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# Short Communication

# Determination of acyclovir by ultrafiltration and high-performance liquid chromatography

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ABSTRACT

A simple, sensitive and rapid high-performance liquid chromatographic (HPLC) procedure to determine total serum acyclovir concentrations is described. The assay involves a heat inactivation step at 56°C to prevent risk of infection, ultrafiltration as a pretreatment step prior to ion-pair reversed-phase liquid chromatography using guanosine as internal standard, and ultraviolet detection at 254 nm. This method has excellent recovery (97–100%), linearity (0.5–100 mg/l) and precision (1.2–8.0% coefficient of variation). The detection limit is 50 μg/l. The assay proved to be suitable for therapeutic drug monitoring of acyclovir.

## INTRODUCTION

Acyclovir or 9-(2-hydroxyethoxymethyl)guanine is a modified nucleoside that demonstrates strong and selective activity against herpes simplex and varicella zoster virus [1]. The mechanism of action of acyclovir, its bioavailability and pharmacokinetics in humans have been well established for both intravenous and oral formulations [2,3].

Various assay procedures have been developed for its determination in biological fluids, for example HPLC [4–7], radioimmunoassay [8,9] and enzyme-linked immunosorbent assay [10]. The immunological methods are sensitive but require lengthy procedures for assay performance. For published HPLC methods a time-consuming sample preparation is required. In this paper a simple, sensitive and rapid HPLC method using a heat inactivation step to avoid the risk of infection and ultrafiltration for sample pretreatment is described for routine analysis.

## EXPERIMENTAL

### *Materials*

Acyclovir was a generous gift from the Wellcome Company (Burgwedel, Germany). The internal standard guanosine was purchased from Aldrich (Steinheim, Germany), the ultrafiltration units Centrisart I (2.5 ml, $M_r$ 5000 cut-off) from Sartorius (Göttingen, Germany) and 1-octanesulfonic acid from Fluka (Neu-Ulm, Germany). All other solvents used were of HPLC grade.

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*Instrumentation*

An HPLC system equipped with an L-6000 HPLC pump, an L-4250 UV–Vis detector and an RP8e column (reversed-phase, end-capped, 125 mm × 4 mm I.D., 5 μm, Merck, Darmstadt, Germany) was used for the chromatographic separation. The mobile phase consisted of 5% methanol and 95% 0.05 mol/l 1-octanesulphonic acid in 0.1 mol/l phosphate buffer pH 3.0 (v/v). The flow-rate was 1.0 ml/min and the effluent was monitored at 254 nm. The column temperature was ambient.

*Preparation of samples*

Before sample preparation the serum specimens were heat-inactivated at 56°C for 1 h. A 500-μl aliquot of serum and 50 μl of internal standard (guanosine 100 mg/l) were mixed prior to ultrafiltration. The samples were ultrafiltered according to the manufacturer's recommendations by Sartorius Centrisart I units. The obtained ultrafiltrate was diluted 1:30 with eluent buffer prior to HPLC analysis. Calibration curves were prepared in the range 0.5–100 mg/l acyclovir.

## RESULTS AND DISCUSSION

Selective extraction of acyclovir from plasma is unsuccessful because of its poor lipid solubility [4]. Published precipitation methods for deproteination of plasma or serum are time-consuming [4,5,7]. For these reasons ultrafiltration has been chosen as a simple pretreatment technique, taking advantage of the low protein binding of acyclovir [2]. Fig. 1 illustrates typical chromatograms obtained in the analysis of blank human serum (A), serum spiked with acyclovir (B), and patient sample (C). In these chromatograms no interfering peaks were observed. The retention times of acyclovir and guanosine were 6.36 and 7.35 min, respectively. Virustatic drugs such as ganciclovir and zidovudine did not interfere in the analysis of acyclovir.

*Recovery and stability*

The recovery of acyclovir was in the range 97–100% using this method. Acyclovir revealed an excellent stability during sample preparation. No breakdown of acyclovir and no change in

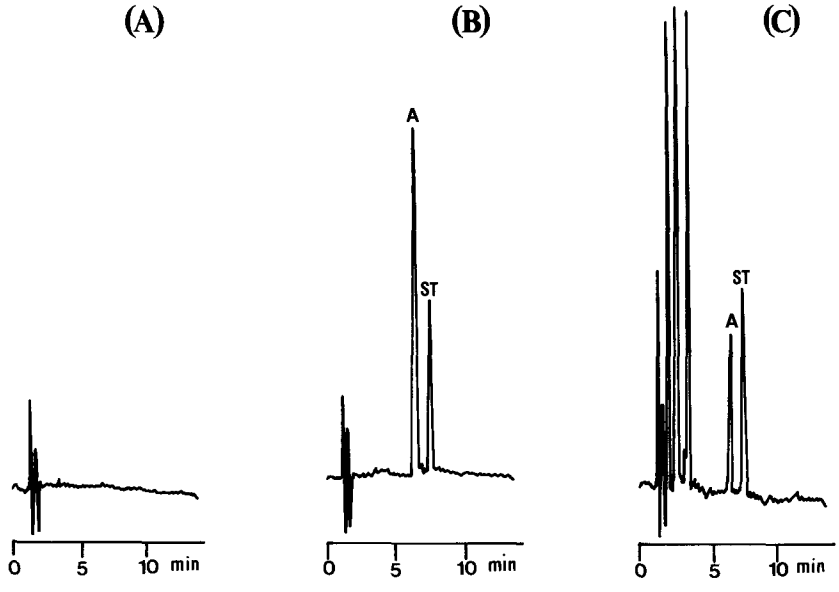


Fig. 1. Typical chromatograms for the analysis of acyclovir in human serum. (A) Blank human serum. (B) Blank human serum spiked with 10 mg/l acyclovir and internal standard. (C) Patient serum sample containing 3.6 mg/l acyclovir. Peaks: A = acyclovir; ST = internal standard, guanosine.

acyclovir concentrations were observed when serum samples were heat-inactivated at 56°C for 1 h. Additionally, the serum and ultrafiltrate specimens were tested for stability by storing them at room temperature and at 4°C for one week. The same values for acyclovir were found as for serum and ultrafiltrate samples processed immediately.

*Linearity and precision*

The standard curves obtained during the analysis of acyclovir were linear up to 100 mg/l. This range is sufficient to measure patient serum acyclovir levels because its therapeutic range is given as 0.5–15 mg/l. The detection limit of this HPLC method was 50 μg/l based on a signal-to-noise ratio of 5:1 and a 1:10 dilution of the ultrafiltrate. The detection limit can be further increased by reducing the dilution factor of the ultrafiltrate.

The precision of the assay was evaluated by determining the coefficients of variation of within- and between-day analyses. Table I shows the analysis data of the within- and between-day variances for human serum samples of four different acyclovir concentrations. For the determination of the intra-assay precision, ten replicates of each concentration were assayed. The coefficients of variation ranged from 1.2 to 6.0%.

The inter-assay precision for serum samples was calculated with the data generated from ten individual assays performed over a period of two weeks. The coefficients of variation were in the range of 1.4–8.0%.

The precision data of the acyclovir assay are valid for total serum acyclovir concentrations. Comparisons between spiked serum and spiked physiological sodium chloride (0.9% NaCl) samples showed minor differences in recovery (less than 5%). Additionally, the effect of dilution before and after the ultrafiltration step was determined for patient serum and spiked serum samples. Minor differences in recovery (less than 5%) were found. These experiments reveal a very low level of acyclovir protein binding.

In conclusion, the HPLC method described has good sensitivity and reliability for the measurement of total acyclovir in a wide range of clinical situations.

TABLE I

INTRA-ASSAY AND INTER-ASSAY ACYCLOVIR CONCENTRATIONS DETERMINED BY HPLC

| Nominal concentration of external controls (mg/l) | Measured concentration (mean ± S.D.) (mg/l) | Coefficient of variation[a] (%) |
|---|---|---|
| *Intra-assay (n = 10)* | | |
| 0.5 | 0.48 ± 0.03 | 6.0 |
| 2.5 | 2.48 ± 0.10 | 4.0 |
| 10 | 9.90 ± 0.19 | 1.9 |
| 20 | 19.89 ± 0.24 | 1.2 |
| *Inter-assay (n = 10)* | | |
| 0.5 | 0.47 ± 0.04 | 8.0 |
| 2.5 | 2.45 ± 0.13 | 5.3 |
| 10 | 9.88 ± 0.24 | 2.4 |
| 20 | 19.85 ± 0.28 | 1.4 |

[a] C.V. (%) = (standard deviation/mean) × 100.

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