Journal of Chromatography, 361 (1986) 285–290
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 631

STABILITY-INDICATING LIQUID CHROMATOGRAPHIC DETERMINATION OF CEPHAPIRIN, DESACETYL CEPHAPIRIN AND CEPHAPIRIN LACTONE IN SODIUM CEPHAPIRIN BULK AND INJECTABLE FORMULATIONS

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(First received January 15th, 1986; revised manuscript received March 10th, 1986)

SUMMARY

A specific, stability-indicating, high-performance liquid chromatographic assay was developed for the determination of cephapirin, desacetyl cephapirin and cephapirin lactone in sodium cephapirin (cefadyl) bulk and injectables. The procedure uses a μBondapak C_{18} column and a mobile phase of dimethylformamide–acetic acid–potassium hydroxide in water. UV detection at 254 nm is used for quantitation with acetanilide used as the internal standard.

The assay is precise, accurate and linear over the range of 100–300 μg/ml for cephapirin and over the range of 2–6 μg/ml for desacetyl cephapirin and cephapirin lactone. The assay is also stability-indicating for the described thermal, acid, base, aqueous and accelerated light degradations.

INTRODUCTION

Sodium cephapirin, 3-[(acetyloxy)methyl]-8-oxo-7-{[2-(4-pyridylthio)acetyl]-amino}-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid monosodium salt, a cephalosporin antibiotic, has been shown to be effective against strains of gram-positive and gram-negative bacteria, including *Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus*[1,2].

The most widely accepted method for the analysis of cephapirin in sodium cephapirin bulk and sodium cephapirin injectable formulations is the microbiological assay; it is the assay method specified by the USP/CFR[3], and it has been covered extensively in the literature[4,5]. Other analytical techniques have also been documented, including potentiometric titration[6], thermogravimetric analysis[7], and spectrophotometry[8,9].

More recently, high-performance liquid chromatographic (HPLC) methods have been reported[10–12] which have the advantage of both selectivity and enhanced sensitivity; others report the separation of cephapirin in mixtures with other cephalosporins[13–16]; however, none of the works noted offer a stability-indicating HPLC assay method, capable of assaying cephapirin, desacetyl cephapirin {3 - (hydroxy-

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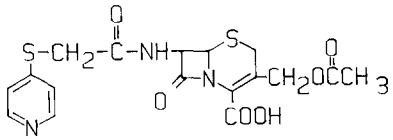
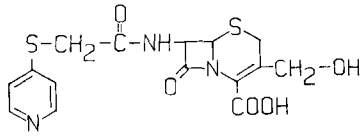
methyl) - 8-oxo-7-{ [2 - (4 - pyridylthio)acetyl]amino} - 5 - thia - 1 - azabicyclo - [4.2.0]oct - 2 - ene - carboxylic acid} and Cephapirin Lactone {1,1-{[(4-pyridylthio)acetyl]amino}-2-aza-8-furo-9,10-oxo-4-thia-tricyclo[7.2.2.0]-undeca-1-ene} in bulk materials and in comerical injectable formulations. The HPLC assay described here is stability-indicating and is capable of monitoring cephapirin, desacetyl cephapirin and cephapirin lactone in the presence of known potential impurities, precursors, and formulation excipients with minimal sample preparation.



CEPHAPIRIN



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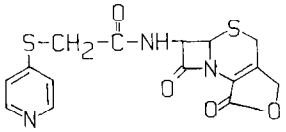

CEPHAPIRIN LACTONE

EXPERIMENTAL

HPLC equipment and operation conditions

The analysis was carried out on a chromatographic system composed of a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.) operated at 2.0 ml/min; Model 440 fixed-wavelength UV detector (Waters Assoc.) equipped to monitor 254 nm at 0.2 a.u.f.s. for cephapirin and 0.02 a.u.f.s. for desacetyl cephapirin and cephapirin lactone; Model 710B automatic injector (Waters Assoc.) programmed to inject 20 μl; and a μBondapak C_{18} analytical column, 30 cm × 3.9 mm I.D. (Waters Assoc.). Chromatograms were recorded using a Model 730 data module (Waters Assoc.). Integration was performed using a Model 3357 laboratory automation computer (Hewlett-Packard, Palo Alto, CA, U.S.A.). HPLC analyses of forced-degradation samples were performed using a Model 1040A diode-array multiwavelength detection system (Hewlett-Packard).

The mobile phase and diluent were composed of 5% dimethylformamide–0.2% acetic acid–0.1% potassium hydroxide solution (45%, w/w) in HPLC-grade water. The mobile phase–diluent was filtered through 0.45-μm filter paper and degassed prior to use.

All chromatographic work was carried out at room temperature. Using the described conditions, the retention times for cephapirin, desacetyl cephapirin and cephapirin lactone were approximately 13.3, 4.0 and 10.4 min, respectively. See Fig. 1.

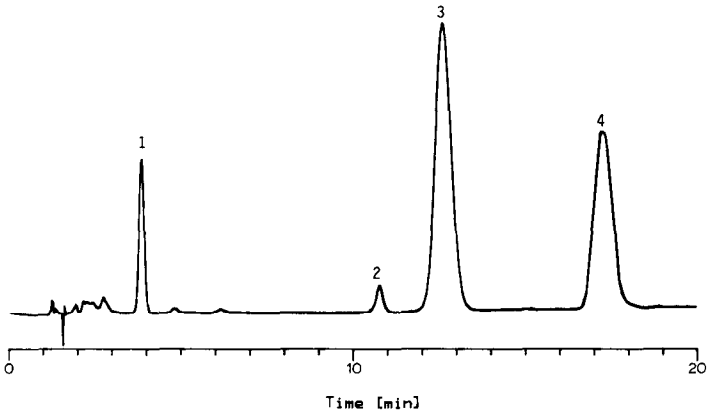

Fig. 1. Typical HPLC chromatogram of a standard solution containing cephapirin, desacetyl cephapirin, cephapirin lactone and acetanilide (internal standard). Peaks: 1 = desacetyl cephapirin; 2 = cephapirin lactone; 3 = cephapirin; 4 = acetanilide. μBondapak C_{18} column; mobile phase, 5% dimethylformamide–0.2% acetic acid–0.1% potassium hydroxide solution (45%, w/w) in water, 2 ml/min.

Reagents

Dimethylformamide, UV grade, was obtained distilled in glass (Burdick and Jackson, Muskegon, MI, U.S.A.). Glacial acetic acid (ACS grade) and potassium hydroxide certified reagent) were products from Fisher Scientific. (Fairlawn, NJ, U.S.A.). Acetanilide was reagent grade (Eastman Organic Chemicals, Rochester, NY, U.S.A.). HPLC-grade water was produced by a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). Sodium cephapirin, desacetyl cephapirin and cephapirin lactone were obtained from the Syracuse Reference Standards Program (Industrial Division, Bristol-Myers), and were used as received. Sodium cephapirin bulk material and formulations were of pharmaceutical quality.

Standard preparation

A solution of acetanilide was prepared in diluent at 2.5 mg/ml (a 5-ml aliquot of dimethylformamide was added prior to the addition of diluent to dissolve the acetanilide). An appropriate aliquot was pipetted into standards and samples prior to diluting with diluent, to produce a final concentration of 125 μg/ml.

The standard solution was prepared to contain the following: 200 μg/ml of cephapirin, 4 μ/ml of desacetyl cephapirin and 4 μg/ml of cephapirin lactone, in diluent.

Sample preparation

Sodium cephapirin bulk. Accurately weighed amounts of sodium cephapirin bulk were prepared in diluent at the 200 μg/ml level.

Sodium cephapirin for injection (500 mg and 1 g vials). The contents of one vial were rinsed into a 100-ml volumetric flask with diluent and diluted to volume. A further dilution was made to obtain a final theoretical concentration of 200 μg/ml for sodium cephapirin.

Sodium cephapirin for injection (2 g, 4 and 20 g vials). The contents of one vial were rinsed into a 200-ml volumetric flask with diluent and diluted to volume. A

TABLE I

LINEARITY OF CEPHAPIRIN AND IMPURITIES

Conditions as in Fig. 1. n = Number of discrete linearity data points.

Compound	*r*	*n*	*Slope****	*Intercept*§
Cepharin*	0.999998	8	4.5677	−0.0031
Desacetyl cephapirin**	0.9998	5	4.5137	0.0002
Cephapirin lactone**	0.998	5	5.1034	0.0015

* Over the 100–300 μg/ml concentration range.
** Over the 2–6 μg/ml concentration range.
*** Peak area units × ml/μg.
§ Peak area units.

further dilution was made to obtain a final theoretical concentration of 200 μg/ml for sodium cephapirin.

RESULTS AND DISCUSSION

Linearity

Linearity of detector response *versus* cephapirin concentration, desacetyl cephapirin concentration and cephapirin lactone concentration was determined over the range of 100–300 μg/ml for cephapirin and over the range of 2–6 μg/ml for desacetyl cephapirin and cephapirin lactone.

A correlation coefficient (r) of more than 0.99999 was obtained for the linear regression plot of the cephapirin peak areas over the range tested. Desacetyl cephapirin and cephapirin lactone produced linear regression plots with correlation coefficients of more than 0.995.

Table I lists the slopes and intercepts of the linear regression equations for the three compounds as well as the correlation coefficients for the three compounds over the ranges tested.

Using single-point standardization at the 100% cephapirin level, the biases calculated for sodium cephapirin at the 50, 75 and 150% levels were acceptable. See Table II.

TABLE II

CEPHAPIRIN ASSAY BIASES

Conditions as in Fig. 1.

Actual conc. (mg/ml)	*Calculated conc.* (mg/ml)*	*%Deviation***
0.3008	0.3008	0.1
0.1506	0.1503	−0.2
0.0989	0.0985	−0.4

* 0.2 mg/ml = 100% level for cephapirin standardization.
** For two replicates at each level.

TABLE III

CHROMATOGRAPHIC RECOVERY AND PRECISION

Conditions as in Fig. 1.

Compound	*Level** *(μg/ml)*	*Recovery (%)*	*Precision** (%)*
Cephapirin bulk	–	–	0.9
Desacetyl cephapirin	2.1	114.2	
	4.4	98.8	1.9
	6.4	111.0	
Cephapirin lactone	2.2	115.9	
	4.4	97.7	3.1
	5.7	100.9	

* For two replicates at each level.
** R.S.D. (%) for six replicate injections.

TABLE IV

ABSORBANCE RATIOS

*Degradation procedure**	*N***	*Mean ratio*	*Ratio maximum*** / Ratio minimum*
254 nm/265 nm			
Undegraded	20	0.892	1.004
Acid degraded	31	0.891	1.004
Base degraded	26	0.892	1.004
Aqueous degraded	27	0.892	1.005
Heat degraded	21	0.891	1.006
Light degraded	37	0.892	1.003
280 nm/265 nm			
Undegraded	21	1.007	1.002
Acid degraded	33	1.000	1.005
Base degraded	31	1.000	1.002
Aqueous degraded	29	1.001	1.004
Heat degraded	22	1.009	1.002
Light degraded	37	1.007	1.003
254 nm/280 nm			
Undegraded	20	0.886	1.003
Acid degraded	31	0.891	1.003
Base degraded	29	0.891	1.007
Aqueous degraded	27	0.892	1.001
Heat degraded	18	0.883	1.005
Light degraded	36	0.886	1.006

* Acid: 1 *M* acetic acid, 80°C, 45 min. Base: 0.1 *M* sodium bicarbonate, 80°C, 90 min. Aqueous: 80°C, 30 min. Heat: 80°C, 11 days. Light: 254 nm, 24 days.
** *N* = Number of data points used to generate the mean ratio.
*** *D* values.

Accuracy was also determined for the impurities by spiking desacetyl cephapirin and cephapirin lactone into 0.2 mg/ml solutions of sodium cephapirin. The accuracy data are reported in Table III. Also Table III reports the chromatographic precision for cephapirin, desacetyl cephapirin and cephapirin lactone obtained from six repetitive samplings of standard solution.

Specificity of the chromatographic method was tested by comparing the retention time of cephapirin to those of synthetic precursors and known impurities. Other investigations included the determination of the consistency of absorbance ratios and spectral slices in the 200–400 nm range for the cephapirin peak in force-degraded samples. Absorbance ratios were calculated at a rate of up to eight calculations per second using 254, 265 and 280 nm absorbance signals. The results of the absorbance ratio study are summarized in Table IV. Homogeneity of the cephapirin peak is indicated based upon the similarity of mean absorbance ratios for a given wavelength, as well as the relatively small range of ratio variation, as reflected in the *D* values, for the non-degraded and degraded samples. No apparent interferences were observed with the cephapirin peak when any of the known impurities or force-degraded samples were chromatographed. To show peak homogeneity, UV spectra were taken over the range of 240–440 nm at discrete time intervals throughout the elution of the cephapirin peak. These spectra were normalized and overlayed for each sample. Apex spectra of the degraded samples and undegraded samples were normalized and overlayed in the same manner. In all cases these spectra were superimposable, showing peak integrity as well as the stability-indicating nature of the assay.

In order to determine the practical limit of detectability for desacetyl cephapirin and cephapirin lactone, solutions of these compounds were prepared at concentration levels lower than those prepared in the linearity experiment. The impurities could be detected to a lower limit of 0.1%. When operating the HPLC system by the described procedure, reliable quantitative results may be obtained down to a lower limit of 0.5% by weight relative to the cephapirin target concentration.

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