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Note

Analysis of diastereomeric 7-ureidoacetamido cephalosporins by high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) is becoming an indispensable tool for qualitative and quantitative analysis of a variety of compounds including β-lactam antibiotics. Both reversed-phase and ion-exchange chromatography have been useful in the separation of cephalosporins from each other[1], from products of degradation[1,2] and metabolism[3–5], and from components in fermentation broths[6,7]. This paper describes the use of reversed-phase chromatography to separate and quantitate diastereomeric mixtures of closely related 7-ureidoacetamido cephalosporins.

EXPERIMENTAL

A high-performance liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.; Model ALC-GPC-202) was equipped with a Rheodyne Model 905-42 septumless injector and a UV detector operating at 254 nm. The column used was a μBondapak C_{18} (300 × 4.0 mm I.D.) custom-packed by Waters Assoc. and the chromatograms were recorded on a Varian A-25 strip chart recorder. The pressure employed was $\leqslant$ 2500 p.s.i. The eluting mixtures were prepared from reagent methanol (Mallinckrodt, St. Louis, Mo., U.S.A.) and diammonium hydrogen phosphate dissolved in water (de-ionized and distilled in glass) and filtered through a Millipore HAWPO4700 filter.

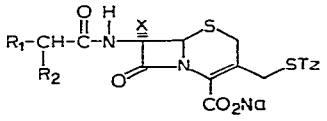
RESULTS AND DISCUSSION

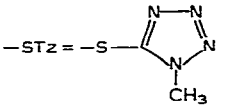
In the synthesis of 7-ureidoacetamido cephems, compounds 1–7* (Table I), varying degrees of epimerization occurred during the coupling of either optically active or racemic side-chain acids with the appropriate 7-aminocephem. Determination of the diastereomeric purity of compounds 1–7 was necessary in order to evaluate the antimicrobial activity of the isomers. Using electrophoresis and thin-layer chromatography (TLC) we were unable to separate the diastereomers routinely; whereas with reversed-phase HPLC, separations were obtained in a convenient time (Table I) using 0.01 *M* aqueous diammonium hydrogen phosphate containing 5–20% methanol.

* The cephalosporins analyzed were synthesized by colleagues at The Squibb Institute for Medical Research and Chemische Fabrik von Heyden.

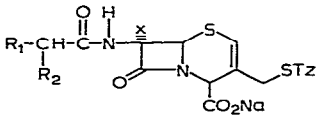
TABLE I

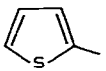
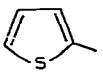
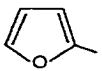
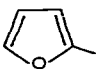
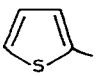
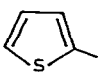
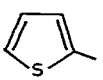
SEPARATION OF DIASTEREOMERIC CEPHALOSPORINS





1-7



Compound	R_1	R_2	X	CH_3OH (%)	*Flow-rate (ml/min)*	L*, *time (min)*	D, *time (min)*
1		NH C=O NHCH$_2$CN	H	20	1.0	11	20
2		NH C=O NHCH$_2$CN	OCH_3	20	0.4	12	14
3		NH C=O NH$_2$	H	10	1.4	7	11
4		NH C=O NH$_2$	OCH_3	5	0.4	18	20
5		NH C=O NH$_2$	H	10	1.5	11	20
6		NH C=O NH$_2$	OCH_3	10	0.8	16	19
7		NH C=O NH$_2$	H	10	1.0	23	44
8		NH C=O NH$_2$	OCH_3	10	0.8	21	31

* The L and D nomenclature designates the stereochemistry of the intermediate arylglycine side chains utilized in the preparation of 1–8.

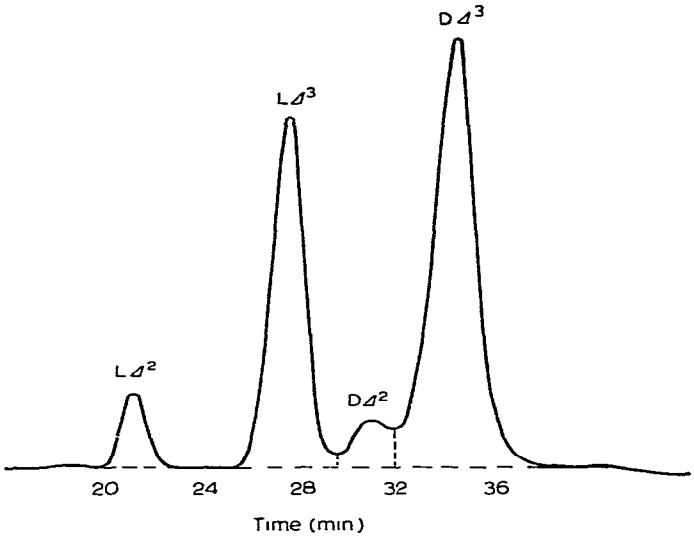


Fig. 1. HPLC separation of diastereomeric cephamycins 6 and 8. Pressure, 1400 p.s.i. Other conditions, see text.

Throughout the entire series the L-isomers were consistently more hydrophilic than the D-isomers, though this difference was not as pronounced with the cephamycin derivatives 2, 4, and 6.

At an intermediate stage in the synthesis of compound 6, partial isomerization of the double bond from the Δ^3 to the Δ^2 position occurred under certain conditions. The final product, therefore, contained four components, the two Δ^3 diastereomers 6 plus the Δ^2 isomers 8. These four isomers were eluted with 10% methanol in aq. 0.01 *M* $(NH_4)_2$ HPO_4 within 40 min (0.8 ml/min) in the following order: L-Δ^2, L-Δ^3, D-Δ^2, and D-Δ^3 (Fig. 1). The isolated DL-Δ^2 and DL-Δ^3 isomers were available as standards. The values obtained by extrapolation for the percentage of the Δ^2 isomers in the known mixtures are in good agreement with the actual values (Table II).

TABLE II

STANDARD MIXTURE OF DIASTEREOMERIC COMPOUNDS 6 AND 8

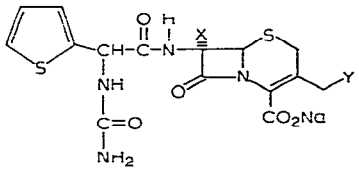
Actual percentage of 8	*Extrapolated value (%)* *
4.2	4.8
4.3	4.8
4.9	5.0
8.7	9.1
9.8	10.1

* No correction was made for the difference (<10%) in the extinction coefficients at 254 nm between compounds 6 and 8.

Depending upon the method of synthesis of cephamycin 6 from 7-amino-cephalosporanic acid, possible impurities present in compound 6 include compounds 5 and 9. Table III shows the retention times of these compounds under uniform conditions.

TABLE III

SEPARATION OF POSSIBLE IMPURITIES IN COMPOUND 6



Compound	*X*	*Y*	*CH_3OH (%)*	*Flow-rate (ml/min)*	L, *time (min)*	D, *time (min)*
5	H	STz	10	1.0	13.5	26.5
6	OCH_3	STz	10	1.0	12.0	14.5
9	OCH_3	$OCCH_3$ (O)	10	1.0	7.5	8.5

Co-injection of compound 6 with compounds 5 and 9 has confirmed the separation of these cephems in the 10% methanol-buffer system.

The diastereomers of 7-ureidoacetamido cephalosporins can efficiently and conveniently be separated and quantitated by reversed-phase HPLC. Quantitation of other cephem contaminants is possible and the method is applicable to both methoxylated and non-methoxylated cephalosporins.

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