Journal of Chromatography, 430 (1988) 420–423
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4251

Note

Simple and rapid high-performance liquid chromatographic assay for zidovudine (azidothymidine) in plasma and urine

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(First received February 10th, 1988; revised manuscript received April 6th, 1988)

Zidovudine (ZDV or azidothymidine) has been shown to be efficacious in the treatment of the acquired immunodeficiency syndrome (AIDS) [1]. ZDV is rapidly eliminated from the body by metabolic conjugation to the glucuronide (about 75% of the dose) and by excretion of the unchanged drug in the urine (about 20% of the dose) [2]. Only the unchanged drug is thought to be the precursor of the active species, zidovudine triphosphate.

The hematological toxicity of ZDV, which has been found to be both dose-dependent and reversible, is of serious concern [3]. For this reason it is likely that plasma concentration of ZDV may, in the future, be monitored in order to individualize drug dosage regimen. Also, animal models are likely to be developed to elucidate the mechanisms of toxicity and the basis for its interaction with other drugs. A high-performance liquid chromatographic (HPLC) method to measure both ZDV and its glucuronide has recently been published in an abstract form [4]. This method uses a solid-phase extraction system which allows quantitation of the major metabolite, the glucuronide, and ZDV. However, when measurement of the glucuronide is not of interest, a simpler HPLC method for measurement of ZDV will suffice. For this reason we have developed a rapid and simple HPLC system for determination of ZDV in both plasma and urine.

EXPERIMENTAL

Sample preparation

Plasma. To a round-bottom screw-cap glass tube (12 cm) were added 25–450 μl of rat plasma, 50 μl of internal standard (I.S.) solution (*p*-hydroxy-

0378-4347/88/$03.50

phenobarbital; Alltech, State College, PA, U.S.A.; 5.0 μg/ml) and an appropriate volume of deionized water so that the total volume of the sample was 500 μl. The sample was then vortexed and extracted with 4 ml of an ethyl acetate–diethyl ether mixture (50:50, v/v) (Baker, Phillipsburg, NJ, U.S.A.) by mixing gently on a tumble mixer for 20 min. After centrifugation (~1000 *g*) for 5 min, the organic layer was transferred to a conical glass tube (15 cm) and dried under a gentle stream of nitrogen at 40°C. The residue was then reconstituted in 300 μl of mobile phase (see below), vortexed, and the entire contents were injected onto the HPLC system.

Urine. Rat urine was first diluted with deionized water, the I.S. (*p*-hydroxyphenobarbital) added (5 μg/ml), and 300 μl of the final mixture were injected directly onto the HPLC system.

Chromatography

The HPLC system consisted of a solvent delivering system (Model 6000A, Waters, Milford, MA, U.S.A.), an injector with a 200-μl loop (Rheodyne, Cotati, CA, U.S.A.), a 10 cm × 0.46 cm precolumn (Co:Pell ODS, 30–38 μm) (Whatman, Clifton, NJ, U.S.A.), and a 25 cm × 0.46 cm C_{18} column (Econosil, 10 μm) (Alltech) connected to a variable-wavelength UV detector (Model 481, Waters). The mobile phase consisted of acetonitrile (HPLC grade; Baker)–0.025 *M* potassium dihydrogenphosphate. (Mallinkrodt, Paris, KY, U.S.A.) (pH adjusted to 2.2) (20:80, v/v). The assay was performed at ambient temperature at a flow-rate of 1 ml/min and ZDV and I.S. were detected at 266 nm. Calibration curves for plasma analysis [peak-height ratio of ZDV to I.S. versus amount of ZDV (ng)] were obtained by analyzing 4% bovine serum albumin spiked with known amounts of ZDV (supplied by Burroughs Wellcome, Research Triangle Park, NC, U.S.A.) and the I.S. The ZDV concentration in unknown plasma samples was calculated by using the calibration equation obtained using weighted (weights = $1/y^2$) linear regression. Calibration curves for urine samples were established by directly injecting deionized water spiked with known amounts of ZDV and I.S. (5 μg/ml *p*-hydroxyphenobarbital).

RESULTS AND DISCUSSION

ZDV and the I.S. elute at 10 and 12 min, respectively (Fig. 1). Chromatograms of both blank rat plasma and diluted (1:1000) blank rat urine indicate no interfering peaks where ZDV and the I.S. are expected to elute (Fig. 1). The plasma calibration curves (peak-height ratio versus amount of ZDV per sample) are linear over the range of 12.5–150 ng of ZDV per sample [$y = 0.0179(\pm 0.0007)x + 0.016(\pm 0.035)$]. The average recovery of ZDV from liquid extraction of plasma is about 65%. Using a more polar extraction mixture, for example 100% ethyl acetate, improved the recovery of ZDV but produced extraneous interfering peaks. ZDV plasma concentration as low as 12.5 ng/ml can be quantitated using this method. Bovine serum albumin (4%) was used as a substitute matrix for all calibration runs since the mean (±S.D.) of the slope and intercept of the calibration curves obtained with 4% bovine serum albumin [slope 0.0173 (±0.0005); intercept 0.081 (±0.063)] was not significantly differ-

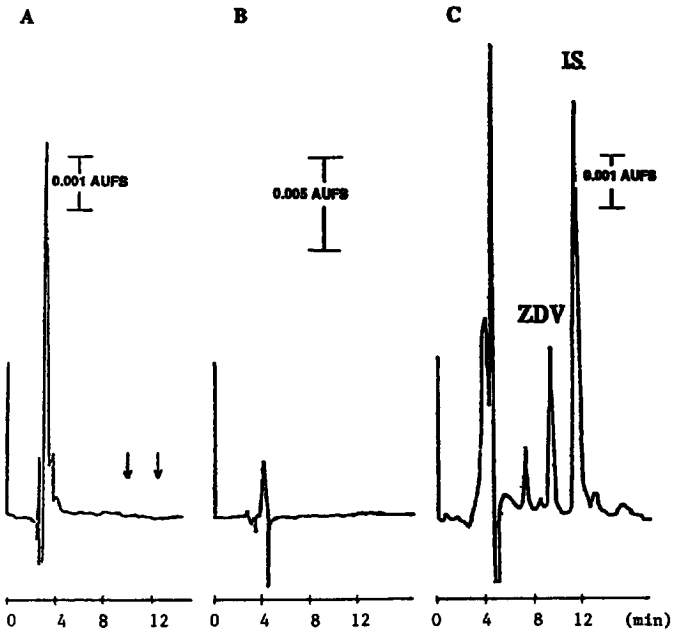

Fig. 1. Chromatograms of (A) extracted blank rat plasma, (B) diluted blank rat urine, and (C) extracted plasma sample obtained at 240 min after administration of 40 mg/kg ZDV to a male Sprague-Dawley rat. The arrows indicate the positions where ZDV and I.S. peaks are expected to elute. For chromatographic conditions see details under Experimental.

TABLE I

WITHIN-DAY COEFFICIENTS OF VARIATION OF THE ASSAY DETERMINED BY ASSAYING BOVINE SERUM ALBUMIN (500 μl) SPIKED WITH DIFFERENT AMOUNTS OF ZDV

Amount of ZDV per sample (ng)	Coefficient of variation ($n=4$) (%)
12.5	2.34
50.0	2.9
150.0	5.59

ent ($P>0.05$) from that obtained with rat plasma [slope 0.0177 ($\pm$0.0007); intercept 0.016 ($\pm$0.035)]. Within-day precision of the assay is excellent, the largest coefficient of variation being about 5.6% (Table I). The day-to-day coefficient of variation in the slopes of the calibration curves is 2.9% indicating good day-to-day reproducibility. The accuray of the assay was determined by processing daily, as described above, samples containing known concentration of ZDV (0.3333 μg/ml) as "unknowns". The accuracy, expressed as the mean absolute percent difference between measured and actual concentrations, was found to be 7.49 ($\pm$5.81)%.

Plasma samples obtained from rats after administration of ZDV as an intravenous bolus were processed as above. A typical ZDV plasma concentration ver-

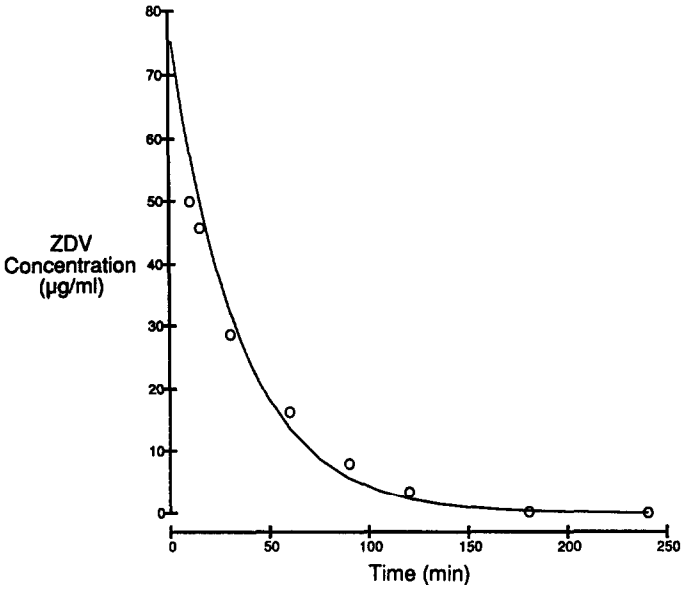

Fig. 2. ZDV plasma concentration–time profile obtained after intravenous administration of 40 mg/kg of ZDV[11] to a male Sprague–Dawley rat. The continuous line is the fit obtained with a one-compartment model.

sus time profile obtained after intravenous administration of 40 mg/kg to a male Sprague–Dawley rat is illustrated in Fig. 2. Analysis of the 24-h urine collection from this animal, after administration of the dose, indicated that the majority of the dose (77%) is recovered unchanged in the urine.

CONCLUSION

The method described here for analysis of ZDV in both rat plasma and urine is rapid, reproducible, reliable, and simple. The method should be readily adaptable for analysis of biological fluids obtained from other animals and human beings.

ACKNOWLEDGEMENTS

Helpful suggestions by Drs. Ainardi and Opheim are gratefully acknowledged. Zidovudine was supplied by Burroughs Wellcome.

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