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STABILITY-INDICATING ASSAY FOR PIVMECILLINAM HYDROCHLORIDE AND PIVMECILLINAM HYDROCHLORIDE CAPSULES

R. B. HAGEL and E. H. WAYSEK

Quality Control Department, Hoffmann-La Roche Inc., 340 Kingsland St., Nutley, N.J. 07110 (U.S.A.)
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SUMMARY

A high-pressure liquid chromatographic assay has been developed for pivmecillinam hydrochloride and pivmecillinam hydrochloride capsules. The method separates five reported degradation products and a number of esterified, related compounds. The accuracy of this method is comparable to the published ultraviolet assay and the relative standard deviation of a series of six replicate assays was better than $\pm$ 0.7%.

INTRODUCTION

Recently we reported a high-pressure liquid chromatographic (HPLC) study of the new β-lactam antibiotic, mecillinam, and some related compounds[1]. We have continued our investigation of the chromatography of some important new 6-amidino penicillins with the study of the pivaloyloxymethyl esters of these compounds. The antibacterial activity of mecillinam has been reported in a symposium dedicated to the drug[2], however, mecillinam is not well absorbed orally and an orally effective derivative was developed as an adjunct to parenteral therapy. This derivative, pivmecillinam hydrochloride (I) does not possess antimicrobial activity but is well absorbed from the gastro-intestinal tract and is rapidly hydrolyzed enzymatically to mecillinam *in situ* making it an excellent candidate for new drug status[3].

Pivmecillinam and some related compounds are listed and described in Tables I and II. A detailed discussion of the stability of pivmecillinam hydrochloride and its routes of degradation in both the solid state and in solution is given by Hattori *et al.*[4]. Under normal conditions, pivmecillinam is reported to be quite stable in the solid state, the main degradation product being the penicilloic acid (IV) formed by the hydrolytic cleavage of the β-lactam. Other minor degradation products reported under accelerated conditions were V, the internal rearrangement product III and the product of the amidino hydrolysis VI. Although it is the desired bioactive hydrolysis product, mecillinam (II) also has been included with the list of degradation products since it is observed as a solution degradation product of pivmecillinam. In solution, the hydrolysis of the piv moiety takes place at moderate rates when samples are buffered between pH 3 and 5 yielding II as well as IV. This hydrolysis is enzymatically and rapidly catalysed *in vivo* to yield the desired antimicrobial active product, II.

TABLE I

STRUCTURES AND CAPACITY FACTORS OF PIVMECILLINAM HYDROCHLORIDE AND RELATED COMPOUNDS

$R = -CH_2O\overset{O}{\overset{\|}{C}}C(CH_3)_3$

Compound	*Structure*	*k'*
I (Pivmecillinam)		8.00
II (Mecillinam)		0.41
III		2.21
IV		0.92
V		10.92
VI		0.46

In addition to these compounds, a series of compounds related to mecillinam (see Table II) also were suggested as potential very low-level impurities ($<0.01\,\%$) in pivmecillinam samples resulting from the hydrolysis of pivmecillinam to mecillinam followed by the degradation of mecillinam as reported by Larsen and Bundgaard[5]. While these compounds have not been observed in current degradation studies of

TABLE II

MECILLINAM RELATED COMPOUNDS AND PIVMECILLINAM METABOLITES

k'(Pivmecillinam) = 8.0.

Compound	*Structure*	k'
VII		0
VIII		0.12
IX		0.12
X		0.14
XI		0.26
XII		0.31
XIII		0.34
XIV		0.54
XV		1.04

pivmecillinam, they were included in this work in order to demonstrate that there was no interference with the HPLC assay.

There are several stability-indicating assay methods described in the literature for pivmecillinam. Larsen and Bundgaard[6] report successful assays using a spectrophotometric procedure to determine a glycine adduct of both mecillinam and pivmecillinam. The reaction is specific for an intact β-lactam ring and an intact amidino side chain. However, the method does not permit the independent determination of pivmecillinam in the presence of mecillinam. Two silica gel HPLC systems and one reversed-phase HPLC system, using 5% XE-60 on silica, are reported by Hattori *et al.*[4]; however, retention times are rather long and peak shapes tend to be too broad for use in a routine analytical procedure. Furthermore, since the purpose of their work was a detailed discussion of the properties of the compound, no report was made of the use of these systems for dosage-form analysis.

It was the objective of our studies to develop a single, rapid, precise HPLC system for the stability analysis of pivmecillinam hydrochloride both as the pure compound and in its capsule dosage form. This analysis is described.

EXPERIMENTAL

Chemicals and reagents

Pivmecillinam hydrochloride and related compounds were provided by Leo Pharmaceutical Products (Copenhagen, Denmark) or by Hoffmann-La Roche Inc. (Nutley, N.J., U.S.A.). Purity criteria and structural confirmation consisted of acceptable spectral and chromatographic data derived from standard methods of analysis. All other reagents were A.C.S. reagent grade or equivalent except that chromatographic-grade solvents were used for all chromatography and were passed through a 0.5-μm filter prior to use.

Sample preparation

All of the pure compounds were dissolved in filtered acetonitrile or aqueous acetonitrile as noted under Results and discussion and injected without further treatment. Samples of pivmecillinam hydrochloride capsule fill were shaken with acetonitrile and filtered prior to analysis.

Chromatographic equipment

The use in this laboratory of component HPLC systems leads to a variety of instrumental combinations. All of the following components were used interchangeably without problem. Pumps: Laboratory Data Control Model 396 mini pump; Waters Assoc. Model 6000A chromatographic pump. Injectors: Water Assoc. Model U6K; Rheodyne Model 7120. Detectors: Tracor Model 970A variable wavelength; Laboratory Data Control Model 1202 variable wavelength.

E.S. Industries 30 cm $\times$ 4.6 mm, 10-μm Chromegabond C_{18} or 15 cm $\times$ 4.6 mm, 5-μm Chromegabond C_8 columns were used throughout, and chromatograms were recorded on a 10-in. strip-chart recorder.

Instrument parameters: eluent flow-rate 2.0 ml/min; detector wavelength 220 nm; detector sensitivity 0.16 a.u.f.s. for analysis of 0.1 mg/ml sample solutions or as required to achieve measurable response.

Mobile phase

The mobile phase consisted of a well-shaken acetonitrile–0.01 *M* sodium phosphate buffer (pH 3.0, adjusted with phosphoric acid) (60:40, v/v) mixture.

RESULTS AND DISCUSSION

A comparison of the UV spectra of pivmecillinam and mecillinam indicate a maximum absorbance at 216 nm for each compound with molar absorptivities of 18,860 and 20,260, respectively. As in the determination of mecillinam, after considering both sensitivity and detector noise, 220 nm was selected as a reasonable wavelength to monitor the HPLC chromatograms of pivmecillinam and its related compounds.

The esterification of mecillinam-type compounds led to a large increase in retention (>60 min) when chromatographed using the systems previously described[1]; however, experience indicated that an increase in the non-polar component of the mobile phase resulted in a significant decrease in elution time. Suitable retention and separation of the various compounds was achieved on both 10-μm particle size C_{18} columns, which were 30 cm long, and on 5-μm particle C_8 columns, 15 cm long, using a 60:40 ratio of acetonitrile to phosphate buffer. The systems were buffered at pH 3 to optimize peak shape and reduce tailing. For the purpose of developing a stability indicating assay for pivmecillinam both as the bulk drug, and in capsule dosage form, it was convenient to select a solvent ratio which would separate potential impurities from the compound as well as provide reasonable retention times for the main component. The 60:40 mixture proved to be the best compromise; however, some of the faster eluting compounds are poorly resolved in this system, especially the unesterified entities described in Table II. When a detailed analysis of these compounds is necessary, the proportion of acetonitrile should be decreased until acceptable separations are achieved. The general effect of decreasing this ratio will be to increase the retention times and separation of all the compounds, and the investigator easily should be able to select a mobile phase which will suit his particular needs. The most rapidly eluting compounds in the series are well retained (>15 min) at a flow-rate of 1.0 ml/min when undiluted phosphate buffer is used.

Based on reported degradation[1,4] and various metabolic studies[7,8], the compounds listed in Tables I and II are expected to be the principle degradation products of pivmecillinam. A chromatogram showing the separation of the most important of these compounds is presented as Fig. 1. Separation parameters for the rest of the compounds are given in the appropriate tables.

The sample solvent of choice for both bulk drug and capsule analyses is acetonitrile. Pivmecillinam hydrochloride is soluble in water, acetonitrile and methanol, however, it has limited stability in alcoholic and aqueous solutions exhibiting as much as 7% degradation after standing for 1 h at 25°. The principal degradation products are V in alcohol and IV in water. Acetonitrile solutions, on the other hand, were stable for at least 16 h when stored under the same conditions. Unfortunately, some of the unesterified compounds discussed in this report are not very soluble in acetonitrile. If it is necessary to analyse mixtures of these compounds, samples can be dissolved in a minimum of water and diluted to appropriate volume with acetonitrile. All aqueous solutions should be analysed immediately after preparation to

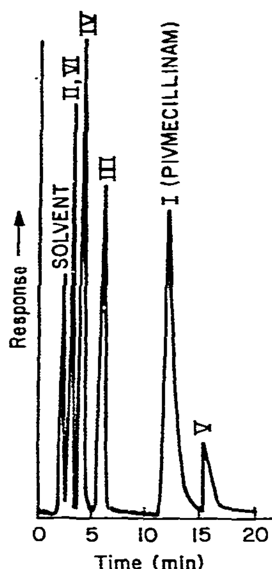

Fig. 1. HPLC chromatogram of pivmecillinam and its major impurities.

minimize subsequent degradation. Normally, one would not expect to observe measurable levels of mecillinam degradation products or enzymatically catalysed reaction products in pivmecillinam hydrochloride samples since they represent either second or third generation reaction products, or in the case of metabolites, compounds that would not arise normally from chemical hydrolysis or reaction. However, they were chromatographed to demonstrate that if present, they would not interfere with the pivmecillinam assay.

Quantitative analysis of pivmecillinam hydrochloride

The precision of the method was tested by making 9 replicate injections of a standard solution of pivmecillinam hydrochloride. An injected weight of 0.456 μg of drug in 20 μl of solution gave an average peak height of 177.5 $\pm$ 0.86 mm with a relative standard deviation of 0.48% at a detector sensitivity of 0.04 a.u.f.s. Linearity was demonstrated for a range of samples from 28.5 to 456 ng of drug injected. Parameters for the line $y = mx + b$ are $m = 0.383$, $b = -5.38$, $r = 0.9916$.

Assay values of samples of pivmecillinam hydrochloride analysed in duplicate by HPLC and the spectrophotometric procedure of Larsen and Bundegaard[6] were in excellent agreement. The results are shown in Table III.

Analysis of pivmecillinam hydrochloride capsules

Capsules of pivmecillinam hydrochloride containing 376 mg of drug (equivalent to 250 mg of mecillinam) were analyzed by combining the contents of 20 capsules, dissolving a weighed portion of the sample in acetonitrile, and filtering off the insoluble excipients. The filtered sample was diluted with acetonitrile to a concentration of approximately 0.1 mg/ml and injected without further treatment. A chromatogram of a typical injection prepared from capsule mix is presented in Fig. 2 with a

TABLE III

COMPARATIVE ASSAY DATA FOR PIVMECILLINAM HYDROCHLORIDE

Sample	*Assay (% pivmecillinam)*	
	HPLC	*Spectrophotometric*
A	100.8	100.9
B	101.5	100.4
C	97.9	98.9

chromatogram of an extract of the capsule excipients for comparison. No interfering peaks were observed.

A comparison of HPLC and spectrophotometric assay data for two samples of capsules showed excellent agreement. Assay values of 385 mg/capsule *versus* 382 mg/capsule for lot 1 and 374 mg/capsule *versus* 374 mg/capsule for lot 2 were obtained for the HPLC and spectrophotometric assays, respectively. Finally, to check the precision of the capsule assay, lot 1 was assayed using six replicate weights on each of three days. Results were 387 $\pm$ 1.8 mg, 383 $\pm$ 2.4 mg, and 385 $\pm$ 2.1 mg for the assays.

Accelerated degradation studies

In addition to the demonstrated separation of postulated degradation products, samples of pivmecillinam also were subjected to solution degradation and thermal degradation under accelerated conditions.

As previously described, solutions of pivmecillinam hydrochloride at concentrations of approximately 1 mg/ml in water, methanol and acetonitrile were allowed to stand at room temperature and checked periodically for five days. Analysis of

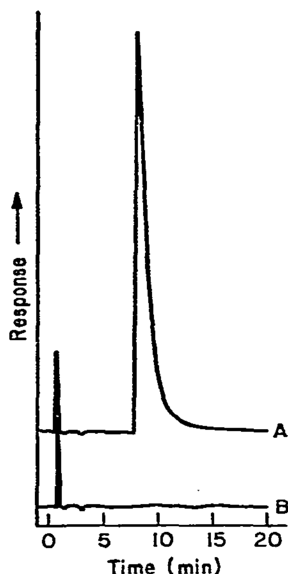

Fig. 2. HPLC chromatogram of pivmecillinam capsule mix (A) and excipient extracts (B).

TABLE IV

SOLUTION DEGRADATION OF PIVMECILLINAM

M — Major product; m = minor product.

Solvent	*Time (days)*	*Compound*				
		II	*III*	*IV*	*V*	*VI*
Water	0.5	m	—	M	—	m
Acetonitrile	5	m	—	M	m	m
Methanol	5	m	m	m	M	—

these solutions indicated the presence of several of the reaction products listed in Table I. No attempt was made at this time to perform quantitative analyses of the impurities because of the unavailability of sufficiently pure reference standards of all of the compounds. Furthermore, comparison of peak heights with that of the main compound is not accurate because of the large differences in UV absorptivities among the various compounds. The principle degradation products for each sample are described in Table IV.

Lastly, a sample of pivmecillinam hydrochloride was heated at 110° for 18 h. A chromatogram of this sample indicated very minor degradation, probably less than 0.5%, with the principle product being IV.

CONCLUSIONS

The HPLC method described in this report can be used to assay pivmecillinam hydrochloride in its pure form and in capsule doses. Separation of expected degradation products and accelerated degradation experiments indicate that the method described is stability indicating for pivmecillinam hydrochloride and can also be used to determine quantitatively the various related compounds described.

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