Waters
Lab Highlights
An Internal Communication
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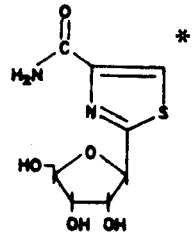
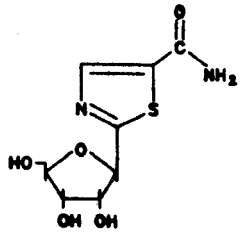
QUANTIFICATION OF TIAZOFURIN IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Tiazofurin (TCAR, 2-β-D-ribofuranosylthiazide-4-carboxamide, Figure 1), a thiazole-c-nucleoside, has shown promise as a new anticancer drug. It has demonstrated antitumor activity against several murine leukemias as well as lung carcinoma.



MW = 260.3
λ_{max} = 238 nm
(254 nm) = 4800



MW = 260.3
λ_{max} = 278 nm
(254 nm) = 2800

Fig. 1. Structures of TCAR (left) and iso-TCAR (right). The asterisk denotes the position of the tritium label on TCAR, when present.

Researchers at the National Cancer Institute have developed an assay for TCAR in serum based on sample pre-purification on SEP-PAK^R (C_{18}) cartridge followed by reversed-phase LC on Radial-PAK^{TM} C_{18} cartridges. The authors chose the radial compression column because of its greater sensitivity and increased column life compared to other LC methods.

Serum samples (0.5 ml) were loaded onto activated SEP-PAK^R cartridges and eluted with water. A TCAR-containing fraction was obtained by eluting the SEP-PAK^R cartridge with methanol. The methanol fraction was evaporated to dryness, redissolved in the mobile phase, and injected into the liquid chromatograph operated at 2 ml/min with a mobile phase of 40 mM acetic acid in 1.5% acetonitrile.

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Baseline resolution of TCAR and iso-TCAR (internal standard) in serum sample is shown in Figure 2. The minimum level of detection for TCAR was 80 ng/ml.

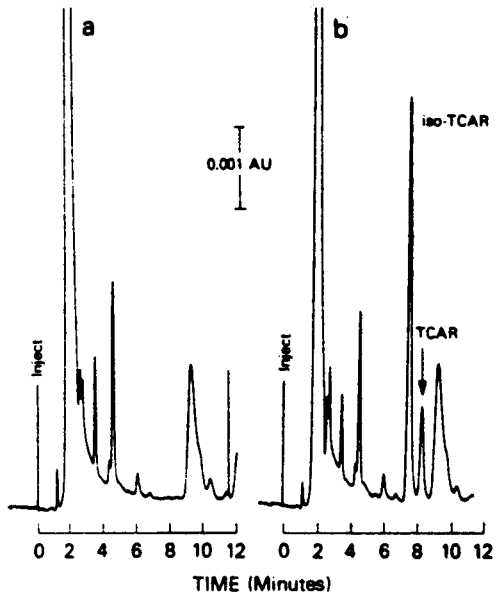

Fig. 2. Chromatograms for 0.5-ml samples of (a) normal human plasma and (b) normal human plasma with TCAR (1 μM) and iso-TCAR (10 μM). The absorbance was measured at 254 nm at an attenuation of 0.01 absorbance units (AU) full-scale. The retention times of TCAR and iso-TCAR were 7.8 min and 8.6 min, respectively. The extracted residue was redissolved in 150 μl HPLC mobile phase, and 50 μl were injected.

Application of this assay procedure to a pharmacokinetic study is shown in Figure 3.

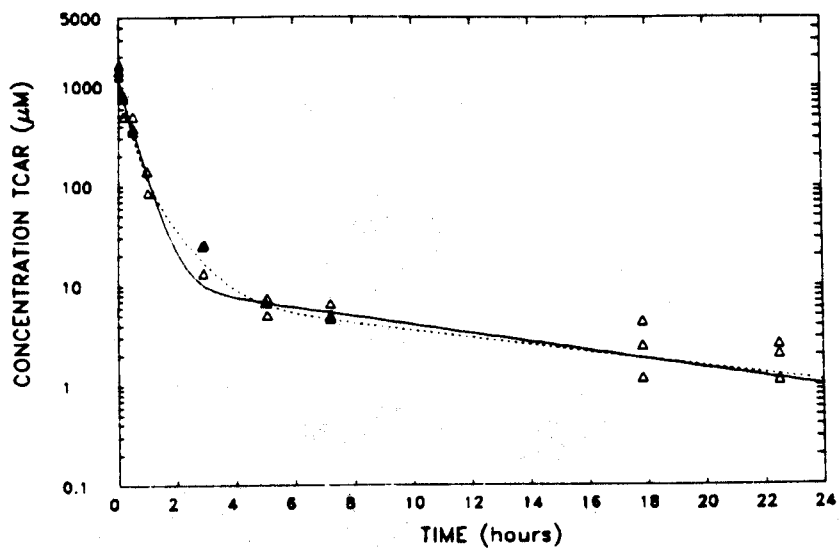

Fig. 3. The disappearance of TCAR in CDF, mouse after intravenous bolus injection (220 mg/kg). The triangles represent the measurement of plasma TCAR in a single mouse. The curves are the non-linear fits of the data for two-compartment (solid) and three-compartment (dashed) models.

1. Raymond W. Klecker and Jerry M. Collins, J. Chromatogr., 307 (1984) 361-369