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Short communication

Simple high-performance liquid chromatographic method for the determination of acyclovir in human plasma using fluorescence detection

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Abstract

A simple high-performance liquid chromatographic method using fluorescence detection was developed for the determination of acyclovir in human plasma. The method entailed direct injection of the plasma sample after deproteination. It is both specific and sensitive with a detection limit of 30 ng/ml at a signal-to-noise ratio of 3:1, and is thus suitable for use in pharmacokinetic studies of acyclovir. The method had a mean absolute recovery of 96%, while the within-day and between-day coefficients of variation and percentages error were all less than 8%. The calibration curve was linear over a concentration range of 62.5–4000 ng/ml.

Keywords: Acyclovir

1. Introduction

Acyclovir is a guanine derivative with strong antiviral activities against herpes simplex and varicella zoster viruses [1,2]. Various methods have been reported for its determination in human plasma. Immunological methods such as radioimmunoassay [3,4], are relatively sensitive but require lengthy procedures. Moreover, problems occasionally arise during sample analysis, possibly due to cross-reactivity with antiserum. Analytical methods based on high-performance liquid chromatography (HPLC) with UV [5,6] or fluorescence [7] detection have been reported. However, these methods have a detection limit of 100 ng/ml or above which may not be sensitive enough for pharmacokinetic investigations in which oral doses of 200–400 mg of acyclovir are administered. More recently, a reversed-phase HPLC method with fluorescence detection, which employed a strongly acidic mobile phase for fluorescence optimization was reported to have a detection limit of 10 ng/ml [8]. Although this method was able to increase the sensitivity, the highly acidic mobile phase could lead to rapid deterioration of the stationary phase, and hence a shortening of the column life. A method using ultrafiltration with a detection limit of 50 ng/ml [9] and an automated method using the Gilson ASPEC system with a quantification limit of 10 ng/ml [10] have also been reported.

In this paper, we report a relatively simple, specific and sensitive HPLC method for the de-

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termination of acyclovir in human plasma using fluorescence detection.

2. Experimental

2.1. Materials

Perchloric acid 60–62% was purchased from BDH Chemicals (Poole, UK). Acyclovir standard was obtained from Chemio Pharm (Milan, Italy). All other solvents used were of analytical reagent grade or of high-performance liquid chromatography grade, purchased from Mallinckrodt (Paris, KY, USA).

2.2. Instrumentation

The HPLC system comprised a Jasco PU-980 pump, a Jasco 821-FP spectrofluorometer detector (gain, ×100; attenuation, 32; band width, 18 nm) (Jasco, Tokyo, Japan) and a Hitachi D-2500 chromato-integrator (Tokyo, Japan). A LiChrosorb RP-8 (E.Merck, Darmstadt, Germany) column (7 μm, 250×4 mm I.D.) fitted with a refillable guard column (Upchurch Scientific, Oak Harbor, WA, USA) packed with Perisorb RP-18 (30-40 μm, pellicular) was used for the chromatographic separation. The mobile phase consisted of 1% acetonitrile in 0.02 *M* disodium hydrogen orthophosphate adjusted to pH 2.5 with 60–62% perchloric acid. The analysis was run at a flow-rate of 1.2 ml/min with the detector operating at an excitation wavelength of 270 nm and an emission wavelength of 380 nm. Samples were quantitated by using peak height.

2.3. Sample preparation

A 250-μl aliquot of plasma sample was measured into an eppendorf microcentrifuge tube and deproteinized by adding 30 μl of 60–62% perchloric acid. The mixture was vortexed for 30 s on a vortex mixer and centrifuged at 12 800 *g* for 25 min. The supernatant was transferred into a new eppendorf microcentrifuge tube and 50 μl were injected onto the column.

2.4. Assay validation

Standard calibration curves were constructed by spiking drug free pooled plasma with a known amount of acyclovir at a concentration range of 62.5–4000 ng/ml. These plasma standards were also used to determine the within-day and between-day accuracy and precision ($n=6$) of the method. In addition, the absolute recovery ($n=6$) was estimated by comparison with directly injected aqueous drug solution of corresponding concentrations.

3. Results and discussion

Extraction of acyclovir from plasma using various organic solvents with sufficient degree of recovery was unsuccessful primarily attributed to its poor lipophilicity. In this regard, the sample preparation procedure which involved direct injection of the sample was thus employed prior to analysis.

Chromatograms obtained with blank plasma and plasma spiked with acyclovir are shown in Fig. 1A Fig. 1B. The acyclovir peak which has a retention time of 9.59 min was well resolved and free of interference from endogenous compounds in the plasma.

The absolute recovery, within-day and between-day accuracy and precision values are presented in Table 1. The average absolute recovery value was approximately 96%. The coefficient of variation (C.V.) of both the within-day and between day precision was found to be less than 8%, while that of accuracy with percent error values of less than 7%.

The standard calibration curve ($n=6$) was found to be linear over the concentration range used. A slope of $9.2729 \cdot 10^{-5}$ with an intercept of 0.0101, and a correlation coefficient of 0.99997 were obtained.

A detection limit of 30 ng/ml was obtained at a signal-to-noise ratio of 3:1. This could be further improved by using a larger volume sample loop. Mascher et al. [8] reported that a strongly acidic mobile phase could further improve the detection limit and a value of 10 ng/ml was reported by this group. The composition of the mobile phase used had a measured pH value of approximately 1.5. However, this method is not recommended as it may lead to rapid deterioration of the column. At a pH of

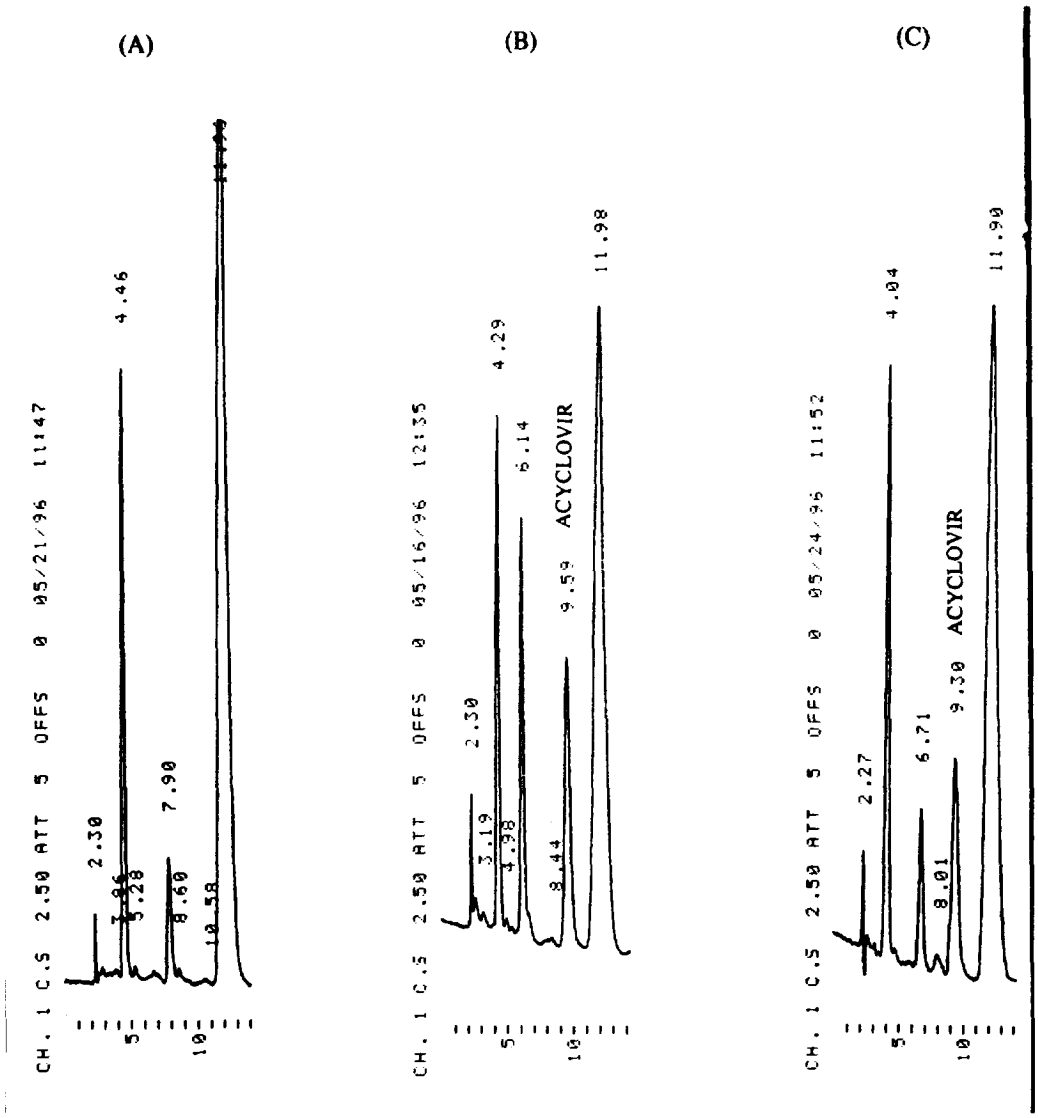

Fig. 1. Chromatograms for the analysis of acyclovir in plasma. (A) Blank plasma. (B) Plasma spiked with 1000 ng/ml acyclovir. (C) A volunteer plasma containing 470.54 ng/ml acyclovir 1 h after oral administration of 400 mg of acyclovir (*y*-axis: attenuation, 5; *x*-axis: chart speed, 2.5 mm/min).

Table 1
Absolute recovery, within-day and between-day precision and accuracy ($n=6$)

Concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	C.V.%	Precision (C.V.%)	Accuracy (% error)	Precision (C.V.%)	Accuracy (% error)
125	96.4	2.8	7.7	6.6	4.9	3.2
500	96.5	7.0	5.1	1.8	6.5	2.4
2000	95.9	3.3	4.8	4.6	4.6	0.5

2.5 used in the present study, the detection limit of 30 ng/ml obtained was deemed satisfactory for the method to be used in pharmacokinetic studies of acyclovir.

The present method has been applied to analyze plasma samples of twelve healthy adult male volunteers from a comparative bioavailability study of two different acyclovir tablet preparations, namely, Zovirax and Hepirax 200, the latter being a generic preparation. Fig. 1C shows a chromatogram obtained from a volunteer 1 h after dosing with 400 mg of acyclovir while Fig. 2 shows the individual plasma concentration–time profiles of the volunteers obtained with the two preparations. It can be seen that for both preparations, acyclovir could still be detected up to at least 14 h and the last detectable level was less than 8% of the peak plasma concentration.

In conclusion, the present HPLC method was simple, specific, sensitive and suitable to be used for determination of plasma acyclovir in pharmacokinetic/bioavailability studies.

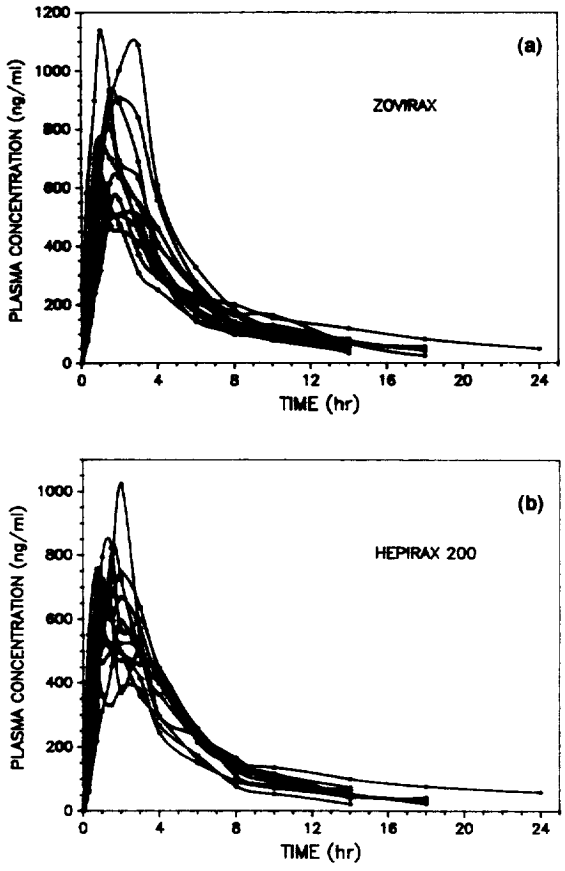

Fig. 2. Plasma acyclovir concentration versus time profiles from twelve volunteers following the oral administration of 400 mg of Zovirax and Hepirax 200.

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