



BDG SYNTHESIS

Certificate of Analysis

BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

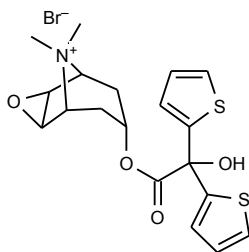
Barry Dent

Barry R. Dent, PhD, Director
10 January 2013

Name: Tiotropium Bromide

CAS Number: 136310-93-5

Structure:



Molecular Weight: $C_{19}H_{22}NO_4S_2 \cdot Br = 472.42$

Lot Number: BDG 6214.1

Appearance: White, crystalline solid

Corrected Purity: 98.8 % (HPLC) - 3.0 % (water) = 95.8 %

Re-test Date: 10 January 2018

Storage and Handling:

Temperature:	refrigerate for prolonged storage; may be handled and shipped at ambient temperature.
Humidity:	not believed to be hygroscopic; may be handled in normal laboratory atmosphere.
Light:	protect from strong sunlight.
Caution:	only experienced laboratory personnel should handle the material.

Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

Residual Solvents: no residual solvents are observed.

Impurities: no significant impurities are evident in the spectrum.

Carbon-13 NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

High-resolution Mass Spectrum (ESI+)

Found m/z 392.0988. $C_{19}H_{22}NO_4S_2$ [M-Br]⁺ requires m/z 392.0985. The deviation of 0.7 ppm is within normally accepted limits for the establishment of identity by HRMS.

HPLC

A sharp, symmetrical peak is observed (98.8 %). Note: in the absence of reference materials for preparing calibration curves, it is assumed that all peaks have the same detector response. Where possible, the conditions of analysis follow a pharmacopeial or literature method, or have been adapted from same.

Elemental Analysis

	Found:	C 47.05, H 4.82, N 2.81 %
$C_{19}H_{22}NO_4S_2 \cdot Br \cdot 0.7H_2O$	Requires:	C 47.05, H 4.86, N 2.89 %
$C_{19}H_{22}NO_4S_2 \cdot Br$	Requires:	C 48.31, H 4.69, N 2.96 %

The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the "best-fit" hydrated molecular formula is given.

Karl-Fischer Analysis

	Found:	H ₂ O 3.0 %
$C_{19}H_{22}NO_4S_2 \cdot Br \cdot 0.7H_2O$	Requires:	H ₂ O 2.6 %

Of necessity, only a small sample could be used and only a single or duplicate analysis performed. We are unable to state what the errors in the reported water content are, but recommend that the result be used, as the best available, when determining corrected purity.

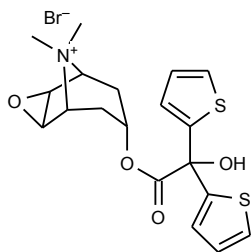
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials often contain higher levels of residual solvents and/or water, and we urge you to use the corrected purity where needed rather than the raw HPLC purity. This compound is intended for use as an analytical reference material and it is not for human administration. Structures are shown with relative stereochemistry unless otherwise specified.

The re-test date is assigned from experience gained with the material in the laboratory and/or on storage. It is not possible to perform formal storage studies because of the small amount of material available.

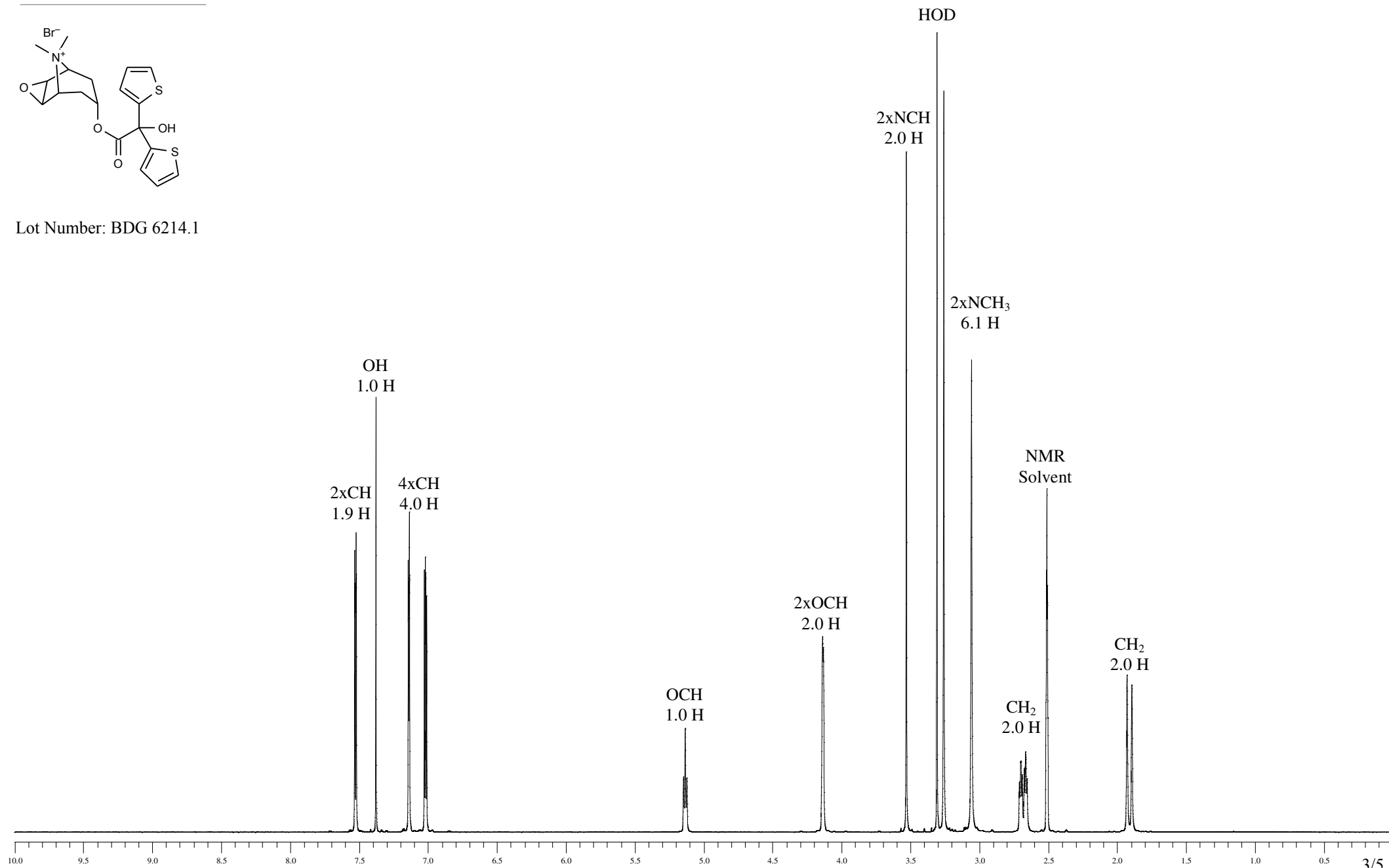


Proton NMR Spectrum of Tiotropium Bromide in DMSO-d₆

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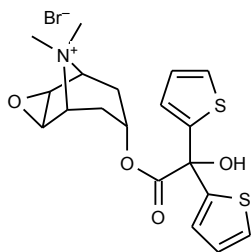
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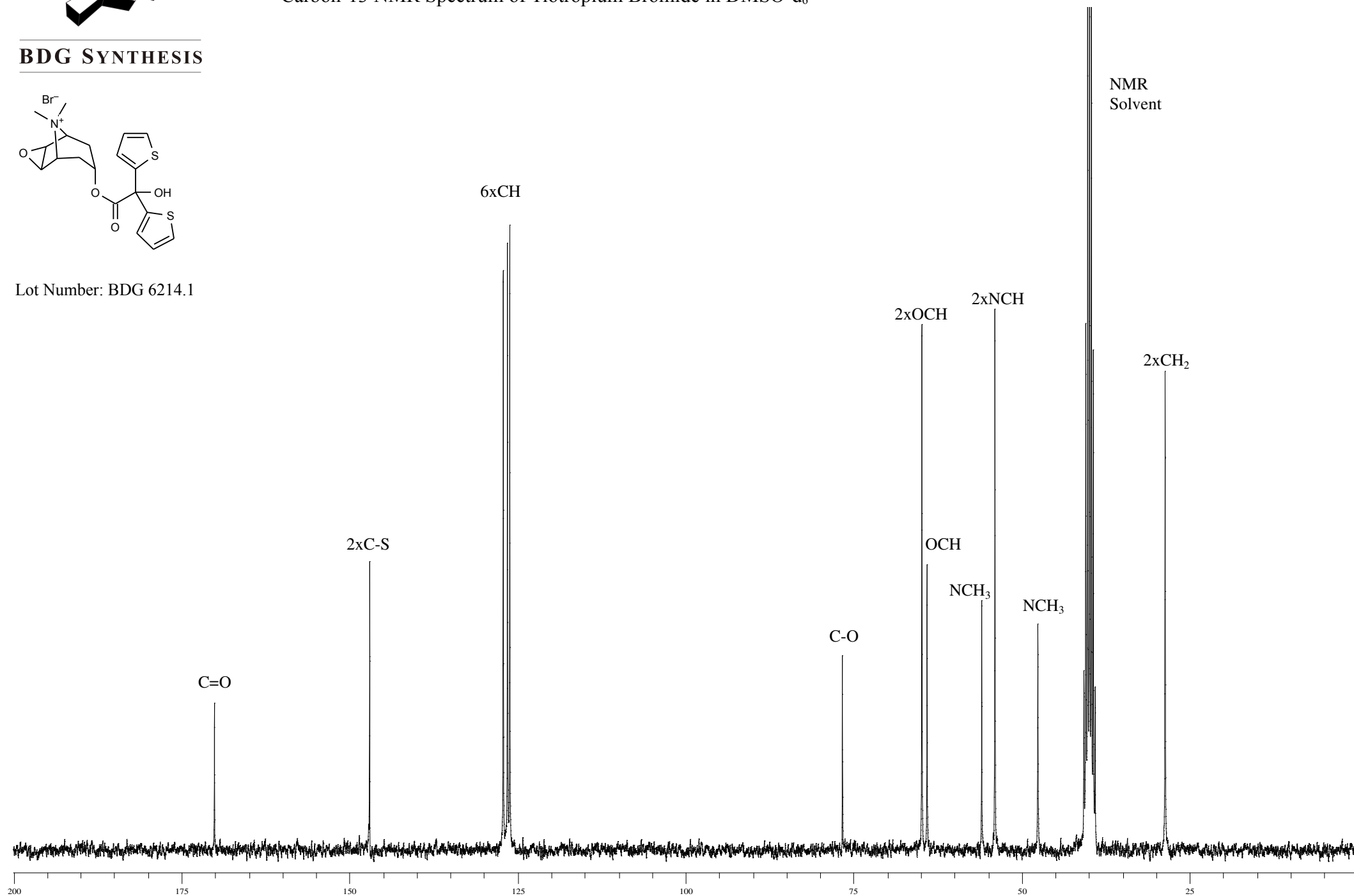


Carbon-13 NMR Spectrum of Tiotropium Bromide in DMSO-d₆

BDG SYNTHESIS



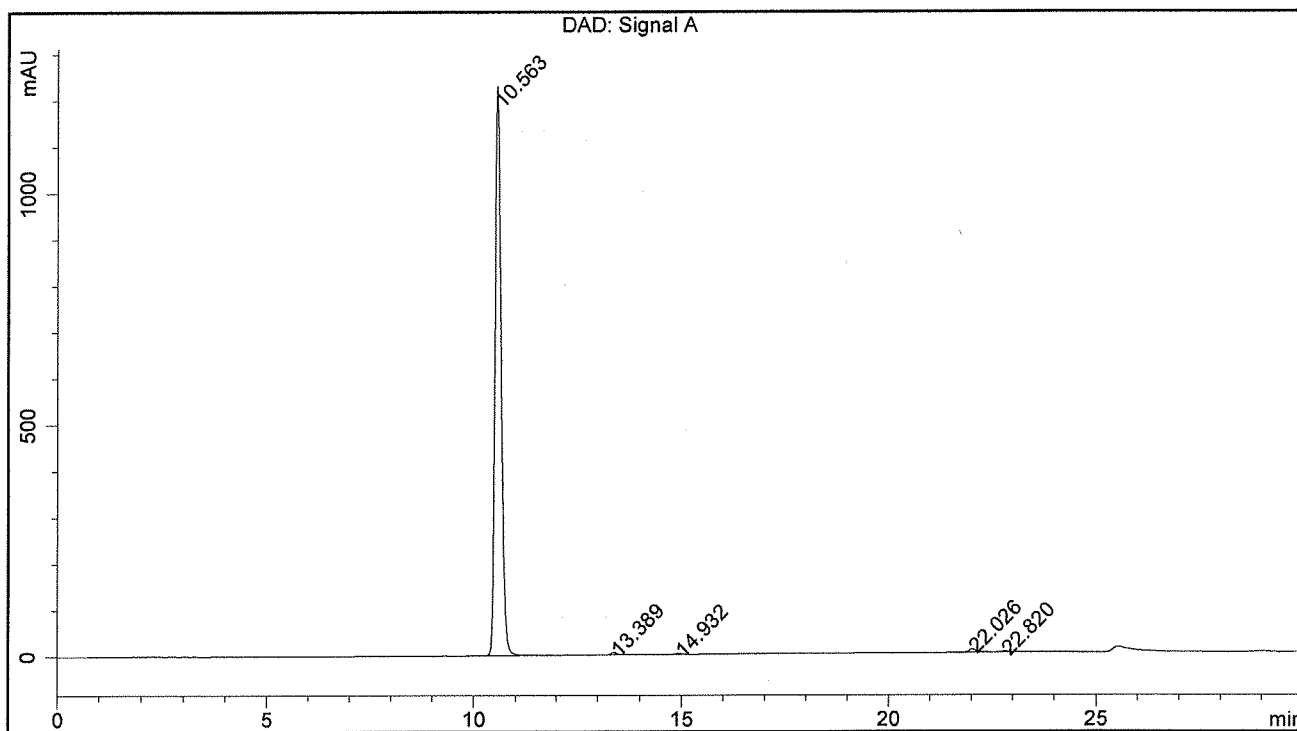
Lot Number: BDG 6214.1



BDG - Analysis of Tiotropium Bromide

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
 Mobile Phase A : 70:30 50 mM KH₂PO₄ + 10 mM Tetrapropylammonium Bromide, to pH 5.50 (KOH) : Methanol
 Mobile Phase B : 40:60 50 mM KH₂PO₄ + 10 mM Tetrapropylammonium Bromide, to pH 5.50 (KOH) : Methanol
 Gradient (A:B) : T0=100:0, T20=0:100, T30=0:100, T32=100:0, T35=100:0
 Flow Rate : 1.0 mL/min
 Sample Solvent : 8:2 Water : Methanol
 Column Temperature : 30C
 Injection Volume : 10 uL
 Detection : UV at 238 nm

Sample Name	BDG 6214.1	Instrument	AnalyticalLC01
Acquisition	10/01/2013, 12:39:46	Method (rev.)	LC10253c (5)
Sequence	BDG_10Jan2012a - Reprocessed	Vial Position	1
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	10.56 min	1229.2626	12798.0937	0.1615 min	98.755 %
2	13.39 min	4.8863	43.6657	0.1383 min	0.337 %
3	14.93 min	1.9646	21.6350	0.1745 min	0.167 %
4	22.03 min	7.1272	81.4730	0.1714 min	0.629 %
5	22.82 min	1.4658	14.5732	0.1580 min	0.112 %