

Caffeine and Metabolites in Serum

Waters Corporation



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

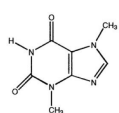
Abstract

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

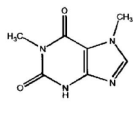
Introduction

The compounds used in this study are –

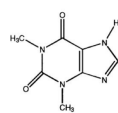
1. Theobromine
2. Paraxanthine
3. Theophylline
4. β -hydroxyethyltheophylline (I.S.)
5. Caffeine



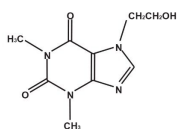
1. Theobromine



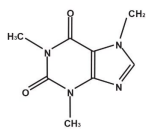
2. Paraxanthine



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:

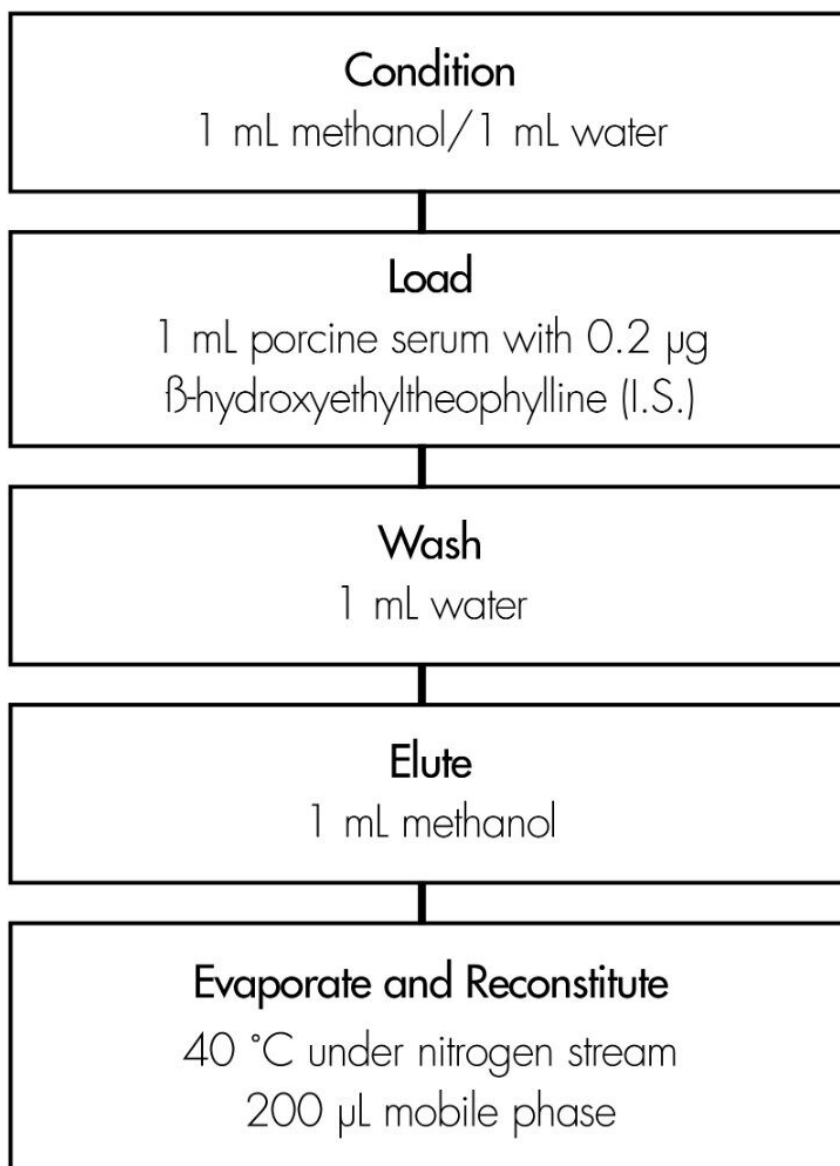
25 °C

Detection:

UV @ 273 nm

Oasis® HLB Extraction Method

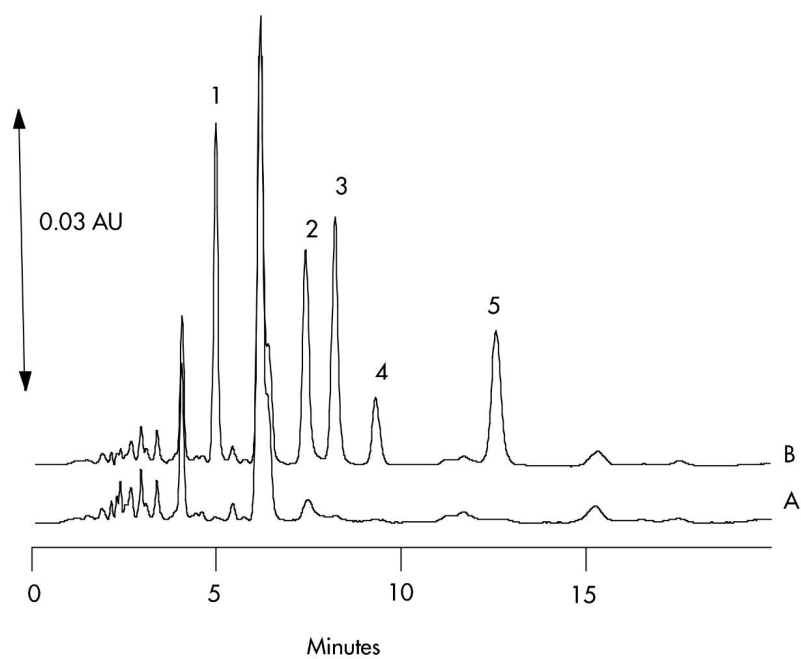
Oasis® HLB 1 cc/30 mg Extraction Cartridge
Part Number WAT094225



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

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