CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Azidodeoxythymidine (AZT) in Serum

Purpose
Determination of AZT (F1, 3'-azido-3'-deoxythymidine) in serum.

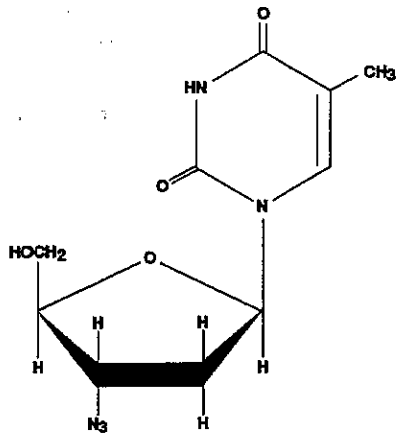

Figure 1. Structure of AZT.

AZT is an experimental antiviral drug currently being evaluated as a treatment for acquired immune deficiency syndrome (AIDS). This disease is caused by a retrovirus (HTLV-III); these RNA viruses use the enzyme reverse transcriptase to make DNA copies of their RNA. AZT has been reported to inhibit the action of reverse transcriptase, and thus may be clinically useful for the treatment of AIDS. Therapeutic concentrations of AZT in serum have not been formally established, but a target value of 1 µmol/L (267 ng/mL) has been used [1].

Existing Methods
Not available (experimental drug)

Conditions
Detector: BAS UV-108 variable wavelength UV detector (266 nm).
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Mobile Phase: 91% 0.03 M H_3PO_4, 7.5 mM KOH, pH 2.2; 9% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: 150 pg injected standard (S/N = 3), 11 ng/mL serum (S/N = 3, 0.2 mL serum sample).
Linear Range: 0.5-100 ng injected standards, 100-500 ng/mL serum.

Sample Preparation
1. Wash Bond-Elut® C_{18} solid-phase extraction columns with 1 mL methanol followed by 1 mL water.

2. Combine the following and load onto each column: 0.2 mL serum, 0.8 mL 0.05 M Na_2HPO_4 (pH 7.5), and standards as appropriate.

3. Wash each column with 1 mL of the phosphate buffer followed by 2 1-mL volumes of water.

4. Elute the samples with 1 mL methanol and dry under a stream of nitrogen or in a rotary evaporator. Redissolve in 0.2 mL mobile phase and inject 20 µL aliquots.

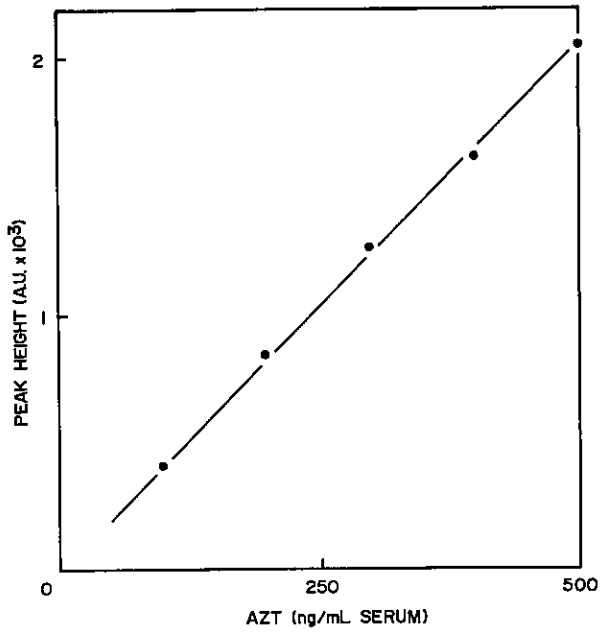

Figure 2. Calibration curve for spiked serum samples.

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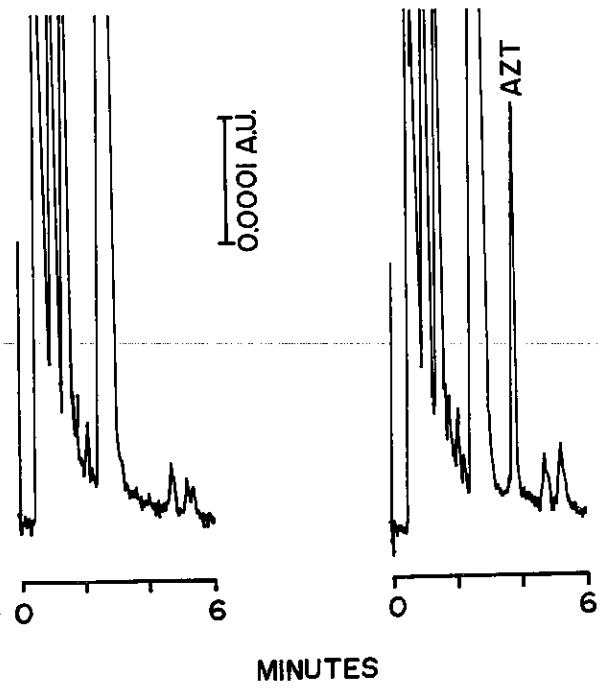

Figure 3. Sample chromatograms of blank (left) and spiked (100 ng AZT/mL) serum.

Notes

A calibration curve for spiked serum samples is presented in F2 and sample chromatograms are shown in F3.

β-hydroxypropyltheophylline and p-hydroxyphenobarbital have been used as internal standards for the determination of AZT.

Reference

1. Klecker, R.W. Jr., J.M. Collins, R. Yarchoan, R. Thomas, J.F. Jenkins, S. Broder and C.E. Myers, Clin. Pharmacol. Ther. 41 (1984) 407-411.