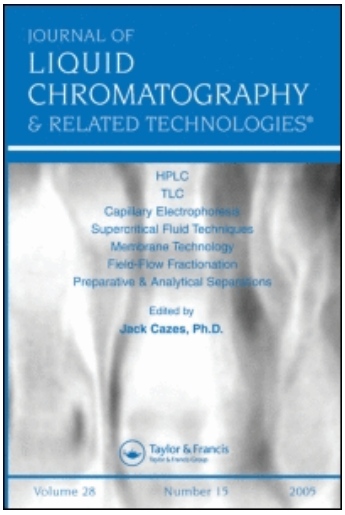

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High-Performance Liquid Chromatographic Determination of Cefoperazone in Serum

D. G. Dupont[a]; R. L. De Jager[a]; Clinique H. J. Tagnon[b]
[a] Service de Medecine et Laboratoire d'Investigation, [b] Laboratoire de Pharmacologie, Clinique Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium

To cite this Article Dupont, D. G. , De Jager, R. L. and Tagnon, Clinique H. J.(1981) 'High-Performance Liquid Chromatographic Determination of Cefoperazone in Serum', Journal of Liquid Chromatography & Related Technologies, 4: 1, 123 — 128
To link to this Article: DOI: 10.1080/01483918108064802
URL: http://dx.doi.org/10.1080/01483918108064802

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(1), 123-128 (1981)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFOPERAZONE IN SERUM

D.G.Dupont and R.L.De Jager[1]

Service de Medecine et Laboratoire d'Investigation
Clinique H.J.Tagnon
Laboratoire de Pharmacologie Clinique
Institut Jules Bordet,Centre des Tumeurs
de l'Université Libre de Bruxelles,Brussels,Belgium.

ABSTRACT

A new HPLC method for the determination of Cefoperazone in serum is described.

INTRODUCTION

Cefoperazone[2] is a new semi-synthetic cephalosporin derivative with broad antibacterial spectrum against aerobic and anaerobic gram positive and gram negative organisms including "Enterobacteriaceae", indole positive "Proteus" and "Pseudomonas aeruginosa".

It is resistant to inactivation by penicillinase and very stable in the presence of cephalosporinase with wide substrate profiles (1).

A rapid HPLC method for the determination of Cefoperazone in serum is described after extraction with SEPPAK cartridges filled with a reverse phase of octadecylsilane (μBondapak C_{18}/Porasil ^{R}B).

MATERIALS AND METHOD

A Hewlett-Packard high-performance liquid chromatograph 1084B and a reverse phase column (μBondapak C_{18} , 10 μm particle size, 250mm x 4,6mm i.d.) were used.
This chromatograph is equiped with a variable wavelength detector (HP 79875 A) and connected to a LC Terminal integrator (HP 79850 B).

SEPPAK cartridges were purchased from Waters Associates (part n°51910). Cefoperazone was supplied by Pfizer Belgium and Methanol P.A. purchased from Merck.

A stock solution of Cefoperazone was prepared in bidistilled water at the concentration of 500 μg/ml and diluted in water or serum in order to prepare a standard curve from 1 to 10 μg/ml.

After prewashing the cartridge with methanol and water, pour 2 ml of sample (or standard) on it, using a syringe, and wash with water to elute the substances unadsorbed on the phase. Thereafter elute the adsorbed Cefoperazone with twice 1 ml of methanol-water (1/1) and inject twenty-five microliters onto the column using methanol-water(1/1) as mobile phase at a flow rate of 1,5 ml/min.(resulting pressure 120 bars). Retention time is 2.30 min. and detection is made at 228 nm.

The concentrations of Cefoperazone were determined using the standard curve assayed following the described method. The detection limit is 0,25 µg/ml.

RESULTS AND DISCUSSION

A standard curve was made at concentrations of 1 - 2 - 3 - 5 - 7,5 and 10 µg/ml.(n=5 for each concentration) (TABLE 1). A graph of the peak area vs. concentration gives a straigh line with a coefficient of correlation of 0,9994.

Recovery from aqueous solutions of Cefoperazone after SEPPAK is 99,6% ± 3,8% (TABLE 2).

Pure serum blank was not adsorbed onto the SEPPAK as controlled by a 97% recovery in the eluate,

TABLE 1

Standard Curve of Cefoperazone

Concentration (µg/ml)	Mean area (range)
10	24792 (24870-25470)
7,5	19200 (19070-19270)
5	12230 (12030-12390)
3	6983 (6916-7300)
2	4624 (4261-4688)
1	2474 (2320-2543)

TABLE 2

Recovery from Aqueous Solutions of Cefoperazone

Concentration (µg/ml)	N	Mean recovery (SD) (%)
4	9	101,6 (± 5,8)
2	5	98,5 (± 2,6)
1	5	98,8 (± 3,0)

measured by a Biuret reaction. Further washing the SEPPAK with methanol-water (1/1) did not result in a peak on the chromatogram that could interfere with Cefoperazone determination.

Recovery from serum solutions of Cefoperazone after SEPPAK was 92% (n = 4 for each concentration from 1 to 10 µg/ml.).

The method described is easy, rapid and reproducible. The limit of sensitivity (0,25 µg/ml.) is adequate for clinical use: A.F.Allaz et al. gave Cefoperazone 2 g every 12 hours I.V. to healthy subjects and measured at 10 hours after the injection a concentration of 3,8 µg/ml (range: 1,5 to 10 µg/ml.) using a biological assay (2).

The SEPPAK extraction method allows the separation from biological fluids of hydrophilic drugs which cannot be extracted by organic solvents (3,4).

Therefore it is very useful and could find wide applications in pharmaceutical and pharmacological analysis.

ACKNOWLEDGMENTS

This work was supported by Le Fonds de la Recherche Scientifique Medicale (Belgium) nº 3.4533.79.

(1): Author to whom correspondence should be addressed

(2): Sodium 7-[D(-)-α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-α-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate.

REFERENCES

1) Mitsuhashi S.,Minami S.,Natsubara N.,Kurashige S. and Saikawa I., In Vitro and in Vivo Antibacterial Activities of Cefoperazone, Proceedings of 1st Intern.Symp.of Cefoperazone-Sodium(T1551), Boston, Massachusettes, October 1979.

2) Allaz AF.,Dayer P.,Fabre J.,Rudhardt M. and Balant L., Pharmacocinétique d'une nouvelle céphalosporine, la céfopérazone, Schweiz.med.Wschr. 109, 1999, 1979.

3) De Jager R.,Dupont D.,Dorlet C., and Lagrange G., Determination of free 2-Formylpyridine Thiosemicarbazone in serum, Anal.Letters 13, in press.

4) Shiu G., and Goehl T., High-performance paired-ion liquid chromatography of Bleomycin A2 in urine , J.Chromatogr.181, 127, 1980.