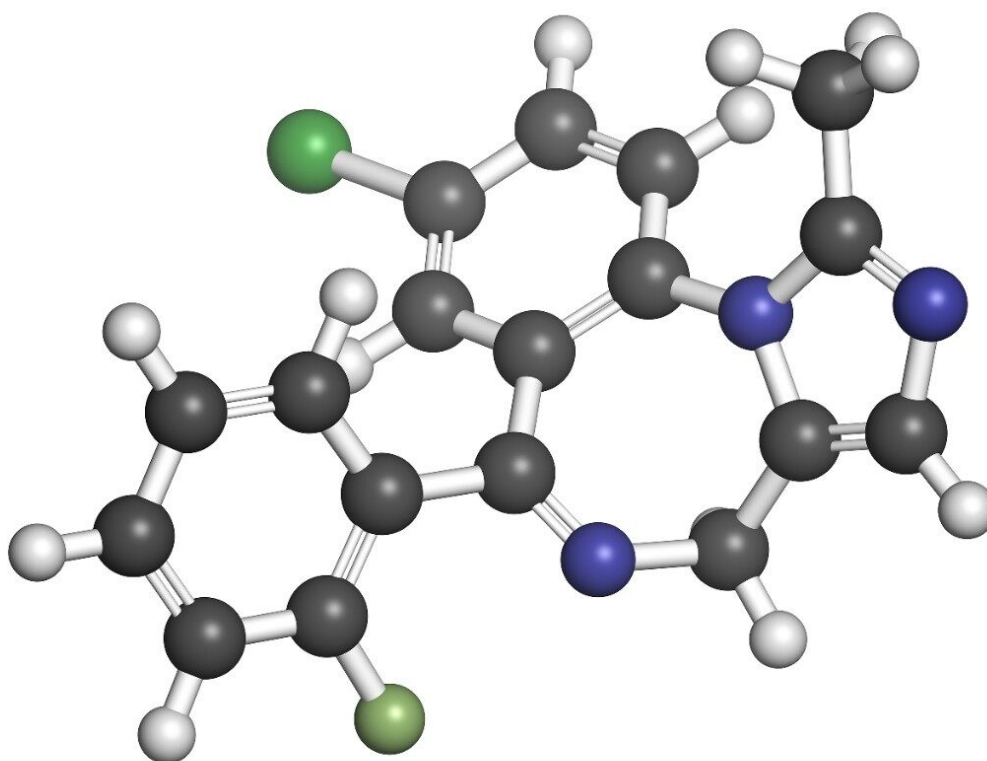


Waters Corporation



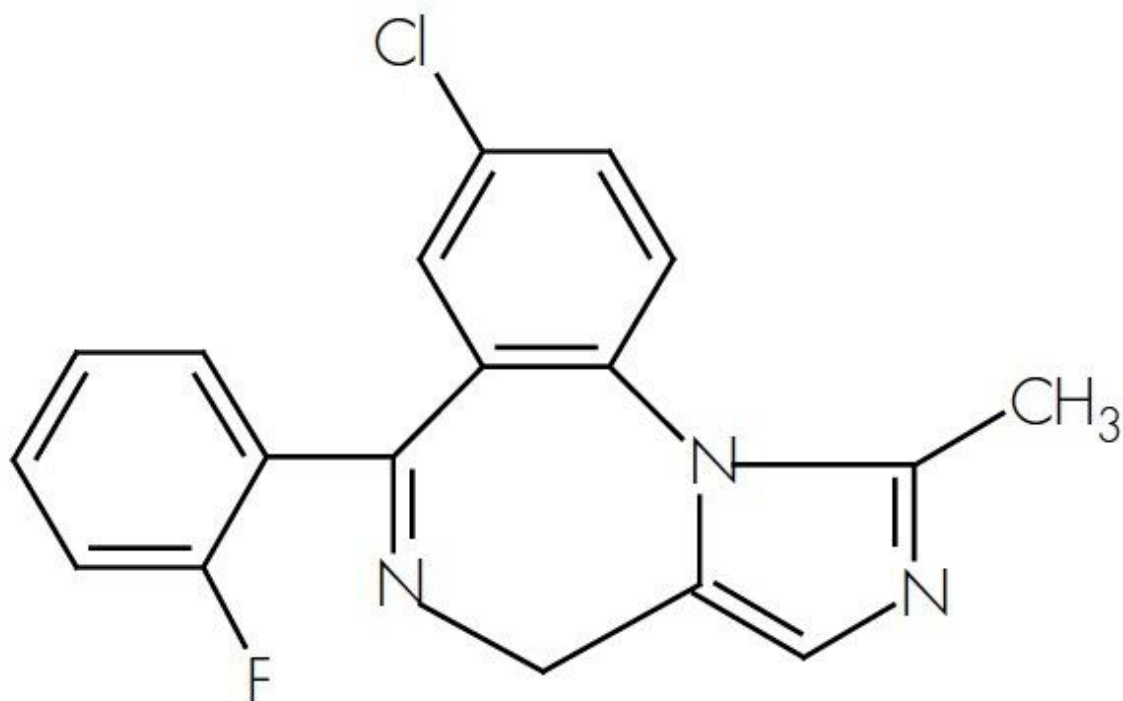
This is an Application Brief and does not contain a detailed Experimental section.

This application brief demonstrates the analysis of midazolam on Oasis MCX.

Introduction

Midazolam is used to produce sleepiness or drowsiness and to relieve anxiety before surgery or certain procedures. In this example, the sample size was limited (less than 100 μ L of plasma) and therefore the Oasis μ Elution plate was used.

Weak Base: $pK_a \sim 6.15$ and therefore Oasis MCX was used.



Midazolam
MW 325.8

Experimental

Test Conditions

Oasis MCX 96-Well μ Elution Plate

Condition:	200 μ L MeOH
Equilibrate:	200 μ L H ₂ O
Load:	100 μ L sample (50 μ L plasma diluted 1:1 with 4% H ₃ PO ₄)
Wash 1:	200 μ L 2% HCOOH
Wash 2:	200 μ L MeOH
Elute:	50 μ L (25 μ L x 2) 5% NH ₄ OH in MeOH
Dilute:	100 μ L H ₂ O or 100 μ L 2% FA in H ₂ O to neutralize
Inject:	10 μ L
Column:	SunFire C ₁₈ 2.1 x 20 mm IS, 3.5 μ m
Mobile phase A:	10 mM CH ₃ COO-NH ₄ ⁺ , pH 5.5
Mobile phase B:	MeOH with 10 mM CH ₃ COO-NH ₄ ⁺ , pH 5.5
Flow rate:	0.4 mL /min
Instrument:	2777 Sample Manager and 1525 μ Binary HPLC Pump

Gradient

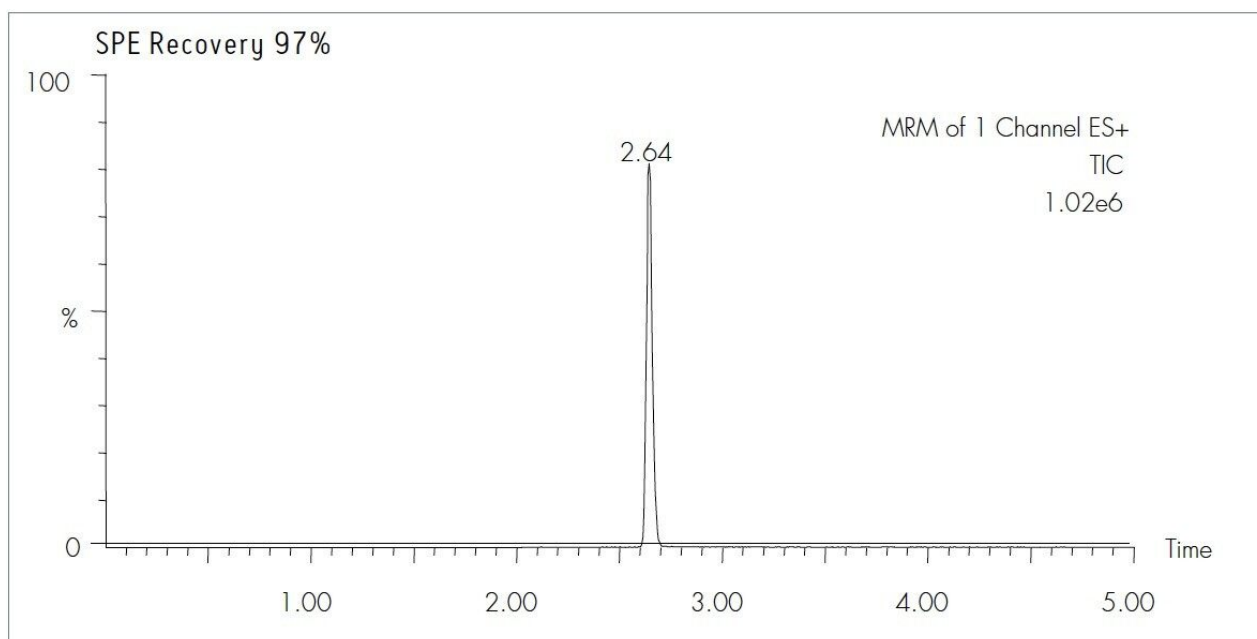
Time(min)	Profile
	<div><div>%A</div><div>%B</div></div>

0.0	95	5
3.0	5	95
4.0	5	95
4.1	95	5
5.0	95	5

Quattro Premier

ESI- source temp:	150 °C
Desolvation temp:	350 °C
Cone gas flow:	50 L /Hr
Desolvation gas flow:	600 L /Hr
Collision cell:	2.2e ⁻³ bar (Ar gas)
Midazolam	MRM Transition
	<i>m/z</i> 326.2 → 291.2

Results and Discussion



WA60087, June 2007