mL arginine. Cefepime sample instability was observed when stored in the autosampler tray at 4 ºC over the 6.1 h time interval needed to run the six replicates of each of the two cefepime-containing samples. NMP peak areas increased 2.7% for the cefepime hydrochloride sample and 3.9% for the Cefepime for Injection simulated sample. Using the IC-based method described in AN 199 will result in faster sample throughput, producing less cefepime decomposition during analysis of a given sample.

CONCLUSION

This work presents the determination of *N*-methylpyrrolidine, a cefepime decomposition product, using a silica-based, weak cation-exchange column coupled with nonsuppressed conductivity detection. Results for LOD, LOQ, linear calibration range, spike recovery, retention time precision, and peak area precision determinations show that this instrumental configuration fulfills acceptance criteria for determining NMP in cefepime hydrochloride and Cefepime for Injection samples. Cefepime decomposition during sample analysis can be decreased and eluent preparation can be simplified by using the method described in AN 199.

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SUPPLIERS

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