

Caffeine and Metabolites in Serum

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

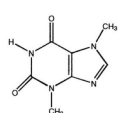
Abstract

This application brief demonstrates analysis of caffeine and metabolites in serum.

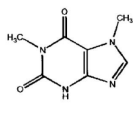
Introduction

The compounds used in this study are –

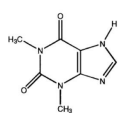
1. Theobromine
2. Paraxanthine
3. Theophylline
4. β -hydroxyethyltheophylline (I.S.)
5. Caffeine



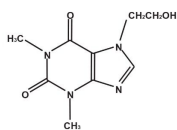
1. Theobromine



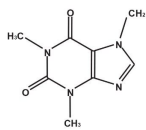
2. Paraxanthine



3. Theophylline



4. β -hydroxyethyltheophylline (I.S.)



5. Caffeine

Experimental

HPLC Method

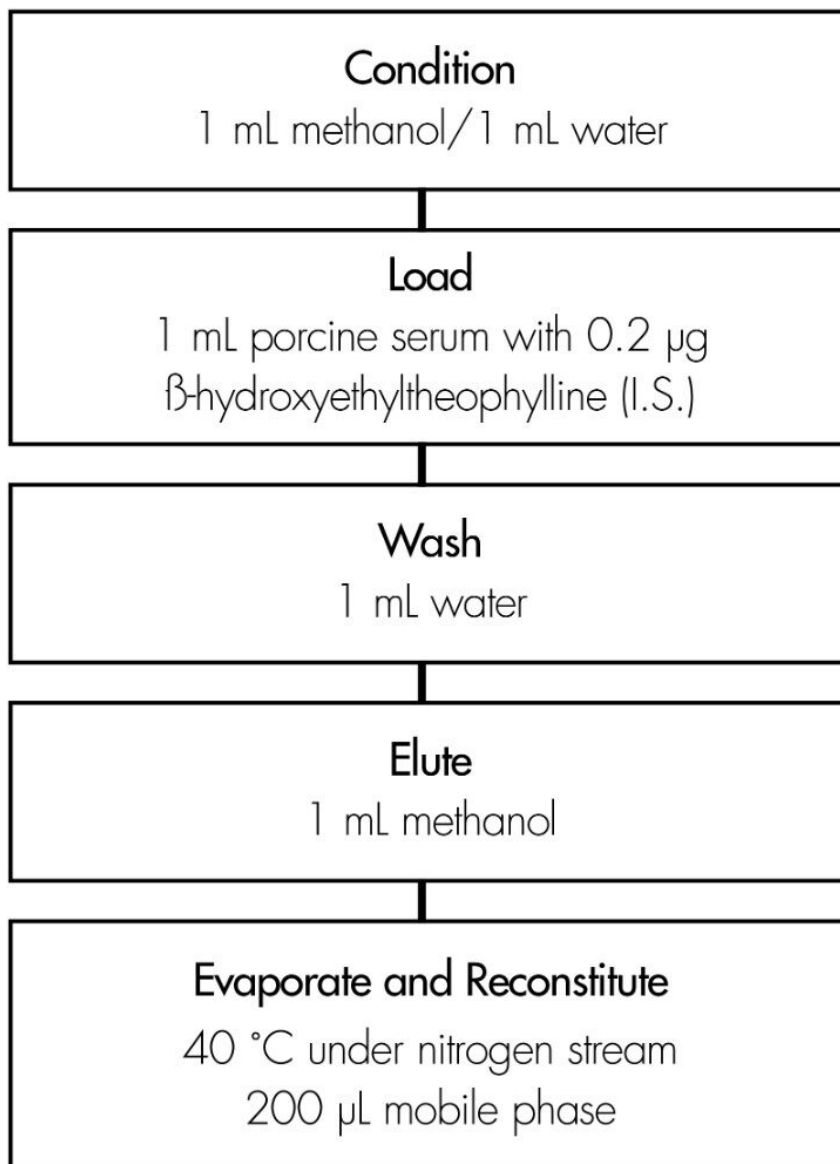
Column:	Symmetry Shield RP ₈ , 3.0 x 150 mm, 5 μ m
Guard column:	Sentry Guard Column, 3.9 x 20 mm, 5 μ m
Part number:	Column - WAT094243, Guard - WAT200675
Mobile phase:	20 mM ammonium acetate, pH 5/acetonitrile, 95:5

Flow rate:	0.6 mL/min
Injection volume:	20 μ L of reconstituted porcine serum extract
Temperature:	25 $^{\circ}$ C
Detection:	UV @ 273 nm

Oasis[®] HLB Extraction Method

Oasis[®] HLB 1 cc/30 mg Extraction Cartridge

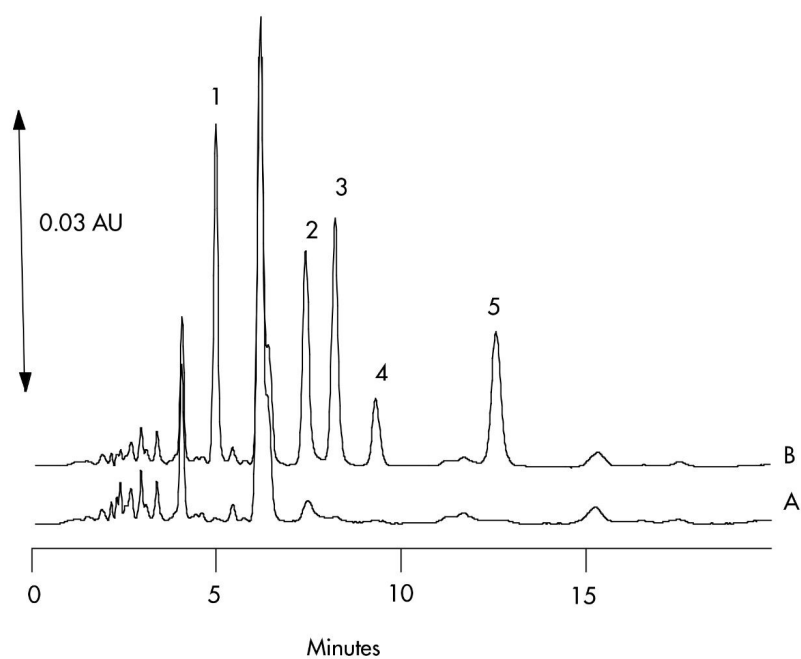
Part Number WAT094225



Results and Discussion

Compound	Concentration µg/mL	% Recovery	%RSD (n-6)
Theobromine	0.100	103.0%	3.4%
	0.500	104.0%	2.4%
Paraxanthine	0.100	101.0%	6.2%
	0.500	98.9%	2.3%
Theophylline	0.100	100.0%	5.2%
	0.500	101.0%	2.1%
Caffeine	0.100	92.6%	4.4%
	0.500	97.5%	3.2%

Chromatogram of Serum Extracts: A) Blank B) Spiked Sample



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WA31763.45, June 2003

