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Short Communication

High-performance liquid chromatographic determination of ganciclovir in plasma

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ABSTRACT

A rapid, selective and sensitive isocratic reversed-phase high-performance liquid chromatographic method for the determination of ganciclovir in plasma samples was developed. This method, which was applied to the analysis of plasma ganciclovir from heart transplant patients under ganciclovir therapy for cytomegalovirus infections, represents a suitable analytical tool for drug monitoring and pharmacokinetic investigations.

INTRODUCTION

Ganciclovir, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, also known as DHPG, is a potent antiviral drug that has shown activity against herpes viruses, including Epstein–Barr and cytomegalovirus [1,2]. Ganciclovir is an effective drug, which should be considered as a first-line therapy in immunocompromised patients with cytomegalovirus infections [3–6].

Some high-performance liquid chromatographic (HPLC) methods [7–9] have been described for the analysis of ganciclovir in plasma. However, these methods appear often complicated or time-consuming, owing to the use of ion-pairing agents [8,9] or gradient elution [7].

This paper describes a rapid, selective and sensitive isocratic reversed-phase HPLC method for the determination of ganciclovir in plasma samples. This method is applied to drug monitoring in heart transplant patients under ganciclovir therapy for severe cytomegalovirus infections.

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EXPERIMENTAL

Reagents

Ganciclovir was purchased from Syntex (Paris, France). 9-Methylxanthine was obtained from Fluka (Buchs, Switzerland). Potassium dihydrogenphosphate, orthophosphoric acid and perchloric acid were obtained from Merck (Nogent-sur-Marne, France).

Apparatus and chromatographic conditions

The chromatographic apparatus consisted of a Model 420 pump equipped with a Model 430 variable-wavelength detector (all from Kontron, St. Quentin-Yvelines, France) and a D-2000 chromato-integrator (Merck). The column (150 mm × 4.6 mm I.D.) was packed with Hypersil ODS 3 μm (Touzart et Matignon, Vitry, France). The mobile phase consisted of 0.02 *M* potassium dihydrogenphosphate, the pH of which was adjusted to 5.25 with potassium hydroxide. The flow-rate was 1.5 ml/min, and the detection wavelength was 254 nm. The analyses were performed at ambient temperature.

Sample collection and treatment

Blood samples were collected in heparinized tubes and centrifuged without delay at low temperature (4°C). Plasma was decanted and stored at −20°C until analysis. Plasma samples (500 μl) were spiked with the internal standard (9-methylxanthine, 4 mg/l) and deproteinized with 50 μl of 35% perchloric acid. The deproteinized samples were centrifuged at 2000 *g* for 15 min at 4°C. Then, the supernatants were removed and 20-μl aliquots were injected on to the column.

RESULTS AND DISCUSSION

Ganciclovir is a purine nucleoside analogue with amino and hydroxyl groups, thus its retention in HPLC can be influenced by the pH and the ionic strength of the mobile phase.

The retention of ganciclovir does not change significantly when the pH of the mobile phase is between 3.5 and 7.0. In this pH range, ganciclovir is in its neutral form according to its pK values (pK_a = 9.4, pK_b = 2.2), so it can be analysed by reversed-phase HPLC. The separation of ganciclovir and 9-methylxanthine (internal standard) can be obtained without interference from endogenous compounds at pH 5.25. At the pH considered, the ionic strength of the buffer over the range 0.01–0.10 *M* does not significantly influence the retention of the compound.

Analytical recoveries for ganciclovir and the internal standard were determined by comparing the peak heights obtained after plasma deproteinization with those obtained by direct injection of aqueous standards. Mean recoveries at concentrations of 1.0 and 7.5 mg/l are given in Table I. The relationship between the concentration and the peak height of ganciclovir and of 9-methylxanthine was

TABLE I

ANALYTICAL RECOVERIES OF GANCICLOVIR AND INTERNAL STANDARD FROM SPIKED PLASMA

Compound	Concentration (mg/l)	Recovery (%)	C.V. (n = 5) (%)
Ganciclovir	1.0	102.6	4.7
	7.5	101.2	1.3
9-Methylxanthine	4.0	98.5	2.8

linear up to 30 mg/l. The minimum detectable amount, defined as a signal-to-noise ratio of 4, was found to be 0.5 ng for ganciclovir. The intra-assay and inter-assay precision and accuracy determined by replicate analysis of plasma samples spiked with ganciclovir are given in Table II.

Under these conditions, the Hypersil ODS column has a long lifetime: *ca.* 600 samples were injected without any deterioration. There was no interference with the compounds of interest by related endogenous compounds, such as uric acid, hypoxanthine, xanthine, guanine and guanosine, or by other drugs, such as acyclovir, allopurinol, oxypurinol, 6-mercaptopurine, azathioprine, caffeine and theophylline.

The method was applied to the analysis of ganciclovir in plasma samples from patients undergoing ganciclovir therapy for severe cytomegalovirus infection. Typical chromatograms of a blank plasma and a plasma sample from a patient who twice daily received 5 mg/kg ganciclovir as a 1-h intravenous infusion are shown in Fig. 1.

The method described is rapid and simple compared with previous HPLC

TABLE II

PRECISION AND ACCURACY

Concentration added (mg/l)	Concentration found (mg/l)	C.V. (%)
Intra-assay (n = 10)		
1.0	1.01	2.7
7.5	7.48	2.8
Inter-assay (n = 10)		
1.0	1.00	3.5
7.5	7.46	2.4

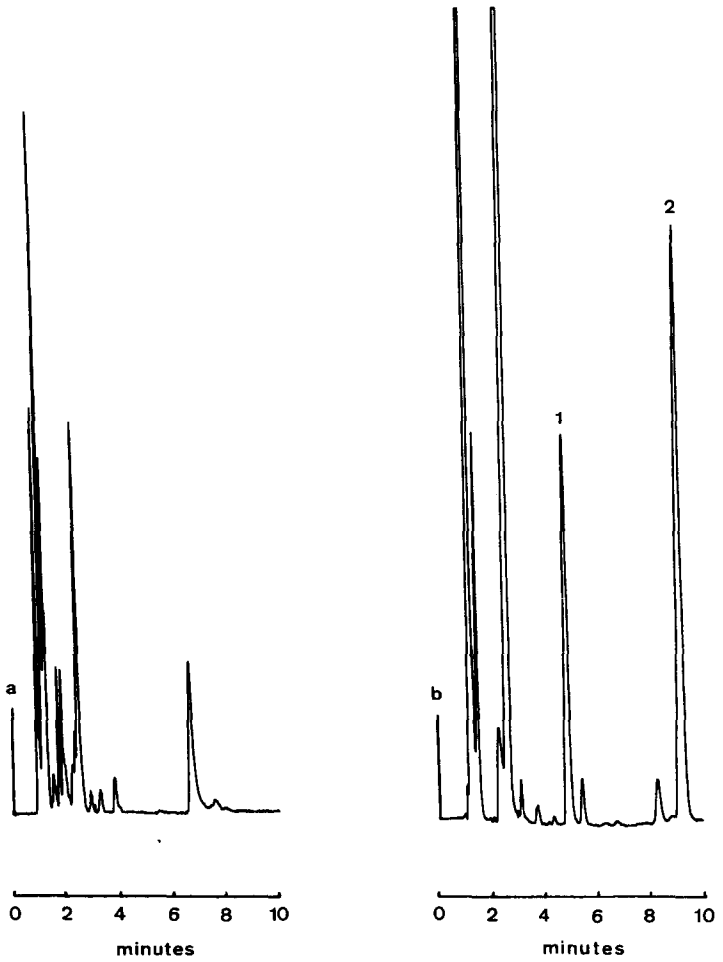

Fig. 1. Chromatograms of (a) a blank plasma and (b) a plasma sample collected 30 min after the end of an intravenous infusion of 5 mg/kg ganciclovir from a heart transplant patient under ganciclovir therapy. Injection volume, 20 μl. Peaks: 1 = 9-methylxanthine (4 mg/l); 2 = gangiclovir (6.1 mg/l).

methods [7–9], which used gradient elution or ion-pairing agents. In contrast with other published methods, we used an internal standard, 9-methylxanthine, a purine derivative never found in biological samples. Furthermore the high detection limit of the method allows the determination of very low plasma levels of ganciclovir with only 20 μl of deproteinized sample injected on to the column. In conclusion, the HPLC method we have developed is a suitable analytical tool for drug monitoring and pharmacokinetic investigation in patients under ganciclovir therapy.

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