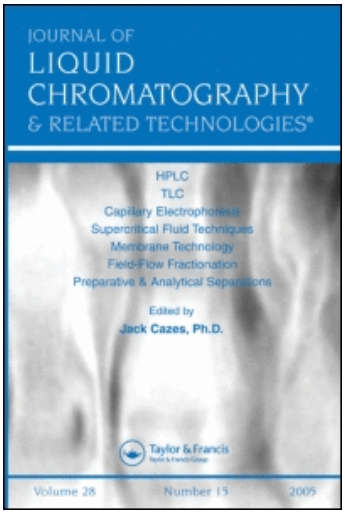

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Determination of Acyclovir in Human Serum by High-Performance Liquid Chromatography

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DETERMINATION OF ACYCLOVIR IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A method was developed for determination of concentrations of acyclovir in serum. Serum proteins were precipitated with equal part of 5% perchloric acid. High-performance liquid chromatography was used for separation from endogenous compounds and detection was done with spectrophotometry. The assay is simple and precise and seems well suited for pharmacokinetic studies.

INTRODUCTION

Acyclovir (9-(2-hydroxyethoxymethyl) guanine) is an antiviral substance effective against herpes simplex and varicella zoster viruses (1). Systemically, it is primarily used in prophylaxis and as a therapeutic agent in immunodeficient patients and in the treatment of herpes simplex encephalitis (2). Different methods

for the analysis of acyclovir concentrations in serum have been developed including radioimmunoassay (RIA) (3, 4), ELISA (5) and high-performance liquid chromatography (HPLC) on ion-exchange or reversed-phase columns (6 - 9).

We have developed an assay, using HPLC, with a simple, one-step sample pretreatment procedure and with good sensitivity, precision and specificity.

EXPERIMENTAL

Reagents: Acyclovir (donated by the Wellcome Foundation, Great Britain) was dissolved in distilled water to obtain a stock solution of 100 ug/ml.

Deionized, distilled water, HPLC grade methanol (Laboratory FSA, Loughbourough, Great Britain) and perchloric acid (E. Merck, Darmstadt, F.R.G.) were used.

Chromatographic conditions: A Waters ALC/GPC 204 liquid chromatograph with a Waters 712 WISP automatic sample injector, a Waters Model 450 variable wavelength detector set at 250 nm and a Scintag recorder (Waters Ass., Gothenburg, Sweden) were employed. The stationary phase consisted of 5 um Nucleosil C18 (Macherey-Nagel, Düren, F.R.G.), slurry packed in a stainless steel column (20 cm x 4 mm i.d.). The mobile phase was a mixture of methanol and water (5:95, vol:vol), with a flow rate of 1.0 ml/min and separations were made at ambient temperature.

Sample preparation: The aqueous stock standard solution of acyclovir was diluted with distilled water or human serum to known concentrations. Serum samples were mixed with 5% perchloric acid (1:1, vol:vol) to precipitate the serum proteins, vortexed briefly and centrifuged for 5 min at 2000 x g. The supernates were filtered through 0.6 um Millipore filters (HVLP) before chromatographic separation.

The volume injected into the chromatograph was 25 ul.

Quantitation of concentrations of acyclovir in serum was based on measurement of the heights of peaks in recorded chromatograms and comparison with the heights of peaks obtained from known standard solutions in pooled sera.

RESULTS

In the above described chromatographic system, acyclovir had a retention time of approximately eight minutes (FIGURE 1). One small endogenous peak eluted closely after the acyclovir peak, but did not change the latter significantly. The height of the acyclovir peak correlated well with the actual concentration in both water and serum (correlation coefficient 0.999) (FIGURE 2).

Recovery from serum was determined by comparing peak heights obtained from serum samples with known concentrations with those from standard water solutions and varied between 80 - 92.4 % (TABLE 1). Thus, recovery was not quantitative and the conclusion is, therefore, to use standards prepared in serum for this assay.

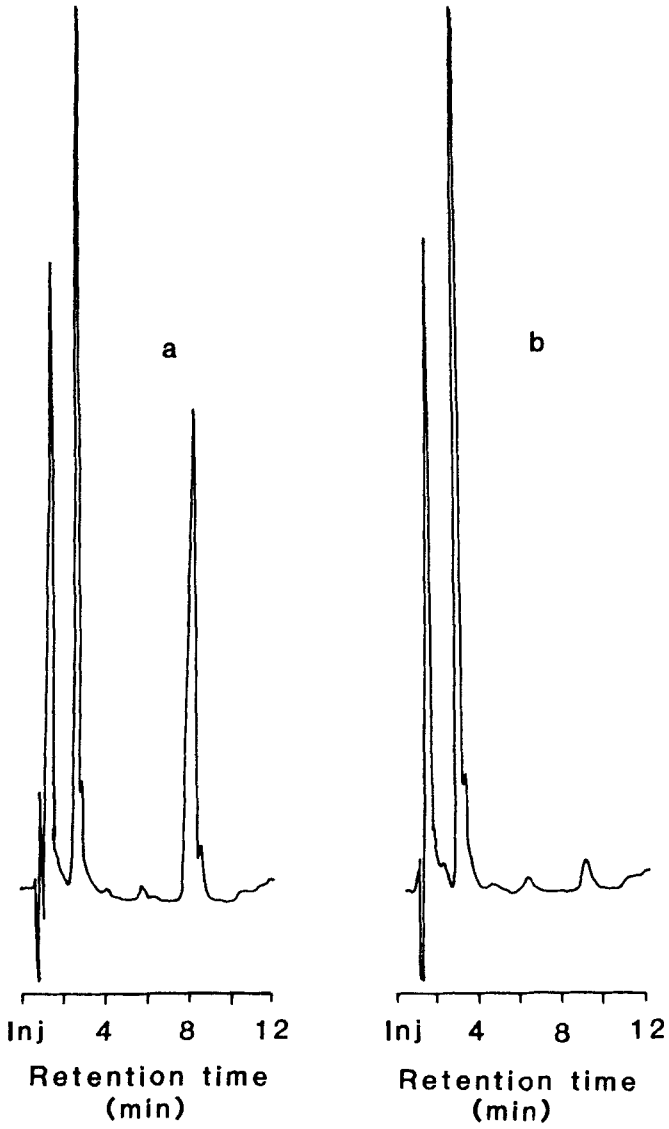

FIGURE 1. Chromatograms of serum samples with and without acyclovir. a. acyclovir 10 ug/ml, b. serum blank. The recorder was set at 10 mV attenuation and the detector attenuation was 0.01 AUFS.

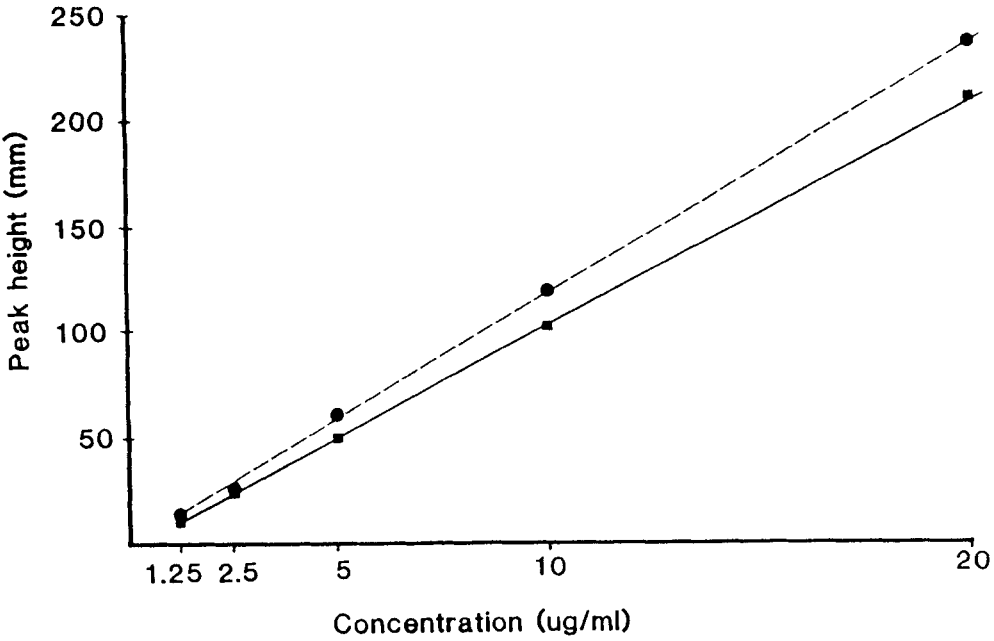

FIGURE 2. Standard curves for samples of acyclovir in water solutions (dots) and in serum (squares).

TABLE 1.
Recovery of Acyclovir from Serum.

Known conc. (ug/ml)	Found conc. (ug/ml)	Recovery (%)
1.25	1.00	80.0
2.50	2.31	92.4
5.00	4.03	80.6
10.00	8.64	86.4
20.00	17.82	89.1

TABLE 2.
Intra- and Interassay Imprecision of the Chromatographic Assay for Acyclovir in Serum.

Concentration (ug/ml)	Intra-assay C.V.* (%)	Inter-assay C.V. (%)
1.00	2.7	4.7
10.00	0.9	2.1

* C.V.: coefficient of variation.

In order to test the intra- and interassay imprecision of the procedure, eight separate analyses of the same serum samples with known concentrations of 1.0 and 10.0 ug/ml were performed during one day and on separate days. As shown in Table 2, coefficients of variation (C.V.) were below 5 % at the tested concentrations.

The sensitivity of this method allows determination of concentrations as low as 0.3 ug/ml which is adequate for clinical and pharmacokinetic purposes.

The specificity of the method was checked by testing serum samples from thirteen patients on treatment with other drugs and antibiotics in the assay system (Table 3). Interference was found only with morphine.

DISCUSSION

We have developed a simple, sensitive and accurate method for measuring concentrations of acyclovir in serum using reversed,

TABLE 3.
Specificity of Assay for Acyclovir in Serum. Drugs Found not to Interfere with Assay.

Antibiotics	Other drugs
Metronidazole	Propranolol
Cefuroxime	Allopurinol
Cloxacillin	Naproxen
Flucloxacillin	Digoxin
Benzyl penicillin	Furosemide
Trimethoprimsulphamethoxazole	Spironolactone
Fusidic acid	Chlorpropamide
Rifampicin	Dicumarol
Isoniazide	Glipizide
Streptomycin	Warfarin sodium
Imipenem-cilastatin	Baclofen
Pyrazinamide	Carbacholinchloride
	Hydrochlortiazide
	Insulin
	Phenytoin
	Heparin
	Diazepam
	Perphenazin
	Ranitidin
	Paracetamol
	Pyridoxinhydrochloride
	Prednisolone
	Ketoprofen

bonded phase chromatography. The procedure involves only one simple pretreatment step and is thus easier to perform than earlier published methods (6 - 8). In the method described by Salamoun et al (9), no pretreatment is performed, plasma is injected directly. Although a guard column is used, this procedure will result in shorter column lives compared to those obtained when proteins are precipitated from samples before

chromatographic separation. This method, furthermore, requires two temperature control steps: separation is performed at 45°C and the effluent is cooled to 2°C before entering the fluorescence detector.

The precision obtained with our assay was excellent; coefficients of variation were below 5 % even at low concentrations where earlier methods have not reported any data or have shown unacceptable imprecision (8). With the exception of morphine, no interfering substances have as yet been encountered. The method should be well suited for use in studies of the pharmacokinetics of acyclovir.

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