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# Note

## Simple and rapid assay for zidovudine and zidovudine glucuronide in plasma using high-performance liquid chromatography

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Zidovudine (3′-azido-3′-deoxythymidine, Retrovir, AZT) is used for the treatment of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC). Available methods [1–3] for the measurement of zidovudine and its main metabolite, zidovudine glucuronide, by high-performance liquid chromatography (HPLC) require multi-step extraction procedures. These methods either do not allow the simultaneous quantification of zidovudine and zidovudine glucuronide [1] or the assay procedure takes a relatively long time [2]. We describe a simple and rapid method for quantification of zidovudine and zidovudine glucuronide in plasma samples by HPLC which involves protein precipitation by trichloroacetic acid followed by a 1-h incubation period at 60°C which is considered to deactivate human immunodeficiency virus (HIV) but ensuring virtually complete recovery of zidovudine and zidovudine glucuronide in the supernatant for subsequent analysis by HPLC.

## EXPERIMENTAL

### *Apparatus*

The chromatographic system employed a Waters pump (Model 510), a Waters sample processor (Model 710B), a Waters data module (Model H730), a Pye-Unicam variable-wavelength UV detector and a reversed-phase $C_{18}$ steel column (5 μm particle size, 15 cm × 4 mm I.D.; FSA Laboratory Supplies, Loughborough, U.K.). Filtration of solvents was carried out using a pyrex holder, pore size 0.45 μm (Millipore, Bedford, MA, U.S.A.).

### *Chemicals*

Zidovudine and zidovudine glucuronide were kindly donated by Wellcome

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Foundation PLC (Beckenham, U.K.). β-Hydroxyethyltheophylline (used as the internal standard) was supplied by Sigma (St. Louis, M.O., U.S.A.). Acetonitrile (HPLC grade) was supplied by FSA Laboratory Supplies. Potassium dihydrogen orthophosphate (Analar), orthophosphoric acid and trichloroacetic acid were supplied by BDH (Poole, U.K.).

*Methods*

All tests were carried out at room temperature (approximately 20°C). Solvents were mixed to the required volume and filtered through a 0.2-μm Millipore filter and the mobile phase was degassed by sonication under vacuum. The column was equilibrated with the mobile phase for at least 30 min prior to analysis of samples. The mobile phase was acetonitrile–potassium dihydrogen orthophosphate (20 mmol/l, pH 2.7) (15:85, v/v) at a flow-rate of 0.4 ml/min (pressure, 20 bar). Zidovudine, zidovudine glucuronide and β-hydroxyethyltheophylline were detected at 267 nm.

*Sample preparation*

Samples of plasma (200 μl) obtained from healthy volunteers were spiked with zidovudine and/or zidovudine glucuronide and were treated with 20% (w/v) trichloroacetic acid (50 μl) and mixed. Plasma samples from AIDS patients receiving zidovudine were treated with trichloroacetic acid in the same way and subsequently heated in a water bath for 1 h at 60°C in order to deactivate HIV [4]. Samples were later centrifuged at 2500 *g* in an Eppendorf centrifuge for 2 min. The resulting supernatant was directly injected into the HPLC apparatus for analysis.

## RESULTS

The mean recovery for zidovudine and zidovudine glucuronide in the supernatant derived from the protein precipitation of the samples of plasma with 20% (w/v) trichloroacetic acid was in excess of 97% (coefficient of variation $< 10\%$) over the range of the standard curve (0.43–97.4 μmol/l for zidovudine and 0.22–43.0 μmol/l for zidovudine glucuronide) when compared to the direct injection of standard samples dissolved in water.

The elution pattern for zidovudine and zidovudine glucuronide in spiked plasma samples is shown in Fig. 1. Changes in the pH of the mobile phase altered the retention time of zidovudine glucuronide to a much greater extent than that of zidovudine. Thus by lowering the pH from 3.2 to 2.7, the retention time for zidovudine glucuronide was increased leading to complete resolution from other peaks. The increase in the retention time of zidovudine glucuronide by lowering the pH of the mobile phase is to be expected, since its $pK_a$ is 3.5 and thus under increasing acidic conditions the compound becomes less ionized and is retained by the reversed-phase column for a longer period of time. Lowering the pH of the

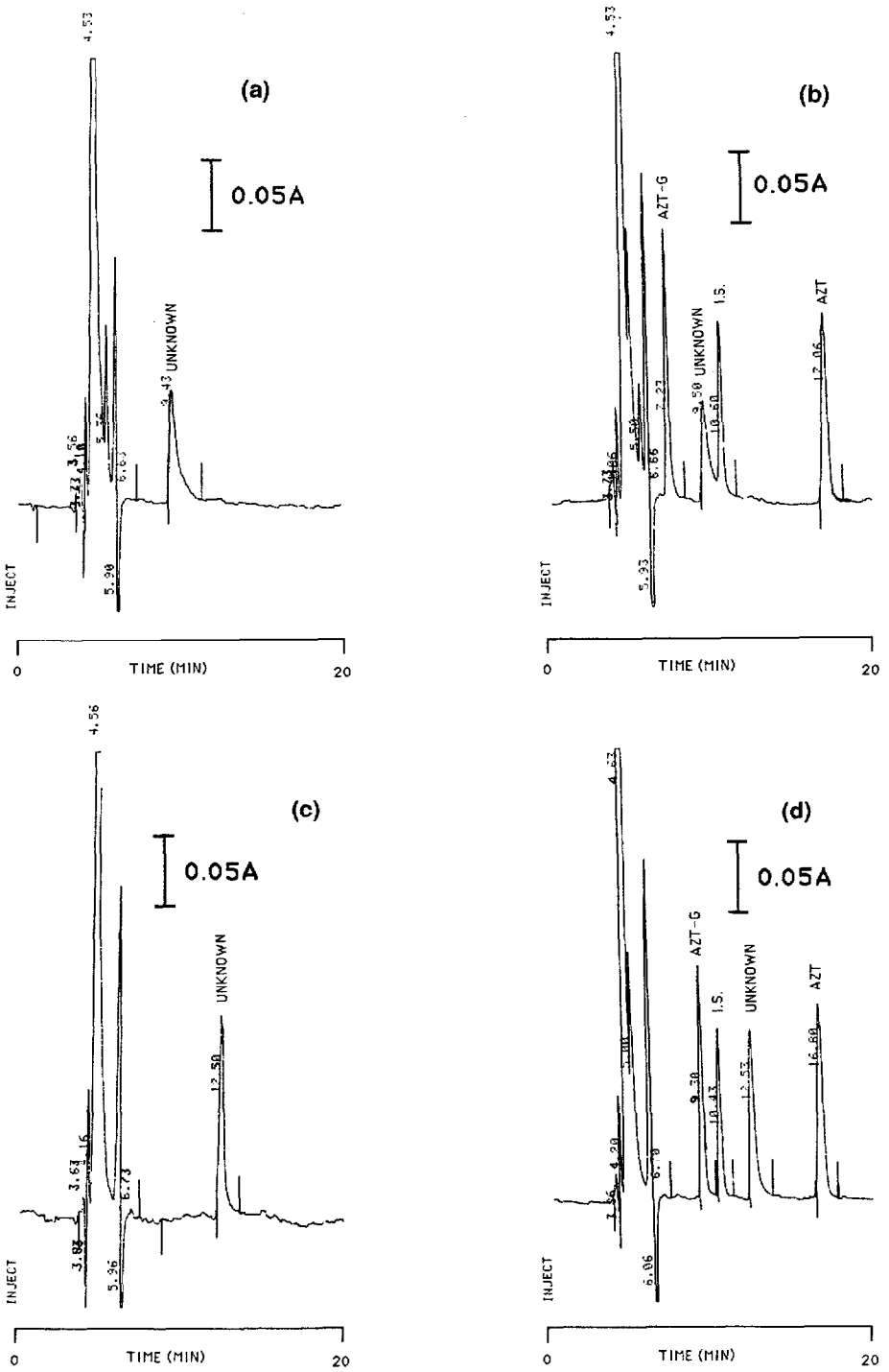


Fig. 1. Elution pattern for zidovudine (AZT) and zidovudine glucuronide (AZT-G). (a) Blank plasma with mobile phase at pH 3.2; (b) plasma spiked with AZT, AZT-G and the internal standard (I.S.) with mobile phase at pH 3.2; (c) blank plasma with mobile phase at pH 2.7; (d) plasma spiked with AZT, AZT-G and I.S. with mobile phase at pH 2.7.

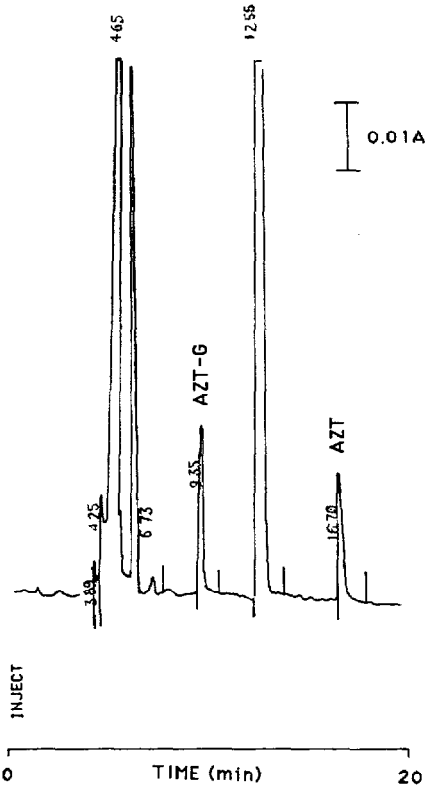


Fig. 2. Elution pattern for zidovudine (AZT) and zidovudine glucuronide (AZT-G) in a plasma sample obtained from a patient 2 h after receiving zidovudine (250 mg).

mobile phase also resulted in an increase in the retention time of an unknown peak present in all plasma samples, resulting in the complete resolution from that of the internal standard (Fig. 1). Consequently, the pH of the mobile phase was maintained at 2.7 for zidovudine and zidovudine glucuronide assays and the retention times for zidovudine and zidovudine glucuronide were 16.8 and 9.30 min, respectively. Fig. 2 shows the elution pattern for zidovudine and zidovudine glucuronide in a plasma sample obtained from an HIV seropositive patient receiving zidovudine, using the mobile phase at pH 2.7 for analysis.

The analysis of the samples showed a linear response by the detector for zidovudine and zidovudine glucuronide over the range 0.43–97.4 μmol/l for zidovudine and 0.22–43.0 μmol/l for zidovudine glucuronide. The limit of detection was 0.04 μmol/l for zidovudine and 0.02 μmol/l for zidovudine glucuronide (signal-to-noise ratio = 2). The reproducibility and precision of the method were established by determining the coefficient of variation of samples of zidovudine and zidovudine glucuronide measured within assay and between assays. The coefficient of variation of six duplicate samples was: within one day, 4.17% for 23.5 μmol/l

zidovudine and 4.81% for 10.9 μmol/l zidovudine glucoronide; between six days, 3.41% for 23.5 μmol/l zidovudine and 5.31% for 10.9 μmol/l zidovudine glucuronide.

*Stability of zidovudine and zidovudine glucuronide in plasma samples following trichloroacetic acid and heat treatment*

The stability of zidovudine and its metabolite in plasma samples treated with trichloroacetic acid and heating at 60°C, in order to inactivate HIV, was investigated. Plasma samples obtained from healthy volunteers were spiked with zidovudine (8.3 and 13.7 μmol/l, final concentration) and zidovudine glucuronide (14.5 and 25.6 μmol/l, final concentration) and treated with trichloroacetic acid as described earlier. One half was kept at room temperature and the other half was incubated for 1 h at 60°C. All the samples were later analysed by HPLC. Following trichloroacetic acid/heat-inactivation, zidovudine concentrations (mean ± S.D.) were 101.1 ± 4.5% ($n = 5$) and 99.44 ± 2.9% ($n = 5$) of corresponding levels from non-heated samples for 8.3 and 3.7 μmol/l zidovudine, respectively. Mean zidovudine glucuronide concentrations were 98.1 ± 2.1% ($n = 5$) and 98.1 ± 4.1% ($n = 5$) of non-heated samples for 14.5 and 25.6 μmol/l zidovudine glucuronide, respectively.

*Interference of other drugs with the zidovudine and zidovudine glucuronide assay*

The possibility of the presence of other drugs in HIV-infected plasma which may interfere with the zidovudine and zidovudine glucuronide assay, as a result of their concomitant administration with zidovudine to AIDS patients, was investigated. Antibacterial drugs such as ketoconazole, sulphamethoxazole, trimethoprim and fluconazole, given to AIDS patients to combat infections, were found not to interfere with the zidovudine and zidovudine glucuronide assay. The interference of mild analgesics such as aspirin and paracetamol was also investigated. Aspirin did not interfere with the assay, paracetamol (retention time = 9.75 min) was detectable by the assay system but was well resolved from that of the zidovudine glucuronide (retention time = 9.30 min) peak.

## CONCLUSION

We describe the development of a simple and rapid HPLC assay for zidovudine and zidovudine glucuronide, the major metabolite of zidovudine in man. The advantages of this method over previous methods are that it enables the simultaneous measurement of zidovudine and zidovudine glucuronide using an isocratic HPLC system which does not require multi-step extraction procedures. Instead it employs a simple method of protein precipitation using trichloroacetic acid. Following treatment of the plasma samples with trichloroacetic acid virtually all zidovudine and zidovudine glucuronide are recovered in the resulting supernatant which can be directly analysed by HPLC. Rapid inactivation of HIV

occurs when plasma samples infected with the virus are heated for 1 h at 60°C [4]. We also found that the pH of the supernatant derived from the treatment of plasma samples with trichloroacetic acid was close to unity. The incubation temperature together with the acidic environment is thought to enhance the inactivation of HIV in plasma samples treated in this way.

REFERENCES

1 R. W. Klecker, Jr., J. M. Collins, R. Yarchoan, R. Thomas, J. F. Jenkins, S. Broder and C. E. Myers, *Clin. Pharmacol. Ther.*, 41 (1987) 407.
2 M. R. Blum, S. H. T. Liao, S. S. Good and P. de Miranda, *Am. J. Med.*, 85 (Suppl. 2A) (1988) 189.
3 S. S. Good, D. J. Reynolds and P. de Miranda, *J. Chromatogr.*, 431 (1988) 123.
4 J. S. McDougal, L. S. Martin, S. P. Cort, M. Mozan and C. M. Heldebrant, *J. Clin. Invest.*, 76 (1985) 875.