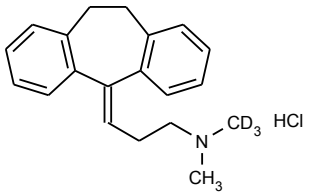
BDG SYNTHESIS

Certificate of Analysis

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

Barry Dent

Barry R. Dent, PhD, Director
5 November 2009

Name:	**Amitriptyline-d_3 HCl**
CAS Number:	549-18-8 (unlabelled)
Structure:	
Molecular Weight:	$C_{20}H_{20}D_3N{\cdot}HCl$ = 316.88
Lot Number:	BDG 5864
Appearance:	White, crystalline solid
Corrected Purity:	99.8 % (HPLC) - 0.5 % (water) = 99.3 %
Isotopic Purity:	Under 0.5 % d_0
Re-test Date:	5 November 2014
Storage and Handling:	Temperature: ambient laboratory temperature; may be refrigerated.
	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.

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Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.
Isotopic Labelling: the signal at the site of deuteration is at half of the intensity compared with what would be expected for unlabelled material, indicating clean deuteration.
Residual Solvents: traces (under 0.1 % *w/w*) of acetone and diethyl ether 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.
Isotopic Labelling: the signal at the site of deuteration is masked by a broadened peak arising from the $N\text{-}CH_3$ group (a phenomenon sometimes observed for groups attached to N-atoms in salts). The spectrum is not helpful in determining the extent of deuteration and the isotopic purity should be assessed in conjunction with the other data available.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 281.2109. $C_{20}H_{21}D_3N$ $[M+H]^+$ requires *m/z* 281.2092. The deviation of 4.5 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for d_0 material was seen (detection limit about 0.5 %).

HPLC

A somewhat broadened, slightly tailing peak is observed (99.8 %). 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 74.06, H 7.11, D 2.03, Cl 11.10, N 4.29 %
$C_{20}H_{20}D_3N{\cdot}HCl{\cdot}0.4H_2O$	Requires:	C 74.12, H 6.78, D 1.86, Cl 10.94, N 4.32 %
$C_{20}H_{20}D_3N{\cdot}HCl$	Requires:	C 75.81, H 6.68, D 1.91, Cl 11.19, N 4.42 %

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_2O 0.5 %
$C_{20}H_{20}D_3N{\cdot}HCl{\cdot}0.4H_2O$	Requires:	H_2O 2.2 %

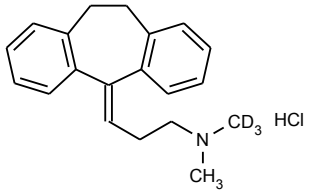
Of necessity, only a small sample could be used and only a single or duplicate analysis performed. We are unable to state what the errors in the reported water content are, but recommend that the result be used, as the best available, when determining corrected purity.

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.

BDG SYNTHESIS

Proton NMR Spectrum of Amitriptyline-d_3 HCl in $CDCl_3$



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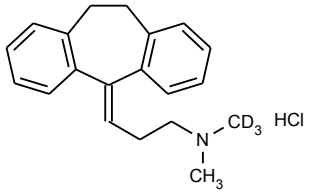
BDG SYNTHESIS

Carbon-13 NMR Spectrum of Amitriptyline-d_3 HCl in $CDCl_3$



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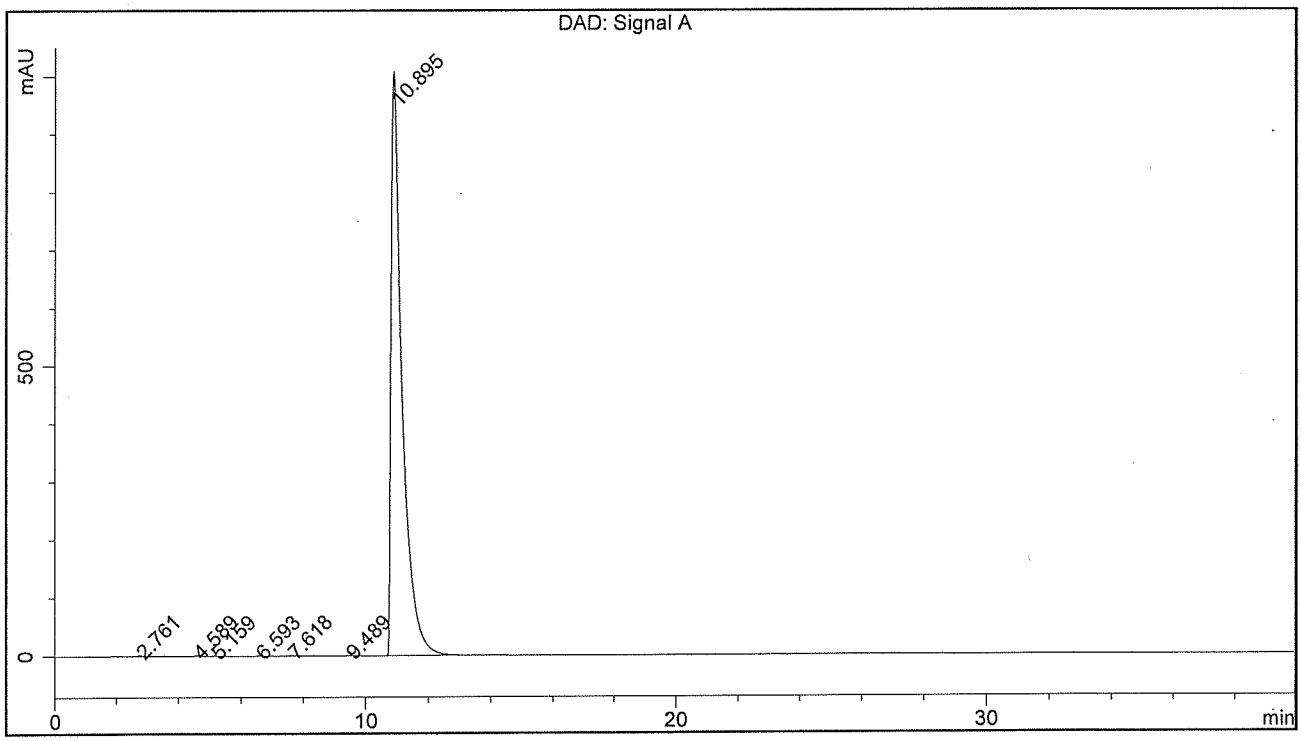
BDG - Analysis of Amitriptyline-d3 Hydrochloride

Column : Phenomenex Gemini-NX C18 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard Gemini-NX C18 4 x 3 mm
Mobile Phase A: 10 mM Ammonium Carbonate pH=10.0
Mobile Phase B: Acetonitrile
Gradient : T0=30:70, T25=20:80, T27=30:70, T30=30:70
Flow Rate : 1.0 mL/min
Sample Solvent : Initial Mobile Phase
Column Temperature : 30C
Injection Volume : 10 uL
Detection : UV at 240 nm

Sample Name	BDG 5864	Instrument	AnalyticalLC01
Acquisition	05/11/2009, 19:03:43	Method (rev.)	LC10281b (8)
Sequence	BDG_05Nov2009f - Reprocessed	Vial Position	1
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	2.76 min	1.7237	11.9933	0.1032 min	0.048 %
2	4.59 min	0.8472	7.8122	0.1336 min	0.031 %
3	5.16 min	0.3340	2.8742	0.1284 min	0.012 %
4	6.59 min	0.3000	3.2419	0.1541 min	0.013 %
5	7.62 min	0.8456	16.3570	0.2917 min	0.066 %
6	9.49 min	0.5612	11.2026	0.2809 min	0.045 %
7	10.90 min	1006.2555	24768.8621	0.3512 min	99.785 %