Waters Lab Highlights

An Internal Communication of Applications and Techniques

LAH 0336 11/86
AN/PA/TD/DR/OT

RAPID ANALYSIS OF CAFFEINE AND METABOLITES FROM PLASMA USING RCSS

The consumption of caffeine (1,3,7-trimethylxanthine) has raised concerns in recent years regarding the health consequences of this drug. These concerns have stimulated considerable interest in assessing exposure to the methylxanthines by measuring levels in biological fluids. The increasing use of caffeine and theophylline (1,3-dimethylxanthine) in the treatment of neonatal apnoea has also focused attention on the methylxanthines. Since caffeine and the dimethylxanthines theophylline and theobromine (3,7-dimethylxanthine) are all pharmacologically active it is essential, whether in monitoring drugs in the treatment of neonatal apnoea or in evaluating the health consequences of the methylxanthines, to be able to determine caffeine and its N-dimethylated metabolites simultaneously in plasma. Several LC assays which permit the simultaneous determination of caffeine and its N-dimethylated metabolites in plasma have been developed (1-3). Hartley and coworkers (4) have reported a method which uses a Radial-Pak™ cartridge with Nova-Pak™ packing material in order to achieve a rapid separation of caffeine and its N-dimethylated metabolites and a fast extraction procedure using solid phase extraction for the plasma.

The chromatographic conditions and representative chromatograms are shown in Figure 1. This method reduces the previously reported analysis time of 10-15 minutes to 8 minutes, and the use of a variable-wavelength detector operating at 273nm allows for increased sensitivity and decreased sample volumes.

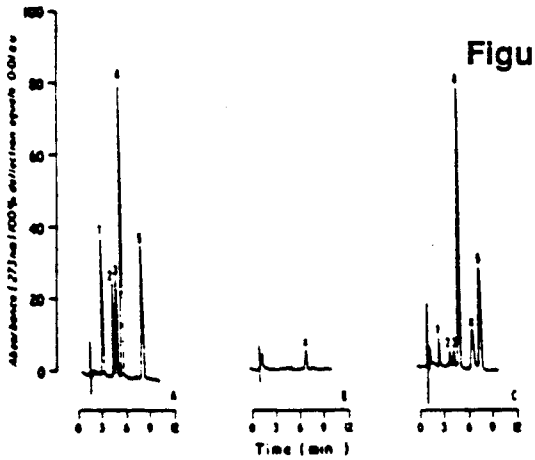

Figure 1

Column:	Radial-Pak™ Radial Compression Cartridge (8mm X 10cm)
Packing:	Nova-Pak™ C_{18}
Holder:	Z-Module™ Radial Compression Separation System
Mobile Phase:	methanol:1% acetic acid (17:83)
Flow Rate:	2.7 ml/min at 1700 psi
Detector:	M481 at 273 nm
Chart Speed:	0.33 cm/min.

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THE PERFORMANCE CHARACTERISTIC FOR THIS PROCEDURE HAS NOT BEEN ESTABLISHED.

Fig. 1. (A) Chromatogram of authentic components in 3% bovine serum albumin. (B) Chromatogram of extracted xanthine-free plasma obtained from an adult male after abstaining from caffeine containing beverages for two weeks. (C) Chromatogram of a typical extracted cord plasma sample obtained at delivery. Concentrations determined (using a 18-µl injection) were 550, 550 and 480 ng/ml and 7.80 µg/ml for theobromine, paraxanthine, theophylline and caffeine, respectively. Peaks: 1 = theobromine, 2 = paraxanthine, 3 = theophylline, 4 = β-hydroxyethyltheophylline, 5 = caffeine; X = endogenous component.

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