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Note

Improved procedure for the determination of the ureidopenicillins azlocillin and mezlocillin in plasma by high-performance liquid chromatography

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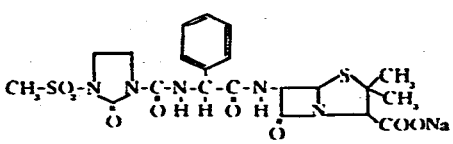
Chemical assay of antibiotics is superior to microbiological determination because of its specificity. In addition, each of the therapeutically used combinations of antimicrobial agents can be determined separately, which is often necessary in clinical situations.

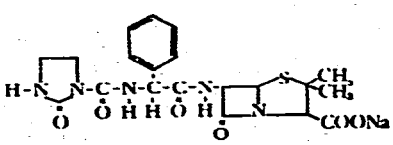
The determination of the ureidopenicillins azlocillin, mezlocillin (Fig. 1) and Bay k 4999 in plasma using high-performance liquid chromatography (HPLC) was described earlier [1]. Two extraction steps are included which are time-consuming and the recovery of the extraction does not exceed 50% which is a

MEZLOCILLIN



mol.wt. 561.6

AZLOCILLIN



mol.wt. 483.5

Fig. 1. Formulae of mezlocillin and azlocillin.

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potential source of variation. Furthermore, both substances were found to undergo chemical degradation at alkaline pH values, which conditions were present during the second extraction step using aqueous sodium bicarbonate solution [2].

In order to overcome these problems, it was necessary to find a method which is able to separate the drugs from plasma proteins and to clean the sample without extraction. In the procedure described below, the plasma samples were pre-cleaned using Sep-Pak C-18 cartridges.

EXPERIMENTAL

In order to prepare the cartridges (Sep-Pak C-18, 37—50 μm particle size, Part. No. 51915; Waters, Königstein, G.F.R.) they were prewetted with 2 ml of methanol followed by 10 ml of distilled water. Then 1 ml of plasma sample was put onto the cartridge. High polar substances were removed by washing the cartridge with 2 × 1 ml of phosphate buffer (0.07 *M*, pH 5) followed by washing with 1 × 1 ml of a mixture of acetonitrile—phosphate buffer (0.07 *M*, pH 5) (1:19, v/v) which eluent was totally removed from the cartridge. The drug was then eluted with 1 ml of a mixture of acetonitrile—phosphate buffer (0.07 *M*, pH 5) (70:30, v/v). The phosphate buffer was chosen since it was shown that the substance was stable under this condition [2].

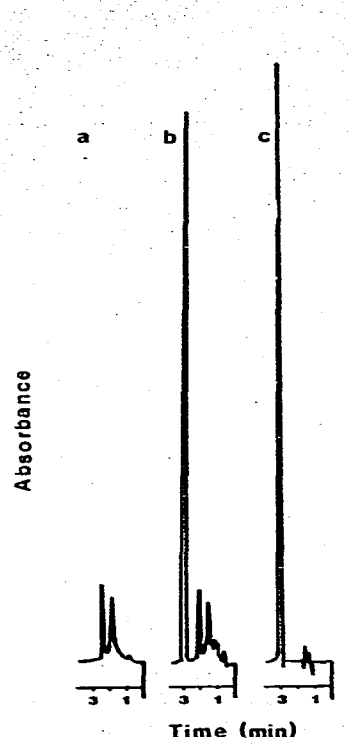
Chromatography was carried out as previously described [1]. In short: the assay was performed on an HPLC system consisting of a Model 6000A solvent delivery system (Waters) combined with a Model U6K injector (Waters) through a prepacked μBondapak C_{18} column (10 μm particle size, 30 cm × 3.9 mm I.D., Waters) to a variable-wavelength UV detector Model 450 (Waters). The chromatographic conditions were as follows: solvent flow-rate 2 ml/min; pressure 138 bar; room temperature; UV detector at 220 nm, sensitivity 0.04 absorbance units; recorder chart speed 5 mm/min; solvent, mixture of acetonitrile—phosphate buffer (0.05 *M*, pH 7) (27:73, v/v).

Plasma standards were prepared by adding freshly diluted aqueous standard solution to human plasma so that concentrations of 100, 50, 20, 10 and 5 μg/ml were achieved. A calibration curve was constructed by plotting peak height versus the concentration of the drugs in aqueous solution and calculating the slope and the intercept by least-squares linear regression analysis.

RESULTS

Figs. 2b and 3b show a chromatogram of plasma samples spiked with azlocillin and mezlocillin, respectively. The plasma blank sample (Figs. 2a and 3a) gave no HPLC peak which would interfere with the peak of the original substances (Figs. 2c and 3c). The mean recovery is 91.9 ± 2.2% and 101.9 ± 2.8% for azlocillin and mezlocillin, respectively, compared to the extraction recovery of 45.0—54.1% which was achieved by the two-step extraction procedure formerly used (Table I).

A further improvement of the method is indicated by the low relative standard deviation of the data used for the calculation of reproducibility (Table I). In addition, assay accuracy could be increased showing differences of 0.5—5.8%



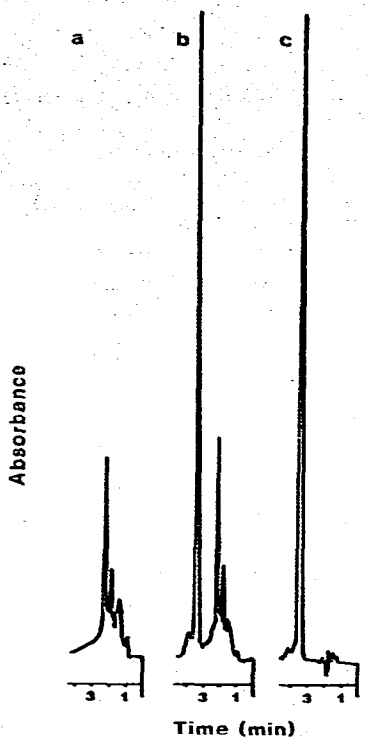


Fig. 2. HPLC traces of (a) plasma blank, (b) plasma spiked with azlocillin, and (c) aqueous solution of azlocillin.

Fig. 3. HPLC traces of (a) plasma blank, (b) plasma spiked with mezlocillin, and (c) aqueous solution of mezlocillin.

TABLE I

RECOVERY AND REPRODUCIBILITY OF THE PROCEDURE

Ureidopenicillin	Concentration (μg/ml)	Percentage recovery (mean ± S.D., $n = 6$)	Calculated* plasma concentration (μg/ml)	
			(mean ± S.D., $n = 6$)	Relative S.D.
Azlocillin	100	90.4 ± 1.8	90.4 ± 1.8	2.0
	50	95.1 ± 3.0	47.6 ± 1.5	3.1
	20	90.0 ± 4.0	18.0 ± 0.8	4.3
	10	93.4 ± 2.2	9.0 ± 0.2	1.8
	5	93.4 ± 6.2	4.7 ± 0.3	6.7
Mean ± S.D.		91.9 ± 2.2		
Mezlocillin	100	99.3 ± 2.9	99.3 ± 2.9	2.9
	50	105.8 ± 1.2	52.9 ± 0.8	1.5
	20	99.5 ± 5.2	19.9 ± 1.2	6.0
	10	101.5 ± 3.4	10.1 ± 0.4	4.0
	5	103.6 ± 5.0	5.2 ± 0.3	5.8
Mean ± S.D.		101.9 ± 2.8		

*By linear regression without correction for recovery.

TABLE II

ASSAY ACCURACY, USING A 4-μl INJECTION VOLUME

Ureidopenicillin	Plasma concentration (μg/ml)		Percentage difference
	Actual*	Calculated**	
Azlocillin	100	100.6	−0.6
	50	52.4	+4.8
	20	20	—
	10	9.6	−0.6
	5	5.3	+4.8
Mezlocillin	100	99.3	−0.7
	50	52.9	+5.8
	20	19.9	−0.5
	10	10.1	+1.0
	5	5.2	+4.0

*Plasma spiked with ureidopenicillins at the indicated concentrations.
**Calculated after correction for recovery.

from the actual value compared to up to 20% in the assay formerly used (Table II). The sensitivity was calculated by linear regression of concentration versus peak height and represents the concentration corresponding to the upper part of the 95% confidence interval of the y-intercept [3]. Using a 4-μl injection volume the value was 1.5 μg/ml (mezlocillin) and 1.3 μg/ml (azlocillin).

With respect to the rapid sample preparation and the short retention time of the substances using the HPLC method described above, serial determinations of plasma samples can be performed within one day. The method is therefore useful for determination of plasma concentrations in pharmacokinetic studies and for monitoring plasma levels in patients treated with the drug.

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