

APPLICATION LIST #401

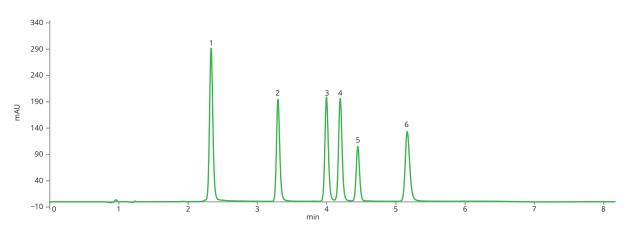
Caffeine metabolites

The HPLC/UV method was developed, optimized and validated for the analysis of caffeine and its selected metabolites (1-methyluric acid, theobromine, paraxanthine, theophylline) in urine. The method is also used for the quantification of caffeine in various food samples.

Substance 1-Methyluric acid, CAS Number 708-79-2

Theobromine, CAS Number 519-41-5 Paraxanthine, CAS Number 611-59-6 Theophylline, CAS Number 58-55-9 Etofylline (IS), CAS Number 519-37-9

Caffeine, CAS Number 58-08-2



Bewerage sample on Arion® HPLC column

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Caffeine metabolites

Column	Arion [®] Plus C18, 5 μm		
Dimensions	150 mm × 3.0 mm		
Part number	ARI-5720-LK30		
Mobile phase	A: 1% acetic acid in Milli-Q water (v/v)		
	B: methanol		
Gradient elution	Retention (min)	%A	%B
	0.0	94	6
	3.0	75	25
	5.0	75	25
	5.5	94	6
	11.0	94	6
Flow rate	1.0 ml/min		
Temperature	35 °C		
Detection	UV @270 nm		
Injection volume	65 µl		
Analytes	1. 1-Methyluric acid		
	2. Theobromine		
	3. Paraxanthine		
	4. Theophylline		
	5. Etofylline (IS)		
	6. Caffeine		



Přírodovědecká Jihočeská univerzita fakulta v Českých Budějovicích Faculty University of South Bohemia of Science in České Budějovice The application has been developed by University of South Bohemia in České budějovice.