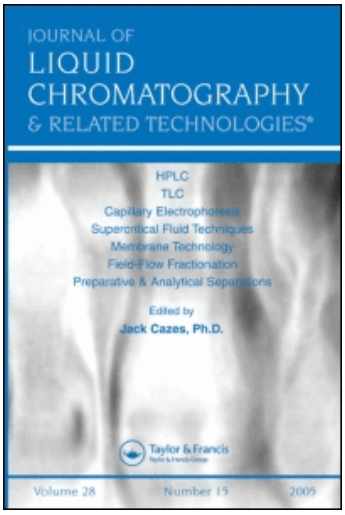

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Piperacillin in Biological Samples by HPLC

A. Zarzuelo[a]; F. G. López[a]; M. Santos[a]; J. M. Lanao[a]
[a] Department of Pharmacy and Pharmaceutical Technology Faculty of Pharmacy, University of Salamanca, Salamanca, Spain

To cite this Article Zarzuelo, A. , López, F. G. , Santos, M. and Lanao, J. M.(1996) 'Determination of Piperacillin in Biological Samples by HPLC', Journal of Liquid Chromatography & Related Technologies, 19: 4, 601 — 610
To link to this Article: DOI: 10.1080/10826079608005523
URL: http://dx.doi.org/10.1080/10826079608005523

J. LIQ. CHROM. & REL. TECHNOL., 19(4), 601–610 (1996)

DETERMINATION OF PIPERACILLIN IN BIOLOGICAL SAMPLES BY HPLC

A. Zarzuelo, F. G. López, M. Santos, J. M. Lanao

Department of Pharmacy and
Pharmaceutical Technology
Faculty of Pharmacy
University of Salamanca
37007 Salamanca, Spain

ABSTRACT

A rapid, specific and reliable technique has been developed for the determination of piperacillin in biological samples by High Performance Liquid Chromatography.

To do so, reverse phase liquid chromatography was used, employing acetonitrile-phosphate buffer 0,1M and pH=6 as the mobile phase (20:80 v/v) and a detector wavelength of 254 nm. Concentrations ranged between 0,5 μg/mL and 200 μg/mL, divided into two calibrations: high concentrations, 15 μg/mL to 200 μg/mL, and low concentrations, 0,5 μg/mL to 15 μg/mL.

The intraday and interday reproducibility of the analytical technique was studied by calculating the variation coefficient of 5 samples analyzed each day and on five consecutive days by ANOVA. The coefficients obtained were 4,02% and 7,11%, respectively, for concentrations ranging between 15-200 μg/mL and 5.71% and 7,54% for concentrations between 0,5 and 15 μg/mL.

INTRODUCTION

Piperacillin is an antibiotic belonging to the β-lactam family and is a semisynthetic penicillin that keep the efficiency of its structural analogs and is also active against new groups of organisms from all evolutionary levels.[1]

In clinical practice the drug has been used successfully in a large variety of situations, including sepsis, pneumonia, intra-abdominal infections, bone and soft tissue infections and gynecological and urinary infections. Its activity against *P.aeruginosa* is striking.

Currently, piperacillin is used in combination with aminoglycoside antibiotics owing to their proved synergism. Use of this combination is particularly important for the treatment of systemic infections due to different species of *Pseudomona*, where the clinical activity of carbenicillin is marginal, and for different types of *Enterobacteriaceae*. Another important aspect of the combination is the potential protective effect of piperacillin against the nephrotoxicity elicited by prolonged treatment with aminoglycoside antibiotics.[2,3]

The aim of the present work was to set up a rapid, specific and reliable analytical technique to determine piperacillin in biological samples by HPLC, both in plasma and in tissues such as renal cortex and medulla.

MATERIAL AND METHODS

Reagents

Sodium piperacillin (Lederle); Sodium Cloxacillin (Antibióticos Farma); HPLC grade acetonitrile (Merck); Trichloroacetic acid (Merck); Potassium Hydroxide (Panreac); Monopotassium phosphate (Panreac); Chloroform (Carlo Erba).

Equipment

Kontron liquid chromatograph, mod.420; Kontron UV detector, mod.430; Kontron MT-1 data treatment station; Crison pH-meter, mod.2001; Reverse phase column (RP-18) with 5 μm particle size, a length of 15 cm and an i.d. of 0,4 cm; Heraeus mod.Labofuge 6000 ultracentrifuge; Selecta ultrasound bath,

mod.513; Supelco vacuum system, mod.5-8068 with 0,45 μm Millipore filters; Super Mixer tube shaker; Nitrogen chamber; Thermostatted sand bath (Kowwel T-1).

Chromatographic Conditions

The mobile phase was composed of a mixture of acetonitrile-phosphate buffer 0,1M adjusted to pH=6 with 19N potassium hydroxide (20:80 v/v). This mobile phase was prepared daily and filtered through a Millipore filter with a 0,45 μm pore diameter and was degassed in an ultrasound bath for 15 min prior to use. Flow rate during the assays was 2 mL/min; detector wavelenght was 254 nm;[4] response time was 0,5 sec., and sensitivity was 0,02.

Sample Preparation

Before the samples were injected into the chromatograph, protein denaturing and precipitation were carried out. Sample treatment was as follows:

One hundred μL of a solution of 0,4 mg/mL of cloxacillin in water -used as internal standard- was added to 150 μL of biological sample. The mixture was vortexed for 30 sec., after which 100 μL of a solution of 10% trichloroacetic acid in water -responsible for protein denaturing- was added and the mixture was vortexed again for 30 sec. and then centrifuged for 5 min. at 3.500 r.p.m. The supernatant was directly injected into the chromatograph with a 200 μL fixed-volume loop.

Tissue concentrations are often below 0,5 μg/mL and hence, although the technique becomes more complex, it is necessary to extract the samples with chloroform and then concentrate them to increase the sensitivity limit. In this case, sample treatment was as follows:

One hundred twenty μL of trichloroacetic acid was added to 1 mL of the problem sample -tissue homogenate in Sörensen's buffer, pH=7,4- and this was vortexed for 30 sec. and then centrifuged for 5 min. at 3.500 r.p.m. Following this, the supernatant was collected, adding 5 mL of chloroform. This mixture was vortexed for 1 min. and centrifuged for 10 min. at 3.500 r.p.m. Then collecting the aqueous phase, on which the same operation was repeated, the oil phase of the both previous steps was brought to dryness in a nitrogen chamber

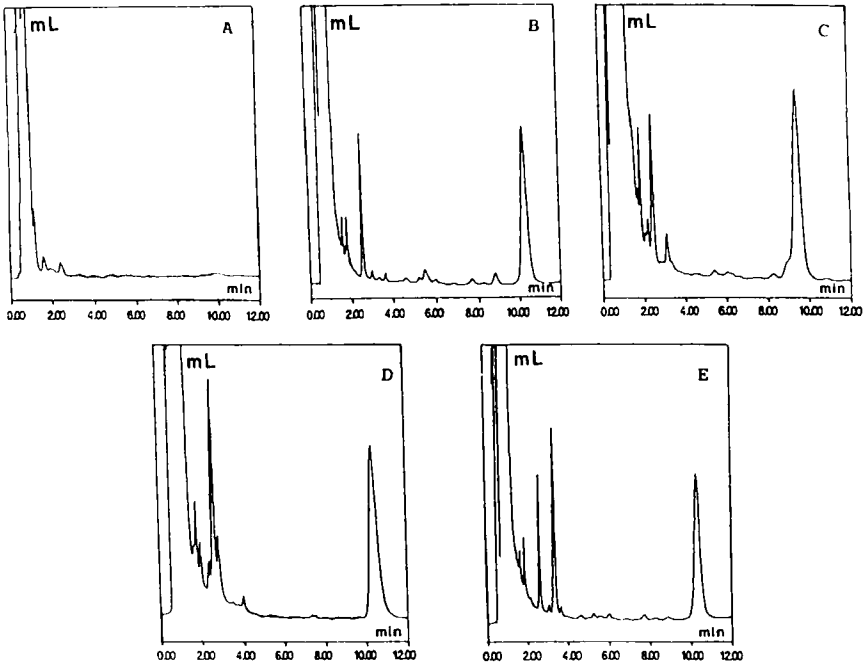

Figure 1. Chromatograms of piperacillin and cloxacillin.

at 37°C, injecting the dry residue dissolved in 250 μL of mobile phase into the chromatograph.

Quantification

The concentration of piperacillin in the problem samples was determined from the following equation:

$$C = (R-A)/B$$

where C is the concentration of piperacillin in μg/mL, A is the ordinate at the origin of the calibration straight line, B is the slope of the calibration line, and R is the peak height ratio (height of piperacillin/height of cloxacillin).

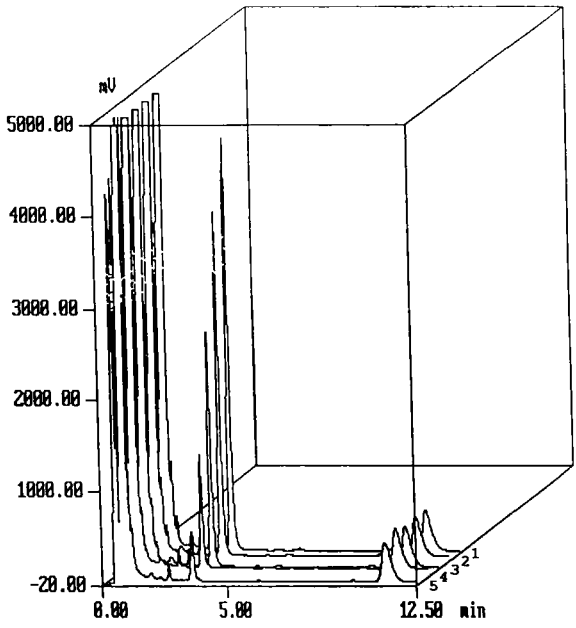

Figure 2. Chromatograms corresponding to the calibration range of piperacillin in plasma:
1. 200 μg/mL; 2. 150 μg/mL; 3. 100 μg/mL; 4. 50 μg/mL; 5. 15 μg/mL

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a tissue blank (A), of a plasma blank with internal standard -0,4 mg/mL- (B) and the chromatograms of piperacillin and cloxacillin in different biological samples (C: cortex, M: medulla, E: plasma). Figure 2 included the whole plasma calibration range of piperacillin using the technique.

Under the above-described conditions, piperacillin and cloxacillin were well separated, their retention times being 3,20 and 10,27 min., respectively.

The linearity of the chromatographic technique can be seen in Figures 3 and 4, corresponding to straight line calibrations for high (200-15 μg/mL) and low (15-0,5 μg/mL) concentrations, respectively, of piperacillin in plasma. A linear relationship was established between the peak height ratio (height of piperacillin/ height of 0,4 mg/mL cloxacillin) and the plasma concentration of

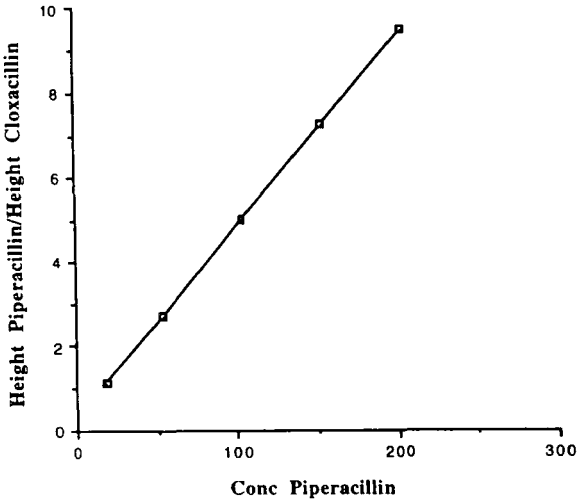

Figure 3. Calibration straight line of piperacillin in plasma for concentrations ranging from 15 to 200 μg/mL.

piperacillin. In both cases, the linear correlation coefficient was 0,999 and regression analysis of the data afforded the following equations:

200-15 μg/mL: $Y = 0{,}0436 + 0{,}0579X$
15-0,5 μg/mL: $Y = 0{,}0045 + 0{,}0412X$

where X and Y are the peak height ratio and piperacillin concentration, respectively.

Statistical Treatment

To study the reproducibility of the analytical technique, its variation coefficients were studied -both intraday and interday- with an ANOVA Test. To do so, five calibration straight lines were analyzed on 5 consecutive days. The data obtained with the ANOVA Test are shown in Tables 1a & 1b.

The resulting variation coefficients were 4,10% for the intra-day study and 7,11% for the inter-day study. A similar study was conducted for low piperacillin plasma concentrations (15-0,5 μg/mL), the variation coefficients

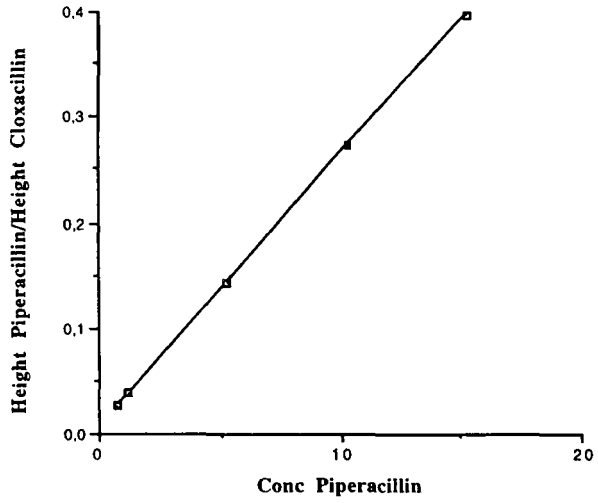

Figure 4. Calibration straight line of piperacillin in plasma for concentrations ranging from 0,5 to 15 μg/mL.

being 5.71% and 7,54% for the intra and inter-day studies, respectively.

CONCLUSIONS

The analytical technique described here is simple and shows good linearity and reproducibility, with a detection limit below 0,5 μg/mL. The technique permits the determination of piperacillin concentrations in biological samples using a small sample volume, which is important when carrying out studies on this type of sample.

All this means that the technique can be routinely used in pharmacokinetic studies with this drug, which are increasingly frequent owing to the importance of the combination of piperacillin with aminoglycosides drugs for the treatment of diverse infections.[5]

With minor modifications, the technique could also be used for the analysis of other β-lactam antibiotics.

Table 1a

Intra and Inter-day ANOVA

Day/Assay	1	2	3	4	5
1	14.75	16.94	16.80	16.79	14.53
2	14.80	14.95	14.80	13.59	13.45
3	15.22	16.38	16.21	13.23	13.89
4	14.67	14.99	15.45	16.53	16.69
5	15.04	16.93	15.04	15.04	15.80
	Mean 15.23 ± 0.98 μg/mL				
1	50.89	49.98	52.95	46.34	55.05
2	47.19	49.10	46.47	51.15	47.46
3	48.44	51.04	54.78	50.15	47.77
4	48.85	51.04	54.78	50.15	47.77
5	49.45	50.40	49.31	50.55	49.55
	Mean 49.26 ± 2.36 μg/mL				
1	99.87	103.21	104.47	102.23	102.42
2	103.18	105.17	97.34	103.31	102.22
3	98.37	98.11	104.31	103.39	104.06
4	95.15	101.26	98.84	98.42	104.02
5	104.84	100.35	99.11	100.10	103.85
	Mean 101.61 ± 2.71 μg/mL				
1	151.20	151.20	149.31	154.41	155.56
2	152.53	147.57	146.82	154.64	149.19
3	143.71	153.02	150.12	149.36	148.20
4	150.51	152.53	153.35	148.22	149.39
5	152.78	149.06	147.48	148.30	149.75
	Mean 150.21 ± 2.91 μg/mL				
1	198.10	205.61	196.57	203.23	201.37
2	199.83	194.94	199.42	194.76	205.64
3	206.59	206.36	204.51	194.92	201.87
4	196.66	206.36	201.53	195.61	197.22
5	203,58	189,01	188,59	201,61	199,85
	Mean 199.81 ± 4.12 μg/mL				

Table 1b

Test ANOVA

Intraday	**SS**	**MS**	**DF**
Regressión	4.904E+02	4.962E+02	1.000E+00
Error	2.211E+00	9.612E+02	2.300E+01
Total	4,926E+02		
Interday			
Regression	1.866E+03	1.883E+03	1.000E+00
Error	1.685E+01	1.719E-01	9.800E+01
Total	1.883E+03		

ACKNOWLEDGEMENTS

Some of the results presented here were obtained with a grant. Project: DGICYT-PB92-0274.

REFERENCES

1. M.F.Parry, S. J. Pancoast, **Antimicrobial Therapy**, Raven Press, Inc., New York, 1984. pp.197-207.

2. T. Hayashi, Y. Watanabe, T. Yasuda, I. Saikawa, J. Katahira, T. Kumada, K. Shimizu, Antimicrobial Agents and Chemotherapy, **32(6)**, 912-918 (1988).

3. W. E. Uber, R. C. Brundage, R. L.White, M. R. Brounley, The Annals of Pharmacotherapy, **25**, 357-359 (1991).

4. D. Debruyne, J. Ph. Ryckelynck, B. H.Ligny, M. Moulin, Journal of Pharmaceutical Sciences, **79**, 99-102 (1990).

5. A.Kucers, M. Bennett, **The Use of Antibiotics**, Heinemann, Inc., London, 1979. pp.171-181.

Received July 23, 1995
Accepted August 16, 1995
Manuscript 3945