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Short communication

A simple and sensitive GC method for determination of *N*-methylpyrrolidine in cefepime and its preparation

Guiliang Chen *, Guyu Liu, Feng Qin, Yiting Wang

Shanghai Institute for Drug Control, Shanghai 200233, China

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Abstract

A new, simple and rapid gas chromatographic method was developed for the determination of *N*-methylpyrrolidine (NMP) in cefepime and its preparation. NMP was extracted with chloroform from cefepime and its preparation. An HP-1 column was maintained at 100 °C. Both the injector and the FI detector were set 250 °C. Pyridine was used as an internal standard. The detector response was linear up to 135 ng. The detection limit was 0.3 ng. The recoveries were 100.2–103.0% at three concentration levels. No interference from organic solvents presented in the synthesis was observed. The proposed method has a potential for application in quality control for cefepime and its preparation.

Keywords: *N*-methylpyrrolidine; Cefepime; Cefepime for injection; Gas chromatography

1. Introduction

Cefepime is a fourth generation, semisynthetic cephalosporin. Its chemical, pharmacokinetic, and clinical characteristics can be found in literature [1,2]. 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 (Scheme 1) is [6*R*-[6α,7β(*Z*)]]-1-[[7-[[(2-amino-4-thiazolyl) (methoxyimino) acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl]methyl]-1-methylpyrrolidinium inner salt. It is synthesized from 7-aminocephalosporanic acid (7-ACA, Scheme 2) with the use of trimethylsilyl iodide and *N*-methylpyrrolidine (NMP) [3]. Cefepime could degrade into NMP in the process of preparation and storage. The determination of NMP is crucial because the NMP level has a direct effect on the purity of cefepime and its preparation, and the NMP in cefepime for injection may also be toxic to patients.

Japanese Pharmacopoeia [4] employs an HPLC method to detect NMP. The analytical HPLC method consists of a strong cation-exchange resin

* Corresponding author. Tel.: +86-2-1648-29531; fax: +86-2-1648-29580.
E-mail address: guiliangc@hotmail.com (G. Chen).

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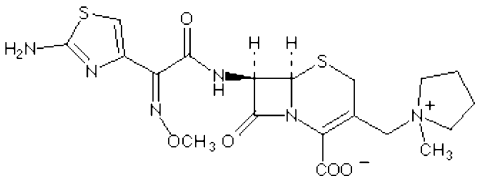
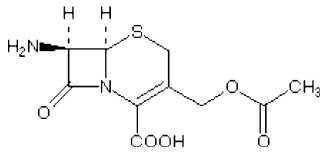


Scheme 1.



Scheme 2.

column, acetonitrile and 0.01 M nitric acid solution (1:99) mobile phase, 0.1 M nitric acid solution as the solvent to prepare the standard and test solutions, and a conductivity detector. The method has several problems. One of its disadvantages is time consuming. It takes 3–4 h to complete one sample analysis. Retention time variation is another problem. The retention time of NMP peak from test solution is more than 10% longer than that from standard solution. Other disadvantages include unpopular detector and unreasonable solvent, i.e. cefepime is unstable in the solution of pH 2 [5]. No other report for determination of NMP in cefepime was found in literature. Here we demonstrate a simple and sensitive GC method for the measurement of NMP in cefepime and its preparation.

2. Materials and methods

2.1. Chemicals and reagents

NMP ($C_5H_{11}N$, Aldrich Chemical Company, Inc., 97%, lot # 03524 JN), pyridine (C_5H_5N, Shanghai Chemical Company, 99.0%, Lot T). Chloroform, methanol, ethanol, trifluorotrichloroethane, dichloromethane and acetone were of analytical grade and were used without further purification.

Cefepime and Cefepime for Injection were obtained from Shanghai Xingya Pharmaceutical Corporation (China).

2.2. Sample preparation

2.2.1. Internal standard solution

A 0.5-ml portion of pyridine was diluted to 1000 ml with chloroform.

2.2.2. Standard solution

A nominal 10-mg portion of NMP was accurately weighed, transferred into a 20 ml of volumetric flask, dissolved and diluted to volume with chloroform. A 1.0 ml of the resulting solution was transferred into a 10 ml of volumetric flask, mixed with 1.0 ml of internal standard solution, diluted to volume with chloroform, and mixed well.

2.2.3. Test solution

A sample equivalent to about 200 mg of cefepime was accurately weighed, triturated with 2 ml of chloroform in a mortar, transferred with chloroform in portions to a 10-ml volumetric flask, mixed with 1.0 ml of internal standard solution, diluted to volume with chloroform, mixed well, filtered through a 0.45 μm filter.

2.3. Instrumentation

The gas chromatographic analyses were performed on a Hewlett–Packard 6890 apparatus equipped with a flame-ionization detector, an automatic injector 7683 (Hewlett–Packard, Wilmington, DE, USA), a Hewlett–Packard VECTRA PC 1990–2000 and Hewlett–Packard CHEMSTATION 3365 software.

The column used was a wide-bore column of 60 m long and 0.53 mm in internal diameter, coated with 5 μm film of 100% polydimethylsiloxane (HP-1, HP Part No. 19095Z-626, Hewlett–Packard, Wilmington, DE, USA). The flow-rates were 40 ml/min for the carrier gas (nitrogen for chromatography) with split ratio 1:5, 4 ml/min for the hydrogen, and 100 ml/min for the air. The temperatures were 100 °C for the column oven, and 250 °C for both the injector and the detector. The injection volume was 1 μl.

Vials, inserts and caps used in the GC system were acquired from Agilent, Shanghai company.

3. Results and discussion

3.1. Specificity

The typical chromatograms are shown in Figs. 1–3 for blank chloroform, standard solution and test solution, respectively. NMP and pyridine are completely separated from each other and from solvent (chloroform). The theoretical plates are 57 000 for NMP and 49 300 for pyridine.

No peaks from the solvent were observed at the loci of NMP and pyridine.

The retention times of some organic solvents used in the synthesis of cefepime were 3.63 min for methanol, 3.86 min for ethanol, 4.06 min for trifluorotrichloroethane, 4.37 min for dichloromethane, and 4.44 min for acetone.

3.2. Precision

The relative standard deviation (R.S.D.) for five replicate injections of a standard solution was 0.12% for the peak-area ratio.

3.3. Linearity and range

A calibration curve was established by analyzing solutions containing 1.12–134.6 μg/ml of NMP, each containing the same amount of pyridine. The area ratios of NMP to pyridine were plotted versus the concentrations. The calibration graph was linear with the regression coefficient of better than 0.9999. The equation was:

$$y = 0.853x - 0.0085$$

here y is peak-area ratio of NMP and the internal standard; x is the corresponding concentration of NMP.

3.4. Limits of quantification and detection

The limit of detection (LOD) was 0.3 ng, based on three times noise. The peak was detected for a solution at a concentration of 0.3 μg/ml.

The limit of quantification (LOQ) was 1.12 ng, based on ten times noise. The R.S.D. for five replicate injections of a solution at 1.12 μg/ml was 2.9%.

3.5. Recovery

The popular GC analytical methods for determining the residue impurities in row materials are by directly injecting the drug solution in a suitable solvent onto the column, by directly injecting the extract from liquid–liquid extraction, or by head-space injection. Cefepime is soluble in water and insoluble in chloroform while NMP is soluble in both water and chloroform. NMP was not detected when aqueous cefepime solution was directly injected onto the column, even when aqueous NMR solution at a concentration of 50 μg/ml was injected. The head-space injection also

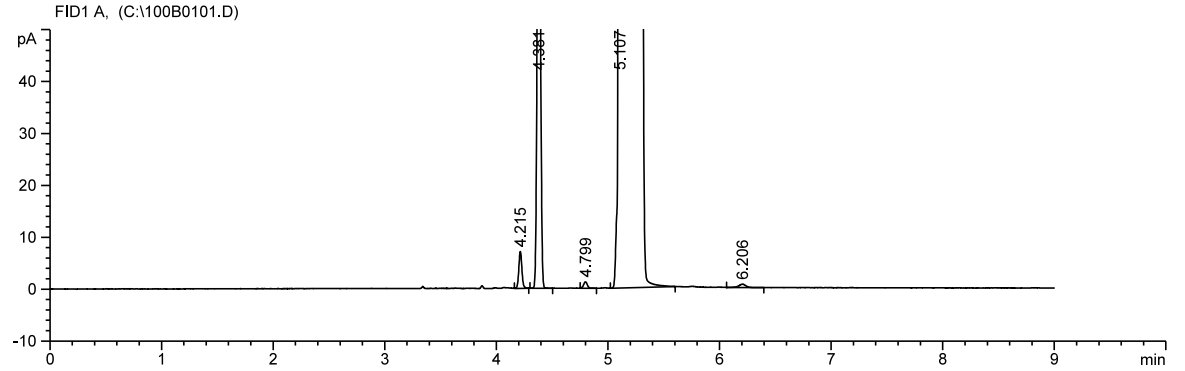


Fig. 1. Chromatogram of solvent (chloroform).

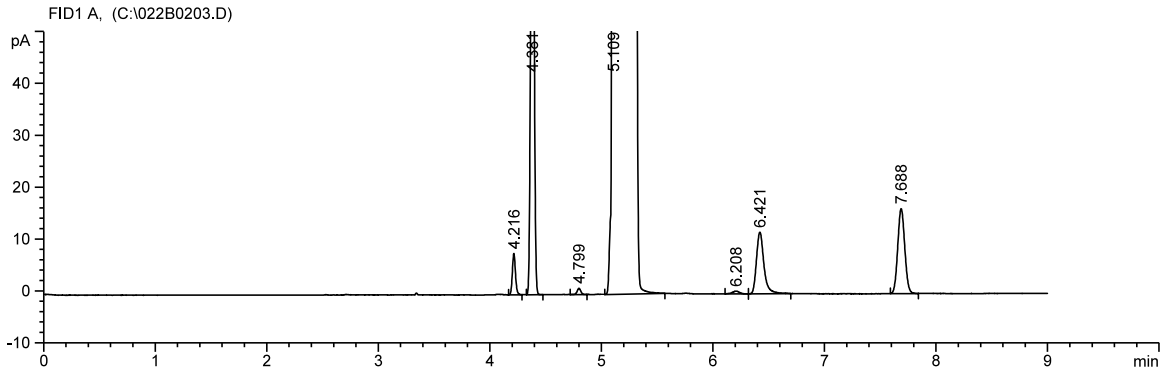


Fig. 2. Chromatogram of standard solution. NMP: 6.421 min, Pyridine: 7.688 min.

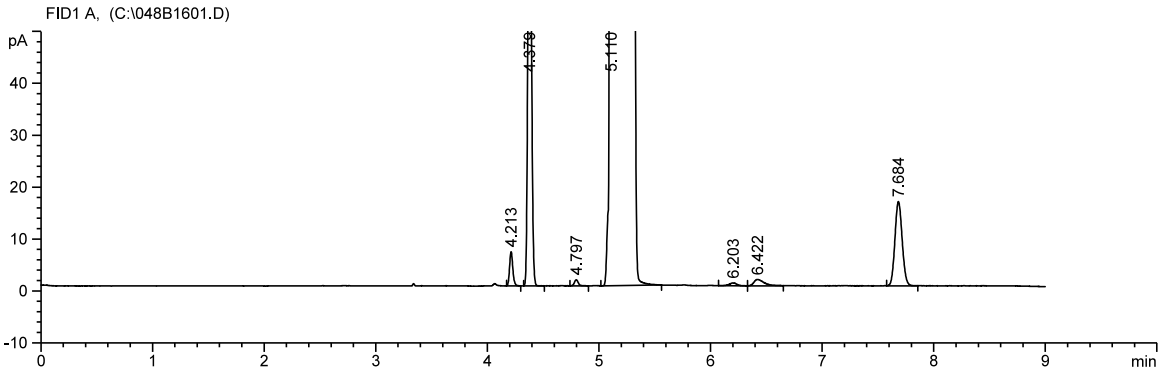


Fig. 3. Chromatogram of test solution. NMP: 6.422 min, Pyridine: 7.684 min.

failed due to degradation of cefepime to NMP during its equilibrium in head-space vials.

The recovery of extracting cefepime powder with chloroform was determined as follows. Triturating procedure increased the efficiency and stability of extraction.

A 0.1156 g portion of NMP was dissolved in 100.0 ml of chloroform to make a stock solution. Three 0.3, 0.5, and 0.7-ml portions (total of nine) of the stock solution were spiked to samples of 200-mg portion of cefepime for injection. The spiked samples were extracted and analyzed as for test solutions. Single injection was made for each sample solution. The NMP recoveries were corrected for the peak areas of existing NMP in cefepime for injection. The validation of the method to the spiked samples prepared by adding known amounts of 33.64, 55.07 and 78.49 ng of NMP yielded mean percent recoveries of 100.2 ± 1.30, 103.0 ± 1.07 and 102.0 ± 1.13, respectively.

Table 1
The results obtained for the determination of NMP in cefepime samples

Samples	Lots number	NMP amount[a] (%)	R.S.D. (%)
Cefepime	010703	0.009	1.2
	010801	0.003	0.8
	010802	0.006	0.8
Cefepime for Injection	010901	0.019	0.6
	011001	0.021	0.7
	011002	0.018	1.0

[a] % (g/g) expresses the number of grams of NMP in 100 g of samples.

Table 2
The results obtained for the determination of NMP in stability samples[a]

Stability conditions	Before stability test (%)	5 days (%)	10 days (%)
Under lights (ca.4500 lx)	0.01	0.25	0.38
At the temperature of 40 °C	0.01	0.58	0.61
At the temperature of 60 °C	0.01	1.40	1.45
In the RH[b] of 75%(25 °C)	0.01	0.98	1.14
In the RH[b] of 92%(25 °C)	0.01	1.30	1.42

[a] % (g/g) expresses the number of grams of NMP in 100 g of samples.
[b] Relative humidity.

3.6. Applications

This method was used to assay three batches of cefepime and three batches of cefepime for injection (containing L-arginine). Three sample solutions were prepared for each batch. Three injections were made for each sample solution. The results are listed in Table 1. All the samples were tested according to *Japanese Pharmacopoeia* [4] and met the requirements.

The method was also used to evaluate the NMP levels in stability samples from a batch of cefepime (Lot No. 010703). Three sample solutions were prepared from each condition, two injections were made for each solution. The results are reported in Table 2.

The results indicate that cefepime is unstable under the conditions of light, heating and humidity.

4. Conclusion

This method for determination of NMP in cefepime and cefepime for injection proved to be simple and reliable. It is useful in manufacture process and quality control. Sensitivity of this method was found to be better than HPLC method [4], thus permitting the determination of small concentrations of NMP as low as 1.12 μg/ml. This proposed method was more advantageous than HPLC method [4] in its stability of retention times, and its saving time and effort, as well as much chemicals.

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