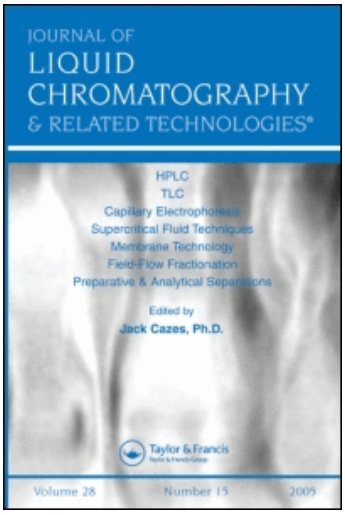

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DETERMINATION OF PENICILLINS AND CEPHALOSPORINS N-PYRROLYLDERIVATIVES BY HPLC

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ABSTRACT

An isocratic reversed-phase liquid chromatographic method for the determination of different types of penicillins and cephalosporins N-pyrrolylderivatives using UV detection at 254 nm is described. The mobile phases were aqueous methanolic (40-60%, v/v), 0.05M potassium phosphate buffer, pH 6.0.

The method is sufficiently sensitive and precise and is thus highly suited for use in the kinetic studies.

INTRODUCTION

The study of the pharmacological properties of some N-pyrrolylderivatives acids, synthesized at our Department (1)(2) have led us to the synthesis and study of the stability in aqueous solutions at 37°C and at ionic strength of 0.5M over the (2.3-11.5) pH range, of a series of penicillins and cephalosporins N-pyrrolylderivatives (3)(4)(5). 4-Thia-1-azabicyclo (3.2.0)heptane-3,3-dimethyl-6-amino-7-oxo-N|2(1H-pyrrolyl)acetyl|2-carboxylic acid (6R,trans) (I), 4-thia-1-azabicyclo (3.2.0) heptane-3,3-dimethyl-6-amino-7-oxo-N-|2-phenyl-2(1H-pyrrolyl)acetyl|2-carboxylic acid (6R,trans) (II),

5-thia-1-azabicyclo (4.2.0) oct-2-ene-3|(acetyloxy)methyl|7-amino-8-oxo-N- |2(1H-pyrrolyl)acetyl|2-carboxylic acid (6R,trans) (III), 5-thia-1-azabicyclo (4.2.0) oct-2-ene-3|(acetyloxy)methyl|7-amino-8-oxo- N-|2-phenyl-2(1H-pyrrolyl)acetyl|2-carboxylic acid (6R,trans) (IV), 5-thia-1-azabicyclo (4.2.0) oct-2-ene-7-amino-3-methyl-8-oxo-N-|2(1H-pyrrolyl)acetyl|2-carboxylic acid (6R,trans) (V) and 5-thia-1-azabicyclo (4.2.0) oct-2-ene-7-amino-3-methyl-8-oxo-N|2-phenyl-2(1H-pyrrolyl)acetyl|2-carboxylic acid (6R,trans) (VI) have been synthesized as potassium salts. All these compouns are β-lactam antibiotics, Figure 1.

MATERIAL AND METHODS

Instruments

The analyses were performed on a LDC Liquid Chromatograph equipped with a UV detector at 254 nm; a Constametric II pump and a Rheodyne 7120 injector fitted with a 20μl loop. Radial compression chromatography (RCM-100 , Waters) was used.

Reagents

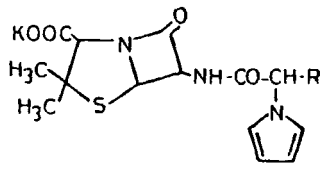
The compounds (I) to (VI) were synthesized by acylation of 6-aminopenicillanic (6-APA), 7-aminocephalosporanic (7-ACA) and 7-aminodeacetoxycephalosporanic (7-ADCA) acids with 2-(1H)-pyrrole acetic acid or 2-(1H)-pyrrole phenylacetic acid using the general mixed anhydride method. All the antibiotics synthesized have been characterized by UV, IR, ^{1}H-NMR and elemental analysis.

Methanol, HPLC grade (Farmitalia, Carlo Erba); water, HPLC grade was obtained from double distillation and filtration through a 0.45μm HA Millipore filter.

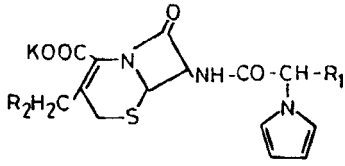
The other chemicals were analytical grade, (MERCK-Schuchardt) and were without further purification.

Chromatographic Procedure

The chromatographic separation was achieved in a (100mm x 8mm) ODS column inside a radial compression RCM-100 Waters. The mobile phases were mixtures of a potassium phosphate buffer (0.05M, pH 6.0) with methanol. The percentage of methanol was 40%, v/v for the penicillins and 60%, v/v for the cephalosporins. In all cases the flow rate was set at 1.0 ml/min. The mixture was degassed by ultrasonication prior to use. The chromatograms were recorded at a chart speed of 0.5 cm/min.



I, R = H
II, R = C_6H_5



III, R_1 = H; R_2 = O - CO - CH_3
IV, R_1 = C_6H_5; R_2 = O - CO - CH_3
V, R_1 = H; R_2 = H
VI, R_1 = C_6H_5; R_2 = H

FIGURE 1.- Chemical structures of N-pyrrolylderivatives antibiotics analyzed.

All the chromatographic analyses were carried out at room temperature.

Sample Preparations

For the kinetic studies, stock solutions of β-lactam antibiotics were prepared by dissolving the compounds in the appropriate buffer systems.

The antibiotic concentrations ranged from 500-50μg/ml for the penicillins and 100-10μg/ml for the cephalosporins.

Calibration Plots

Eight concentrations of standard solutions ranging from 1000-10μg/ml for the penicillins and 200-5μg/ml for the cephalosporins were prepared in water. Triplicate injections of each concentration were made.

Reproducibility

Reproducibility of the method was stablished by analyzing 20μl replicate portions of a solution that contained antibiotics in concentrations appropriates to the kinetic studies.

Study of the Interferences of the Degradation Products

Solutions of the antibiotics were prepared in 0.01N NaOH, pH 11.5 and were kept at 37°C for 1h until total degradation.

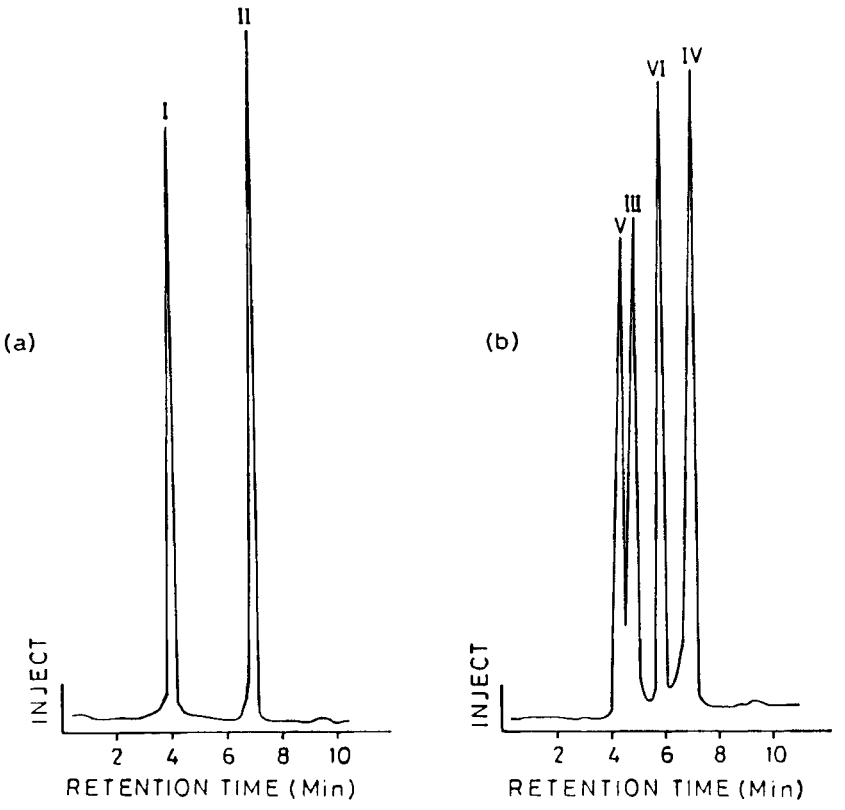


FIGURE 2.- Chromatographic separation of compounds. (a) = I and II. (b) = III to VI.

Recovery was determined by addition of appropriate aliquots of a just made solution of compounds (I-VI) to the above solutions previously neutralized with 2N HCl, to produce concentrations ranging from 750-20 μg/ml for the penicillins (I and II) and 150-5 μg/ml for the cephalosporins (III-VI).

The peak heights obtained were compared with those from injections of standard solutions and the percentage recovery determined.

RESULTS AND DISCUSSION

Calibration plots were obtained by plotting the mean peak height versus the respective antibiotic concentrations and were li-

near over the range studied. In all cases the correlations coefficients were found to be greater than 0.999.

The relative standard deviations for five injections of a sample were less than 2.0%.

The chromatograms shown in Figure 2 indicate the possibility of the separation of the penicillins (I) and (II) and the cephalosporins (III-VI). Retention times under the experimental conditions of the N-pyrrolylderivatives antibiotics (I-VI) were 4 min, 7 min, 5 min, 7 min, 4.5 min and 6 min respectively.

The recovery from the degradation products gave reproducible results with a mean accuracy of 100.9% for (I); 101.1% for (II); 101.2% for (III); 101.0% for (IV); 100.8% for (V) and 100.5% for (VI), with a relative standard deviations in the range of 1-2%.

No interference due to the degradation products was detected. Table 1.

The results obtained from the chromatographic study allows us to select the most appropriate chromatographic conditions to analyze the antibiotics N-pyrrolylderivatives by HPLC.

TABLE 1

Recovery of Compounds I and IV from the Degradation Products

(I)			(IV)		
Conc (μg/ml)		Recovery	Conc (μg/ml)		Recovery
Added	Determined	%	Added	Determined	%
750	741.75	98.9	150	141.5	98.7
600	598.80	99.8	120	119.88	99.9
500	505.00	101.0	100	101.10	101.1
200	203.00	101.5	40	40.52	101.3
80	81.36	101.7	20	20.46	102.3
20	20.50	102.5	5	5.12	102.5
Mean		100.9	Mean		101.0
$\pm$ SD		1.3	$\pm$ SD		1.4
RSD		1.3	RSD		1.4

This method has been applied to the kinetic studies (6)(7) and allows a direct monitoring of the amounts of the unreacted antibiotic present at any given time and avoids interferences from the degradation products.

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REFERENCES

1. Salto, F; Costa, L.; Fernández, J. L. and Fernández, J. M., U.S. Patent, 4,563,477; Chem. Abstr., 100, 85589, 1983.

2. Salto, F.; Negro, A. and Martin, J. "N-pyrrolylderivatives Acids Chromatography", J. Liquid. Chromatogr., 9, 2442, 1986.

3. Alemany, M. T. and Salto, F. "DL-α-(N-pirrolil)fenilacetamidopenicilina)I. Síntesis y Propiedades cinetoquímicas", An. Fac. Vet. León., 25, 19, 1979. Chem. Abstr., 95, 42977r, 1981.

4. Alemany, M. T.; Salto, F.; Costa, L.; fernández, J. L. and Fernández, J. M., Span ES Patent 492,589; Chem Abstr., 96, 217578m, 1982.

5. Arín, M. J.; Díez, M. T. and Salto, F. "Síntesis y propiedades cinetoquímicas de la N-pirrolilacetamidopenicilina, de la N-pirrolilacetamidocefalosporina y de la N-pirrolilacetamidodesacetoxicefalosporina", An. Fac. Vet. León., 29, 113, 1983.

6. Arín, M. J., Díez, M. T. and Salto, F. "The Comparative Stability of Different Types of Penicillins and Cephalosporins N-pyrryl derivatives", Pharmazie,,43, H.1, 1988.

7. Salto, F.; Díez, M. T.; Arín, M. J. and Negro, A. "Cinéticas de descomposición de α-(N-pirrolil)fenilacetamidocefalosporinas en disolución acuosa, An. Quím., 83, C, 5, 1987.