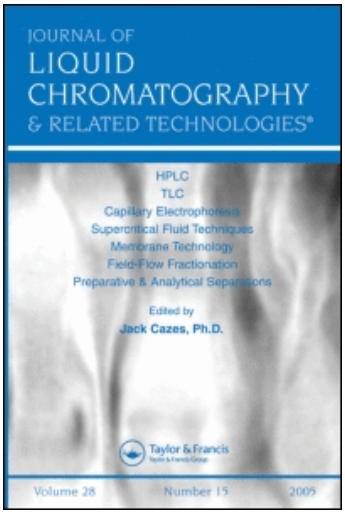

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Analysis of Penicillin V Solid Dosage Forms

M. J. Lebelle[a]; G. Lauriault[a]; W. L. Wilson[a]
[a] Drug Research Laboratories Health Protection Branch Health and Welfare Canada, Ottawa, Canada

To cite this Article Lebelle, M. J. , Lauriault, G. and Wilson, W. L.(1980) 'High Performance Liquid Chromatographic Analysis of Penicillin V Solid Dosage Forms', Journal of Liquid Chromatography & Related Technologies, 3: 10, 1573 — 1578
To link to this Article: DOI: 10.1080/01483918008062797
URL: http://dx.doi.org/10.1080/01483918008062797

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(10), 1573-1578 (1980)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF PENICILLIN V SOLID DOSAGE FORMS

M.J. LEBELLE*, G. LAURIAULT, and W.L. WILSON

Drug Research Laboratories
Health Protection Branch
Health and Welfare Canada
Ottawa, Canada, K1A 0L2

ABSTRACT

An isocratic high performance liquid chromatographic (HPLC) method for the determination of Penicillin V in solid dosage forms is described. A reverse phase RP-8 column and a mobile phase of 52% methanol in 0.05 M phosphate buffer (pH 3.3) were employed. Detection was effected at 254 nm. The results obtained are compared with those from the iodometric method.

INTRODUCTION

Recently, we reported a high performance liquid chromatographic (HPLC) method for the determination of penicillin V in penicillin V benzathine oral suspensions (1).

We have now extended the method to the analysis of penicillin V and penicillin V potassium solid oral dosage forms. This method is shorter and provides more information on product purity than the iodometric method (2). Moreover, it is more specific, as p-hydroxyphenoxymethyl penicillin, which would interfere in the official method, is separated by HPLC.

*To whom all correspondence should be addressed.

EXPERIMENTAL

Instrumentation

A Spectra-Physics HPLC-data system, SP8000, Spectra-Physics, (Santa Clara, Ca.) equipped with an SP 8300 fixed wavelength (254 nm) detector was employed. A commercially available 10 µ RP-8 column (4.6 mmx25 cm), Brownlee Laboratories (Santa Clara, Ca.) was used.

Chemicals and Reagents

Acetonitrile and methanol, HPLC grade, were obtained from Fisher Scientific Company (Fair Lawn, N.J.). The internal standard, 1,3,5-trimethoxybenzene was obtained from Aldrich Chemical Co. (Milwaukee, Wis.).

Mobile Phase

Phosphate buffer pH 3.3 - To 0.05 M aqueous sodium dihydrogen phosphate was added 0.05 M aqueous phosphoric acid until a pH of 3.3 was obtained. To 520 ml of methanol was added 480 ml of phosphate buffer pH 3.3.

Chromatographic Parameters

A flow rate of 1.5 ml/min. was employed. Ten microlitre injections of all solutions were made. A range setting of 0.16 Aufs which produced a full scale deflection for the internal standard peak was used.

PROCEDURE

Internal Standard Solution

A solution of 1,3,5-trimethoxybenzene (15 mg/ml) in 50% aqueous acetonitrile was prepared by dissolving initially in acetonitrile and diluting with an appropriate amount of water.

Standard and Bulk Drug Solutions

To an amount of bulk drug material equivalent to 150 mg of penicillin V was added 15.0 ml of internal standard solution and the volume made to 25.0 ml with 50% acetonitrile-water.

Sample Solutions

Into a 50 ml centrifuge tube, was weighed an amount of tablet or capsule material equivalent to 150 mg of penicillin V. Internal standard solution (15.0 ml) and 50% acetonitrile-water (10.0 ml) was added. The tube was then shaken for 15-20 minutes and insoluble materials allowed to settle. A portion of the supernatant was filtered prior to injection.

RESULTS AND DISCUSSION

Optimum separation of penicillin V from its impurities and the internal standard was achieved with the mobile phase specified above. The pH of the phosphate buffer (3.3) is important in achieving reproducable baseline separation between penicillin V and the internal standard. A typical chromatogram of a formulation is shown in Figure 1.

Sample solutions were prepared by diluting with acetonitrile-water to prevent decomposition. Dissolution in methanol leads to the rapid (< 10 min.) formation of the methyl penicilloate of penicillin V with some formulations.

Common degradation products such as the penicilloic, penilloic and penicillenic acids of penicillin V as well as the congener, penicillin G and the precursor, phenoxyacetic acid, did not interfere. Table 1 lists the retention times of these compounds in the system described.

The linearity of the system was confirmed by injection of five solutions of penicillin V (3-8 mg/ml). A graph of the ratio of the area counts of the penicillin V peak divided by the area counts of the internal standard peak versus the concentration of the penicillin V (mg/ml) yielded a straight line, coefficient of correlation, 0.9997, ($y=0.098x-0.002$).

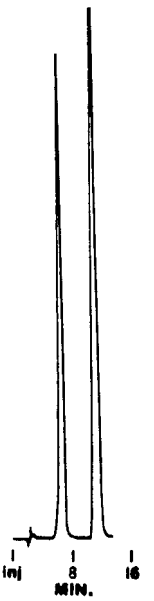

Figure 1. HPLC chromatogram of a penicillin V tablet. Column: RP-8, 4.6 mm x 25 cm; Mobile phase: 52% methanol in 0.05 M phosphate (pH3.3). Flow rate: 1.5 ml/min. Penicillin V (7.2 min.) and 1,3,5-trimethoxybenzene, internal standard (12.3 min.)

System reproducibility was verified by multiple injections (3-5) of three separate solutions of a penicillin V bulk drug. A coefficient of variation, C.V., of 0.35% was obtained. Multiple analyses of a tablet and capsule formulation yielded C.V. values of about 1% (Table 2).

The USP penicillin V reference standard and the penicillin V working standard employed in this work were the same as those used in our previous study. These standards contained small amounts of an impurity which we previously identified as p-hydroxyphenoxymethyl penicillin (1). The results from the analysis of penicillin V formulations by the HPLC and iodometric methods are shown in Table 2. An excellent correlation between the two methods was obtained. None of these formulations examined contained detectable amounts of extraneous materials.

TABLE 1

Retention Times of Penicillin V and Related Compounds

Compound	Retention Times (min.)
p-hydroxyphenoxyacetic acid	3.0
phenoxyacetic acid	3.2
penicillin V penicilloic acid	3.6
penicillin V penilloic acid	4.6
penicillin G	5.2
penicillin V	7.2
methyl phenoxymethylpenicilloate	8.5
penicillenic acid	11.0
1,3,5-trimethoxybenzene (internal standard)	12.3

TABLE 2

Analysis of Penicillin V Formulations[a]

Formulation	% Penicillin V	
	HPLC	Iodometry
Capsules		
Penicillin V Potassium	100.7 (c.v. = 1.10)[b]	100.2
Tablets		
Penicillin V Potassium	105.8	103.4
Penicillin V[c]	108.5 (c.v. = 0.76)[b]	108.6
Penicillin V[c]	100.5	99.9

[a] Average of two determinations

[b] Average of five determinations

[c] Different manufacturers

This HPLC method is a rapid, precise and accurate method for the determination of penicillin V and penicillin V potassium in solid oral dosage forms.

REFERENCES

(1) LeBelle, M., Graham, K., and Wilson, W.L., J. Pharm. Sci., 68, 555 (1979).

(2) "Code of Federal Regulations", Title 21, 440. 171-440. 173 (1979).