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Short Communication

Determination of serum zidovudine by ultrafiltration and high-performance liquid chromatography

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Introduction

Zidovudine (3-azido-3-deoxythymidine, AZT), a thymidine analogue, is the most common medicament in the treatment of AIDS and AIDS-related complex. The clinical efficacy of zidovudine against AIDS, its pharmacokinetics and bioavailability in humans have been well established [1, 2]. However, zidovudine displays limiting toxic effects at the level of the bone marrow [3, 4]. To minimize toxic side effects an accurate and specific method for monitoring the concentration of zidovudine in serum is required. Several HPLC zidovudine methods had been published but either long analysis times, complicated column switching methods [5], solid-phase [5–7] or liquid–liquid extraction [8] procedures or protein precipitation steps [5, 9] for sample pre-treatment were required. In this paper a simple, rapid and sensitive HPLC method for the determination of zidovudine in serum using ultrafiltration as the pre-treatment step is described.

Experimental

Materials

Zidovudine was a generous gift from the Wellcome Company (Burgwedel, Germany). The internal standard adenine was purchased from Aldrich Chemicals (Steinheim, Germany), 1-octanesulphonic acid from Fluka (Neu-Ulm, Germany), the ultrafiltraiton units Centrisart I (2.5 ml, 5000 dalton) from Sartorius AG (Göttingen, Germany). All other chemicals were of analytical grade. The 1 mg ml^{-1} stock solutions of zidovudine and adenine in methanol were stable for at least 1 month. The working solutions were prepared daily.

Sample preparation

Before sample preparation, serum samples were heat inactivated for 1 h at 56°C in order to avoid risk of infection. Prior to ultrafiltration, 500 μl of serum and 50 μl of internal standard solution (adenine 100 mg l^{-1}) were mixed. The ultrafiltration was performed according to the manufacturer's recommendations. The obtained ultrafiltrate was diluted 1:20 with HPLC eluent buffer and 50 μl were injected for analysis. Standards were prepared by adding known amounts of zidovudine to blank human serum.

Chromatography

The LC system consisted of a L-6000 pump, a L-4250 UV–vis detector, a Lichrospher RP8e column (reversed-phase, endcapped, 125 × 4 mm i.d.) of 5 μm size and a D-2500 integrator (Merck, Darmstadt, Germany). The mobile phase consisted of methanol–octanesulphonic acid (50 mM) in phosphate buffer (pH 2.3, 0.1 M; 20:80, v/v). The flow rate was 1.0 ml min^{-1}, the column temperature ambient and the effluent was monitored at 268 nm.

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Results and Discussion

Sample preparation and chromatography

Zidovudine is a rather polar compound with low extraction capacities in organic solvents [10]. Several sample preparation methods involving protein precipitation by acetonitrile [9] or perchloric acid [5], solid-phase one- or two-step extraction procedures [6, 7] were applied for zidovudine enrichment from biological specimens. These procedures were either time consuming or impurity problems in the chromatogram were observed due to other co-extracted unidentified components. To overcome these problems a simple ultrafiltration technique was applied, taking advantage of the low protein binding of zidovudine and of its low molecular weight.

The chromatograms obtained using this simple and rapid ultrafiltration pre-treatment technique of human blank and spiked zidovudine serum samples revealed no interference by endogenous compounds [Fig. 1(A,B)]. The retention time of the internal standard adenine and zidovudine was 3.28 and 6.46 min, respectively. This technique was applied to serum samples of AIDS patients who had been administered zidovudine in order to verify this method. No interferences were found in the analysis of zidovudine [Fig. 1(C)].

Analytical validation

The recovery of this method was in the range of 98–100% for the tested concentrations of 1, 5 and 25 mg l^{-1}. Standard curves obtained during the analysis of zidovudine were linear up to 100 mg l^{-1} and, consequently, were linear over the range of concentrations observed clinically during the therapy with zidovudine. The mean calibration curve (n = 5) was $y = 0.084x + 0.01$, where y = peak-height ratio and x = sample concentration ($r > 0.990$). The therapeutic range of zidovudine is considered to be 0.1–15 mg l^{-1}. Under the assay conditions a detection limit of 0.05 mg l^{-1} was achieved with a signal to noise ratio of five for the 1:20 dilution of the ultrafilitrate. The detection limit could be further increased by a lower dilution factor of the ultrafiltrate.

Zidovudine possesses satisfactory chemical stability. The stability of zidovudine at 56 and −20°C has already been described [6]. The heat inactivation step is necessary to avoid risk of infection. Additionally, the stability of serum specimens containing zidovudine was demonstrated at room temperature and 4°C. No decomposition of zidovudine was observed if serum specimens were stored for 1 week under these conditions. The ultrafiltrate obtained after sample preparation showed the same stability.

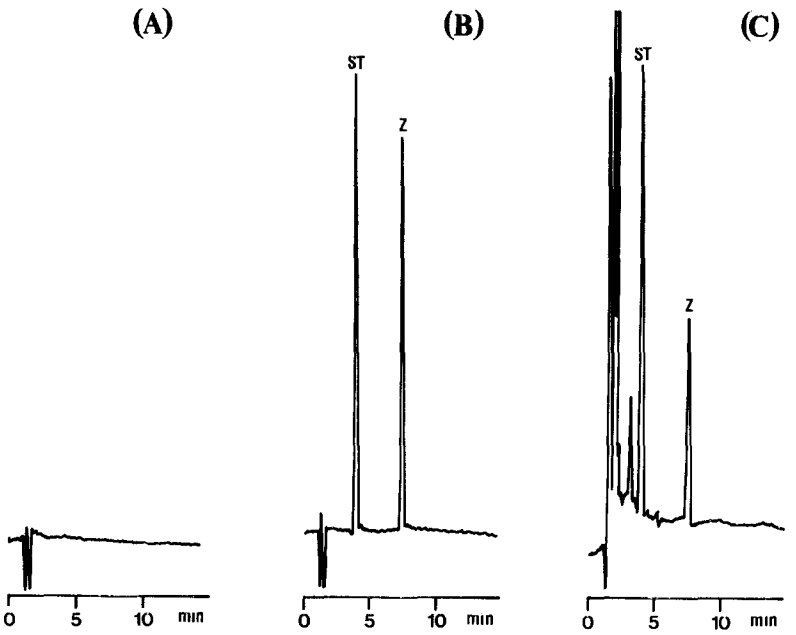

Figure 1
Typical chromatograms for the analysis of zidovudine (Z) in human serum. (A) Blank human serum; (B) blank human serum spiked with 10 mg l^{-1} zidovudine and internal standard (ST); and (C) patient serum sample containing 5.3 mg l^{-1} zidovudine.

Table 1
Intra-day and inter-day precision data for the determination of zidovudine in human serum

Nominal concentration of zidovudine ($mg.l^{-1}$)	Intra-day precision* (n = 10)		Inter-day precision† (n = 10)	
	Measured concentration ± SD	% RSD	Measured concentration ± SD	% RSD
2.0	1.89 ± 0.13	6.9	1.85 ± 0.15	8.1
5.0	4.84 ± 0.18	3.7	4.75 ± 0.20	4.3
10.0	9.70 ± 0.22	2.3	9.61 ± 0.31	3.2

* Analysed on the same day.
† Analysed on 10 different days during 5 weeks.
SD, standard deviation; RSD, relative standard deviation.

The overall reproducibility of the method was excellent. The intra-day precision was 2.3–6.9% and the inter-day precision was 3.2–8.1% for this described method (Table 1).

Conclusion

A simple, sensitive, rapid and specific HPLC method for the reliable determination of zidovudine in serum samples in a wide range of clinical situations is described.

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