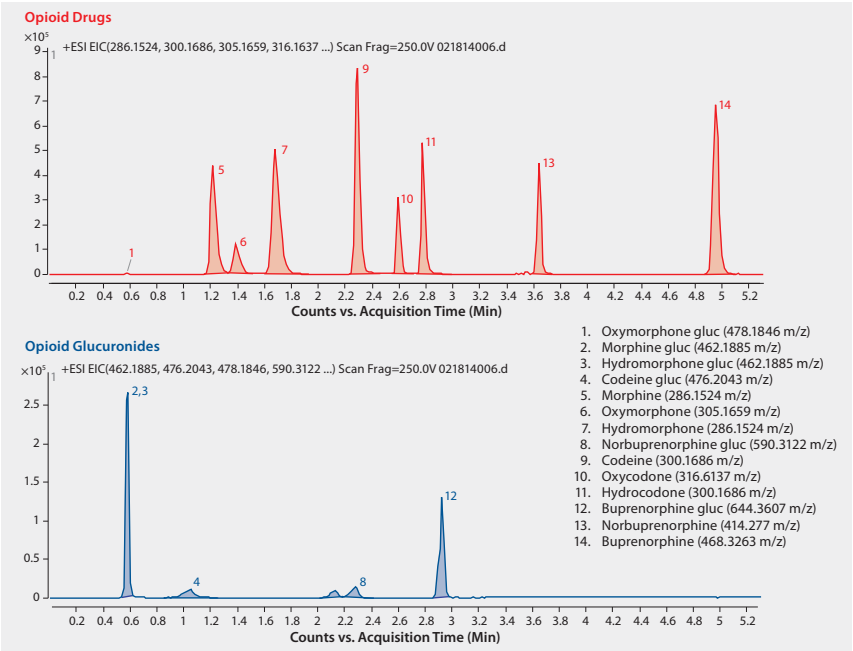
Email: techserv@sial.com

LC/MS Analysis of Opioid Glucuronide Metabolites in Urine on Ascentis® Express 2.7 µm F5 after Solid Phase Extraction (SPE) using Supel™-Select HLB

Sample pretreatment: β-glucuronidase hydrolysis

The workflow for the LC/MS analysis of native drugs and associated metabolites from urine is shown here. The convenient liquid form of beta-glucuronidase from Sigma was used for hydrolysis of the glucuronide metabolites. SPE using Supel-Select HLB yielded clean extracts. The Ascentis Express F5 columns gave highly efficient, well-resolved peaks and rapid separation. LC-MS Ultra CHROMASOLV solvents and additives provided trouble-free operation, and Cerilliant CRMs gave reliable quantification.

market focus	Clinical; Forensics and Toxicology
sample preparation	Solid Phase Extraction
sample/matrix	urine spiked with drugs and metabolites at 300 ng/mL
SPE well plate	Supel-Select HLB 96-well plate, 60 mg/well (575662-U)
condition	1 mL methanol followed by 1 mL water
sample addition	1 mL hydrolyzed urine
washing	1 mL water followed by 1 mL 25% methanol
elution	1 mL acetonitrile
column	Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase	[A] 5 mM ammonium formate, pH 4.2 (adjusted with formic acid); [B] 5 mM ammonium formate in (95:5 acetonitrile:water) pH 5.2 (adjusted with formic acid)
gradient	15% B held for 1 min, to 70% B in 2 min, held at 70% B for 7 min
flow rate	0.4 mL/min
column temp.	35 °C
detector	ESI(+) TOF, full scan, m/z 100-1000
injection	3.0 µL
sample	300 ng/mL
Application No.	G006299



Related Products	Related Products	Related Products
analytical column	**standard**	Morphine solution (Cerilliant M-030)
Ascentis® Express F5, 2.7 Micron HPLC Column (Supelco 53569-U)	Buprenorphine solution (Cerilliant B-044)	Morphine-3-β-D-glucuronide solution (Cerilliant M-031)
eluent	Buprenorphine glucuronide solution (Cerilliant B-035)	Norbuprenorphine solution (Cerilliant N-059)
Acetonitrile (Sigma-Aldrich 34998)	Codeine solution (Cerilliant C-015)	Norbuprenorphine glucuronide solution (Cerilliant N-045)
Ammonium formate (Fluka 14266)	Codeine-6-β-D-glucuronide solution (Cerilliant C-087)	Oxycodone solution (Cerilliant O-002)
Water (Sigma-Aldrich 34877)	Hydrocodone solution (Cerilliant H-003)	Oxymorphone-D_3 solution (Cerilliant O-003)
SPE tube or plate	Hydromorphone solution (Cerilliant H-004)	Oxymorphone-3-β-D-glucuronide solution (Cerilliant O-030)
Supel™ - Select HLB SPE 96-well Plate (Supelco 575662-U)	Hydromorphone-3-β-D-glucuronide (Cerilliant H-051)	