High-Performance Liquid Chromatographic Analysis of Penicillin V Benzathine Oral Suspensions

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Abstract □ A rapid high-performance liquid chromatographic assay for penicillin V content of penicillin V benzathine bulk drug and oral suspensions is described. Dilution of the oral suspension with methanol containing 1,3,5-trimethoxybenzene as the internal standard allowed for direct analysis on a reversed-phase column with a mobile phase of 53% methanol in 0.05 *M* aqueous pH 3.5 phosphate buffer. A relative standard deviation of less than 1% was obtained on commercial formulations, and the system was linear over a range of 10–40 μg injected.

Keyphrases □ Penicillin V—analysis in penicillin V benzathine bulk drug and oral suspensions, high-performance liquid chromatography □ Penicillin V benzathine—high-performance liquid chromatographic analysis of penicillin V content in bulk drug and oral suspensions □ High-performance liquid chromatography—penicillin V analysis in penicillin V benzathine bulk drug and oral suspensions

The iodometric assay (1–3) is the official designated potency method for penicillin V benzathine drug substance and formulations (4). Normally in this method, a sample and reference standard of the same chemical species are analyzed under identical conditions. With the benzathine salt of penicillin V, however, the drug is assayed against a penicillin V acid standard. Moreover, the inactivated and blank solutions are titrated at a different pH. Under these conditions, benzathine was reported to react with iodine in the sample blank solution (5), resulting in reduced thiosulfate titers and values for the penicillin V content that are 5–10% low.

An absorptivity test is also specified in the "Code of Federal Regulations" (4) for the penicillin V benzathine bulk drug. This method is nonspecific since a known impurity, *p*-hydroxyphenoxymethylpenicillin, interferes (6).

Because of these problems, a new analytical approach was needed. High-performance liquid chromatography (HPLC) has been used for the analysis of other penicillins (7–11), and its application as a specific, rapid, and accurate assay for penicillin V benzathine products was examined.

EXPERIMENTAL

Apparatus—The liquid chromatograph was equipped with two constant-flow pumps[1], a solvent programer[2], a septumless injector[3], and a commercial 4.6-mm × 25-cm prepacked reversed-phase column[4]. A variable-wavelength spectrophotometer[5] operating at 274 nm and 0.20 aufs was used for detection. Peak areas and calculations were computed using a chromatographic data system[6].

Reagents—A low UV cutoff, distilled-in-glass grade of methanol[7] was employed. All other chemicals were reagent grade.

Chromatographic Parameters—The mobile phase, 53% methanol in phosphate buffer (v/v), was prepared by adding 53% solution of methanol in 0.05 *M* aqueous phosphoric acid to 53% methanol in 0.05 *M* sodium dihydrogen phosphate until pH 3.5 was obtained. A flow rate of 1.0 ml/min (600 psi) was employed, and injection volumes were 5 μl.

Internal Standard Solution—A stock solution of 1,3,5-trimethoxybenzene[8] (45 mg/ml) was prepared in methanol.

Standard Preparation—European Pharmacopoeia[9] (EP) reference standard phenoxymethylpenicillin CRS (labeled 100% potency) and USP phenoxymethylpenicillin reference standard[10] (labeled 98.06% potency) were dried as required before use. Penicillin V USP bulk drug powder[11] was employed as a working standard and used as received. Solutions were

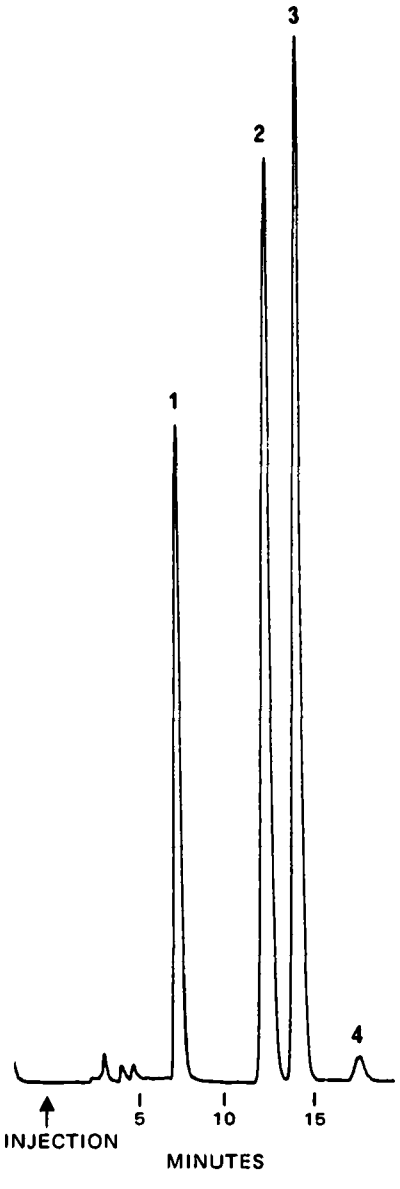

Figure 1—*HPLC analysis of a commercial penicillin V benzathine oral suspension. Key: 1, methylparaben and sodium benzoate; 2, penicillin V; 3, the internal standard; and 4, propylparaben.*

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model 660, Waters Associates, Milford, Mass.
[3] Model U6K, Waters Associates, Milford, Mass.
[4] RP-8, 10 μm, Brownlee Laboratories, Santa Clara, Calif.
[5] Model 155-40, Altex Scientific Inc., Berkeley, Calif.
[6] SP4000, Spectra-Physics, Santa Clara, Calif.
[7] Burdick & Jackson Laboratories, Muskegon, Mich.
[8] Aldrich Chemical Co., Milwaukee, Wis.
[9] European Pharmacopoeia Commission, Strasbourg, France.
[10] Lot H2.
[11] Wyeth Ltd., Windsor, Canada.

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Table I—Retention Times of Various Common Excipients and Penicillin V Impurities

Compound	Retention Time, min
FD&C Red No. 2	2.7
FD&C Yellow No. 6	3.0
p-Hydroxyphenoxymethylpenicillin	4.9
Phenoxyacetic acid	5.6
Benzathine	6.0
Penicillin V penicilloic acid	6.4
Penicillin V penilloic acid	7.2
Sodium benzoate	7.7
Methylparaben	8.1
Penicillin G	8.3
Penicillin V	12.3
Propylparaben	16.0
Penicillin V penicillenic acid	18.2

prepared in methanol to contain 9 mg/ml of the internal standard and either 3 or 6 mg/ml of penicillin V.

Sample Preparation—*Penicillin V Benzathine Bulk Drug*—Solutions (6 mg/ml) were prepared in the same way as for the standards.

Penicillin V Benzathine Oral Suspension—Approximately 2.5 ml of suspension was weighed accurately into a 25-ml volumetric flask. Internal standard solution, 5.0 ml, and about 15 ml of methanol were added. The flasks were swirled to disperse the suspension, methanol was added to volume, and insoluble materials were allowed to settle.

RESULTS AND DISCUSSION

The EP phenoxymethylpenicillin standard, with a labeled potency of 100%, chromatographed as a single peak in this HPLC system and was used as a reference standard. Penicillin V benzathine chromatographed as two separate entities. A typical chromatogram of a commercial formulation is shown in Fig. 1. The concentration of methanol in the mobile phase was critical to achieve baseline separation between penicillin V (peak 2) and the internal standard (peak 3). The benzathine portion of the molecule was eluted prior to peak 1 but was not detected at the wavelength used. With a conventional fixed-wavelength detector (254 nm), the benzathine was visible as a broad peak eluting with a retention time of about 6 min. This type of detector may also be used.

Figure 1 shows that common formulation excipients such as methylparaben and propylparaben, sodium benzoate, and coloring agents did not interfere. Methylparaben and sodium benzoate had nearly identical retention times (peak 1). Common degradation products such as the penicillenic, penicilloic, and penilloic acids, the congener penicillin G, and the precursor phenoxyacetic acid were resolved from the penicillin V peak. Table I lists the retention times obtained for these compounds in the system described.

Both the USP and working standard penicillin V contained a small amount of an impurity with a retention time of 4.9 min. Relative to the penicillin V peak and based on relative peak areas, 1 and 3% of this product were found in the USP and working standard, respectively. This impurity was collected from HPLC using 50% methanol–water as the mobile phase, and rechromatography indicated its integrity. This material had an absorbance maximum at 287 nm, which underwent a phenolic shift in base to 303 nm, suggesting that the product was *p*-hydroxyphenoxymethylpenicillin, an impurity known to be occasionally present in penicillin V (6, 12, 13). PMR spectrometry[12] confirmed this structure.

With six solutions (2–8 mg/ml), a linear calibration curve ($r = 0.9995$) was obtained over the range tested (10–40 μg injected) with a linear regression of $y = 0.0303x + 0.005$.

The precision of the method was determined by analysis of 10 aliquots of a commercial sample of a 150-mg/5 ml penicillin V benzathine suspension. A mean assay of 24.89 mg/g of suspension and a relative standard deviation of 0.94% was obtained (Table II).

There was good agreement between the HPLC and iodometric values for both the USP and working standard penicillin V (Table III). Similarly, excellent agreement between the HPLC and official (4) absorptivity assays for penicillin V content of the penicillin V benzathine bulk drug was obtained. The iodometric assay of this product exhibited the interference previously mentioned (5).

[12] WP-80, Bruker Spectrospin Ltd., Mississauga, Canada.

Table II—Precision of HPLC Determination of Penicillin V in a Penicillin V Benzathine Oral Suspension (150 mg/5 ml)

Determination	Amount of Penicillin V[a]
1	24.93
2	24.46
3	24.76
4	24.72
5	25.13
6	25.00
7	25.01
8	25.25
9	24.80
10	24.91
Mean	24.89
RSD, %	0.94

[a] Results are expressed as milligrams of penicillin V per gram of suspension.

Table III—Analysis of Penicillin V and Penicillin V Benzathine Bulk Drug and Oral Suspension

Product	Penicillin V, %	
	HPLC	Official[a]
USP penicillin V reference standard (98.06% label claim)	98.7	97.6
Penicillin V working standard	95.9	97.8
Penicillin V benzathine bulk drug	66.0	65.9
Oral Suspension A (300 mg/5 ml)	54.7, 54.0[b]	49.0, 49.2[b]
Oral Suspension B (300 mg/5 ml)	54.2, 54.7[b]	49.8, 50.3[b]

[a] Iodometric assay except for penicillin V benzathine bulk drug which is the UV absorptivity method. [b] Results are expressed as milligrams of penicillin V per gram of suspension.

Table III also shows a comparison of the assay results using the two methods for two commercial formulations of penicillin V benzathine (300 mg/5 ml) from different manufacturers. The values listed are expressed as milligrams of penicillin V per gram of suspension. For potency determination, conversion to milligrams per dose is required and is effected by determination of the specific gravity of the suspension, because problems are encountered when pipetting such viscous preparations. The difference between the HPLC and iodometric assay values for these oral suspensions is due to the interference by the benzathine in the iodometric method (5).

The HPLC procedure can be used for the analysis of other penicillin V products and as a combined identity and purity test to control the quality of these antibiotics.

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(12) "European Pharmacopoeia, 1971," Maisonneuve S. A., France, 1971, p. 339.

(13) "British Pharmacopoeia, 1973," Her Majesty's Stationery Office, London, England, 1973, p. 362.

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