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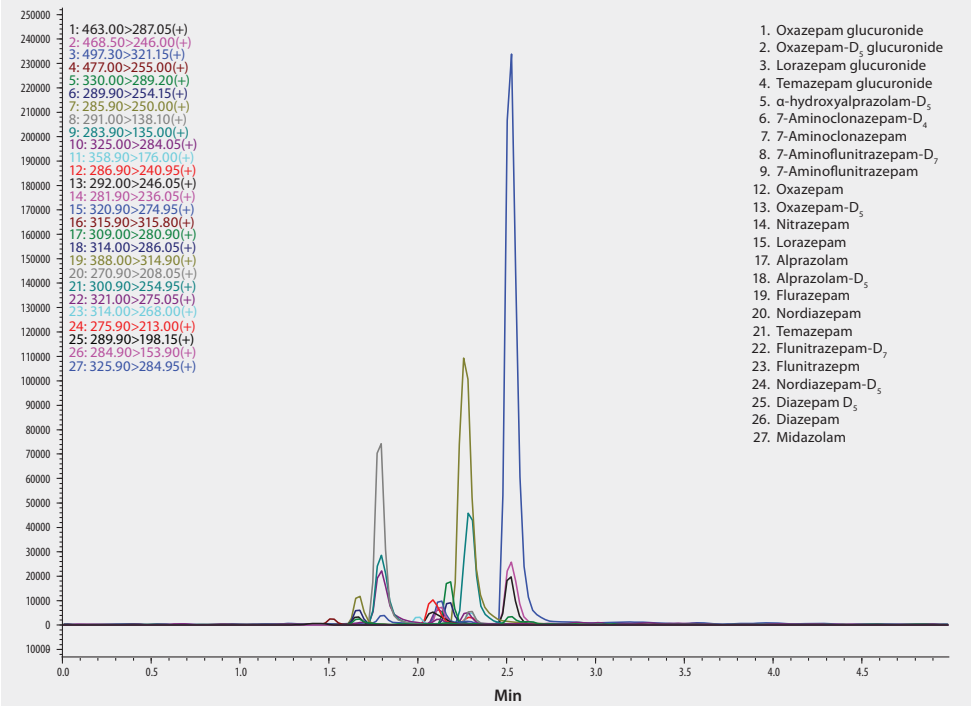
UHPLC/MS/MS Analysis of Benzodiazepines and Glucuronide Metabolites in Urine on Titan™ C18 after SPE using Supel™-Select HLB, β-Glucuronidase Enzyme Digestion

Urine samples were spiked with various glucuronide metabolites of benzodiazepines along with the parent drugs. The glucuronide metabolites were converted back to the parent drug using β-glucuronidase, then processed using SPE prior to LC/MS analysis. The Supel-Select HLB provided clean extracts and the Titan C18 provided rapid resolution of parent drugs and metabolites. Cerilliant CRMs provided reliable quantitation.

market focus	Forensics and Toxicology
sample preparation	SPE (Solid Phase Extraction)
SPE well plate	Supel-Select HLB 96-well plate, 60 mg/well (575662-U)
condition	1 mL acetonitrile, 1 mL water
sample addition	1.1 mL enzyme-digested urine
washing	1 mL water, 1 mL 25% methanol
elution	1 mL acetonitrile
column	Titan C18, 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U)
mobile phase	[A]: 5 mM ammonium formate [B]: 5 mM ammmonium formate in acetonitrile:water (95:5)
gradient	10 to 90% B in 2.5 min, held for 0.5 min
flow rate	0.3 mL/min
column temp.	35 °C
detector	ESI(+), MRM
injection	2.0 µL
Application No.	G006291



Components	Components	Related Products
Oxazepam glucuronide solution (Cerilliant O-023)	Lorazepam solution (Cerilliant L-901)	**analytical column**
Oxazepam-d_5 glucuronide (Cerilliant O-038)	Alprazolam solution (Cerilliant A-903)	Titan™ C18 UHPLC Column, 1.9 micron (Supelco 577122-U)
Lorazepam glucuronide solution (Cerilliant L-021)	Alprazolam-d_5 solution (Cerilliant A-902)	**eluent**
Diazepam solution (Cerilliant D-907)	Flurazepam solution (Cerilliant F-003)	Acetonitrile (Fluka 14261)
alpha-Hydroxyalprazolam solution (Cerilliant A-905)	Nordiazepam solution (Cerilliant N-905)	Ammonium formate (Fluka 14266)
7-Aminoclonazepam-d_4 solution (Cerilliant A-917)	Temazepam solution (Cerilliant T-907)	Water (Fluka 14263)
7-Aminoclonazepam solution (Cerilliant A-916)	Flunitrazepam-d_7 (Cerilliant F-915)	**enzyme**
7-Aminoflunitrazepam-d_7 solution (Cerilliant A-925)	Flunitrazepam solution (Cerilliant F-907)	β-Glucuronidase from limpets (*Patella vulgata*) (Sigma G2174)
7-Aminoflunitrazepam solution (Cerilliant A-911)	Nordiazepam-d_5 solution (Cerilliant N-911)	**SPE tube or plate**
Oxazepam solution (Cerilliant O-902)	Diazepam-d_5 solution (Cerilliant D-902)	Supel™ - Select HLB SPE 96-well Plate (Supelco 575662-U)
Oxazepam-d_5 solution (Cerilliant O-901)	Diazepam solution (Cerilliant D-907)	
Nitrazepam solution (Cerilliant N-906)	Midazolam solution (Cerilliant M-908)	