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Short communication

Determination of acyclovir in human serum by high-performance liquid chromatography using liquid–liquid extraction and its application in pharmacokinetic studies

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Abstract

A fast, simple and sensitive high performance liquid chromatographic (HPLC) method has been described for determination of acyclovir in human serum. Since acyclovir is a polar compound and soluble in aqueous medium and practically insoluble in most of organic solvents, its analysis in biological fluids in currently published HPLC methods, involve pre-treatment of acyclovir plasma sample including deproteinization or solid phase extraction. In present method liquid–liquid extraction of acyclovir and internal standard (vanillin) is achieved using dichloromethane-isopropyl alcohol (1:1, v/v) as an extracting solvent. Analysis was carried out on ODS column using methanol-phosphate buffer (0.05 M) containing sodium dodecyl sulfate (200 mg/L) and triethylamine (2 mL/L, v/v) as mobile phase (pH = 2.3; 5:95, v/v) at flow rate of 2 ml/min. The method was shown to be selective and linear into the concentration range of 10–2560 ng/mL. Accuracy and precision of the method were also studied. The limit of quantitation was evaluated to be 10 ng/mL. This method was applied in bioequivalence study of two different acyclovir preparations after administration of 400 mg in 12 healthy volunteers.

Keywords: Reverse phase chromatography; HPLC; Acyclovir; Serum; Bioequivalence study

1. Introduction

Acyclovir, 9-[(2-hydroxyethoxy)-methyl]methyl]-guanosine, is an acyclic guanosine derivative which exhibits a selective inhibition of herpes viruses replication with potent clinical antiviral activity against the herpes simplex and varicella-zoster viruses [1,2]. As acyclovir is structurally similar to endogenous substances, its analysis in human serum is complicated and requires high selective analytical methods. Immunological techniques [3–5] and HPLC are the most common used methods for determination of acyclovir in biological samples. Radioimmunoassay methods are sensitive but require antiserum or monoclonal antibodies development and are expensive. Several HPLC methods [6–21] have been published for determination of acyclovir in human serum using UV or fluorescence detection. Since acyclovir is a polar compound and soluble in aqueous medium and practically insoluble in most of organic solvents, protein precipitation with perchloric acid [6–8,12,16,17] or solid phase extraction [9–11,14,15,20] are applied for pre-treatment of the drug in serum samples. While the sensitivity of analysis is significantly reduced due to dilution of the samples after deproteinization, injection of the acid supernatant after precipitation of proteins by perchloric acid leads to numerous late-eluting peaks and significant reduction of the lifetime of analytical column. Time consuming gradient elution is needed for removing of the late-eluting peaks, and deterioration of column performance significantly reduces the number of samples which can be analyzed. Solid phase extraction is expensive and moreover about 1 ml of solvent is required for elution of the drug from the cartridges. How-

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ever, in solid phase extraction methods, the drug is eluted by application of aqueous solvents. As these solvents cannot easily be evaporated, dilution of the samples reduces the method sensitivity. Different limits of quantification (LOQ) ranging from 10 to 200 ng/mL of serum have been reported in published methods, however; LOQ of less than 50 ng/mL has been achieved in these methods by either increasing the injection volume [6,8,9,11,13] and/or application of highly acidic mobile phase [12] and at the expense of rapid deterioration of the analytical column. Limit of quantification of 10 ng/mL has been obtained in method described by Swart et al. [9] using specific polymeric Oasis^{TM} extraction column and injection volume of 130 μl. However, the corresponding recovery of acyclovir in this method in which calibration curves were linear between the ranges of 5–1200 ng/mL was less than 50%. Their extraction procedure needs to expensive specific polymeric Oasis^{TM} extraction column and is more time consuming than the deproteinization technique. A protein precipitation method (8) with LOQ of 20 ng/mL and injection volume of 100 μl has also been reported. However, this method involves long retention time of acyclovir and insufficient sensitivity for analysis of the drug up to 24 h following single dose bioequivalence studies.

Present paper describes a simple, economic and yet sensitive HPLC method for determination of acyclovir in human serum using liquid–liquid extraction. This method in which the LOQ has been improved without the using of highly acidic mobile phase or large injection volume has been applied in a bioequivalence study.

2. Experimental

2.1. Reagent and chemicals

Acyclovir was from Sigma (Sigma, St. Louis, MO, USA) and kindly provided by Bakhtar Bioshimi pharmaceutical company (Kermanshah, Iran). HPLC-grade methanol, monobasic sodium phosphate, phosphoric acid, triethylamine, sodium dodecyl sulfate, vanillin (I.S.), 2-propanol and dichloromethane purchased from Merck (Darmstadt, Germany). Water was glass-double distilled and further purified for HPLC with a maxima purification system (USF ELGA, England).

2.2. Preparation of standard solutions

Stock solutions of acyclovir (200 μg/ml) and vanillin (25 μg/ml) were prepared by dissolving the drug in deionized water and stored in a refrigerator at 4 °C. It remained stable for at least one month. Acyclovir stock solution was further diluted with methanol to obtain the different working solutions ranging from 10 ng/mL to 2560 ng/mL.

For preparation of serum calibration curves samples, 100 μl each of working standard acyclovir solutions within the concentration range of 10–2560 ng/ml were evaporated in glass tubes (16 mm × 100 mm) under gentle stream of nitrogen at 50 °C, after addition of 1 ml human blank serum and mixing for 10 s on a vortex mixer, the samples were subjected to extraction and analysis.

2.3. Apparatus and chromatographic condition

The chromatographic analysis performed on a LC-10A (Shimadzu, Kyoto, Japan). System consisted of two pumps (LC-10A), a system controller (SCL 10AD), a UV–VIS spectrophotometric detector operated at 250 nm (SPD-10A), and rheodyne injection valve with a 20 μl filling loop, a column oven (CTO-10A) set at 62 °C, a degasser (DGU-3A) and a data processor (C-R4A) all from Shimadzu, Kyoto, Japan. The analysis was performed on a reverse phase column (150 mm × 6 mm i.d.) which was packed with 5 μm particles of ODS packing material (Shimpack-CLC-ODS). A guard column (4.0 mm i.d. × 1 cm; Shim-pack G-ODS, Shimadzu Japan) packed with the same packing material was placed before the inlet of the analytical column. The mixture of methanol-phosphate buffer (0.05 M) containing sodium dodecyl sulfate (200 mg/L) and triethylamine (2 mL/L, v/v) was used as mobile phase (pH = 2.3; 5:95, v/v). The eluent was filtered through a 0.45 μm filter (Milipore, Bedford, MA, USA), degassed and pumped at flow rate of 2 ml/min.

2.4. Sample preparation

To 1 ml serum samples in glass tubes (16 mm × 100 mm) 100 μl of the I.S. (25 μg/ml) and 5 ml dichloromethane-isopropyl alcohol (1–1, v/v) were added. After mixing for 30 s on a vortex mixer and centrifugation (5 min at 6000 × *g*), the organic phase was removed and evaporated to dryness under stream of nitrogen at 50 °C. The residue was reconstituted with 100 μl of water, syringe filtered using 0.45 μm syringe filter and a volume of 20 μl was injected into the HPLC system.

2.5. Calibration and method validation

Serum samples obtained from healthy volunteers were used for method validation and linearity studies. Calibration curves (unweighted regression line) were obtained by linear least-squares regression analysis plotting of peak-area ratios (acyclovir/I.S.) versus the acyclovir concentrations. The presence of disturbing endogenous peaks was examined on twelve human serum samples from different volunteers. These samples were pretreated according to the sample preparation procedure except from the addition of the I.S. The recoveries of acyclovir at the concentration range of calibration curve as well as the I.S. at applied concentration were calculated by comparing peak areas obtained after extraction of known amounts of acyclovir from serum, with peak areas obtained from the same amounts of unextracted acyclovir. Intra and inter day variations were determined by repeated analysis ($n = 6$) of different acyclovir concentrations within the range