

Determination of *N*-Methylpyrrolidine in Cefepime Using Ion Chromatography

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ABSTRACT

Cefepime (Figure 1A) is a semi-synthetic, fourth generation cephalosporin that is commonly prescribed for the treatment of urinary tract infections, pneumonia, febrile neutropenia, skin or soft-tissue infections, and abdominal infections. During the preparation and storage of cefepime, degradation of the beta-lactam ring can occur to yield *N*-methylpyrrolidine (NMP). The primary concerns with degradation are loss of potency and the potential toxicity of degradation products to patients. The US Pharmacopeia (USP) specifies a limit of <0.3% NMP (Figure 1B) in cefepime hydrochloride and <1% in cefepime for injection.

The current USP method, using cation-exchange chromatography with direct conductivity detection for determining the limit of NMP in cefepime, requires 3–4 h per injection and has poor retention stability between the standards and samples. In this poster, we demonstrate an improved method that significantly reduces the time between injections, thereby increasing sample throughput, and improves retention time stability. The linearity, detection limits, precision, and accuracy of NMP in cefepime hydrochloride are discussed.

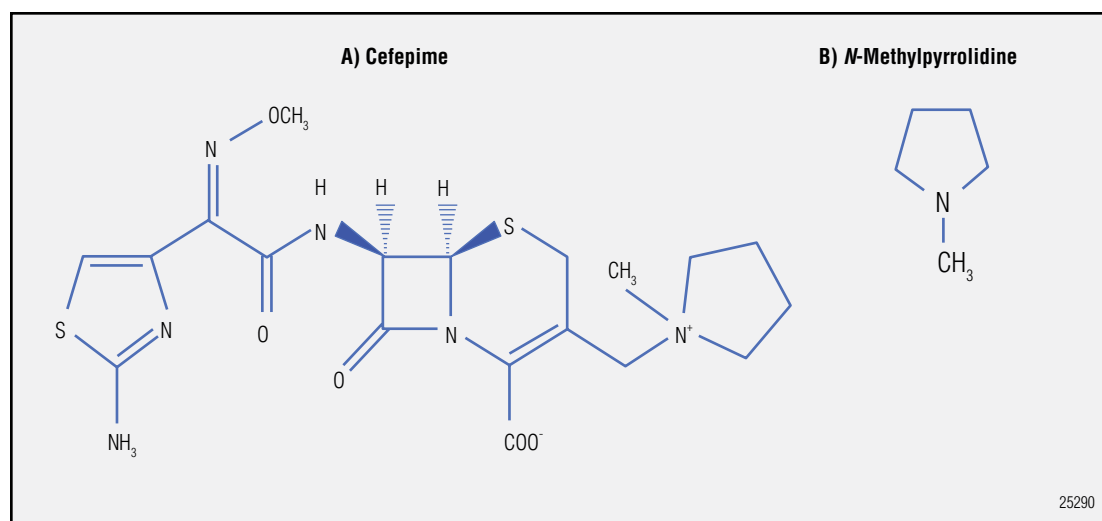


Figure 1. Chemical structures of A) cefepime and B) *N*-methylpyrrolidine.

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EXPERIMENTAL

- Dionex ICS-3000 Reagent-Free™ ion chromatography system consisting of a DP dual gradient pump, EG Eluent Generator with an EGC II MSA cartridge and CR-CTC II trap column, and a DC detector compartment with dual temperature zones.
- IonPac® CG17 guard (2 × 50 mm) and CS17 analytical (2 × 250 mm) columns with a 5 µL injection volume and flow rate of 0.40 mL/min at a column temperature of 40 °C for the assay and 50 °C for the system suitability test.
- Analytes were detected by suppressed conductivity detection using a CSRS® 300 (2 mm) suppressor in the recycle mode with a current setting of 100 mA.

Sample Preparation

- A 10 mg/mL solution of cefepime hydrochloride was prepared by dissolving 100 mg cefepime in 10 mL of deionized water.
- A simulated cefepime for injection solution was prepared by combining 100 mg cefepime hydrochloride with 72.5 mg of arginine and dissolving in 10 mL of deionized water.
- The cefepime system suitability sample was prepared by dissolving 10 mg of the sample in 1 mL of deionized water. This solution was diluted to a final concentration of 1.4 mg/mL prior to analysis.

RESULTS AND DISCUSSION

Separation

The IonPac CS17 column is a hydrophilic, moderate capacity (363 µeq/column, 2 × 250 mm) carboxylate-functionalized cation exchange column specifically developed for the separation of hydrophobic and polyvalent amines. Due to the very low hydrophobic character of the column, strongly retained compounds such as cefepime are eluted from the column more rapidly. Therefore, the use of the IonPac CS17 column for this method significantly reduces the time between injections relative to the current USP method. Table 1 compares the proposed method, presented in this poster, to the USP method for the determination of NMP in cefepime.

Table 1. Comparison between the Proposed Method and the Current USP Method for Determination of NMP in Cefepime Hydrochloride

| | Proposed Method | USP Method |
|--------------------------------------|------------------------|------------------------------------|
| Separation phase | Cation exchange | Cation exchange |
| Eluent | Methanesulfonic acid | 10 mM Nitric acid/1% acetonitrile |
| Column rinse solution | Not required | 40 mM Nitric acid/50% acetonitrile |
| NMP retention time (min) | 5.3 | ≥ 8 |
| Cefepime retention time (min) | 12 | 55 |
| Time per sample (min) | 35 | 180–240 |

Figure 2 compares the separation of a 25 µg/mL NMP standard to a 10 mg/mL cefepime hydrochloride solution. The NMP retention time was about 5.3 min in the standard and sample solution. An additional 5 min equilibration time was added before each analysis, resulting in a total analysis time of 35 min per sample.

Linearity, Limit of Detection, and Limit of Quantitation

NMP was calibrated between 0.45 and 200 µg/mL using a least squares regression fit, which produced a correlation coefficient (r^2) of 0.9999. To determine the limit of detection (LOD) and quantitation (LOQ), the baseline noise was measured in a representative 1 min segment of the baseline where no peaks elute. Typical baseline noise for this method using the CSRS 300 suppressor in the recycle mode is 0.2–0.4 nS/min. The LOD for NMP was estimated to be 0.03 µg/mL based on a signal-to-noise ratio of 3 ($S/N = 3$). The LOQ for NMP was estimated to be 0.10 µg/mL ($S/N = 10$), which represents 0.001% NMP in a 10 mg/mL cefepime hydrochloride solution.

Accuracy and Precision

The method was used to assay three independently prepared 10 mg/mL cefepime hydrochloride solutions from a single USP lot over three consecutive days. The average NMP concentration detected in cefepime was $0.236 \pm 0.003\%$, which meets the $<0.3\%$ NMP specification for cefepime hydrochloride according to USP 31-NF 26. The intraday retention time and peak area precisions ($n = 10$) were $\leq 0.3\%$ and $\leq 1.3\%$, respectively. The interday retention time and peak area precisions ($n = 30$) over three consecutive days were 0.5% and 1.5%, respectively. Table 2 summarizes the concentration of NMP determined in cefepime over three days and the retention time and peak area precisions.

The method accuracy was determined by spiking the samples with 0.26, 0.52, and 1.0% NMP, which produced average recoveries of $102.0 \pm 3.2\%$, $100.6 \pm 0.8\%$, and $97.8 \pm 0.4\%$, respectively, suggesting the method is accurate.

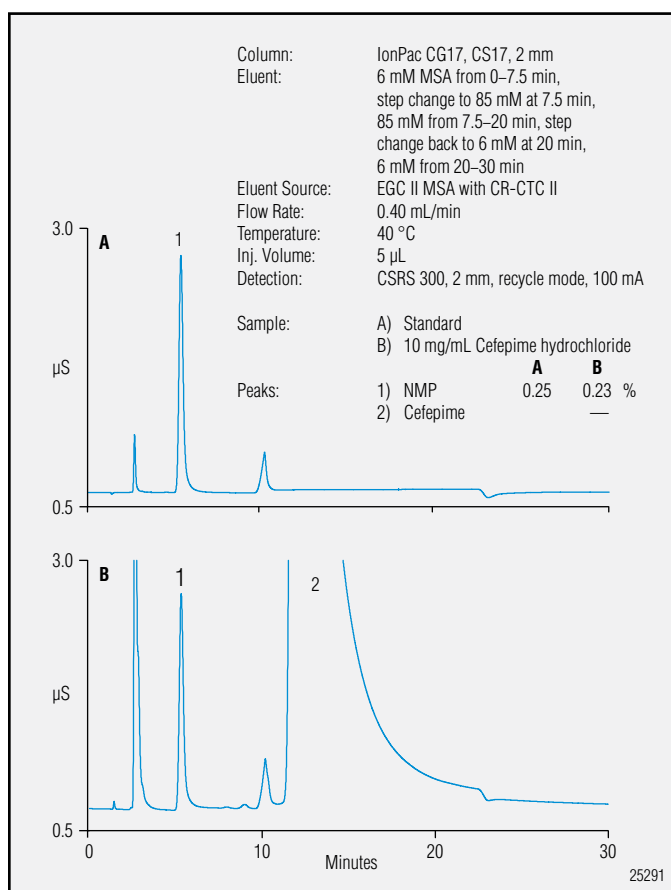


Figure 2. Comparison of the separation of NMP in A) a standard solution and B) cefepime hydrochloride.

| Table 2. Summary of NMP Determined in Independently Prepared Solutions of 10 mg/mL Cefepime Hydrochloride over Three Consecutive Days | | | | | |
|---|----|-----------------|------------------------------|--------------------|---------------|
| Day | n | Average NMP (%) | Average Retention Time (min) | Retention Time RSD | Peak Area RSD |
| 1 | 10 | 0.232 | 5.3 | 0.3 | 1.36 |
| 2 | 10 | 0.239 | 5.3 | 0.8 | 0.99 |
| 3 | 10 | 0.236 | 5.3 | 0.1 | 1.01 |

Cefepime for Injection

This sample is a mixture of cefepime hydrochloride and a sufficient amount of L-arginine to obtain a reconstituted pH between 4 and 6. Figure 3 shows an example chromatogram of 10 mg/mL cefepime with 7.25 mg/mL arginine. As shown, NMP was well-resolved from arginine and the concentration of NMP was nearly equivalent to previous cefepime samples. The retention time and peak area precisions for triplicate injections of the simulated cefepime for injection sample were 0.6% and 1.1%, respectively.

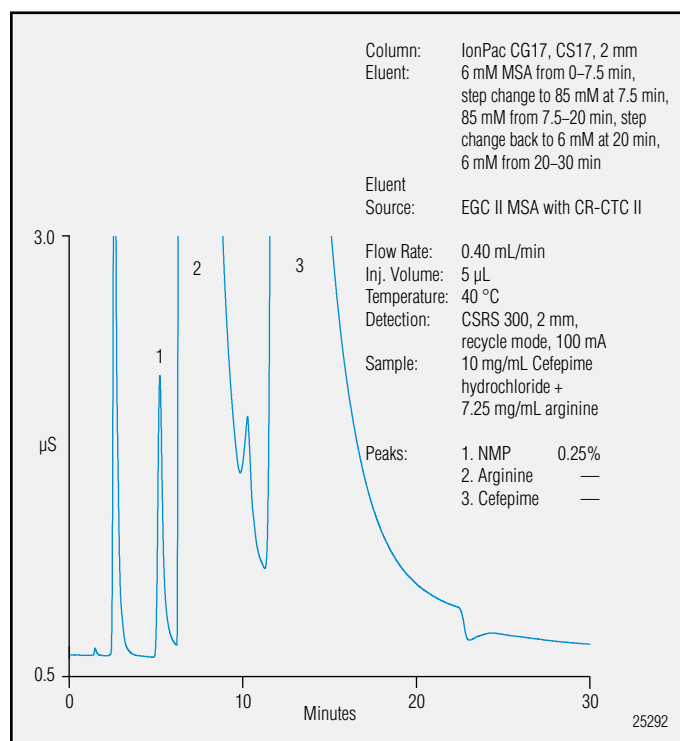


Figure 3. Determination of NMP in a simulated cefepime for injection sample.

USP Cefepime System Suitability Sample

This sample is a mixture of 93.8% cefepime hydrochloride, 0.9% cefepime related compound A, and 1.4% cefepime related compound B. The eluent conditions and column temperature were modified for this sample to improve the resolution between NMP and an unidentified peak, which is most likely derived from the cefepime related compounds. Figure 4 demonstrates the separation of NMP in the system suitability sample using optimized conditions.

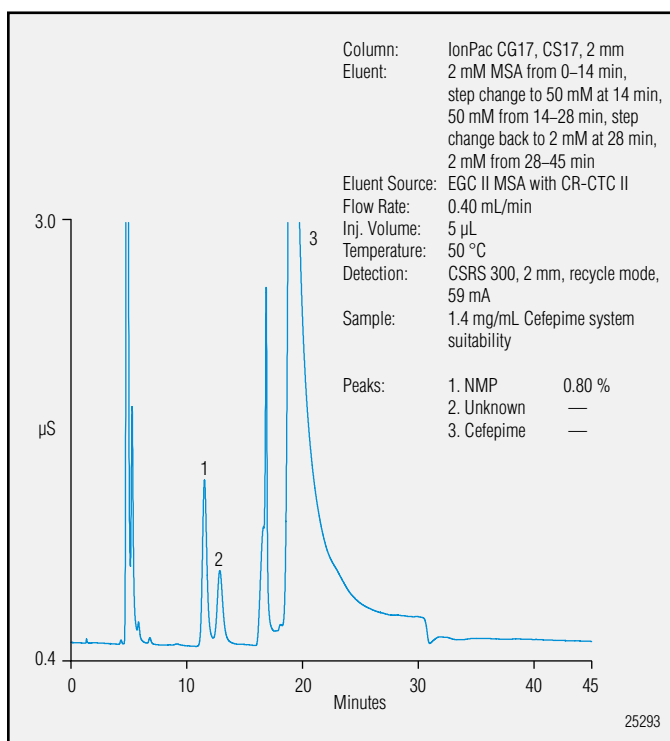


Figure 4. Determination of NMP in USP Cefepime System Suitability Sample.

The average NMP concentration for three independently prepared solutions analyzed over three different days was $0.80 \pm 0.02\%$ (Table 3). Due to the presence of additional cefepime related compounds, which also contain NMP as part of their chemical structures, the NMP concentration was higher than detected in the assay. The intraday retention time and peak area precisions ($n = 6$) were $<0.1\%$ and $\leq 0.7\%$, respectively. The interday retention time and peak area precisions over three different non-consecutive days ($n = 18$) were 0.3% and 2.7% , respectively. Table 3 summarizes the data for NMP determined in three independently prepared system suitability samples.

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Table 3. Summary of NMP Determined in Independently Prepared Solutions of 1.4 mg/mL Cefepime System Suitability Sample over Three Days

| Day | n | Average NMP (%) | Average Retention Time (min) | Retention Time RSD | Peak Area RSD |
|-----|---|-----------------|------------------------------|--------------------|---------------|
| 1 | 6 | 0.81 | 11.5 | 0.03 | 0.39 |
| 2 | 6 | 0.82 | 11.5 | 0.02 | 0.74 |
| 5 | 6 | 0.77 | 11.5 | 0.04 | 0.35 |

Sample Stability

The stability of NMP in cefepime stored at -17 °C, 4 °C, and 25 °C for up to four consecutive days was evaluated in this study. Figure 5 demonstrates a significant increase in the concentration of NMP when a 10 mg/mL cefepime hydrochloride sample was stored at 25 °C over four days. In one hour, NMP increased from 0.23 to 0.27% and continued to increase to nearly 2% over the next three days. For cefepime stored at 4 °C, NMP did not significantly increase within 6 h, but increased from 0.22 to 0.29% within 24 h. No significant increase in NMP was observed over 96 h of storage at -17 °C.

CONCLUSIONS

- The IonPac CS17 column with an electrolytically-generated MSA eluent and suppressed conductivity detection was successfully used to determine NMP in cefepime hydrochloride, cefepime for injection, and cefepime system suitability samples.
- The column separated NMP in <10 min and efficiently removed cefepime from the column, which enabled shorter analysis times and good retention time stability relative to the current USP method.
- The proposed method required a simple MSA eluent, without the organic solvent required in the USP method, which reduced time needed to optimize the separation of NMP from an unidentified peak in the system suitability sample.
- The exceptionally low background and noise using suppressed conductivity detection enabled the quantification of 0.001% NMP in cefepime hydrochloride.

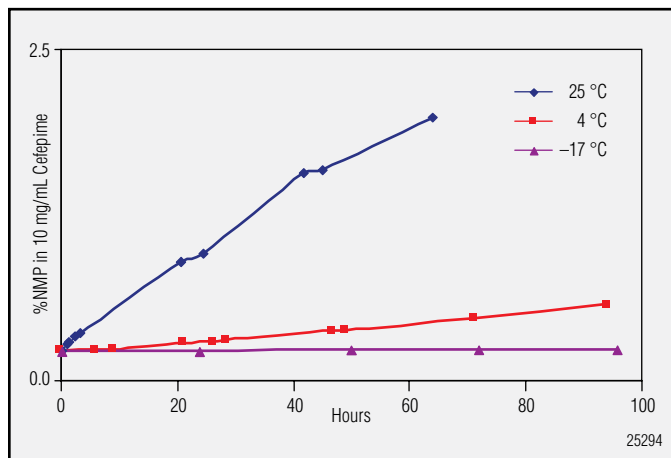


Figure 5. Stability of NMP in USP RS cefepime hydrochloride.

REFERENCES

1. United States Pharmacopeia. The National Formulary. Cefepime Hydrochloride. USP 31, NF 26, 2008, p. 1669.
2. United States Pharmacopeia. The National Formulary. Cefepime for Injection. USP 31, NF 26, 2008, p. 1670.
3. Dionex Corporation. Determination of N-Methylpyrrolidine in Cefepime Using a Reagent-Free Ion Chromatography System; Application Note 199, LPN 2005. Sunnyvale, CA, 2008.

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