ELSEVIER

Journal of Chromatography B, 672 (1995) 160–164

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

High-performance liquid chromatographic determination of tazobactam by precolumn derivatization

E. Peyrin, Y. Guillaume, C. Guinchard*

Laboratoire de Chimie analytique, UFR des Sciences Médicales et Pharmaceutiques, Place St Jacques, 25030 Besancon Cedex, France

First received 7 March 1995; revised manuscript received 2 May 1995; accepted 2 May 1995

Abstract

A new reversed-phase high-performance liquid chromatography (RP-HPLC) method for the detection and quantification of tazobactam in serum and haemofiltration fluid is described. The assay for these biological fluids involves an extraction with diethyl ether followed by derivatization using 1,2,4-triazole. The mobile phase consisted of phosphate buffer–methanol and the detection wavelength was 325 nm. The limit of detection was 0.05 μg/ml in the two fluids and the calibration curves were linear over the range 0.1–50 μg/ml. For a tazobactam concentration equal to 1, 5 or 20 μg ml^{-1}, the coefficients of variation were less than 5%. The assay was successfully applied to the analysis of samples from drug monitoring in a patient with renal insufficiency undergoing continuous venovenous haemofiltration (CVVH).

1. Introduction

Since the introduction of β-lactam antibiotics, many organisms previously susceptible to penicillins have developed resistance. The resistance is often due to the presence of β-lactamase which is able to catalyze the hydrolysis of the β-lactam ring of penicillins. One way to restore the sensitivity of resistant strains of bacteria to the antibiotic is to coadminister a β-lactamase inhibitor.

Tazobactam is a new β-lactamase inhibitor belonging to the class of penicillanic acid sulfones (Fig. 1). It is being codeveloped as an intravenous combination with piperacillin [1]. Recently, a new method has been described for the rapid determination of sulbactam and tazobactam in human serum by RP-HPLC [2]. Tazobactam can be detected by RP-HPLC by direct absorption at 220 nm [3,4], but the chromatograms show interferences from UV-absorbing endogenous proteins. The use of a precolumn derivatizing reagent eliminates this problem and enables the measurement at trace levels in body

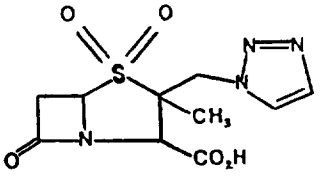

Fig. 1. Chemical structure of tazobactam.

* Corresponding author.

0378-4347/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0378-4347(95)00193-X

fluids. Precolumn derivatization by imidazole and 1,2,4-triazole has been used to measure the two other β-lactamase inhibitors, clavulanic acid and sulbactam, in serum, plasma, urine and tissue [5–7]. In this paper, the development of the first precolumn derivatization by 1,2,4-triazole for the analysis of tazobactam is described.

2. Experimental

2.1. Reagents

All chemicals were of the analytical grade. The following substances were used: tazobactam (sodium salt) (Lederle, Ourlins, France), 1,2,4 triazole (Aldrich, Strasbourg, France), sulfuric acid and phosphoric acid from Merck (Darmstadt, Germany); diethyl ether and diammonium hydrogen phosphate from Prolabo (Paris, France); sodium hydroxide (OSI, Dijon, France), methanol and mercuric chloride (Carlo Erba, Rueil-Malmaison, France).

2.2. Preparation of derivatizing reagent

The derivatizing reagent was prepared by dissolving 4 g of 1,2,4-triazole in water, adding 10 *M* aqueous sodium hydroxyde to bring the solution to pH 9.0 and diluting to 20 ml with water.

2.3. Sample preparation

To 1 ml of serum or haemofiltrate, 100 μl of 3 *M* sulfuric acid and 4 ml of diethyl ether were added. After vortex-mixing for 1 min and centrifugation at 5500 *g* for 10 min, the organic layer was transferred to a clean glass tube and then evaporated to dryness at 37°C under a stream of nitrogen. The residue was dissolved in 100 μl of 1,2,4-triazole reagent solution and 10 μl of 1% (v/v) mercuric chloride in water solution was added to the mixture. The specimen was incubated for 30 min at 37°C. A 20-μl portion was injected into the chromatograph with a Hamilton syringe.

2.4. Apparatus

The analyses were carried out with a HPLC Waters pump 501 (Saint Quentin en Yvelines, France), an Interchim Rheodyne injection valve Model 7161 (Montluçon, France) with a 20-μl sample loop, a Merck L 4000 variable wavelength UV spectrophotometer detector or a Merck 4500 diode array detector (Nogent sur Marne, France). A Merck Lichrospher RP (18) column (125 mm × 4 mm I.D. 5-μm particle size) (Nogent sur Marne, France) was used. The mobile phase consisted of a diammonium hydrogen phosphate–methanol (99.5:0.5, v/v) mixture. The pH of the solution was adjusted to 6.00 with phosphoric acid and the solution was degassed by sonication for 10 min. The flow-rate was 2.2 ml/min and the detection was performed at 325 nm. The chromatographic separations were carried out at ambient temperature.

2.5. Assay validation

The standard curves for serum and haemofiltrate were obtained by adding tazobactam to drug-free biological fluids, to obtain a concentration of 0.1–50 μg/ml. Standard serum and haemofiltrates were prepared under operating conditions as described above.

Accuracy and precision of the tazobactam assay in the two biological fluids were determined by analyzing four replicates at each of three concentration levels (1, 5 and 20 μg/ml).

2.6. Derivatization and HPLC separation

A single liquid–liquid extraction procedure followed by evaporation of the organic phase and reconstitution of the residue with 1,2,4-triazole solution was used. Our study indicates that the derivatizing reaction was optimal in terms of detection sensitivity with a basic reagent solution for a pH equal to 9.00. As an $HgCl_2$ solution was needed for the stable formation of the chromophoric derivative of sulbactam with 1,2,4-triazole [8], this metallic ion was used by analogy

and this was successful. The derivatized product of tazobactam showed UV absorption maxima at 325 nm. The chromatograms of serum and haemofiltrate from a subject receiving tazobactam and blank serum and haemofiltrate are shown in Fig. 2.

The analysis time for each sample was less than 5 min. No chromatographic interference from the blank samples was found. The specificity of the assay was demonstrated against the coadministered antibiotic piperacillin.

3. Results and discussion

3.1. Linearity, detection limit, accuracy and precision

The calibration curves for tazobactam were linear over the concentration range of 0.1 to 50 μg/ml. The equations determined from 5 different concentrations (experiments repeated twice) were $y = -1.11 + 38461x$ ($r^2 = 0.981$) for standard serum and $y = -4.05 + 42559x$ ($r^2 = 0.989$)

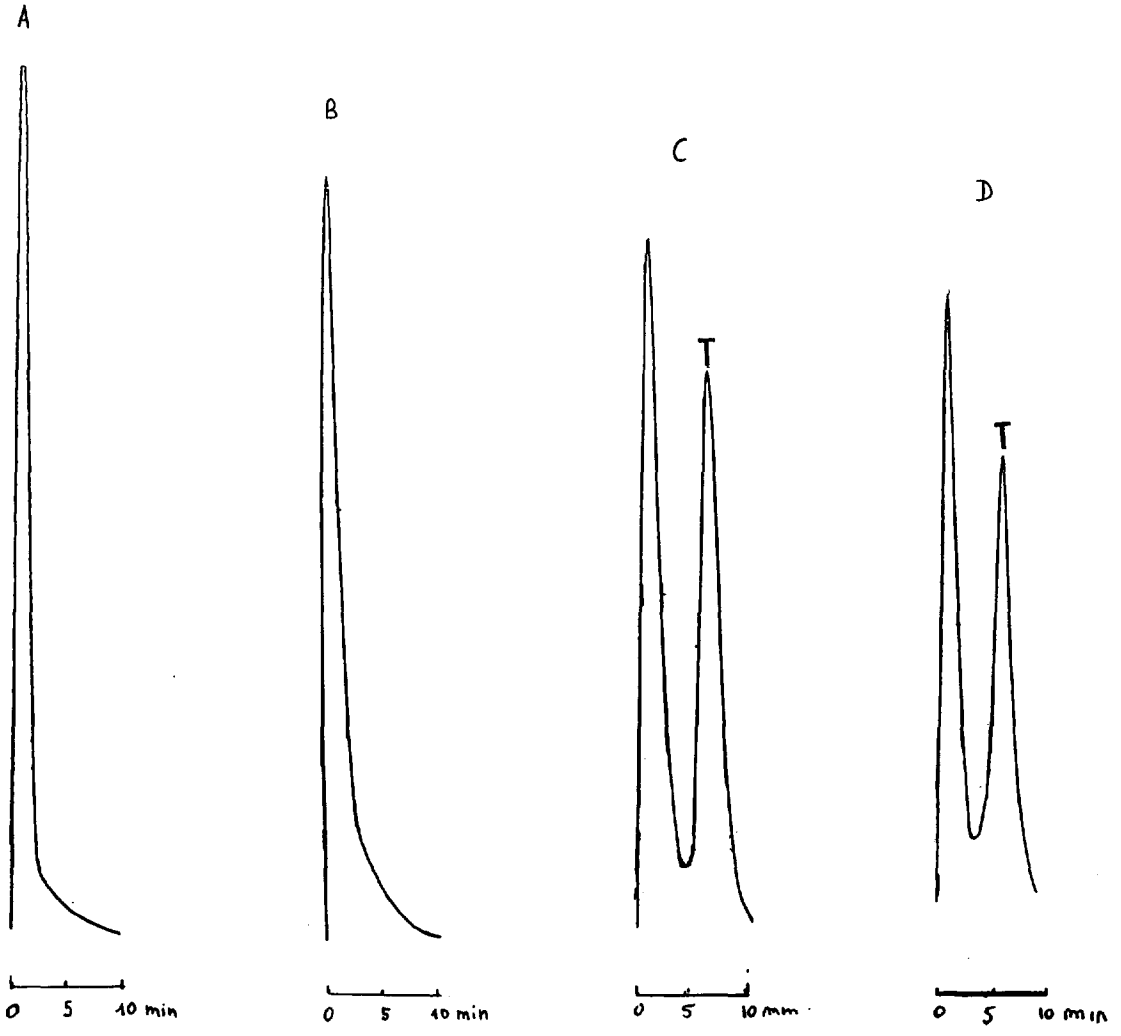

Fig. 2. Chromatograms of (A) blank serum, (B) blank haemofiltrate, (C) serum with 29.7 μg/ml of tazobactam, (D) haemofiltrate with 12.6 μg/ml of tazobactam.

Table 1
Precision and accuracy in serum ($n = 4$)

Spiked concentration (μg/ml)	C.V. (%)	Accuracy (%)
1	4.7	95.9
5	4.2	97.1
20	2.4	101.3

for standard haemofiltrate where y is the peak area for tazobactam and x is the concentration of tazobactam in μg/ml, r is the correlation coefficient. The detection limit of the method in the two biological fluids was 0.05 μg/ml. Our method is more sensitive than previously reported RP-HPLC methods without derivatization [4]. Tables 1 and 2 show the precision (expressed as coefficient of variation: C.V.) and accuracy of the tazobactam assays in serum and hemofiltrate. The coefficients of variation were less than 5% ($n = 4$) over the concentration range 1–20 μg/ml. The accuracy was between 95.9 and 102.0%.

3.2. Application

A patient undergoing CVVH received piperacillin (4 g every 8 h) and tazobactam (1.5 g every 8 h) for an infectious disease. The continuous haemofiltration is an extracorporeal technique that is used to remove fluid, electrolytes and other waste products from the blood supply of critically ill patients with acute renal failure. The determination of tazobactam concentrations in serum and haemofiltration fluid of the subject was carried out to ascertain the impact of CVVH on drug elimination. Blood samples (7 ml) were collected just before (0 h) and 1.5 h after each dose. Three haemofiltrate samples (10 ml) were collected at regular intervals between each dose. The haemofiltrate concentration (12.6 to 34 μg/ml) demonstrated the convective trans-membrane movement of tazobactam, but the clearance via haemofiltration could be significantly lower than normal renal clearance because drug accumulation was reported. Peak and residual subtherapeutic serum concentrations are shown in Table 3. These results involved dosage reduction of tazobactam to obtain the required drug concentration (5 μg/ml).

Table 2
Precision and accuracy in haemofiltrate ($n = 4$)

Spiked concentration (μg/ml)	C.V. (%)	Accuracy (%)
1	3.6	99.3
5	4.5	102.0
20	2.9	98.8

Table 3
Peak and residual (RS) serum concentration of a patient undergoing CVVH

	Time (h)	Concentration (μg/ml)
RS	0	14.0
Peak	1.5	29.7
RS	8	20.7
Peak	9.5	33.8

These data must be extrapolated to the individual clinical situation with caution because of large interindividual variation in the clinical status of the subjects and the fact that this analysis only reports one patient.

4. Conclusion

This newly developed RP-HPLC method for tazobactam using precolumn derivatization is highly sensitive and selective and has good reproducibility. It can be used to measure concentrations of tazobactam at trace levels for pharmacokinetic studies or in serum for optimal clinical management of patients.

References

[1] [1] R.N. Jones, M.A. Pfaller, P.C. Fuchs, K. Aldridge, S.D. Allen and E.H. Gerlach, Diagn. Microbiol. Infect. Dis., 12 (1989) 489.
[2] Y. Guillaume, E. Peyrin and C. Guinchard, J. Chromatogr., in press.

[3] M. Kinzig, F. Sorgel, B. Brisman and E. Nord, Antimicrob. Agents Chemother., 36 (1992) 1997.
[4] A.P. Ocampo, K.D. Hoyt, N. Wadgaonkar, A.H. Carver and C.V. Puglisi, J. Chromatogr., 496 (1989) 167.
[5] R.E. Bawdon and P.O. Madsen, Antimicrob. Agents Chemother., 30 (1986) 231.
[6] J. Martin and R. Mendez, J. Liq. Chromatogr., 11 (1988) 1697.
[7] A.J. Shah, M.W. Adlatd and J.D. Stride, J. Pharm. Biomed. Anal., 8 (1990) 437.
[8] J. Haginaka, J. Wakai, H. Yasuda, T. Uno and T. Nakagawa, J. Chromatogr., 341 (1985) 115.