



## Caffeine and Metabolites in Serum

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Waters Corporation



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

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### Abstract

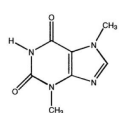
This application brief demonstrates analysis of caffeine and metabolites in serum.

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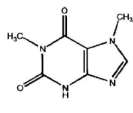
## Introduction

The compounds used in this study are –

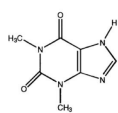
1. Theobromine
2. Paraxanthine
3. Theophylline
4.  $\beta$ -hydroxyethyltheophylline (I.S.)
5. Caffeine



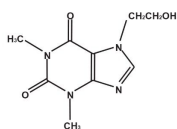
**1. Theobromine**



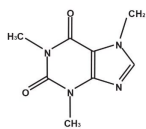
**2. Paraxanthine**



**3. Theophylline**



**4.  $\beta$ -hydroxyethyltheophylline (I.S.)**



**5. Caffeine**

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## Experimental

### HPLC Method

Column:	Symmetry Shield RP <sub>8</sub> , 3.0 x 150 mm, 5 $\mu$ m
Guard column:	Sentry Guard Column, 3.9 x 20 mm, 5 $\mu$ 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  $\mu$ L of reconstituted porcine serum extract

Temperature:

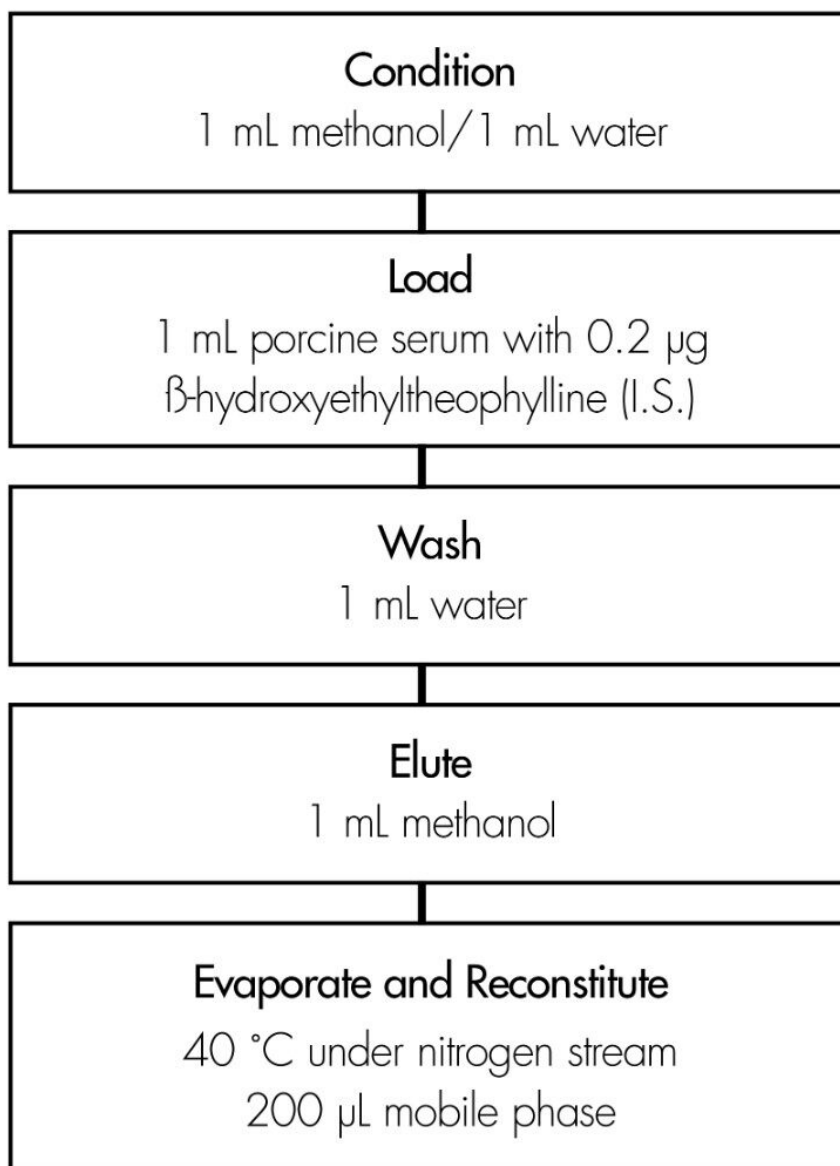
25 °C

Detection:

UV @ 273 nm

# Oasis® HLB Extraction Method

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

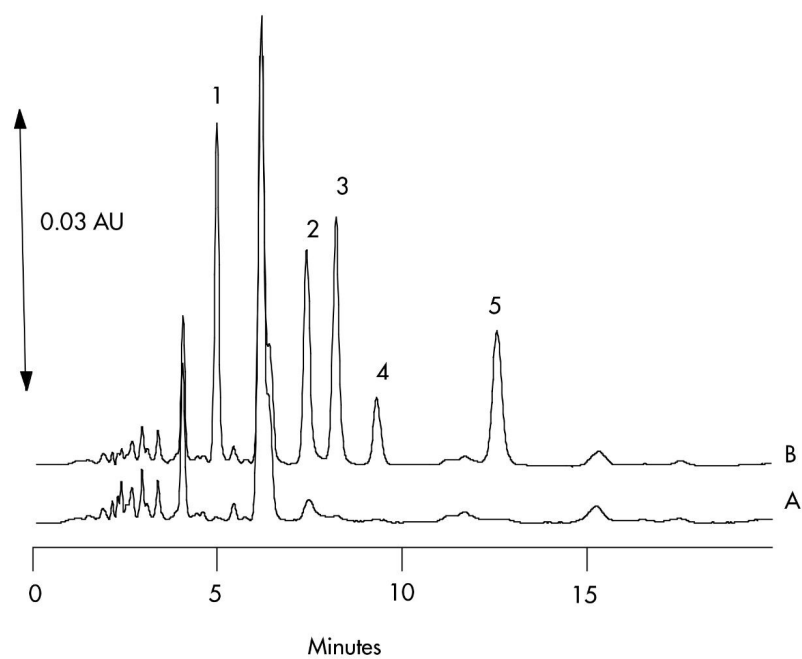


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Results and Discussion

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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