

# What is the Best Ion Chromatography Method for Determining *N*-Methylpyrrolidine in Cefepime?

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## INTRODUCTION

Cefepime (Figure 1A) is a semi-synthetic, fourth generation cephalosporin 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, Figure 1B). The primary concern with degradation products is their potential toxicity to patients and their effect on the purity of the pharmaceutical product, which can decrease the potency of the active component.

The US Pharmacopeia (USP) specifies a limit of < 0.3% NMP in cefepime hydrochloride and < 1% in Cefepime for Injection. The USP is proposing an improvement to the current method for determining the limit of NMP by eliminating the column rinse and re-equilibration steps, which increases sample throughput from 3–4 h to 60 min per sample injection. However, this proposed method still uses cation-exchange chromatography with direct conductivity detection for the separation and detection of NMP. Direct conductivity detection imposes several limitations, such as the requirement of low-capacity resins with dilute eluents, higher background conductivity, higher baseline noise, and therefore higher detection limits relative to suppressed conductivity detection.

Here, the authors compare suppressed conductivity and direct conductivity detections for the determination of NMP in cefepime and Cefepime for Injection. The method using suppressed conductivity was found to increase sample throughput by decreasing the time from 60 min to 35 min per injection. In addition, sensitivity for NMP was improved with suppressed conductivity with an LOQ of 0.10 µg/mL relative to 1.6 µg/mL using direct conductivity detection. Therefore, lower cefepime concentrations are required for the analysis, which improves cost efficiency. The authors compare the two methods for linearity, detection limits, precision, and recovery of NMP in cefepime hydrochloride.

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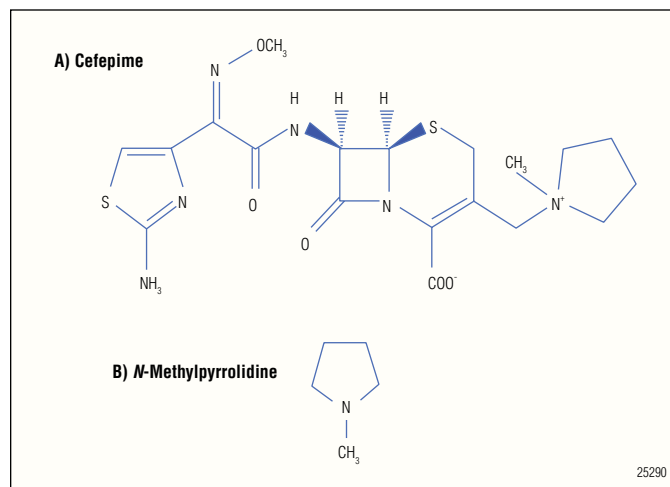


Figure 1. Chemical structures of A) cefepime and B) *N*-methylpyrrolidine.

## EXPERIMENTAL

### Instrument (Suppressed IC)

Dionex ICS-3000 Reagent-Free™ Ion Chromatography (RFIC™) system with Eluent Generation (RFIC-EG™) including:  
 SP Single Pump or DP Dual Pump module  
 EG Eluent Generator with EluGen EGC II MSA cartridge  
 Continuously-Regenerated Cation Trap Column, CR-CTC II  
 DC Detector/Chromatography module (dual-temperature zone configuration)  
 AS Autosampler with sample tray temperature control  
 Chromeleon® Chromatography Data System (CDS)

### Instrument (Nonsuppressed IC)

Dionex ICS-2100 ion chromatography system including:  
 Single isocratic pump with vacuum degas  
 Column heater enclosure  
 AS Autosampler with sample tray temperature control  
 Chromeleon Chromatography Data System

## METHOD CONDITIONS

	Suppressed IC	Non-suppressed IC
Columns	IonPac® CG17, CS17, 2 mm	IonPac SCSG 1, SCS 1, 4 mm
Eluent	6 mM MSA from 0–7.5 min, step to 85 mM at 7.5 min, 85 mM from 7.5–20 min, step to 6 mM at 20 min, 6 mM from 20–30 min	10 mM HNO <sub>3</sub> /5% CH <sub>3</sub> CN
Eluent Source	EGC II MSA with CR-CTC II	Manually prepared
Inj. Volume	5 µL	10 µL
Temperature	40 °C	30 °C
Detection	Suppressed conductivity, CSRS® 300, 2 mm, recycle mode, 100 mA	Nonsuppressed conductivity

## Samples and Standards

For the suppressed conductivity detection method, the *N*-methylpyrrolidine standards, cefepime hydrochloride (USP, Rockville, MD) sample, and simulated Cefepime for Injection sample was prepared in deionized (DI) water. For the nonsuppressed conductivity detection method, the same standards and samples were prepared in a 2 mM HNO<sub>3</sub> solution. Simulated Cefepime for Injection samples were prepared by combining cefepime hydrochloride and arginine in either DI water or 2 mM HNO<sub>3</sub>.

## RESULTS AND DISCUSSION

### Separation of NMP: Suppressed Conductivity IC Method

The determination of NMP in cefepime using suppressed conductivity detection was developed using an IonPac CS17 column, which is a hydrophilic, moderate capacity cation-exchange column with carboxylated functional groups. Due to the low hydrophobic character of the column, strongly retained compounds such as cefepime are eluted more rapidly. Therefore, the analysis time for determining NMP in cefepime is significantly reduced relative to the current and proposed USP methods. Figure 2 compares the separation of 25 µg/mL NMP standard to a 10 mg/mL cefepime hydrochloride sample. The retention time of NMP was approximately 5.3 min in the standard and sample solutions. At 7.5 min, the MSA concentration was increased from 6 mM to 85 mM to remove cefepime from the column to produce a run time of 30 min. An additional 5 min equilibration time was added before each analysis, which resulted in a total analysis time of 35 min per sample.

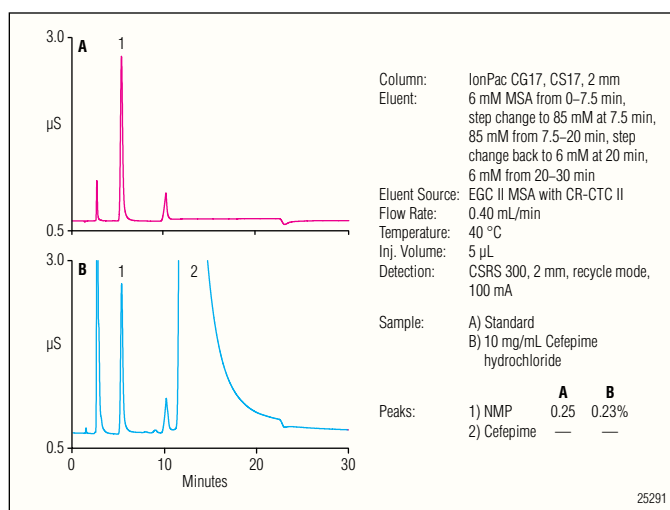


Figure 2. Comparison of the separation of NMP in A) a standard solution and B) cefepime hydrochloride.

### Separation of NMP: Nonsuppressed Conductivity IC Method

The determination of NMP in cefepime using nonsuppressed conductivity detection was developed using a silica-based IonPac SCS-1 column, which is a low capacity cation-exchange column functionalized with carboxylic acid groups. This column is nearly equivalent to the column described in the proposed USP method for NMP and therefore was used in this application to reproduce the proposed method. The USP describes an eluent composition containing 10 mM nitric acid with 5% acetonitrile. Figure 3 compares the separation of spiked and unspiked NMP in cefepime using the conditions described in the proposed monograph. As shown, NMP is eluted in approximately 10 min, while cefepime elutes in less than 40 min with a total run time of 60 min.

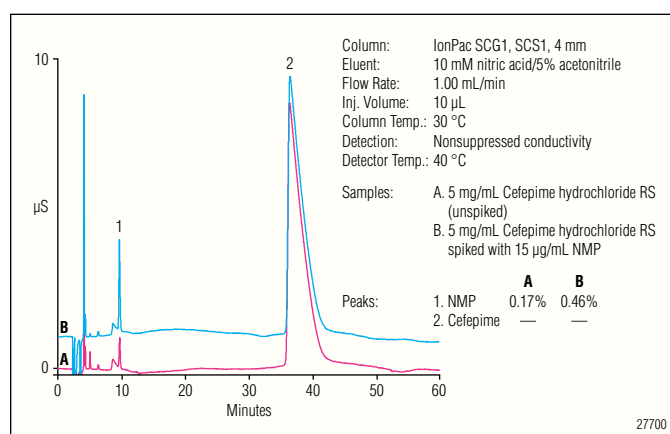


Figure 3. Comparison of A) unspiked and B) 15 µg/mL (0.3%) NMP-spiked solutions of 5 mg/mL cefepime in 2 mM nitric acid.

## Comparison of Method Performance

The performance of the two methods was initially evaluated by determining the linearity, LOD, and LOQ for NMP. For the method using suppressed conductivity detection, NMP was calibrated from 0.45–200 µg/mL compared to 5–100 µg/mL using nonsuppressed conductivity detection. To determine the detection and quantification limits of NMP, the baseline noise was measured in a representative one-minute segment where no peaks elute. The measured noise using non-suppressed conductivity detection was 18 nS, which is significantly higher than the 0.2–0.4 nS noise measured using suppressed conductivity detection. The typical background conductivity using the USP eluent conditions with nonsuppressed conductivity detection is > 3000 µS compared to < 0.7 µS with eluent suppression. Therefore, the background conductivity without suppression produces a significantly higher baseline and therefore higher detection limit. The calculated LODs and LOQs are compared in Table 1. As shown, the suppressed conductivity IC method produced LODs and LOQs for NMP that were more than an order in magnitude better than nonsuppressed conductivity IC. The improved sensitivity with eluent suppression therefore would require lower cefepime concentrations, which translates into a more cost efficient method.

**Table 1. Comparison of Suppressed and Non-Suppressed Conductivity IC for Linearity, LODs, and LOQs for NMP**

Method	Analyte	Range (µg/mL)	Corr. Coeff. (r <sup>2</sup> )	LOD <sup>a</sup> (µg/mL)	LOQ <sup>b</sup> (µg/mL)
Suppressed IC	NMP	0.45–200	0.9999	0.03	0.1
Nonsuppressed IC	NMP	5–100	0.9996	0.5	1.6

<sup>a</sup>S/N = 3

<sup>b</sup>S/N = 10

The performance of the two methods was also evaluated based on the precision from replicate injections of standards and samples, and from the recovery of known NMP concentrations added to cefepime hydrochloride samples. The relative standard deviations (RSDs) of the retention times and measured peak areas for replicate injections of 25 µg/mL NMP using suppressed conductivity was < 0.1% and 1.2%, respectively. For the nonsuppressed conductivity method, 15 µg/mL NMP was used for this analysis, which produced retention time and peak area RSDs of 0.02% and 0.14%, respectively. For all cefepime samples analyzed in these studies, the NMP concentration was less than the 0.3% USP specification, regardless of the detection method used. Table 2 compares retention time and peak area RSDs for NMP in

**Table 2. Comparison of Retention Time and Peak Areas Precisions for NMP in Cefepime**

Sample	Suppressed IC				Nonsuppressed IC			
	N	Avg. NMP Conc.	Retention Time RSD	Peak Area RSD	N	Avg. NMP Conc.	Retention Time RSD	Peak Area RSD
NMP standard	10	25 µg/L	<0.1	1.2	6	15 µg/L	0.02	0.14
Cefepime HCl	10	0.23%	0.3	1.4	6	0.18%	0.06	1.1
Cefepime for Injection	10	0.25%	0.6	1.1	6	0.19%	0.13	1.6

cefepime and cefepime for injection using either suppressed or nonsuppressed conductivity detection. As shown, the results obtained by either detection method were comparable. However, the suppressed conductivity method produces analysis times of 35 min relative to 60 min using nonsuppressed conductivity. This is a critical difference between the two methods because the longer analysis time may yield higher NMP concentrations due to the rapid degradation of cefepime.

The accuracy for both methods was investigated by adding a known concentration of NMP to the cefepime hydrochloride samples. For each method, the recovery of NMP in cefepime was nearly 100%, suggesting that both methods are accurate.

Cefepime for injection was analyzed using suppressed and nonsuppressed conductivity detection. This sample contains a mixture of the hydrochloride salt and *L*-arginine to provide a reconstituted sample pH between 4 and 6. Figures 4 and 5 compares the separation of NMP in Cefepime for Injection with and without suppression, respectively. On the IonPac CS17 column, arginine elutes just after NMP compared to before NMP on the IonPac SCS-1 column. However, arginine is resolved from NMP using both methods and the accuracy of determining NMP in this sample was not affected based on the concentration of NMP determined relative to previous sample preparations.

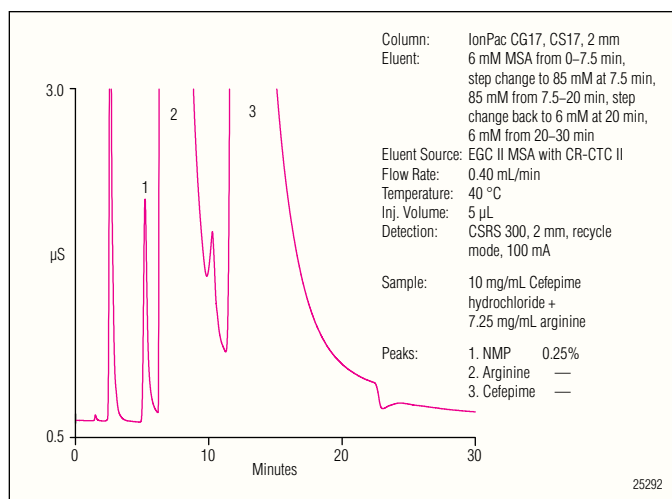


Figure 4. Determination of NMP in a simulated Cefepime for Injection sample.

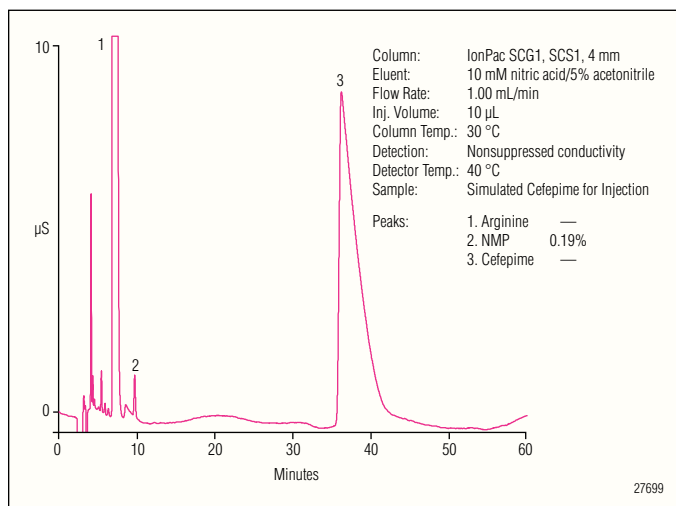


Figure 5. Determination of NMP in simulated Cefepime for Injection sample.

## CONCLUSIONS

- The IonPac CS17 column with electrolytically-generated MSA eluent and suppressed conductivity detection was compared to the IonPac SCS1 column with manually prepared HNO<sub>3</sub>/acetonitrile eluent and nonsuppressed conductivity detection for the determination of NMP in cefepime.
- The suppressed conductivity IC method provides several benefits over the nonsuppressed IC method, such as lower LODs, significantly lower analysis times; also the method uses an electrolytically-generated MSA eluent that does not require the addition of organic solvent.
- The lower LODs produced by eluent suppression enables the analysis of lower cefepime concentration and therefore a more cost efficient method. The shorter run time also minimizes cefepime degradation for a given sample.
- The accuracy and precision for NMP in cefepime were comparable for each method.
- For more information on the methods described here, see Dionex Application Note 1991 (suppressed IC method) or Application Note 259 (nonsuppressed IC method).

## REFERENCES

1. Dionex Corporation. *Determination of N-Methylpyrrolidine in Cefepime Using a Reagent-Free Ion Chromatography System*. Application Note 199, LPN 2005, Sunnyvale, CA, 2008.
2. Dionex Corporation. *Determination of N-Methylpyrrolidine in Cefepime with Nonsuppressed Conductivity Detection*. Application Note 259, LPN 2586, Sunnyvale, CA, In Press.

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