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HPLC determination of azlocillin sodium for stability studies

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Summary

A new method is described for the determination of the broad-spectrum antibiotic Azlocillin sodium by HPLC. This method is useful for stability studies, since it allows the separation of Azlocillin from its degradation products. This has been checked by TLC.

Introduction

Azlocillin sodium (Fig. 1) belongs to a semisynthetic penicillin group with a broad spectrum of antibacterial activity. It is used mainly in hospitals for parenteral administration by slow intravenous injection or by intravenous infusion agains *Pseudomonas aeruginosa* infections (Fu and Neu, 1978; Ellis et al., 1979; Bergan et al., 1982; Sanders, 1983).

Several HPLC methods for the determination of azlocillin sodium have been described in the literature (Gundert-Remy and De vries, 1979; Hildebrant and Gundert-Remy, 1982; Fasching and Peterson, 1983) but in spite of the advantages of this technique, none of the methods described permits the isolation of azlocillin sodium from its degradation products.

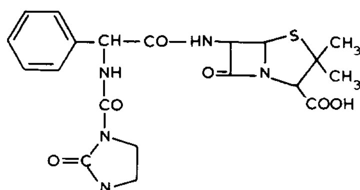

Fig. 1. Structure of azlocillin sodium.

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Materials and Methods

Chemicals

Azlocillin sodium was a gift from Bayer S.A. (Barcelona, Spain), and HPLC grade acetonitrile was supplied by SDS S.A. (Barcelona). All other chemicals were of analytical grade and were used as received from the various sources. Double-distilled water was used after filtration in a Millipore system (France) and a second vacuum filtration in a helium atmosphere.

HPLC method

This procedure was carried out in a liquid chromatograph with a constant-flow-rate pump and diode array detector (model HP-1090, Hewlett

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Packard, U.S.A.), integrator (model HP-3396D, Hewlett Packard) and Hewlett Packard thin jet printer. Samples were chromatographed using an analytical ODS-Hypersil column (Hewlett Packard) of 10 cm length, 4.6 mm internal diameter and 5 μm particle size. The mobile phase had a flow rate of 2.0 ml/min under isocratic conditions of 86% 0.008 M borax buffer at ph 7.0 (Tablas Cientifficas Documenta Geigy, 1965) and 14% acetonitrile. The UV detector was set at 210 nm. Each sample was injected in triplicate and the results were averaged to obtain the value of the concentration. Statistical calculations were performed using a Tektronix 4051 (Tectronix, France).

The samples were an azlocillin sodium solution of 200 μg/ml in water and a series of 20 dilutions of this were prepared. All samples were chromatographed in order to assess the reproducibility and linearity of the method. Linearity of the LC assay was determined by linear regression; peak areas for the antibiotic were plotted on a linear scale vs. drug concentration. Reproducibility of assays was ascertained by analysis of variance.

TLC

TLC was carried out on TLC aluminium plate silica gel 60 F_{254} 20 × 20 cm and layer thickness 0.2 mm (Merck, F.R.G.). The plates were spotted with a Hamilton syringe and the spots left to dry. The plates were then placed in a developing chamber containing the eluent system, a mixture of acetone-acetic acid (45 : 3, v/v), until the solvent front reached the top of the plate. Once dried, the plates were placed under a Uvaton ultraviolet lamp (Atom) at 254 nm in order to visualize the spots.

The first sample was a 1.5% aqueous solution of azlocillin sodium. This sample was put into a

TABLE 1

Peak areas obtained for different concentrations of azlocillin in water, by the HPLC method

Concentration (g/ml)	Samples (areas)			$\bar{x}$	Confidence interval 95% (±)
	1	2	3		
0	0	0	0	0	–
10	122582	126041	142129	130250	4212
20	285423	272782	291808	283337	8424
30	433420	444748	458368	445512	12636
40	576571	588808	606434	590604	16848
50	771771	778398	708894	753021	21060
60	907436	936194	929521	924383	25272
70	1088695	1098248	1083031	1089991	29484
80	1248968	1262108	1248256	1253110	33696
90	1427398	1425508	1413082	1421996	37908
100	1581809	1568239	1564454	1571500	42120
110	1793235	1764973	1806699	1788302	46332
120	1916520	1920313	1930549	1922460	50544
130	2019718	2014453	2093020	2042397	54756
140	2221378	2232800	2247336	2233838	58968
150	2427714	2439946	2455106	2440922	63180
160	2627362	2589026	2604518	2606968	67392
170	2787800	2796506	2792150	2792152	71604
180	2944378	2933472	2969653	2949167	75816
190	3134510	3140973	3125294	3133592	80028
200	3304973	3270890	3309437	3295100	84240
Coefficient of correlation			1.00*E*0		
Standard deviation			28896		
Prob > *F*			1.83*E*-9		

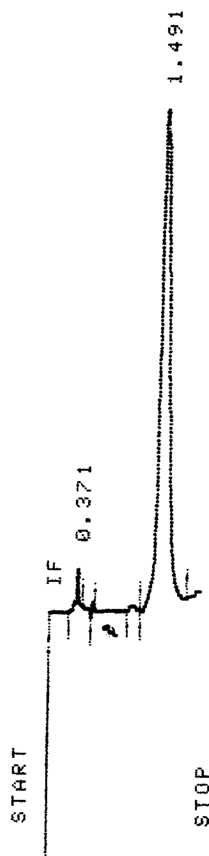

Fig. 2. Sample chromatogram of azlocillin from HPLC, column ODS-Hypersil (10 cm×4.6 mm), 5 μm particle size. Mobile phase 0.008 M borax buffer at pH 7.0 and acetonitrile (86:14).

thermostat at 50 °c to obtain the azlocillin degradation products. A series of samples were removed at random times. All of these were chromatographed by TLC to obtain an R_f table for all of the spots, being simultaneously diluted (1.3:100) and subjected to HPLC in order to determine the T_r values of the different products. After the extraction of each spot with ethanol (Bolós et al., 1977), UV spectra were recorded on a Perkin Elmer 356 dual-wavelength spectrophotometer (Perkin Elmer, U.S.A.) which permits the reading of turbid samples. These samples were filtered using a Millex-HV filter (Millipore, France) and chromatographed by HPLC to obtain a table of T_r values for all spots and their UV spectra (diode array detector HP-1090). Each assay was carried out in duplicate.

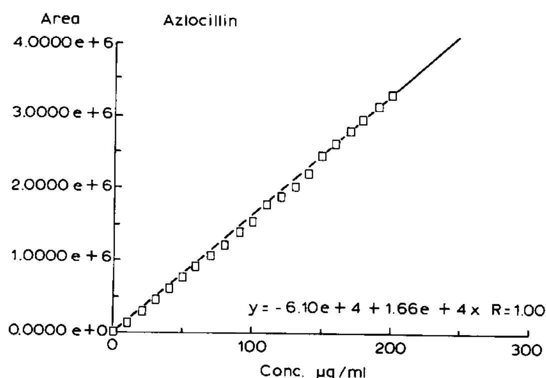

Fig. 3. Detector response to azlocillin concentration in sample.

Results and Discussion

The retention time for azlocillin was found to be 1.49 ± 5% min, its chromatogram being shown in Fig. 2. Azlocillin showed a linear detector response throughout the range tested (r = 1.00E0) as demonstrated in Fig. 3 and presented a good standard deviation (σ). All values are listed in Table 1.

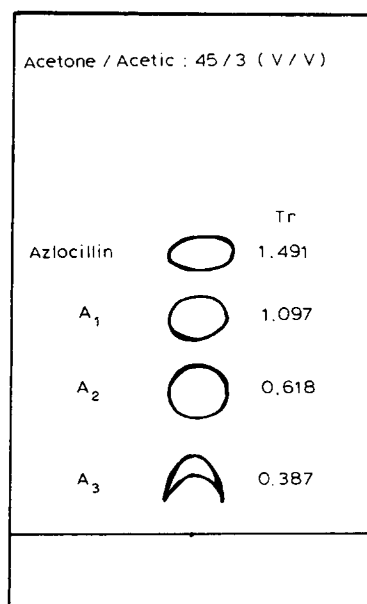

Fig. 4. Sample chromatogram of azlocillin from TLC after 5 days at 50 ° C.

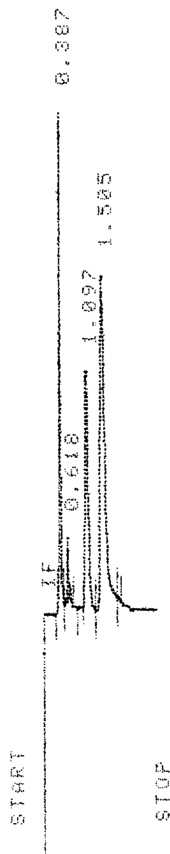

Fig. 5. Sample chromatogram of azlocillin from HPLC after 3 days at 50 ° C.

Fig. 4 shows a TL chromatogram of an azlocillin sample after 5 days at 50 ° C. More than 10% of the antibiotic had been degraded and three degradation products appeared (A_1–A_3), all of which have a lower R_f value than that of azlocillin. Fig. 5 shows an HPLC chromatogram of the same sample, three peaks being observed with a lower T_r value than that of azlocillin ($T_{rA_1} = 1.097$, $T_{rA_2} = 0.618$ and $T_{rA_3} = 0.387$).

When the azlocillin degradation products were extracted from the plates and chromatographed individually by the HPLC method, the same retention times and identical ultraviolet spectra were obtained for A_1–A_3.

Based upon the above results, it can be stated that the method described herein is suitable for azlocillin stability studies and represents the first step in identification of the degradation products of azlocillin.

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