Diazepam in Serum - LC/MS

HPLC Method

Column:	Symmetry® C_{18}, 2.1 x 100 mm, 3.5 µm
Part number:	WAT058965
Mobile phase:	2 mM Ammonium Acetate/Acetonitrile/Formic Acid 65:35:0.1
Flow rate:	200 µL/min
Injection volume:	10 µL
MS:	Micromass Quattro LC
Ion Mode:	ES+
Cone Voltage:	45 V
Collision Energy:	25 eV

Oasis® HLB Extraction Method

Oasis® HLB 1 cc/30mg Extraction Cartridge
Part Number WAT04225

Condition:
1 mL methanol/1 mL water

Load:
1 mL porcine serum

Wash:
1 mL 5% methanol in water

Elute:
1 mL methanol

Evaporate and Reconstitute:
40° C under nitrogen stream,
200 µL mobile phase

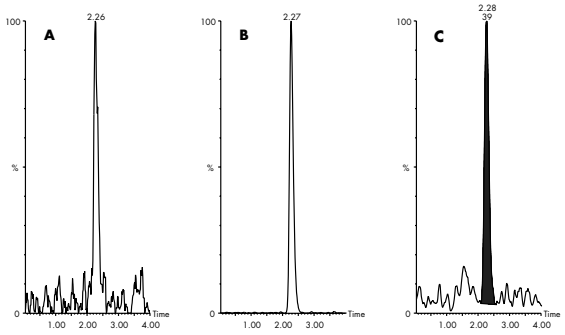
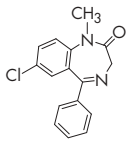


Figure 4: MRM Chromatograms under optimum conditions of pure diazepam standard at (A) 0.2ng/mL (LOD) and (B) 5.0ng/mL and (C) a processed human plasma sample with a low concentration of diazepam (calculated as 0.75ng/mL)

Diazepam



Compound 3 name: Diazepam
Coefficent of Determination: 0.998439
Calibration curve: 150.210* x + -0.398957
Response type: External Std., Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x Axis trans: None

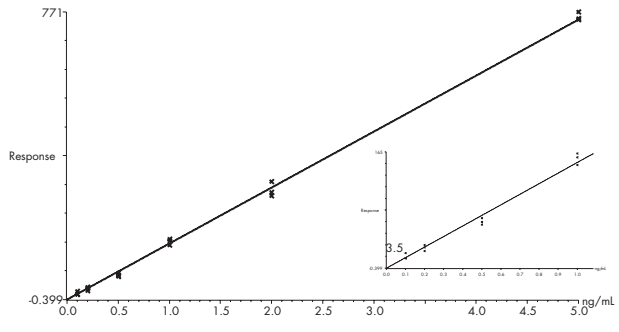


Figure 3: Calibration curve with triplicate injections for each point and demonstrating LOQ at 0.2ng/mL

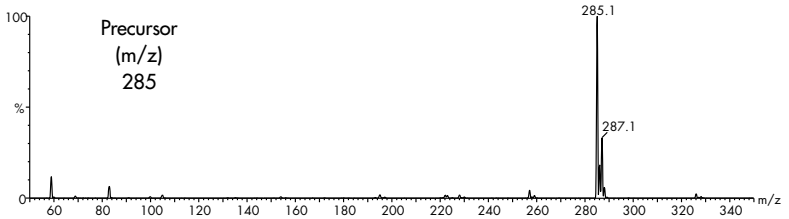


Figure 1: Background-subtracted electrospray mass spectrum of pure diazepam standard (5µg), under optimum conditions. Chromatographed as described above to remove contaminants.

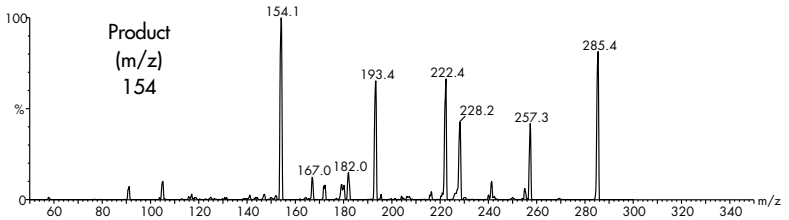


Figure 2: Background-subtracted electrospray product tion spectrum of pure diazepam standard (5µg), under optimum conditions. Chromatographed as described above to remove contaminants.