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QUANTITATION OF ACYCLOVIR IN PHARMACEUTICAL DOSAGE FORMS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A stability-indicating high performance liquid chromatography method for the quantitation of acyclovir in pharmaceutical dosage forms (capsules, ointment and injection) has been developed. The method is accurate and precise with a percent relative standard deviation of 1.2 based on 5 readings. The excipients present in the dosage forms did not interfere with the assay method. The recovery from the synthetic mixtures was quantitative. The samples decomposed under drastic conditions showed a new peak in the chromatogram. Acyclovir appears to be more stable in the alkaline than in the acidic solution. There appears to be a distribution/decomposition problem with the ointment sample being marketed in certain types of tubes used previously and still on the market.

INTRODUCTION

Acyclovir (Figure 1) is an antiviral drug which is extensively used for the treatment of initial episodes and the management of recurrent episodes of genital herpes. Three dosage forms, capsules, ointment, and lyophilized powder of sodium salt for injection, are available. In spite of its extensive use, there is very little information available concerning its quantitation and stability in

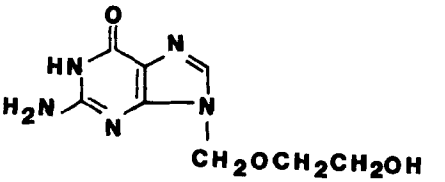

Figure 1 - Structure of acyclovir.

dosage forms. The purpose of these investigations was to develop a stability-indicating high-performance liquid chromatography method for the quantitation of acyclovir in pharmaceutical dosage forms.

METHODOLOGY

Chemicals and Reagents - All the chemicals and reagents were USP-NF or ACS quality and used without further purification. Acyclovir powder was generously supplied by Burroughs Wellcome Co. and used as such. All the dosage forms were of commercial lots.

Equipments - A high-pressure liquid chromatograph (ALC 202, Waters Associates) equipped with an injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF 770, Applied Biosystems) and a recorder (Omniscribe 5312-12, Houston Instruments) was used. A C_{18} column (Microbondapak, 30 cm x 3.9 mm i.d., Waters Associates) was the stationary phase.

Chromatographic Conditions - The mobile phase contained 3% V/V of acetonitrile in 0.01M KH_2PO_4 aqueous buffer solution. The flow rate was 2.0 ml/min, the sensitivity was 0.04 AUFS (at 252 nm, the wavelength of maximum absorption of acyclovir), the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Solutions - A 0.02% stock solution of acyclovir in water was prepared fresh daily. A 0.2% stock solution of salicylic acid (the internal standard) was prepared by dissolving 200 mg of the acid in 2 ml of acetonitrile and then bringing to volume (100.0 ml) with water. These stock solutions were mixed and diluted further with water as needed. The most commonly used standard solution contained 6.4 μg/ml of acyclovir and 240 μg/ml of salicylic acid.

Extraction Procedure from the Capsules - The contents of 10 capsules were weighed accurately and a quantity of the powder representing 20 mg of acyclovir was mixed with 90 ml of water and stirred for 10 minutes using a magnetic bar. The mixture was brought to volume (100.0 ml) in a volumetric flask, shaken, filtered (Fisher's 9-803-5-E filter paper), first 20 ml of the filtrate was rejected and then collected for further dilution. A 10.0 ml of the clear filtrate was diluted to 100.0 ml with water. A 8.0 ml quantity of the diluted solution was mixed with 3.0 ml quantity of the stock solution of salicylic acid (the internal standard) and then brought to volume (25.0 ml) with water.

Extraction Procedure from the Ointment - A 200 mg quantity of the ointment was dissolved in enough water to make 100.0 ml of the solution. A 10.0 ml of the solution was diluted to 50.0 ml with water. A 8.0 ml quantity of the diluted solution was mixed with the internal standard and brought to volume as given above under capsules.

From Injection - According to information on the label, the powder for injection contained 500 mg of acyclovir in the form of acyclovir sodium salt. From B lot of the injection, a quantity of the powder

representing 20.0 mg of acyclovir was accurately weighed and dissolved in enough water to make 100.0 ml of the solution. The rest of the procedure was the same as given under the capsules except that filtration was not necessary since it did not contain any excipients. The lot A of the injection was assayed by dissolving the whole contents of the vials (label claim 500 mg of acyclovir) in enough water to make 500.0 ml of the solution. A 10.0 ml quantity of this solution was diluted to 50.0 ml with water. The rest of the procedure was same as given under capsules starting with 1 in 10 dilution.

Decomposition of Acyclovir - A 8.0 ml quantity of the solution of acyclovir (20.0 μg/ml in water- see first dilution of the stock solution) was mixed with either 1 ml of ~1N H_2SO_4 or 1 ml of ~1N NaOH solution in a 150 ml beaker. A 10 ml quantity of water was added and the mixture heated to boiling for 10 minutes using a hot plate (more water was added if needed to prevent splashing). The solution was cooled to room temperature, neutralized using 1N H_2SO_4 or 1N NaOH solution, brought to volume (25.0 ml) with water, and assayed. The internal standard was not added in order to detect new peaks in the chromatograms.

Assay Procedure - A 30.0 μl quantity of the assay solution was injected into the chromatograph using the conditions described above. For purpose of comparison, an identical volume of the standard solution was injected after the assay sample eluted. The standard solution contained identical concentrations of acyclovir (based on the label claim) and the internal standard.

Calculations - Since preliminary investigations indicated that the

ratio of peak heights (acyclovir/salicylic acid) were directly related to the concentrations of acyclovir (range tested ± 50% of the standard concentration), therefore, the results were calculated using a simple equation:

$$\frac{(R_{ph})a}{(R_{ph})s} \times 100 = \text{Percent of the label claim found}$$

where $(R_{ph})a$ is the ratio of the peak heights of the assay sample and $(R_{ph})s$ that of the standard solution. In the case of the decomposed solutions, the results were estimated by direct comparison of peak heights (assay/standard) of acyclovir since no internal standard was added.

RESULTS AND DISCUSSION

The results indicate (Table 1) that the developed method can be used to quantify acyclovir in pharmaceutical dosage forms. The method is accurate and precise with a percent relative standard deviation of 1.2 based on 5 readings. The separation of salicylic acid (peak 2 in Figure 2) from acyclovir (peak 1 in Figure 2) was complete. The method is stability-indicating since the product of decomposition gave a new peak (peak 2, Figure 3) in the chromatograms. Acyclovir appears to be much more stable in the alkaline solution versus the acidic solution. For example, on 10 minute boiling with sulfuric acid, the potency of acyclovir decreased to about 88% versus 95% on boiling with sodium hydroxide. This may be one reason why the powder for injection (the sodium salt) has a high pH value of ∿11 on reconstitution. The water solubility of acyclovir in the alkaline

TABLE 1
ASSAY RESULTS

Dosage Form	Claim Acyclovir	Percent of the Label Label Claim Found	Remarks
Capsules A	200 mg/cap	101.8	
Capsules B	200 mg/cap	103.2	
Injection A	500 mg/vial	106.3	Whole powder taken for assay.
Injection B	500 mg/vial	98.7	Powder representing 20.0 mg of acyclovir taken for assay.
Ointment A (old tube 1)	50 mg/g	79.7 96.0	Initial sample. Second sample (see discussion)
Ointment A (old tube 2)	50 mg/g	86.1	Initial sample (see discussion)
Ointment B (new tube)	50 mg/g	104.3 103.8	Initial sample. Second sample
Synthetic Mixture A	20 mg drug in 250 mg of lactose	99.9	
Synthetic Mixture B	20 mg drug in 250 mg of dextrose	99.7	

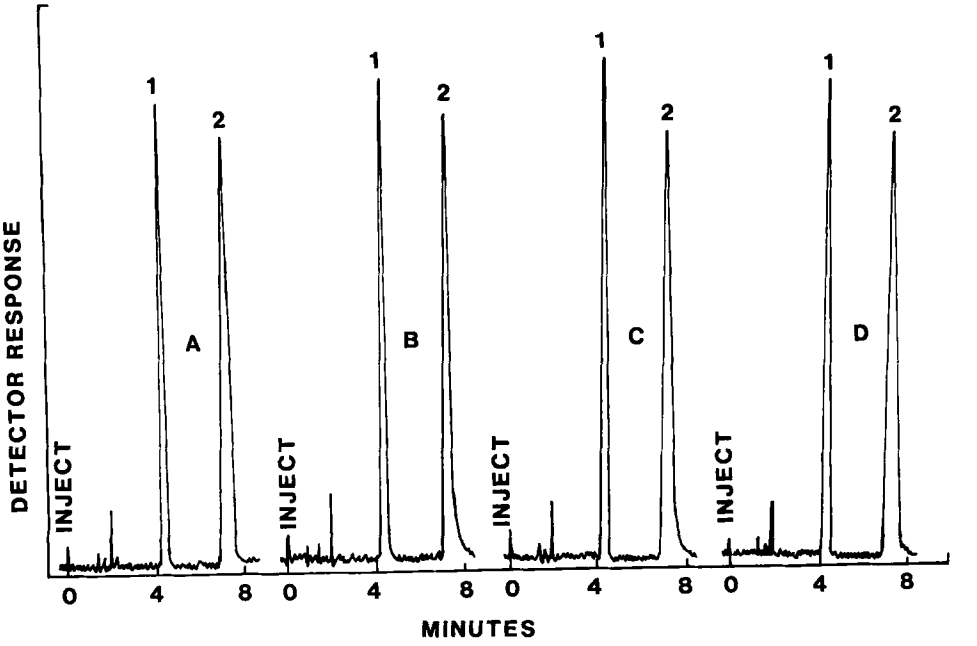

Figure 2 - Sample chromatograms. Peaks 1-2 are from acyclovir and salicylic acid (the internal standard), respectively. Chromatogram A is from a standard solution; B from capsules; C from an injection (whole powder was used) and D from an ointment (lot B in newer tube, expiry 7/92). For chromatographic conditions, see text.

solutions is also greatly increased. Overall, acyclovir appears to be a very stable compound.

Extraction Procedures from the Dosage Forms - The extraction procedures are very simple and there was no interference from the excipients present (Figure 2). The capsules also contained corn starch, lactose, magnesium stearate and sodium lauryl sulfate. The ointment base was polyethylene glycol, a completely water soluble base. The

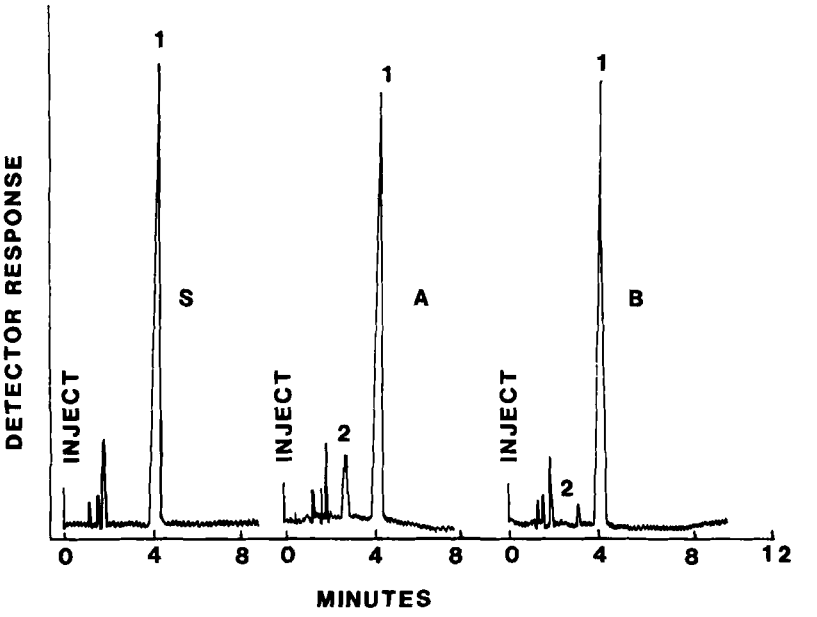

Figure 3 - Sample chromatograms. Peak 1-2 are from acyclovir and the product of decomposition, respectively. Chromatogram S is from a standard solution; A from an acid decomposed sample and B from a base decomposed sample (see text). For chromatographic conditions, see text.

injection did not contain any excipients. The recovery from the synthetic mixtures was quantitative (Table 1).

When the whole powder from an injection vial was assayed, the results were high because it is a common practice to fill the vials with an overage. In the case of the ointments, there were two different lots assayed. Lot A (expiry date 9/90) was in tube which was 7.3" long and 3.4" wide at the crimp with a white cap. Lot B (expiry date 7/92), the tube was 8.2" long and 2.9" wide at the crimp with a black cap. Apparently, lot B tube was the newer one being

used to market the ointment. The results obtained from lot B tube were slightly high (Table 1) probably because of the addition of an overage which is normal. However, on lot A tubes, the results of the first sample drawn from the tubes were lower (79.7% from tube 1 and 86.1% from tube 2) due to either a problem of decomposition near the tip or poor distribution. The first sample drawn also indicated part of the ointment was liquified. This may be the reason why the company has started using the new tubes. However, the old tubes (expiry date 9/90) are still on the market. It should be pointed out that a second sample from tube 1 had assayed 96% which is within the accepted limits. This potency was still lower by 7-8% when compared with results from lot B (newer tube, expiry date 7/92) ointment (Table 1).