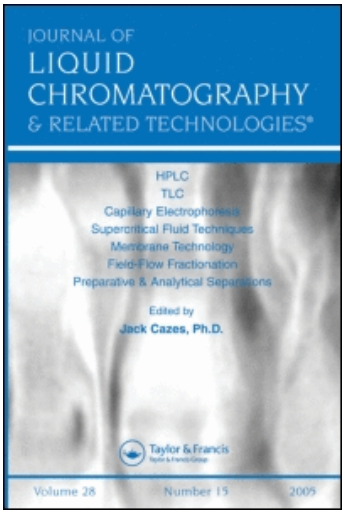


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Determination of Ceftazidime and Pyridine by HPLC: Application to a Viscous Eye Drop Formulation

A. R. Barnes[ab]

[a] Pharmaceutical Sciences Institute Aston University Birmingham, United Kingdom [b] Regional Quality Control Laboratory Department of Pharmacy and Pharmaceutical Science City Hospital Birmingham, United Kingdom

**To cite this Article** Barnes, A. R.(1995) 'Determination of Ceftazidime and Pyridine by HPLC: Application to a Viscous Eye Drop Formulation', Journal of Liquid Chromatography & Related Technologies, 18: 15, 3117 — 3128

**To link to this Article: DOI:** 10.1080/10826079508010437

**URL:** http://dx.doi.org/10.1080/10826079508010437

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(15), 3117-3128 (1995)

# DETERMINATION OF CEFTAZIDIME AND PYRIDINE BY HPLC: APPLICATION TO A VISCOUS EYE DROP FORMULATION

**A. R. BARNES**
*Pharmaceutical Sciences Institute*
*Aston University*
*Birmingham B4 7ET, United Kingdom*
*and Regional Quality Control Laboratory*
*Department of Pharmacy and Pharmaceutical Science*
*City Hospital*
*Birmingham B18 7QH, United Kingdom*



## ABSTRACT

A stability-indicating high performance liquid chromatographic (HPLC) method was developed to determine the cephalosporin antibiotic ceftazidime and its degradation product pyridine. The method was applied to analysis of an aseptically prepared viscous ceftazidime eye drop formulation (ceftazidime 5% w/v in *Sno Tears*). The chromatographic conditions were: Spherisorb 5$\mu$m hexyl column, 100 x 4.6mm; mobile phase acetonitrile:aqueous ammonium acetate 0.05M (7:93); flow rate 2ml/min; detector wavelength 254nm. The retention times of ceftazidime and pyridine were 1.3min and 3.8min respectively. The chromatographic precision of the method was typically 0.3-0.5% relative standard deviation (RSD) for ceftazidime. The recovery of ceftazidime from the eye drop was 101.3% and the within-day precision was 0.8% RSD. The limit of detection for pyridine was 2.4ng. Ceftazidime degraded by 7% over 7 days in the eye drops, with the production of pyridine accounting for 50% of the degraded antibiotic.

## INTRODUCTION

The stability and compatibility of the third-generation cephalosporin eye drop ceftazidime has been studied in injection formulations by various reversed-phase HPLC methods. Hwang *et al.*[1] described a method for the analysis of ceftazidime in biological fluids which used a phenyl-bonded column. A number of authors have adopted this method for the analysis of ceftazidime in formulations[2-4].

The United States Pharmacopeia method for the analysis of ceftazidime injection[5] employs an ODS column. Various other methods employ this column type[6-9]. Other methods have used phenyl[10] and hexyl[11] columns.

A major decomposition pathway for cephalosporins is scission of the C-3 side chain[12]. In the case of ceftazidime, this would lead to the formation of pyridine. This product is potentially toxic and control of its levels in reconstituted injections and other formulations is desirable. None of the above methods allow its simultaneous determination with ceftazidime. Fabre and Kok[13] described an HPLC method for the detection of cephalosporins, including ceftazidime, and their degradation products. This depends upon post-column oxidation of the C-3 side-chain and would not be applicable to the determination of pyridine.

Cephalosporin or penicillin antibiotics have been used in eye drop formulations[14-16]. In the case of cefuroxime[15], a viscous artificial tears solution (*Sno Tears*) has been used as a vehicle in order to increase corneal residence time of the antibiotic.

This paper describes a reversed-phase HPLC method for the simultaneous determination of ceftazidime and pyridine and its application to a viscous ophthalmic formulation.

## MATERIALS AND METHODS

### Materials

Ceftazidime injection 1g (Fortum lots B1962NB and B3263BA) and ceftazidime pentahydrate standard lot AWS27C (84.8%w/v on a dried basis) were from Glaxo Laboratories, Uxbridge, UK. *Sno Tears* eye drops, containing polyvinyl alcohol 1.4%w/v (lots 209024 and 303038) were from Smith & Nephew Medical, Romford, UK.

Acetate buffers were prepared by mixing solutions of 0.05M ammonium acetate and acetic acid to the required pH.

All other chemicals and solvents were of analytical or HPLC grade.



### Chromatographic Conditions

The HPLC system consisted of a CE1100 pump (Cecil Instruments, Cambridge, UK), SA6500 variable wavelength UV detector (Severn Analytical, Macclesfield, UK) and Peakmaster 3.2 data system (Harley Systems, Princes Risborough, UK). Samples were injected by an ASI-4 autosampler equipped with a Rheodyne 7010 injection valve (Talbot Instruments, Alderley Edge, UK).

A 100 x 4.6mm Spherisorb 5$\mu$m hexyl reversed-phase column was used, with a 10 x 4.6mm guard column of the same material (Hichrom, Reading, UK). The mobile phase was acetonitrile:0.05M ammonium acetate (7:93) at a flow rate of 2ml/min. The injection volume was 10$\mu$l and the detector wavelength was 254nm.

### Sample Preparation

Samples were allowed to reach ambient laboratory temperature before processing. A 100$\mu$l sample of viscous ceftazidime eye drops was transferred with

an air-displacement pipette to a 25ml volumetric flask. The pipette tip was removed and rinsed into the flask with a jet of water. The flask was diluted to volume with water and the diluted solution sealed in an autosampler vial.

Standard Preparation

Ceftazidime pentahydrate standard, 100mg, was weighed into a 5ml volumetric flask. To this was added 1ml of a 1.2%w/v aqueous solution of sodium carbonate. When the ceftazidime pentahydrate had dissolved, the flask was diluted to volume with water. A 100$\mu$l aliquot of this solution was transferred, in duplicate, to 10ml volumetric flasks with the air-displacement pipette. The pipette tips were rinsed into each flask with water as for the sample. The flasks were diluted to volume with water and the solutions sealed in autosampler vials.

Ceftazidime was quantified by peak area measurement from duplicate injections of sample and standard solutions. Pyridine was estimated by measurement of its peak area from samples, calibrated with the corresponding ceftazidime peak and corrected using the relative response factor for pyridine with respect to ceftazidime. This factor was determined to be 1.4 in this chromatographic system.

System Suitability Tests

Each time the method was performed, the precision of chromatography was checked by chromatographing six replicates of a standard. The peak area should not have greater than 2% RSD. The mean peak area of duplicate injections of each of the two standards should not differ by more than 3%. A degraded solution was prepared by diluting 1ml of the initial standard solution with water; this was boiled

for approximately 5min and cooled. This solution was chromatographed at the start of each run to allow peaks to be identified and ensure adequate separation of degradation products from ceftazidime.

Preparation of Ceftazidime 5% w/v Eye Drops

The contents of a 1g vial of ceftazidime injection were dissolved in 20ml of either *Sno Tears* or water for injection, using aseptic technique in a laminar flow cabinet.

Eye drops for use in the recovery study were however prepared by mixing the contents of a 1g vial of ceftazidime injection and accurately weighing approximately half of the contents into a 10ml volumetric flask. This was dissolved in and diluted to volume with *Sno Tears*. The other half of the vial's contents was used as the standard: this was accurately weighed into a 10ml volumetric flask, then dissolved in and diluted to volume with water. Both solutions were further diluted as for preparation of the sample.



Forced Degradation

The stability-indicating potential of the method was assessed with solutions of 1.25mg/ml ceftazidime pentahydrate, from an injection vial, degraded in various aqueous solutions. The degradation conditions were: 1%w/v sodium carbonate, heated at 60°C for 5min; 0.1M sodium hydroxide maintained at ambient temperature for 30min; phosphoric acid, added dropwise to an aqueous ceftazidime solution, to give a pH of 1.0, heated at 60°C for 5min. The solutions were diluted 1:250 with 0.05M ammonium acetate solution before injection onto the chromatograph. Also, a simple aqueous solution of ceftazidime pentahydrate was boiled for 5min and was diluted 1:250 with water before chromatographing.

Confirmation of the stability-indicating capability of the method for use with viscous eye drops was obtained by chromatographing samples of formulation stored at 25°C for up to 17 days.

Stopped-Flow Scanning

The stopped-flow UV-spectra of the ceftazidime and pyridine peaks were obtained with a SA6503 detector (Severn Analytical, Macclesfield, UK).

## RESULTS AND DISCUSSION



The degradation of the ceftazidime by any of the conditions employed lead to the production of a peak corresponding to pyridine and two other major degradation products which were not identified (Figure 1).

In the mobile-phase chosen for the method, the ceftazidime and pyridine peaks both had $_{max}$, by stop-flow scanning, of 254nm. This was chosen as the analytical wavelength.

A mobile-phase with an aqueous component pH of approximately 7.0, *i.e.* ammonium acetate solution with no pH modification, gave the best separation between ceftazidime and its degradation products (Figure 2). Increasing the concentration of buffer from 0.05M to 0.1M made no observable difference to peak shape or resolution.

Increasing flow rate caused decreased resolution between the ceftazidime and degradation product peaks (Table 1). However the symmetry of the ceftazidime peak increased with increasing flow-rate. A 2ml/min flow-rate was adopted since this gave adequate resolution of ceftazidime with a short analysis time, minimising sample degradation.

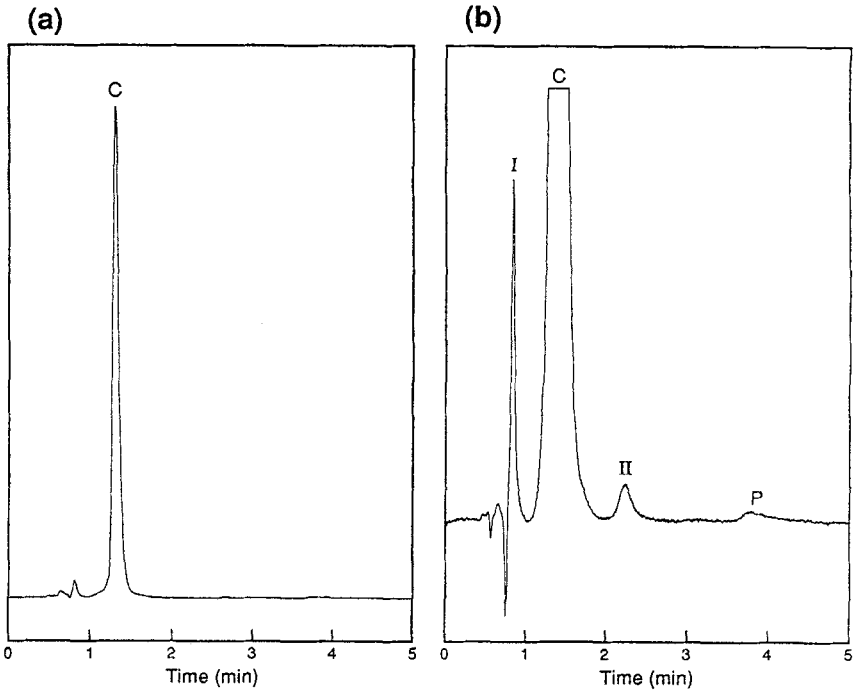


FIGURE 1: Chromatograms of (a) ceftazidime in eye drop formulation stored for 7 days at 7°C, (b) aqueous solution of ceftazidime, degraded by boiling. C = ceftazidime; P = pyridine; I, I I = unidentified degradation products.



Pyridine was confirmed as a degradation product of ceftazidime (Figure 3) from the retention time of the peak from a solution of ceftazidime degraded by boiling and from its stopped-flow UV spectrum, by comparison with the peak from an authentic pyridine standard.

Ceftazidime pentahydrate standard dissolved slowly in water. The addition of sodium carbonate to the standard solution caused rapid dissolution of the ceftazidime, giving a solution with a pH of approximately 7.0.

The chromatographic precision of the ceftazidime peak area, from system suitability testing, was typically in the range 0.3-0.5% RSD.

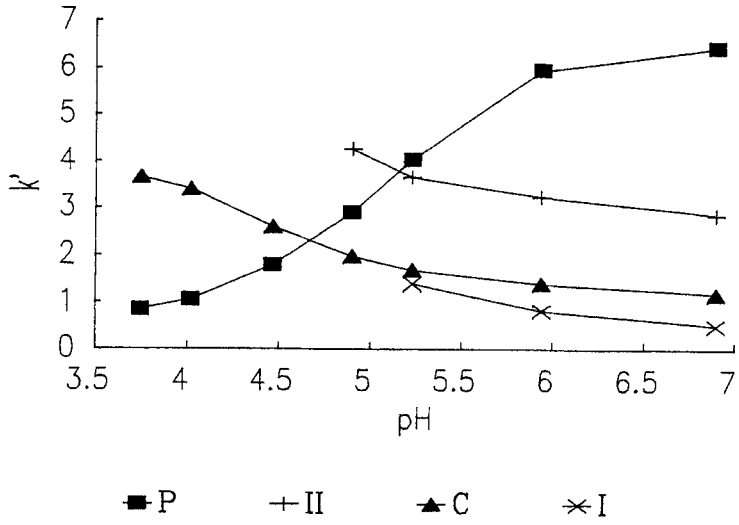


FIGURE 2: Effect of mobile phase pH on capacity factor (k').
C = ceftazidime; P = pyridine; I, I I = unidentified degradation products.



**Table 1. Effect of Flow Rate on Peak Resolution and Symmetry.**

| Flow rate (ml/min) | Resolution factor[a] between ceftazidime and I[b] | Resolution factor[a] between ceftazidime and II[b] | Symmetry factor[a] |
|---|---|---|---|
| 0.5 | 4.83 | 4.98 | 1.88 |
| 1.0 | 4.72 | 4.08 | 1.92 |
| 1.5 | 4.05 | 4.24 | 1.58 |
| 2.0 | 3.08 | 3.84 | 1.42 |

[a] As defined in British Pharmacopoeia 1993[17]

[b] See Figure 1

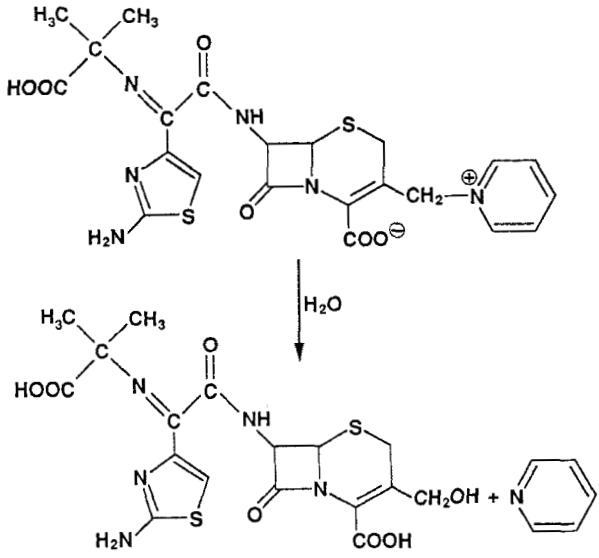

FIGURE 3: Potential scheme for the degradation of ceftazidime, forming pyridine.



The chromatography was linear for ceftazidime in the range 0.2-5.0$\mu$g injected; y = mx + c: m = $6.81 \times 10^5$ ($\pm 6.4 \times 10^3$, 95% confidence limit); c = $-3.7 \times 10^3$ ($\pm 1.8 \times 10^4$, 95% confidence limit); n = 16; r = 0.9999. The chromatography was linear for pyridine in the range 0.0028-0.0282$\mu$g injected; y = mx + c: m = $4.53 \times 10^5$ ($\pm 1.1 \times 10^4$, 95% confidence limit); c = $-2.35 \times 10^2$ ($\pm 1.9 \times 10^2$, 95% confidence limit); r = 0.9994. These data were obtained on separate days. In order to obtain an accurate estimate of the relative response factor of pyridine with respect to ceftazidime, a pyridine calibration curve was performed with concurrent calibration with a ceftazidime standard. This gave a value of 1.4.

The recovery of ceftazidime from the eye drops was 101.3%, and the within-day precision was 0.8% RSD.

The recovery of pyridine, spiked in the range equivalent to the degradation of 0.8-9.4% of the ceftazidime in the eye drops, was 93.1%, assessed by comparison of the slopes of the calibration curves for pyridine spiked into the formulation and for aqueous pyridine solutions. The detection limit for pyridine, judged on the basis of a peak of height four times greater than the baseline noise, was 2.4ng.

Diluted standard solutions degraded by 0.35%/h at room temperature and 0.20%/h in a refrigerator. Chromatography should therefore be performed immediately after solution preparation.

Storage of eye drops at 7°C for 7 days lead to a 7% reduction in the initial ceftazidime concentration. Pyridine levels increased to 500$\mu$g/ml, accounting for approximately 50% of the degraded ceftazidime. An unacceptable yellow colour developed after 7 days storage.



## CONCLUSION

The method is rapid and stability-indicating and is accurate and precise enough for the determination of ceftazidime and pyridine in viscous eye drops. It is capable of adaptation to other formulations of ceftazidime.

## ACKNOWLEDGEMENTS

The author thanks S Nash and G Sihre for their technical contribution, W J Irwin for advice and Glaxo Laboratories for the gift of ceftazidime pentahydrate standard.

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Received: March 12, 1995
Accepted: April 2, 1995