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Determination of ticarcillin in human plasma and in urine by reversed-phase LC

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Abstract: A liquid chromatographic assay for ticarcillin (ticar.) in plasma and urine is described. For analysis, the internal standard cefoperazone (cfp) is dissolved in acetonitrile, which is used for precipitating the protein. The supernatant is evaporated, reconstituted in running mobile phase and injected directly onto the reversed-phase C_{18} column, with detection at 205 nm. The mobile phase is composed of water–acetonitrile-*o*-phosphoric acid–tetramethylammonium chloride (TMA). Coefficients of variation for reproducibility were in the range of 2.2–15.5% for extra-low, low, medium and high controls. Limits of detection were 0.5 μg ml^{-1} for plasma and 1 μg ml^{-1} for urine. No interference from other cephalosporins or other antibiotics was noted. This liquid chromatographic assay is simple, accurate, requires no extraction and overcomes previous problems related to the drug's peak splitting due to isomerization.

Keywords: *Ticarcillin; cefoperazone; liquid chromatography; antibiotics; plasma; urine.*

Introduction

Ticarcillin (Fig. 1, alpha-carboxy-3-thienylmethylpenicillin) is a semisynthetic penicillin formed by racemic side-chain derivatization of 6-aminopenicillanic acid (6-APA). This derivatization of the basic penicillin structure increases activity against Gram-negative organisms by increasing the affinity of penicillin binding to bacterial proteins [1–5]. Ticarcillin is frequently coadministered with aminoglycosides and/or cephalosporins, and its efficacy compares favourably with other, semisynthetic penicillins [6].

Penicillins have been quantified in biological fluids by microbiological assay procedures [2, 3, 7–14], which suffer from lack of specificity and precision, as well as requiring long periods of time for incubation. A wide variety of chemical and physical assays have also been developed, including spectrophotometric [15–17], colorimetric [18–20] and iodometric [21–24]. These procedures lack specificity and/or sensitivity. Rapid and specific liquid chromatography (LC) techniques have been employed for the measurement of ticarcillin in sterile fluids [6, 25–28] and biological fluids [29–34]. All of these assays either use no internal standard, or involve extraction of the drug or require pre-column derivatization. We have developed an LC assay that is fast, specific, sensitive and requires no extraction or derivatization prior to assay.

Experimental

Apparatus

The LC system used consisted of a Waters M-45 pump (Waters Assoc., Milford, MA, USA), and WISP 710B (Waters), and a Lambda-max 481 variable UV detector (Waters). A Sergovar 120 strip-chart recorder (BBC-Metrawatt/Goerz, Edison, NJ, USA) or HP 3990 integrator (Hewlett–Packard, Avondale, PA, USA) was used for quantitation of chromatographic data.

The detector wavelength and sensitivity were set at 205 nm and 0.05 aufs, respectively. Separation was achieved with an Adsorbosphere-C_{18}, 5 μm, 250 × 4.6 mm reversed-phase column (Alltech Assoc., Deerfield, MI, USA).

Reagents

Nanopure deionized–distilled water, glass-distilled UV grade acetonitrile (CH_3CN)

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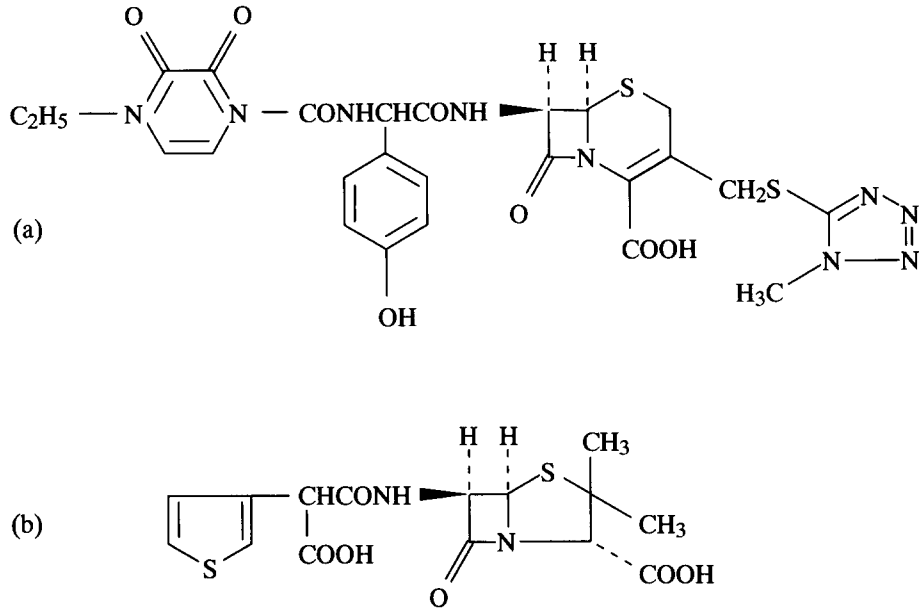

Figure 1
Chemical structures of (a) cefoperazone and (b) ticarcillin.

(Burdick & Jackson, product no. 015, Muskegon, MI, USA), *o*-phosphoric acid (85%, Mallinckrodt, lot KTGY, Paris, KY, USA), tetramethylammonium chloride (TMA) Fluka Chemical Corp., lot 241574684, Waupaugh, NY, USA), ticarcillin disodium (Beecham Laboratories, lot BK 3822, Bristol, TN, USA), cefoperazone dihydrate (Pfizer, lot 2J039-35QCS, NY, USA) were used as received. Blank plasma was obtained from blood donated by healthy volunteers which was collected in heparinized tubes and separated by centrifugation. Additional plasma was obtained from Irwin Memorial Blood Bank, San Francisco, CA, USA. All quality assurance (QA)-plasma samples and stock solutions were stored at −20°C.

Mobile phase

The mobile phase was composed of water–acetonitrile-*o*-phosphoric acid–tetramethylammonium chloride (10%), (696:300:1:3, v/v/v/v). Mobile phase was prepared in 2 l quantities in the following manner. Six hundred millilitres of CH_3CN were transfered to a 2-l graduated cylinder. "Nanopure" water was then poured into the cylinder until the fluid level reached the 1900 ml mark. Two millilitres of *o*-phosphoric acid were added along with 6 ml of previously prepared 10% TMA (1 g TMA salt/10 ml water) solution. Water was then added to the 2-l mark, and the contents filtered under vacuum through a 0.45 μm membrane filter while degassing. Isocratic flow rate was 1.0 ml min^{-1}.

Sample preparation and analysis

Ticarcillin disodium (20.0 mg) was dissolved in 10.0 ml deionized water to give 2000 μg ml^{-1} stock solution. Using this solution, extra-low, low mid and high QA-controls were prepared spiking 20.0, 100.0, 300.0 and 710.0 μl into 20 ml blank plasma aliquots, respectively. Final concentrations of extra-low, low, mid and high were 1.67, 8.33, 25.0 and 59.0 μg ml^{-1} (purity factor 83.3%), respectively. Two hundred-microlitre aliquots were stored at −20°C. For the calibration curve, using a 200.0 μg ml^{-1} ticarcillin solution, 0.5, 1, 2, 5, 10, 20, 30, 50, 70 and 100 μl were spiked into plasma aliquots (0.2 ml) to give a 0.42, 0.82, 1.64, 4.11, 8.21, 16.42, 24.63, 41.05, 57.47 and 82.1 μg ml^{-1} concentrations, respectively. Plasma protein in all samples was precipitated with 0.5 ml of CH_3CN containing the internal standard cfp (2.0 μg ml^{-1}).

Urine-QA samples were prepared by spiking ticarcillin (30.0, 150.0, 450.0, 900.0 μl) from the same stock solution used for plasma-QA sample preparation, into 20-μl aliquots of blank urine to give the final concentrations of 30.0, 150.0, 450.0 and 900.0 μg ml^{-1} of extra-low, low, mid and high, respectively. For the calibration curve in urine, 0.75, 1.5, 3.0, 7.5,

15.0, 30.0, 45.0, 75.0, 105.0 and 150.0 μl from 200 μg ml^{-1} ticarcillin solution were spiked into 0.2-ml aliquots of blank urine to give concentration of 0.63, 1.25, 2.5, 6.25, 12.5, 25.0, 37.5, 62.5, 87.5 and 125.0 μg ml^{-1}, respectively.

After the plasma samples were vortexed for 20 s and centrifuged at 3000 rpm for 10 min, the resulting supernatant was transferred into glass vials and evaporated under nitrogen. The residue was reconstituted in running mobile phase (0.2 ml) and 10–15 μl samples were directly injected onto the column for analysis. Urine samples (10–20 μl) were injected onto the column without any sample preparation.

Results

Separation

Figure 2(a, b and c) represents typical chromatograms of blank plasma, the internal standard in blank plasma and the ticarcillin together with the internal standard in human plasma, respectively. Figure 3(a, b and c) represents the chromatograms of blank urine, the internal standards in blank urine and ticarcillin together with the internal standard, respectively. The mean retention times for internal standard and ticarcillin were 6.8 and 8.8 min, respectively. Usual running time for a sample was 12 min.

Linearity

The calibration curve data for the drug in plasma are presented in Table 1. Linear

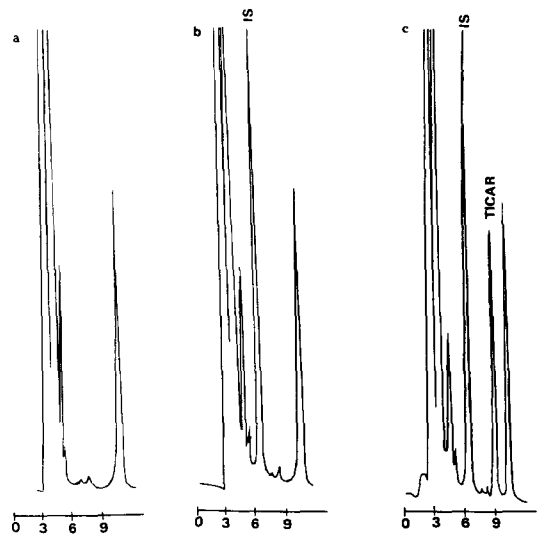

Figure 2
Chromatograms of: (a) blank human plasma; (b) internal standard (cfp) in blank plasma; and (c) ticarcillin and internal standard (cfp) in plasma.

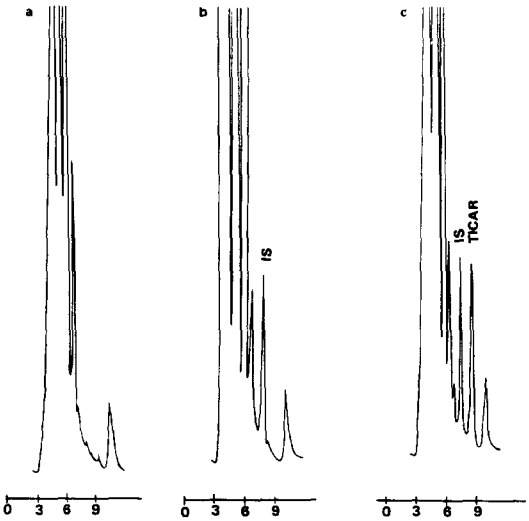

Figure 3
Chromatograms of: (a) blank human urine; (b) internal standard (cfp) in blank urine; and (c) ticarcillin and internal standard (cfp) in urine.

Table 1
Standard curve-data of ticarcillin in human plasma

Ticar. conc. μg ml^{-1}	Peak height ratio (Ticar/cfp)	Calc. conc. μg ml^{-1}
0.42	0.04	0.31
0.84	0.09	0.81
1.67	0.19	1.79
4.18	0.45	4.35
8.35	0.85	8.30
16.70	1.70	16.70
r = 0.9997	Slope = 0.1015	Int. = 0.0081
16.70	1.70	16.30
25.10	2.52	24.80
41.80	4.35	43.50
58.50	5.68	57.10
83.50	8.26	83.70
r = 0.9983	Slope = 0.0974	Int. = 0.1054

Table 2
Standard curve-data of ticarcillin in human urine

Ticar. conc. μg ml^{-1}	Peak height ratio (Ticar/cfp)	Calc. conc. μg ml^{-1}
0.63	0.07	0.74
1.25	0.12	1.60
2.50	0.16	2.28
6.25	0.42	6.73
12.50	0.74	12.50
r = 0.9986	Slope = 0.0585	Int. = 0.0266
12.50	0.74	12.16
25.00	1.49	25.00
37.50	2.25	36.80
62.50	3.95	63.20
87.50	5.53	87.80
125.00	7.90	124.00
r = 0.9998	Slope = 0.0644	Int. = 0.1199

regression of peak height ratio vs concentration gives a typical coefficient of determination (r^2) of 0.999. The standard curve is broken into two linear regression equations (concentrations of 0.42–16.7 and 16.7–83.5 μg ml^{-1}) to allow for better evaluation of lower points. Similar results for urine are shown in Table 2. Concentration ranges for urine are 0.63–12.5 and 12.5–125 μg ml^{-1}.

Specificity

Under the above chromatographic conditions, no endogenous peaks interfered with ticarcillin or cfp in either plasma or urine. Four to five different lots of plasma and urine were used for the study. Blank plasma from volunteers also indicated that xanthines, acetylsalicylic acid and acetaminophen had no influence on ticarcillin quantitation.

Some drugs that may be coadministered with ticarcillin were tested for interference. Included were a number of cephalosporins, penicillins, beta-agonists and benzodiazepines and they showed no interference with either ticarcillin or cefoperazone.

Variation

Interday and intraday variation of the method for extra-low, low, medium and high plasma and urine concentrations were determined using frozen controls. Five samples from each concentrations were assayed for both interday and intraday variability studies. For interday studies, six calibration curves on six consecutive days were used and one calibration curve was used for the intraday studies. The ranges for the relative standard deviations (RSDs) were between 4.18 and 11.9% for plasma (Table 3) and from 2.2 to 15.5% for urine (Table 4).

Recovery

Assay percentage recovery for ticarcillin in plasma vs water was measured by comparing the peak heights. The results are shown in Table 5. Spiked samples in both plasma and

Table 3
Interday and intraday variation of ticarcillin in human plasma

		Concentration* (μg ml^{-1})			
		Extra-low (1.67)	Low (8.33)	Medium (25.00)	High (59.00)
Inter day	Mean	1.76	8.44	25.60	55.04†
	SD	0.21	0.41	1.38	3.24
	RSD	11.90	4.90	5.40	9.73
	Rel. error	5.30	1.32	2.40	−6.71
Intraday	Mean	1.75	9.13	24.87	48.37†
	SD	0.13	0.70	1.04	3.41
	RSD	7.41	7.67	4.18	7.05
	Rel. error	4.79	9.60	−0.52	−18.00

*Each value represents mean of $n = 5$.
†These values represent mean of $n = 4$.

Table 4
Interday and intraday variation of ticarcillin in human urine

		Concentration* (μg ml^{-1})			
		Extra-low (2.5)	Low (12.5)	Medium (37.5)	High (75.00)
Inter day	Mean	2.08	12.42	37.55	74.02
	SD	0.48	0.80	1.32	1.64
	RSD	15.55	6.41	3.51	2.21
	Rel. error	16.80	0.64	0.13	1.31
Intraday	Mean	2.65	12.60	37.78	73.40
	SD	0.23	0.82	1.73	2.14
	RSD	8.67	6.49	4.58	2.91
	Rel. error	6.00	0.80	0.75	2.13

*Each value represents mean of $n = 5$.

water were prepared as described above with the exception that cfp was not added until after centrifugation. The percent recovery was determined as follows

$$\% \text{ Recovery} = \frac{(\text{Peak height ratio of ticar/cfp in plasma})}{(\text{Peak height ratio of ticar/cfp in water})} \times 100 \quad (1)$$

The mean recovery was 83.6% in plasma.

Stability

Stability of ticarcillin at −20°C over a period of 4 weeks for plasma and 3 weeks for urine was evaluated. Data was obtained from frozen quality assurance controls which were prepared on day 1 and stored at −20°C until analysis. The results are presented in Tables 6 and 7. While the drug did not show relevant degradation in urine over the period covered, plasma samples showed as much as 17% degradation over 18 days. Degradation increased more rapidly from 18 to 36 d, showing 50% degradation in medium-QA control samples.

Clinical studies

This assay has been utilized for the determination of ticarcillin in clinical samples for a pharmacokinetic study [35]. Figure 4 represents a plot of concentration vs time in plasma, and Fig. 5 is a plot of excretion rate vs time in urine, both from a healthy volunteer administered ticarcillin.

Table 5
Recovery of ticarcillin from human plasma

Conc. ticar. μg ml^{-1}	Peak height ratio		Recovery (%)
	Plasma	Water	
1.67	0.11	0.15	73.3
8.33	0.96	1.12	85.7
25.00	2.92	3.25	89.8
59.00	6.63	7.73	85.8
Overall recovery of ticarcillin			83.6%

Table 6
Storage stability of ticarcillin (at −20°C) in human plasma

Day	Concentration* (μg ml^{-1})			
	Extra-low (1.67)	Low (8.33)	Medium (25.00)	High (59.00)
0	1.84	9.06	28.20	64.70
4	2.00	7.00	25.00	71.20
5	1.78	7.60	25.10	nd†
17	1.82	5.91	23.60	64.40
18	1.65	nd†	23.30	48.80
30	1.24	3.43	13.50	41.00

*Each value represents mean of $n = 5$.
†Not determined.

Discussion

Ticarcillin, being a bifunctional molecule with a very few absorbing chromophores, has inherent problems with both detection limits and peak-splitting. The first problem was solved using a low-wavelength setting of 205 nm in the detector. Fixed-wavelength detectors at 214 nm were not sensitive enough for our projected clinical lower limit of quantitation of 0.5 μg ml^{-1}. It has been found, for this assay, the detectors with deuterium lamps are better in both sensitivity and selectivity, than xenon and mercury lamp detectors.

Peak splitting is a common problem encountered in chromatography with compounds such as ticarcillin. The use of the ion-pairing reagent tetramethylammonium chloride (TMA) prevented the peak splitting of ticarcillin when TMA was at least 0.01% and the acetonitrile content was kept above 28%. The use of 0.03% TMA for this assay allowed for optimum separation of both ticarcillin and the internal standard cefoperazone (cfp) from endogenous plasma peaks.

Though not chemically related, the use of cfp as an internal standard can be justified because the possibility of interference from other drugs was less in this method. No extraction is required and precision as well as recovery are sufficient to allow for a high degree of confidence in the use of this assay for clinical samples. The assay is simple, fast,

Table 7
Storage stability of ticarcillin (at −20°C) in human urine

Day	Concentration* (μg ml^{-1})			
	Extra-low (2.50)	Low (12.50)	Medium (37.50)	High (75.00)
0	2.62	11.50	36.50	71.50
12	2.59	12.00	36.90	72.90
13	3.16	12.60	39.00	72.40
14	3.93	12.20	40.60	80.70
23	3.22	14.50	33.40	69.00

*Each value represents mean of $n = 5$.

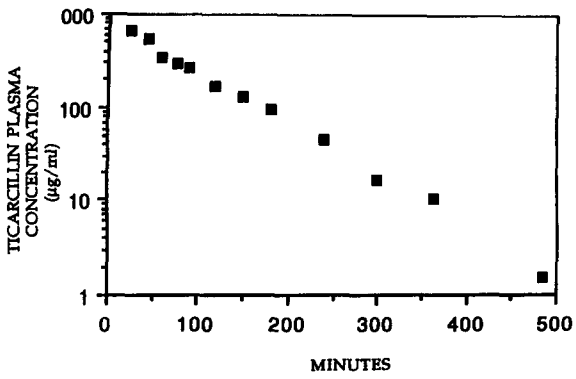

Figure 4
Concentration vs time of ticarcillin following a single intravenous dose of 50 mg kg^{-1} in a healthy volunteer.

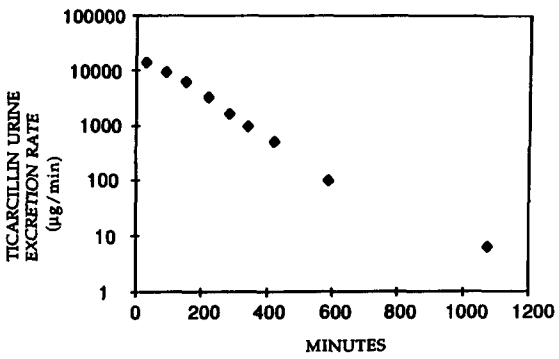

Figure 5
Urine excretion rate of ticarcillin following a single intravenous dose of 50 mg kg^{-1} in a healthy volunteer.

accurate and inexpensive when compared to other methods of quantitation. We feel this assay offers significant advantages over other methods currently available in the literature.

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