

## Diazepam in Serum by LC-MS

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Waters Corporation

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

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### Abstract

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

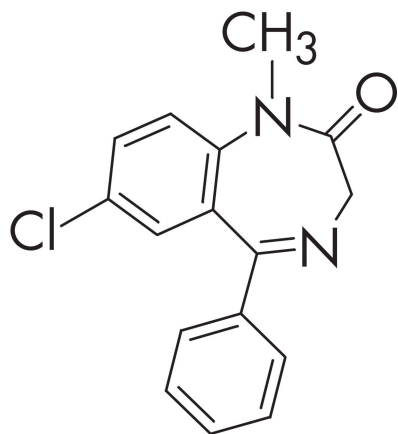
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### Introduction

The compound analyzed in this study is diazepam.

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# Diazepam



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## Experimental

### HPLC Method

Column:	Symmetry C <sub>18</sub> , 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+

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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

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## Results and Discussion

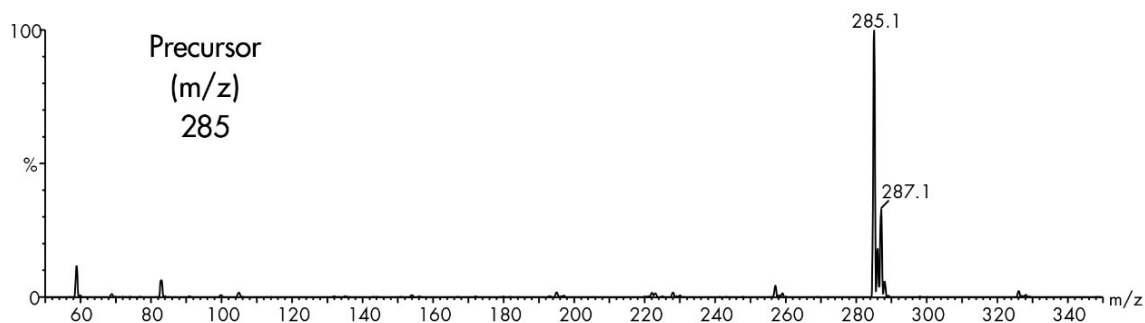


Figure 1. Background-subtracted electrospray mass spectrum of pure diazepam standard (5 $\mu$ g), under optimum conditions. Chromatographed as described above to remove contaminants.

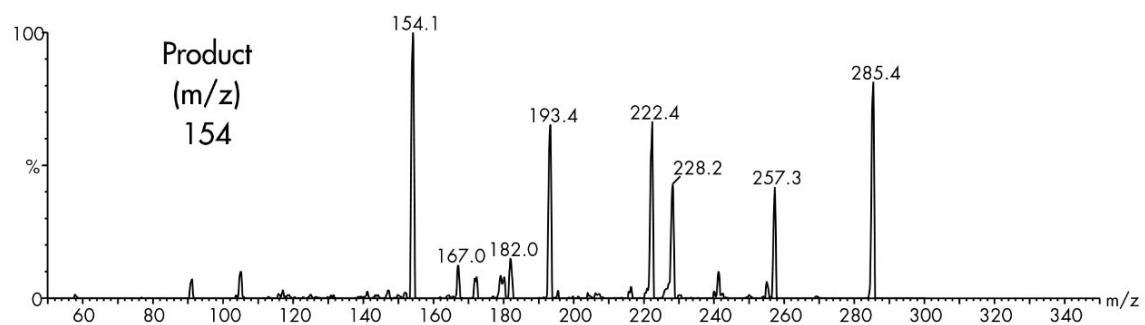


Figure 2. Background-subtracted electrospray product ion spectrum of pure diazepam standard (5 $\mu$ g), under optimum conditions. Chromatographed as described above to remove contaminants.

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

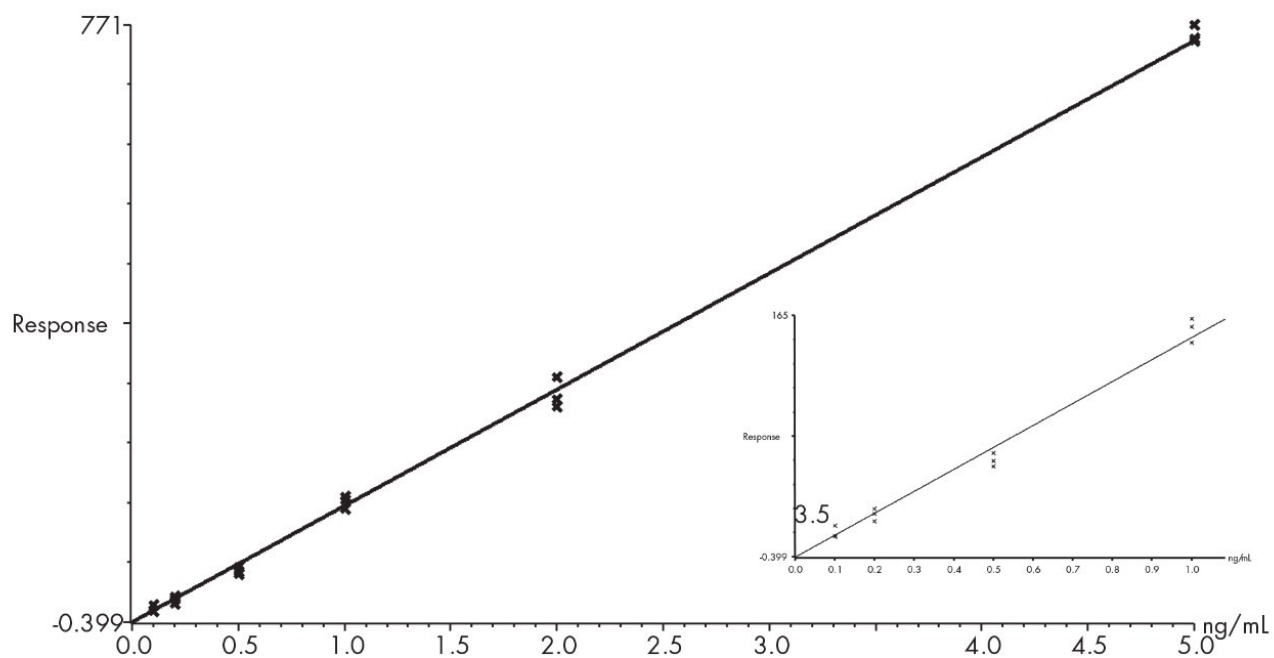


Figure 3. Calibration curve with triplicate injections for each point and demonstrating LOQ at 0.2ng/mL.

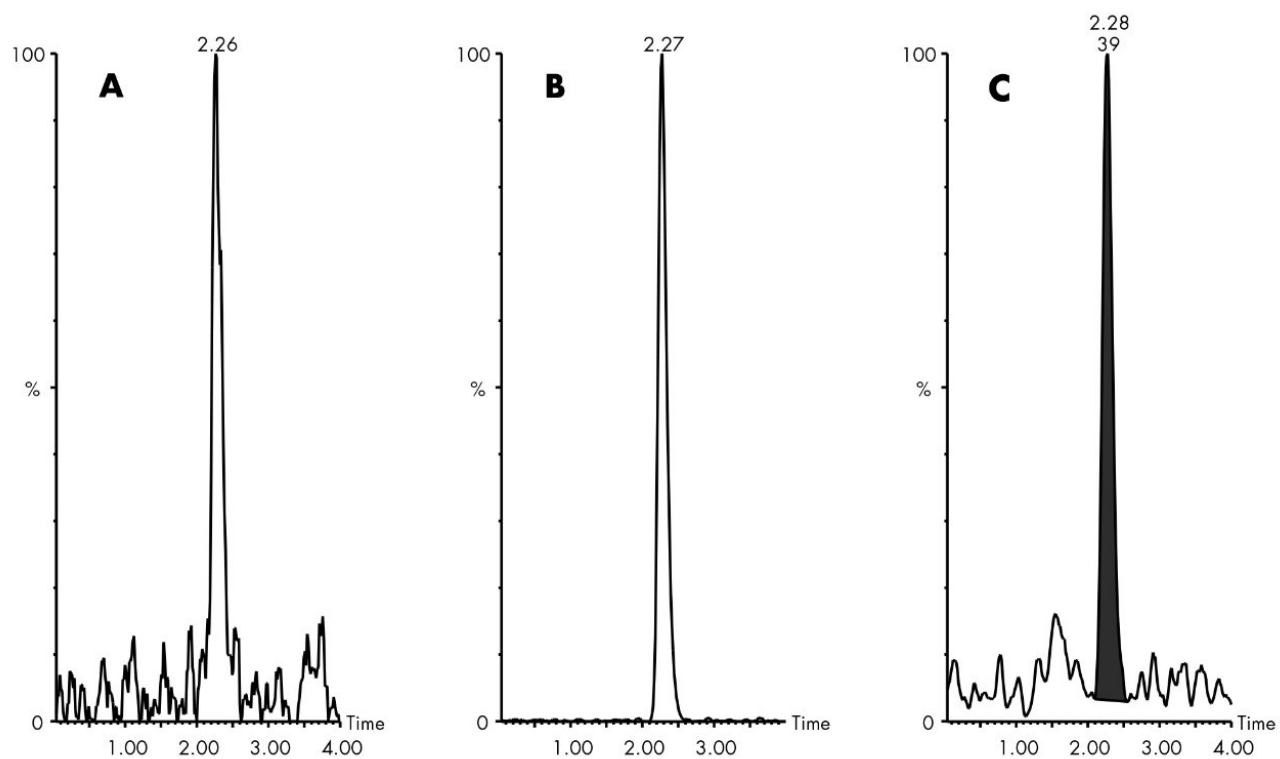


Figure 4. MRM Chromatograms under optimum conditions of pure diazepam standard at (A) 0.2ng/mL (LOD) and (B) 5.0ng/mL and (C) a processed human plasma sample with a low concentration of diazepam (calculated as 0.75ng/mL).

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