BDG SYNTHESIS

Certificate of Analysis

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

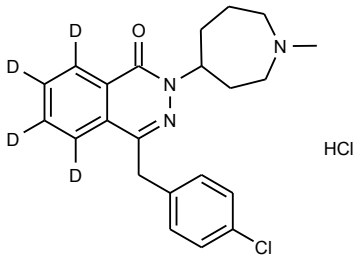
Barry Dent

Barry R. Dent, PhD, Director
22 May 2012

Name: **Azelastine-d_4 HCl**

CAS Number: 79307-93-0 (unlabelled)

Structure:



Molecular Weight:	$C_{22}H_{20}D_4ClN_3O \cdot HCl = 422.38$	
Lot Number:	BDG 3927.6	
Appearance:	Off-white, crystalline solid	
Corrected Purity:	99.9 % (HPLC) - 4.1 % (2-propanol) = 95.8 %	
Isotopic Purity:	Under 0.5 % d_0	
Re-test Date:	22 May 2017	
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.

Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available. The spectrum is complicated by the presence of two conformers in approximately a 2:1 ratio.
Isotopic Labelling: signals at the sites of deuteration are absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a small amount of 2-propanol (4.1 % *w/w*) is 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. The spectrum is complicated by the presence of two conformers in approximately a 2:1 ratio.
Isotopic Labelling: signals at the sites of deuteration have collapsed to small multiplets compared with the spectrum of unlabelled material, indicating clean deuteration.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 386.1927. $C_{22}H_{21}D_4ClN_3O$ $[M+H]^+$ requires *m/z* 386.1933. The deviation of 1.5 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

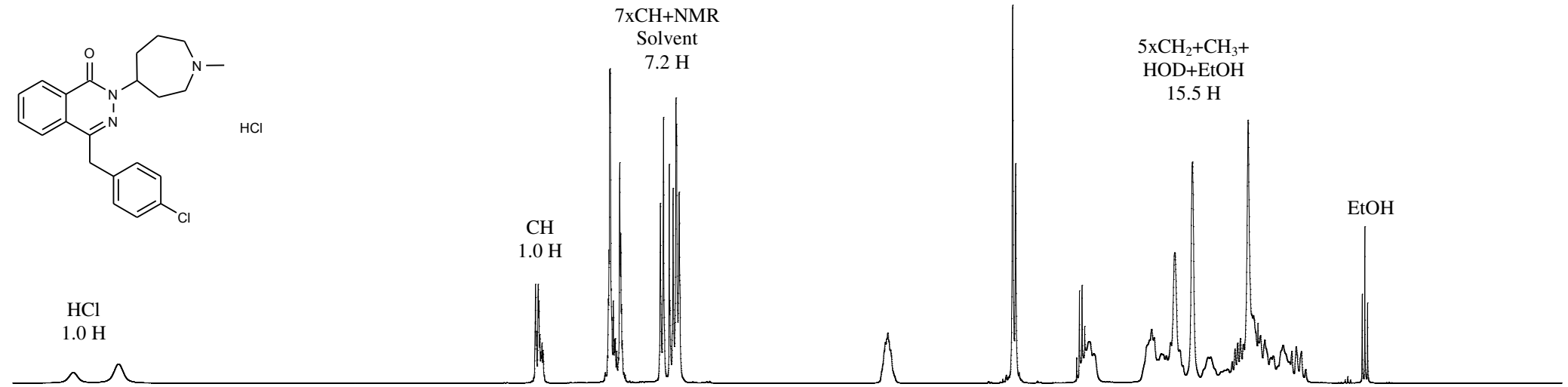
A somewhat broadened, symmetrical peak is observed (99.9 %). Note: in the absence of reference materials for preparing calibration curves, it is assumed that all peaks have the same detector response. Where possible, the conditions of analysis follow a pharmacopeial or literature method, or have been adapted from same.

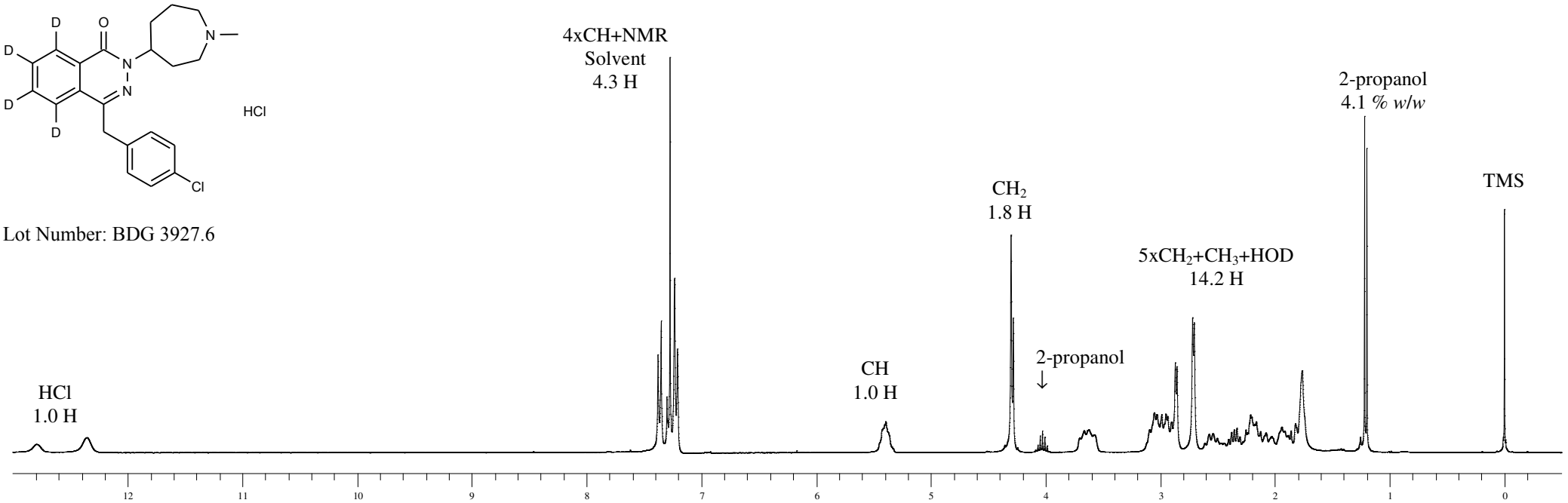
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 Azelastine HCl (top) and Azelastine-d_4 HCl (bottom) in $CDCl_3$

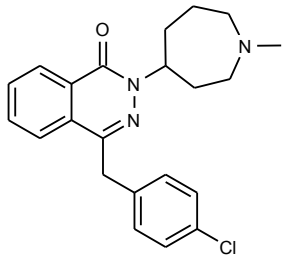




Lot Number: BDG 3927.6

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Azelastine HCl (top) and Azelastine-d_4 HCl (bottom) in $CDCl_3$



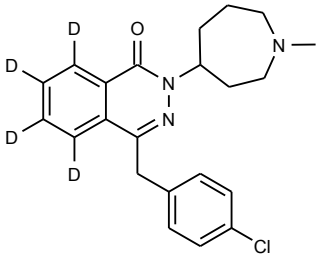
HCl

NMR
Solvent

4xCH

2xCH

2xCH



HCl

Lot Number: BDG 3927.6

4xCH

2xC

2xC

C=O

C

CD

2-propanol

2-propanol

$2xCH_2$+CH

CH_3

CH_2

$2xCH_2$

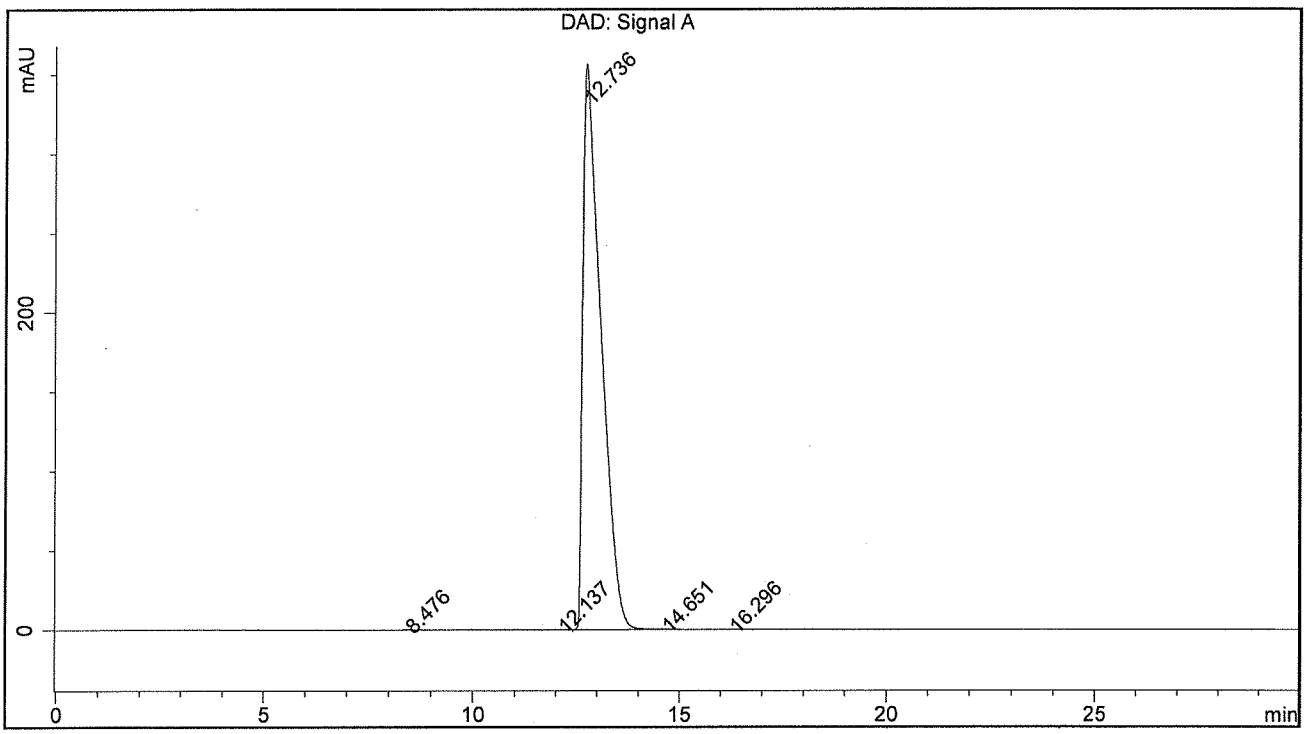
CH_2

200 175 150 125 100 75 50 25

BDG - Analysis of Azelastine-d4 HCl

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 4 x 3 mm
Mobile Phase : 65:35 20mM KH2PO4 + 5mM Heptanesulfonic Acid Sodium Salt to pH=3.0 : Acetonitrile
Flow Rate : 1.0 mL/min
Sample Solvent : Mobile Phase
Column Temperature : 20C
Injection Volume : 10 uL
Detection : UV at 288 nm

Sample Name	BDG 3927.6	**Instrument**	AnalyticalLC01
Acquisition	22/05/2012, 18:58:36	**Method (rev.)**	LC10514a (5)
Sequence	BDG_22May2012c - Reprocessed	**Vial Position**	1
Operator	solvation010\cerityadmin	**Injection**	1 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	8.48 min	0.1477	1.7875	0.1692 min	0.016 %
2	12.14 min	0.1854	2.3278	0.1605 min	0.021 %
3	12.74 min	356.5594	10869.0827	0.4256 min	99.915 %
4	14.65 min	0.1651	2.7769	0.2102 min	0.026 %
5	16.30 min	0.1037	2.3093	0.2838 min	0.021 %