

Application Note

Diazepam in Serum by LC-MS

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

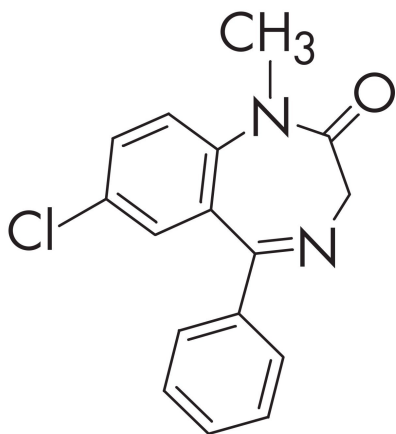
Abstract

This application brief demonstrates the analysis of diazepam by LC-MS.

Introduction

The compound analyzed in this study is diazepam.

Diazepam



Experimental

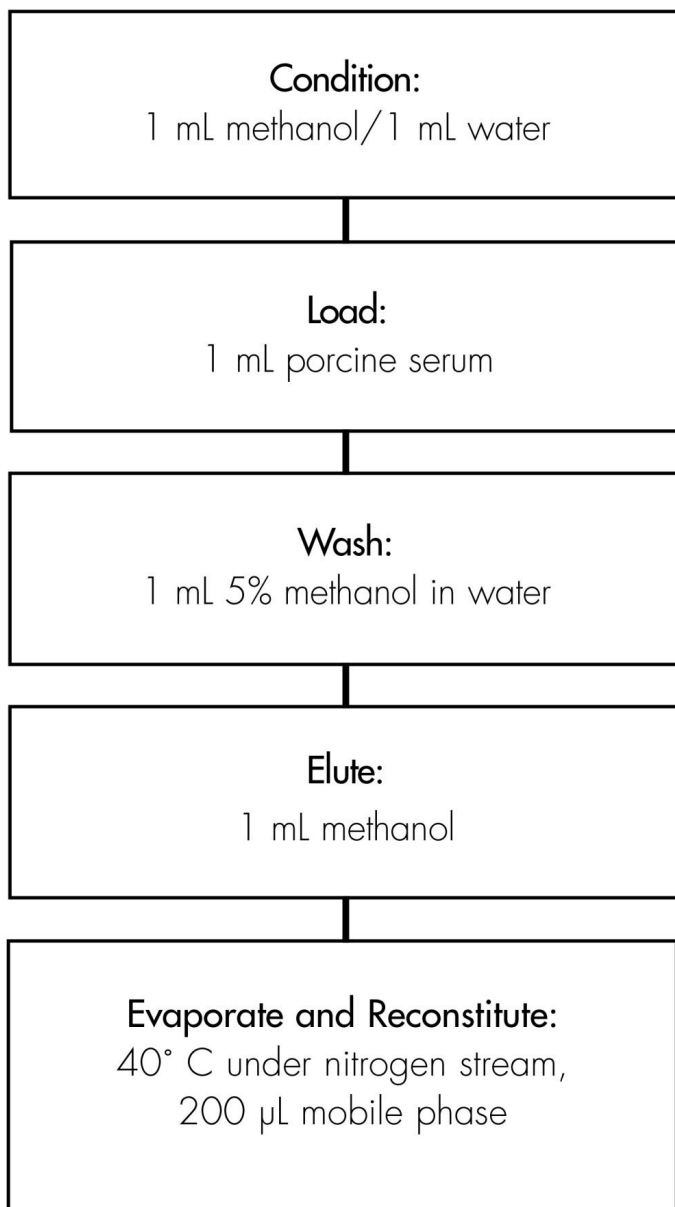
HPLC Method

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



Results and Discussion

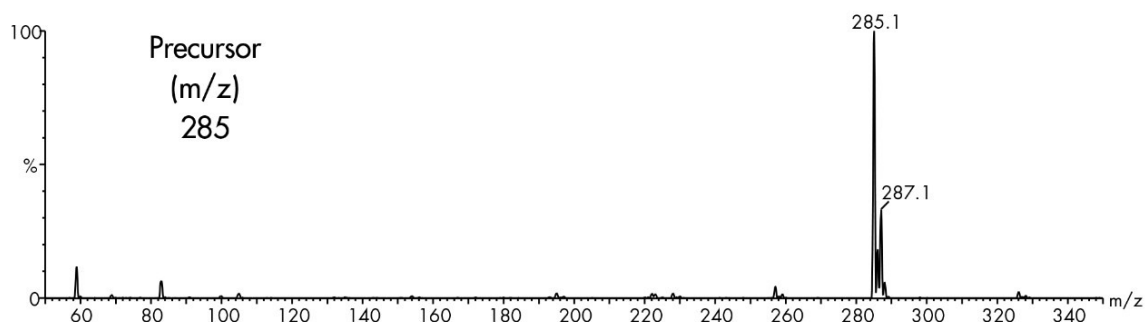


Figure 1. Background-subtracted electrospray mass spectrum of pure diazepam standard (5µg), under optimum conditions. Chromatographed as described above to remove contaminants.

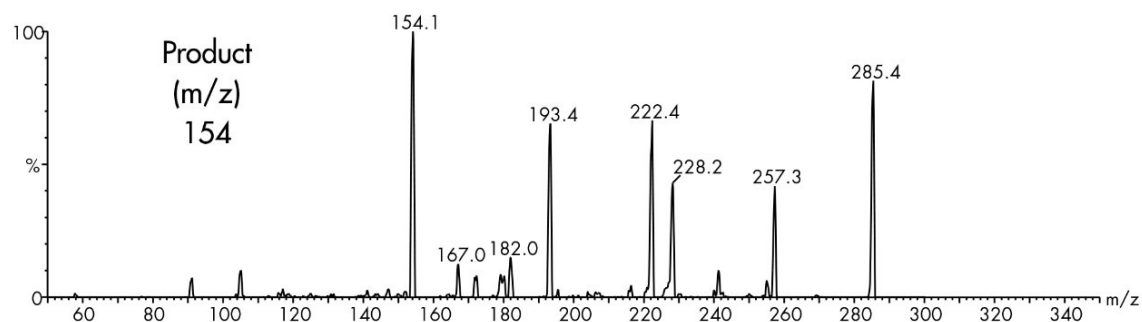


Figure 2. Background-subtracted electrospray product ion spectrum of pure diazepam standard (5 µg), under optimum conditions. Chromatographed as described above to remove contaminants.

Compound 3 name: Diazepam

Coefficient of Determination: 0.998439

Calibration curve: $150.210 \cdot x + -0.398957$

Response type: External Std., Area

Curve type: Linear, Origin: Exclude, Weighting: 1/x Axis trans: None

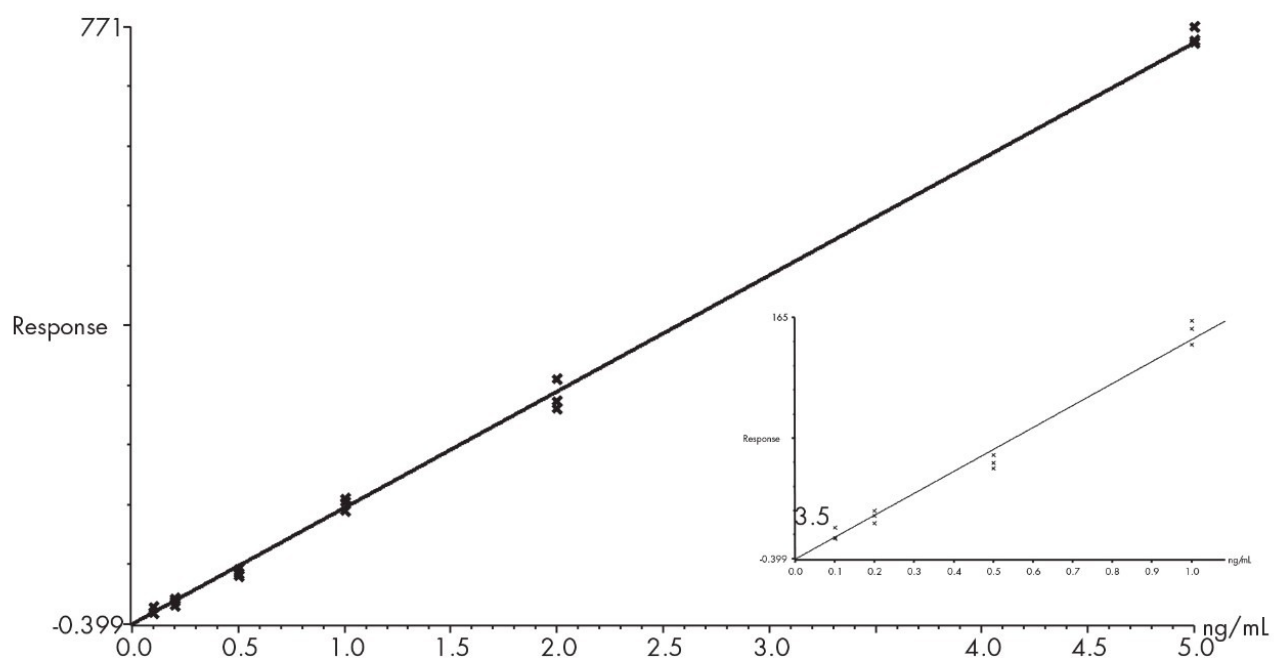


Figure 3. Calibration curve with triplicate injections for each point and demonstrating LOQ at 0.2ng/mL.

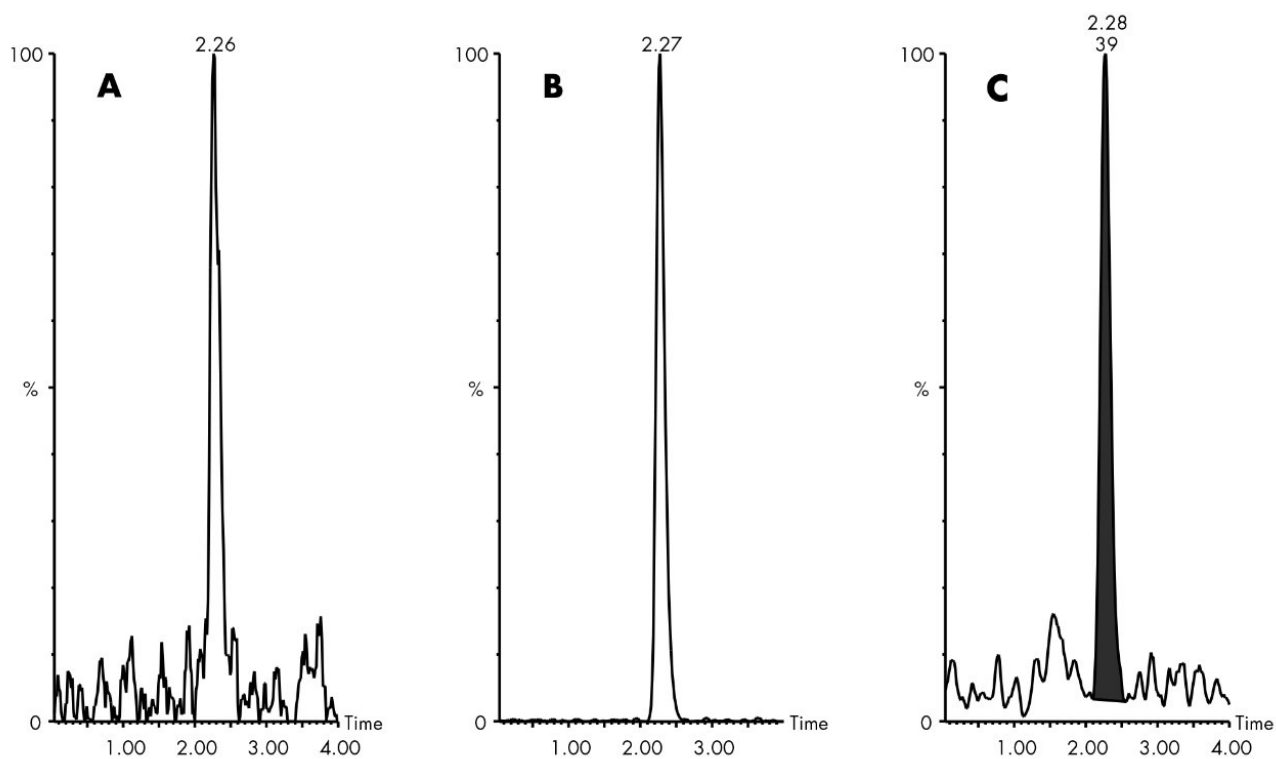


Figure 4. MRM Chromatograms under optimum conditions of pure diazepam standard at (A) 0.2ng/mL (LOD) and (B) 5.0ng/mL and (C) a processed human plasma sample with a low concentration of diazepam (calculated as 0.75ng/mL).

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