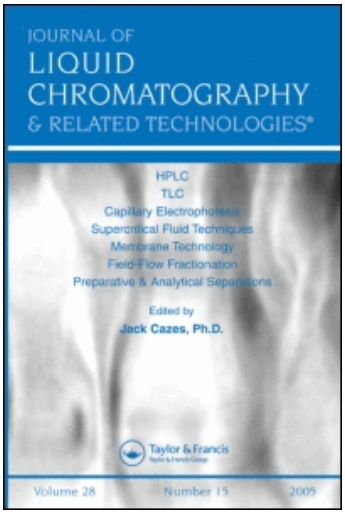
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

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

Rapid Determination of Teicoplanin in Human Plasma by High Performance Liquid Chromatography

G. Chapelle[a]; S. Bouquet[ab]; G. Rivet[a]; L. Barrier[a]; E. Rochard[b]; Ph. Courtois[b]
[a] Laboratoire de Pharmacocinétique, CHRU La Milétrie, Poitiers, France [b] Institut du Medicament UFR de Medecine et Pharmacie, Poitiers, France

To cite this Article Chapelle, G. , Bouquet, S. , Rivet, G. , Barrier, L. , Rochard, E. and Courtois, Ph.(1991) 'Rapid Determination of Teicoplanin in Human Plasma by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 14: 11, 2157 — 2167
To link to this Article: DOI: 10.1080/01483919108049681
URL: http://dx.doi.org/10.1080/01483919108049681

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(11), 2157-2167 (1991)

RAPID DETERMINATION OF TEICOPLANIN IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

G. CHAPELLE[1], S. BOUQUET[1,2],
G. RIVET[1], L. BARRIER[1],
E. ROCHARD[2], PH. COURTOIS[2]
[1]*Laboratoire de Pharmacocinétique*
CHRU La Milétrie
BP 577, 86021 Poitiers, France
[2]*Institut du Medicament*
UFR de Medecine et Pharmacie
34 Rue du Jardin des Plantes
86000 Poitiers, France

Downloaded At: 10:02 25 January 2011

ABSTRACT

A rapid isocratic reversed-phase HPLC method for the determination of the major component of teicoplanin in plasma is described.
By using solid phase extraction C18 and UV detection at 240 nm, this method is specific, and sufficiently sensitive .
We found a good linearity over the range: 5 - 40 mg/l of teicoplanin plasma concentrations. The coefficients of variation did not exceed 5.4 % both within-day and between-day assays.
With the small plasma sample required for the analysis (250 µl) and the good accuracy, this rapid procedure appears to be suitable for the therapeutic drug monitoring.

INTRODUCTION

Teicoplanin is a new glycopeptide antibiotic with chemical structure related to the ristocetin-vancomycin group (figure 1)

Copyright © 1991 by Marcel Dekker, Inc.

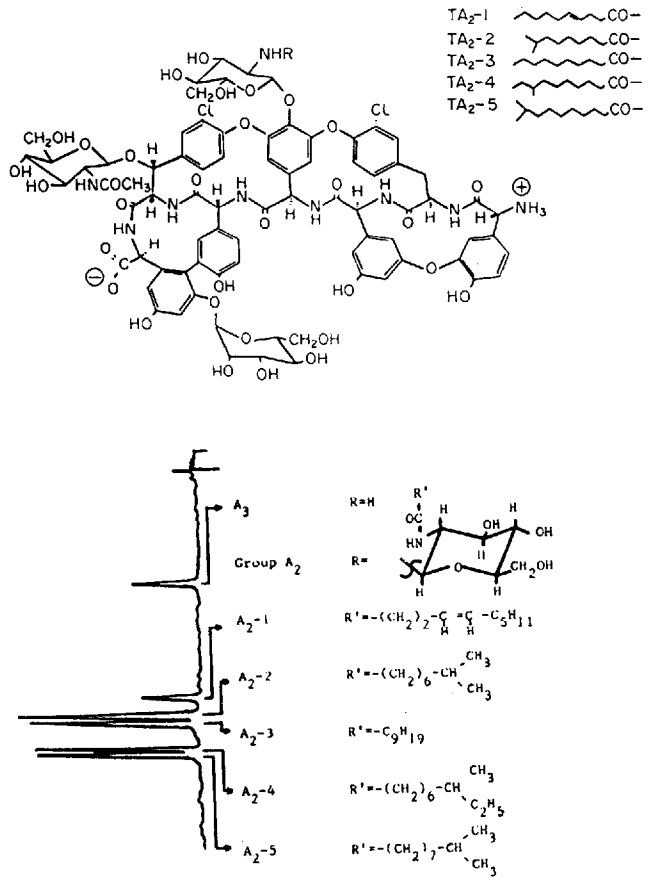


FIGURE 1: Chemical structures of the teicoplanin components and their relationship to the HPLC chromatogram as reported by Riva E. et al (4).

This drug is active against Gram positive bacteria, including meticillin resistant staphylococci which are involved in severe hospital infections (1,2).

The determination of teicoplanin concentrations in plasma, could be important for this antibiotic in order to maintain the levels in a therapeutic range at steady-state (3).

This antibiotic agent is constituted by six major components: AIII, AII.1, AII.2, AII.3, AII.4, AII.5 (Figure 1).

Downloaded At: 10:02 25 January 2011

The methods which used gradient elution chromatography (4,5) are time consuming to resolve the six major peaks . One used derivatisation and fluorimetric detection (6) and another detected only one unidentified fraction (7).
We describe a rapid HPLC method using Extra-Sep (C18) solid-phase extraction of plasma samples with isocratic reversed-phase chromatography and UV detection. We determine the plasma levels of teicoplanin with a good accuracy in therapeutic drug monitoring.

MATERIALS AND METHOD

Chemicals

Teicoplanin and carbamazepine (internal standard, IS) were supplied by Merell Dow Institute and Ciba-Geigy laboratories, respectively.
HPLC grade acetonitrile and methanol were obtained from Carlo-Erba (Milan, Italy).
Distilled water was provided from Biosedra (Malakoff, France)
Analytical grade sodium dihydrogen phosphate was obtained from Merck (Darmstadt,Germany)
Sample clean up cartridges (C18, 100 mg, 1 ml), Extra-Sep column were obtained from Lida Manufacturing Corp.(Bensenville, USA).

Downloaded At: 10:02 25 January 2011

HPLC equipment

The liquid Chromatograph consisted of a model L 6000 pump MERCK and a GILSON 231 automatic sample processor.
The detection was performed with a model 484 UV absorbance detector at a wavelength of 240 nm (WATERS Associates Inc.)
Data recording were undertaken by a Chromatopac CR6A SHIMADZU integrator.

Chromatographic conditions

HPLC was performed at room temperature by using reversed- phase RP 8 select B column (150 x 4.2 mm i.d., 5 μm particle size, MERCK.)

The mobile phase A was a mixture of aqueous sodium dihydrogen phosphate solution (0.01 M, PH 6), acetonitrile, 80/20,V/V.
The mobile phase B was a mixture of the same aqueous dihydrogen phosphate solution, acetonitrile, 75/25, V/V.
These solvents were filtered through a 0.45µm Millipore filter and carried through the column at 1.1 ml/mn at ambient temperature.

Standard solutions

Stock solution of teicoplanin was weekly prepared in distilled water at a concentration of 400 mg/l and stored at -70°C during one month. Calibration standards (5, 10, 20, 40 mg/l) were daily made in pooled human plasma.
A working solution of carbamazepine (IS) at 20 mg/l was prepared in methanol and kept during 30 days at +4°C.

Downloaded At: 10:02 25 January 2011

Sample preparation

Teicoplanin was isolated from plasma sample with Extra-Sep cartridge packed with C18 silica particles placed on the SUPELCO Inc extraction unit.
Each cartridge was prepared prior to use by washing with 1ml of methanol and 1 ml of distilled water.
A 0.25 ml of plasma sample containing 0.5 µg of internal standard was loaded on the cartridge.
The column was washed with 1 ml of 5% acetonitrile in distilled water, and the mixture was discarded. Finally, the six components of teicoplanin and internal standard were eluted with 0.5 ml of methanol. The effluent was evaporated to dryness at ambient temperature under a gentle stream of nitrogen gas. The dry residue was reconstituted in 100 µl of mobile phase and an aliquot of 20 µl was injected into the chromatographic system.

RESULTS AND DISCUSSION

Chromatograms

The figure 2 shows the chromatograms obtained from aqueous solutions spiked with teicoplanin using mobile phase A and

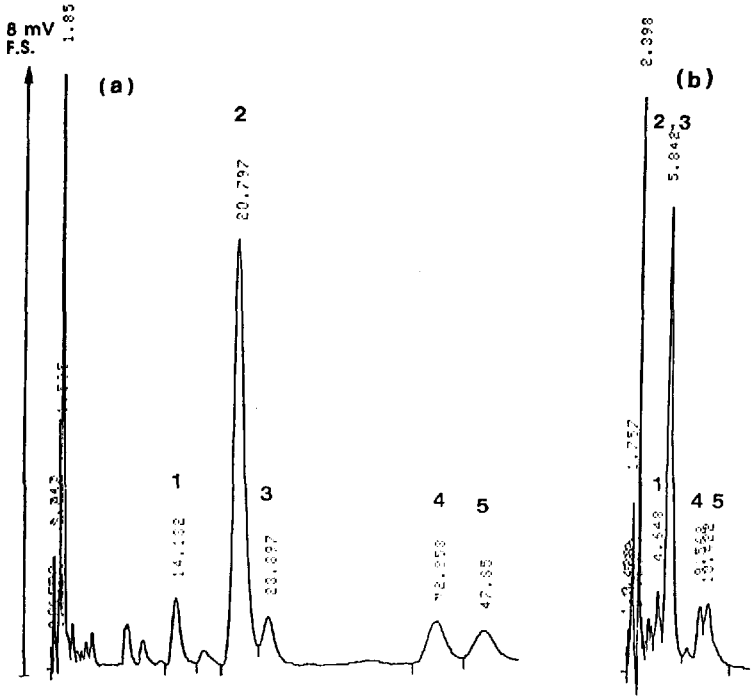


FIGURE 2: Chromatograms obtained from standard solutions of teicoplanin containing:
(a) 40 mg/l with mobile phase A
(b) 20 mg/l with mobile phase B
Peaks: 1: AII.1, 2: AII.2, 3: AII.3, 4: AII.4, 5: AII.5
Conditions: flow rate : 1.1 ml/min, inj. vol.= 20 µl, sensitivity: 8 mv full scale

mobile phase B; injection 20 µl, 8mV full scale.

With mobile phase A (figure 2a), five main components of teicoplanin were separated within 50 min.

With mobile phase B (figure 2b), all components were eluted in less 12 mn, with co-elution of AII.2 and AII.3 fractions at 5.8 min.

These last chromatographic conditions (mobile phase B), were used in routine determination of teicoplanin plasma levels.

The co-eluted peaks AII.2 and AII.3 represent more than 60% of total teicoplanin (Table 1).

Downloaded At: 10:02 25 January 2011

TABLE 1: Retention time and distribution of the six major components of teicoplanin using mobile phase A and B.

	Mobile phase A		Mobile phase B	
component	RT min	Percentage of 5 fractions areas[1]	RT min	Percentage of 5 fractions areas[1]
AIII	ND [2]	ND	ND	ND
AII.1	14.1	7.2	4.6	5.2
AII.2	20.8	63.0	5.8	72.1
AII.3	23.9	8.7		
AII.4	42.8	11.4	9.5	9.2
AII.5	47.8	9.4	10.4	13.3

(1): AII.1, AII.2, AII.3, AII.4 and AII.5.
(2): no detectable

Downloaded At: 10:02 25 January 2011

The results described by Falcoz et al (8) showed that the five major components of AII group were not affected by various diseases. In this way, we have made the determination of teicoplanin from the main peak (retention time: RT= 5.3 min. in figure 3a) corresponding to both AII.2 and AII.3 fractions.
The figure 3 shows chromatograms of teicoplanin obtained from extracts of blank plasma spiked with 10 mg/l (figure 3a) and patient plasma (figure 3b).
Concentrations were calculated comparing the ratio of peak heights of samples (major peak 5.3 min./ IS), with calibration curve.
Carbamazepine (IS) was eluted in 15.7 min., after AII.4 and AII.5 peaks of teicoplanin in isocratic conditions. This is the main advantage of its choice as internal standard.

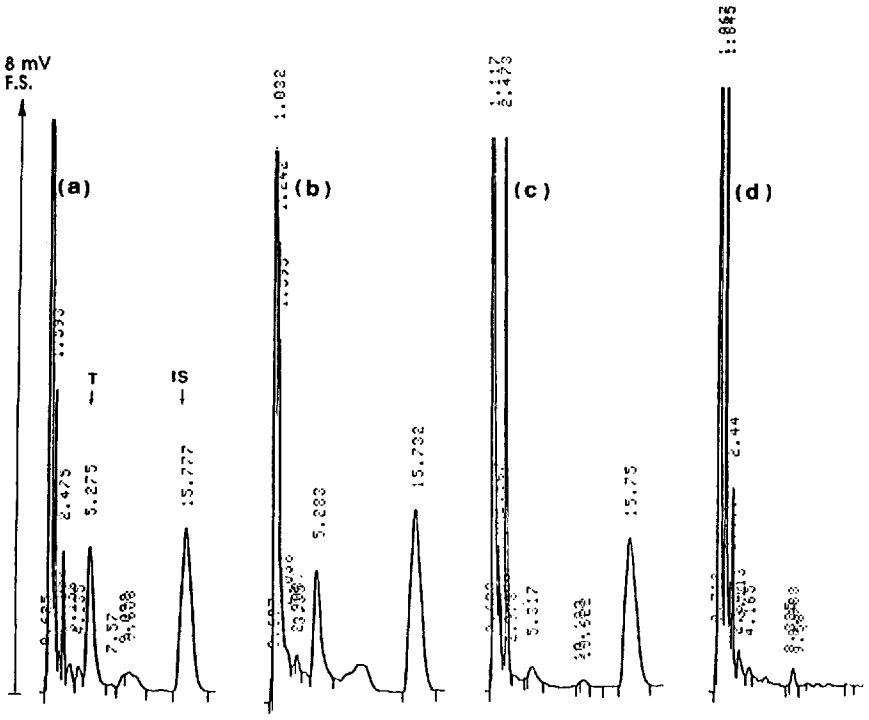


FIGURE 3: Chromatograms obtained from extract of: (a) plasma sample spiked with teicoplanin (10 mg/l) and internal standard (IS); (b) patient plasma corresponding to through level (7.3 mg/l) in chronic treatment with teicoplanin (3 mg/kg/d); (c) plasma samples spiked with teicoplanin (1 mg/l) and (d) drug free
conditions: mobile phase B, flow rate 1.1 ml/min., inj.vol.= 20 µl, sensitivity : 8mv full scale.

Recovery

The extraction efficiences of teicoplanin were determined by extracting samples containing 5 and 20 mg/l in triplicate and comparing them to unextracted samples prepared by dilution with water. The recoveries of the major peak of teicoplanin were included in the range 74-80 %, 76-80 %, respectively.

This rapid method seems in accordance with a good

Downloaded At: 10:02 25 January 2011

TABLE 2: Within and between-day variability in measured teicoplanin concentrations in plasma.

concentration spiked mg/l	concentration found mg/l mean ± SD	accuracy (%)	coefficient of variation (%)
Within-day variability			
5 (n=6)	5.09 ± 0.22	101.8	4.32
20 (n=6)	20.36 ± 0.82	101.7	4.05
40 (n=6)	39.40 ± 2.10	98.5	5.32
Between-day variability			
5 (n=6)	4.78 ± 0.24	95.6	5.02
10 (n=6)	10.15 ± 0.55	101.5	5.41
20 (n=6)	19.76 ± 0.93	98.8	4.70

Downloaded At: 10:02 25 January 2011

reproductibility of extraction compared to acetonitrile precipitation (9).

Linearity

The linear curve of teicoplanin (AII.2, AII.3) was linear over the range 0 - 40 mg/l in plasma and can be expressed by the equation : $y = 0.0387 x + 0.00345$ ($R=0.997$, $n=6$).

Accuracy

The high, medium and low quality control samples were assayed six times on the same day and on different days over one month period, to calculate the precision of the assay.
As shown in table 2, the within-day precision of the method was

illustrated by the coefficients of variation: 5.3%, 4.0%, 4.3% for 40, 20, 5 mg/l, respectively.
The between-day coefficients of variation of the plasma control samples were: 4.7%, 5.4%, 5.0% for 20, 10 and 5 mg/l, respectively.
The accuracy was greater than 95% for both within-day and between-day analysis. These results may be explain by the use of an internal standard and the small volume of plasma loaded on the cartridge.
The limit of detection was 1.0 mg/l, allowing a signal-noise ratio of 4, when 0.25 ml of plasma was used (figure 3c). This sensitivity is sufficient for the plasma monitoring of this antibiotic.

Downloaded At: 10:02 25 January 2011

Cartridges reusability

Plasma samples spiked with 5 and 20 mg/l of teicoplanin were prepared once daily and extracted on the same cartridge for a total of five days. After each use, the cartridges were washed by three milliliters of methanol. The solid-phase columns were reused indiscriminately among the samples. During this time, no decrease of the ratio of peak heights teicoplanin/IS, was observed.

Specificity

We have found a good specificity of the method at 240 nm. No interference on AII.2, AII.3 peaks was detected in the control human samples or in the plasma samples from patients who received concomitant drugs such as: paracetamol, acetylsalicylic acid, gentamicin, amikacin, netilmicin, amoxicillin, cloxacillin, mezlocillin, cloxacillin, cefotaxime, ceftriaxone, aztreonam, rifampicin, vancomycin, fosfomycin, erythromycin, pefloxacin, ofloxacin, ciprofloxacin, isoniazid, cotrimoxazole, ketoconazole, fluconazole, acyclovir, theophylline, diazepam, nordiazepam, clonazepam, clobazam.
This HPLC method is now intensively used in our laboratory and more than 350 samples collected from patients receiving parenteral doses of teicoplanin in chronic treatment, have been

analysed with the same analytical column. There is not established therapeutic plasma concentration range of this antibiotic, although a minimum through concentration of 10 mg/l is needed for the treatment of severe infections caused by gram positive species (10).

CONCLUSION

The present HPLC procedure for rapid determination of teicoplanin is sufficiently sensitive, specific and suitable to be applied in routine analysis.
This method is easily applicable to the monitoring of plasma concentrations of drug in patients with severe gram positive infections or when a renal failure appears.

Downloaded At: 10:02 25 January 2011

AKNOWLEDGMENTS

The authors are much obliged to Dr Durrande, Merrell Dow laboratories for supplying teicoplanin and Mrs Garandeau M.C., Deshoullieres M.P. and Dalloul H., for their technical assistance.

REFERENCES

1. Greenwood D., microbiological properties of teicoplanin, J.of antimicrob. Chemother., 21, Suppl.A, 1, (1988).
2. Williams A.M., Grüneberg R.N., teicoplanin, J. of Antimicrob. Chemother., 14, 441, (1984).
3. Lewis P., Garaud J.J., Parenti F., a multicentre open clinical trial of teicoplanin in infections caused by Gram-positive bacteria, J. of Antimicrob. Chemother., 21, Suppl.A, 61, (1988).
4. Riva E., Ferry N., Cometti A., Cuisinaud G., Gallo G.G., Sassard J., determination of teicoplanin human plasma and urine by affinity and reversed-phase high-performance liquid chromatography, J. Chromatogr., 421, 99, (1987).
5. Jehl F., Monteil H., Tarral A., HPLC quantitation of the six main components of teicoplanin in biological fluids, J. of Antimicrob. Chemother., 21, Suppl.A, 53, (1988).

6. Joos B., Lüthy R., determination of teicoplanin concentrations in serum by high-pressure liquid chromatography, Antimicrob. Agents Chemother., 31, 1222, (1987).
7. Georgopoulos A., Czjeka M.J., Starzengruber N., Jäger W., Lackner H., high-performance liquid chromatographic determination of teicoplanin in plasma: comparison with a microbiological assay, J. Chromatogr., 494, 340, (1989).
8. Falcoz C., Ferry N., Pozet N., Cuisinaud G., Zech P.Y., pharmacokinetics of teicoplanin in renal failure, Antimicrob. Agents Chemother., 31, 1255, (1987).
9. Levy J., Loan Truong B., Goignau H., Van Laethem Y., Butzler J.P., Bourdoux P., high pressure liquid chromatographic quantitation of teicoplanin in human serum, J. of Antimicrob. Chemother., 19, 533, (1987).
10 Leport C., Perronne C., Massip P., Decazes J.M., Canton P., Bernard E., Vilde J.L., evaluation de la teicoplanin dans le traitement des endocardites bacteriennes, La Lettre de l'infectiologue, 20 , Suppl.III, 23, (1988).

Downloaded At: 10:02 25 January 2011