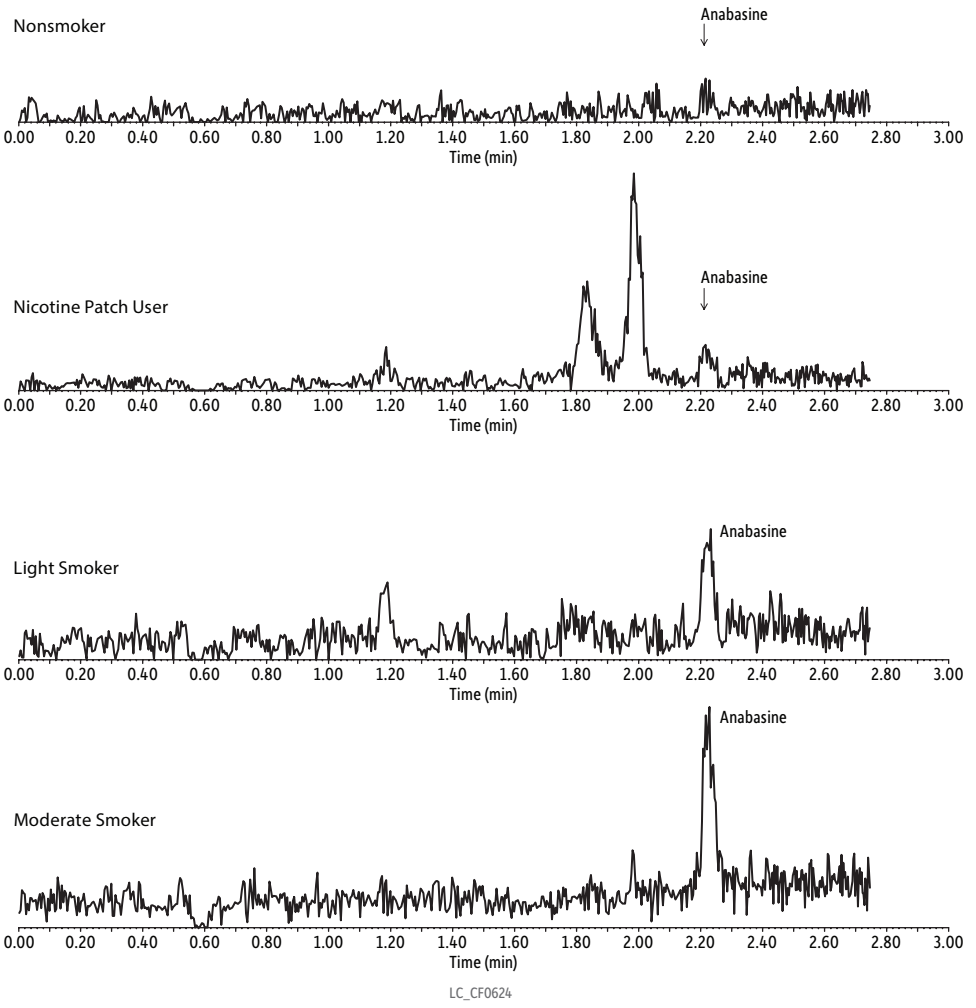
Specific Anabasine Detection in the Urine of Tobacco Users on Raptor Biphenyl



LC_CF0624

Column	Raptor Biphenyl (cat.# 9309512)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	5 µm
Pore Size:	90 Å
Guard Column:	Raptor Biphenyl EXP guard cartridge 5 mm, 2.1 mm ID, 5 µm (cat.# 930950252)
Temp.:	30 °C
Standard/Sample	
Diluent:	Water
Conc.:	Unknown concentration in human urine sample
Inj. Vol.:	5 µL
Mobile Phase	
A:	0.1% Formic acid, 5 mM ammonium formate in water
B:	0.1% Formic acid in methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
1	0.4	90	10
2	0.4	70	30
3	0.4	30	70
3.01	0.4	90	10
5	0.4	90	10

Detector	MS/MS
Ion Source:	Waters Zspray ESI
Ion Mode:	ESI+
Instrument	UHPLC
Sample Preparation	Samples were processed with a liquid-liquid extraction (LLE) procedure. An aliquot of 250 µL urine was mixed with 40 µL of internal standard solution (250 ng/mL in methanol) and 50 µL of 5N sodium hydroxide in a 4 mL glass vial. Extraction was performed by adding 1.5 mL of 50:50 methylene chloride:diethyl ether and stirring for 1.5 minutes. After centrifugation at 4000 rpm for 5 minutes, 1 mL of the organic phase was transferred to a 1.5 mL HPLC vial and mixed with 10 µL of 0.25 N hydrochloric acid before evaporating to dryness at 35 °C under a gentle stream of nitrogen. The dried extract was reconstituted with 200 µL of water.