

Caffeine and Metabolites in Serum

HPLC Method

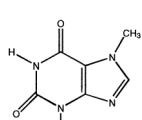
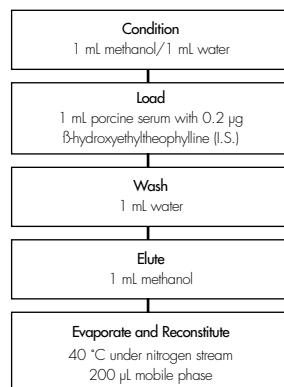
Column: SymmetryShield™ RP₈, 3.0 x 150 mm, 5 µm
Guard Column: Sentry™ guard column, 3.9 x 20 mm, 5 µm
Part numbers: Column - WAT094243, Guard - WAT200675
Mobile phase: 20 mM ammonium acetate, pH 5/acetonitrile, 95:5
Flow rate: 0.6 mL/min
Injection volume: 20 µL of reconstituted porcine serum extract
Temperature: 25°C
Detection: UV @ 273 nm

Compounds:

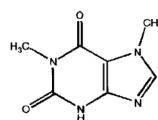
- 1: Theobromine
- 2: Paraxanthine
- 3: Theophylline
- 4: β-hydroxyethyltheophylline (I.S.)
- 5: Caffeine

Oasis® HLB Extraction Method

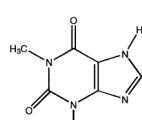
Oasis® HLB 1 cc/30 mg Extraction Cartridge
 Part Number WAT094225



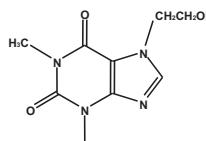
1. Theobromine



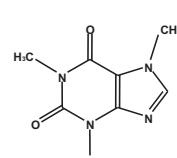
2. Paraxanthine



3. Theophylline

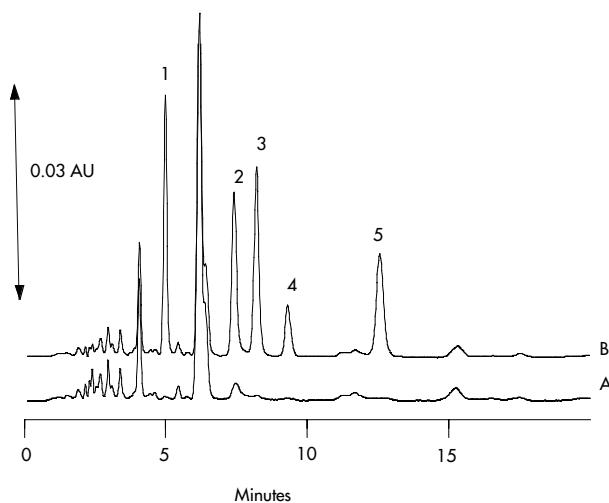


4. β-hydroxyethyltheophylline (I.S.)



5. Caffeine

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



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%