*Journal of Chromatography*, 181 (1980) 120—122
*Biomedical Applications*


CHROMBIO. 426

## Note

# Reversed-phase high-performance liquid chromatographic assay for cefoxitin in proteinaceous biological samples

MARK G. TORCHIA* and RUDY G. DANZINGER

*University of Manitoba, Surgical G-I Research Laboratory, St. Boniface General Hospital, 409 Tache Ave., Winnipeg, Manitoba R2C 2L3 (Canada)*

(Received June 19th, 1979)

Cefoxitin is a semi-synthetic derivative of cephamycin C (q.v.) under government control (Canada) as an experimental antibiotic agent. It is a 7-α-methoxyl, 7-thienyl-acetamido cephalosporanic acid. The methoxy group at the 7-α position of the 3-cephem nucleus gives cefoxitin an increased resistance to β-lactamase produced by some bacteria. Organisms that are sensitive to cefoxitin but resistant to most other cephalosporins (at concentrations of less than 50 mg/ml), include indole-positive *Proteus morganii* and *Serratia marcescens* [1].

This method was developed to accurately quantitate low concentrations of cefoxitin in canine serum and cerebrospinal fluid (CSF). The method described also works well for accurate determinations of cefoxitin in small samples of human serum (100 μl). Other methods have been published for cefoxitin [2, 3] but none have included measurements in proteinaceous fluids.

## EXPERIMENTAL

### *Apparatus*

The high-performance liquid chromatographic (HPLC) system used was the Waters Assoc. (Milford, Mass., U.S.A.) Model 6000A solvent delivery system, Model 450 variable wavelength detector, Model U6K injector, and a Hamilton 25-μl syringe (No. 805). A μBondapak $C_{18}$ (10-μm particle size) reversed-phase column (30 cm × 3.9 mm I.D.) was used, with a Bondapak $C_{18}$/Corasil guard column in series (Waters Assoc.). The detector was connected to a Hewlett-Packard Model 3380A integrator.

* To whom correspondence should be addressed.

*Materials*

Methanol (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Cefoxitin standard was produced by Merck, Sharpe & Dohme Research Labs. (West Point, Pa., U.S.A.) as a sterile preparation of the sodium salt (mefoxin) and supplied by the Department of Pharmacy, St. Boniface General Hospital.

The purity of the cefoxitin was assayed by injecting 20 $\mu$l of the 100 $\mu$g/ml stock solution onto the column under assay conditions. After 45 min no other significant peaks other than that of the cefoxitin were found.

Samples of the canine sera and CSF were supplied by Dr. G. Stiver, Director of Infectious Disease Section, Dept. of Medicine, St. Boniface General Hospital (supported by grants from Merck Frosst Labs., Point Claire-Dorval, PQ. and Manitoba Medical Services Foundation Inc.).

*Procedure*

Methanol was added to an aqueous solution of 0.03% ammonium carbonate (15:85, v/v). This mobile phase was filtered through a membrane filter (0.2-$\mu$m pores) (GA-8 cellulose triacetate; Gelman, Ann Arbor, Mich., U.S.A.) and degassed by mixing with a magnetic stirrer while applying a vacuum. This mobile phase was stored at 4° and allowed to come to room temperature before use.

Stock solutions of cefoxitin were prepared in water to concentrations of 10 $\mu$g/ml and 100 $\mu$g/ml and stored frozen. Experimental standards were prepared by adding the appropriate amount of stock solution to blank samples to concentrations of 1, 10, 25, 50, and 100 $\mu$g/ml.

To one volume of the sample were added 3 volumes of anhydrous ethanol. The solution was mixed on a vortex mixer for 2 min and allowed to stand for 15 min at room temperature, during which time the mixture was again shaken twice. The sample was centrifuged at 3000 *g* for 15 min and the supernatant removed. The supernatant (20 $\mu$l) was injected onto the column. If the protein layer was disturbed during the removal of the supernatant, the entire mixture was re-centrifuged, as the protein particles quickly clog the microparticulate columns.

The flow-rate was 2.0 ml/min, detector wavelength 238 nm, with a sensitivity of 0.1 a.u.f.s. and a back pressure of 900 p.s.i. The integrator settings were: attenuation, 1; chart speed, 0.5 cm/min; area reject, $10^3$ $\mu$V sec and slope sensitivity, 0.1 mV/min.

The peak areas were integrated in $\mu$V sec units and were plotted against the concentrations of the standards to give the calibration curve.

## RESULTS

Chromatograms obtained from serum and CSF are shown in Fig. 1. Retention time for the cefoxitin was 4.8 min. No interfering peaks occurred near the cefoxitin peak in normal blank serum. In all cases the peaks were symmetrical, sharp, and well augmented from the baseline.

The concentration range of 1—100 $\mu$g/ml was linear with respect to the area (absorbance). The minimum detectable amount of cefoxitin was 2 ng in 20 $\mu$l, at this detector range.

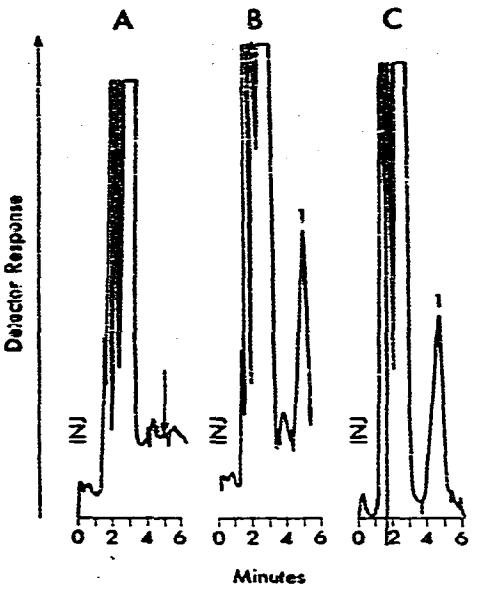


Fig. 1. Chromatograms of cefoxitin in canine serum and CSF. A, blank CSF sample (arrow indicates cefoxitin peak); B, serum sample and C, CSF sample. Cefoxitin concentration for B and C was 15 μg/ml.

Recovery from human serum was 92.5% ± 0.9% based on 10 samples for each standard concentration or 50 samples in total. Recovery studies on the canine samples could not be completed due to lack of samples but initial studies show a recovery of greater than 85%.

Ethanol was used to deproteinize the samples because it did not precipitate any of the protein-bound cefoxitin. Trichloroacetic acid is an excellent deproteinizing agent but because it lowers the pH the cefoxitin also precipitates out.

A reversed-phase HPLC method has been described for the assay of cefoxitin in human serum, canine serum, and canine CSF.

ACKNOWLEDGEMENTS

We are grateful to Mr. Dennis Cote, Dept. of Pharmacy, St. Boniface General Hospital for helpful discussions and Judi Doell for preparing the typewritten manuscript. The authors are supported by Medical Research Council of Canada Grant No. 321-3143-06 and funds from the Department of Surgery, University of Manitoba Faculty of Medicine.

REFERENCES

1 L.D. Thrupp, Annu. Rev. Pharmacol., 14 (1974) 435.
2 R.P. Buhs, T.E. Maxim, N. Allen, T.A. Jacob and F.J. Wolf, J. Chromatogr., 99 (1974) 609.
3 M.A. Carrol, E.R. White, Z. Jancsik and J.E. Zarembo, J. Antibiot., 30 (1977) 397.