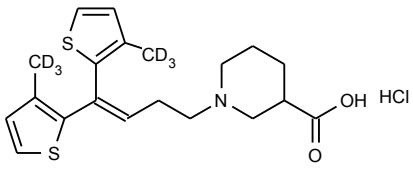
BDG SYNTHESIS

Certificate of Analysis

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

Neil Beare, PhD, Director
30 August 2015

Name:	**Tiagabine-d_6 HCl**
CAS Number:	145821-59-6 (unlabelled)
Structure:	
Molecular Weight:	$C_{20}H_{19}D_6NO_2S_2 \cdot HCl = 418.05$
Lot Number:	BDG 6047.5
Appearance:	White, crystalline solid
Corrected Purity:	98.6 % (HPLC) - 0.5 % (dioxane) - 1.3 % (acetone) - 4.5 % (water) = 92.3 %
Isotopic Purity:	Under 0.5 % d_0
Re-test Date:	30 August 2020

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.

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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: signals at the sites of deuteration are absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: small amounts of dioxane (0.5 % *w/w*) and acetone (1.3 % *w/w*) 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: 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* 382.1763. $C_{20}H_{20}D_6NO_2S_2$ $[M+H]^+$ requires *m/z* 382.1775. The deviation of 3.2 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 sharp, symmetrical peak is observed (98.6 %). 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 52.90, H 4.92, D 2.46, N 3.04 %
$C_{20}H_{19}D_6NO_2S_2{\cdot}HCl{\cdot}2.0H_2O$	Requires:	C 52.90, H 5.33, D 2.66, N 3.08 %
$C_{20}H_{19}D_6NO_2S_2{\cdot}HCl$	Requires:	C 57.46, H 4.82, D 2.89, N 3.35 %

The elemental analyses fall substantially 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 4.5 %
$C_{20}H_{19}D_6NO_2S_2{\cdot}HCl{\cdot}2.0H_2O$	Requires:	H_2O 7.9 %

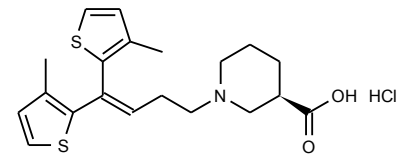
Of necessity, only a small sample could be used and only a single or duplicate analysis performed. We are unable to state what the errors in the reported water content are, but recommend that the result be used, as the best available, when determining corrected purity.

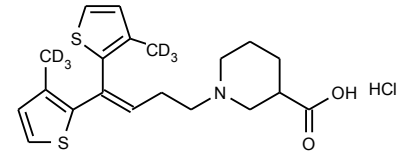
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 Tiagabine HCl (top) and Tiagabine-d_6 HCl (bottom) in DMSO-d_6

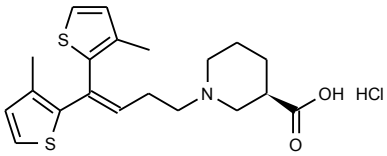


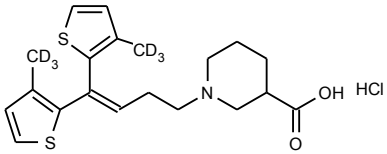


Lot Number: BDG 6047.5

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Tiagabine HCl (top) and Tiagabine-d_6 HCl (bottom) in DMSO-d_6



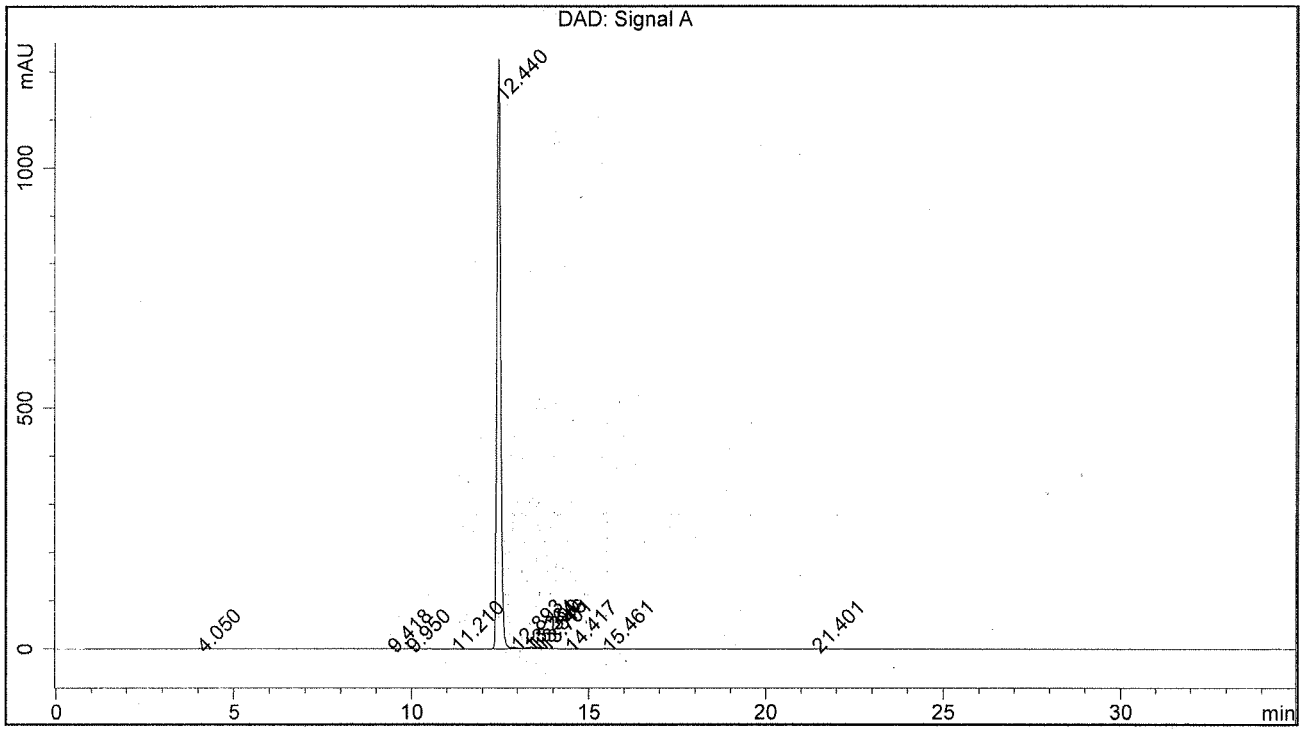


Lot Number: BDG 6047.5

BDG - Analysis of Tiagabine-d6 Hydrochloride

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase A : 25 mM Potassium diHydrogen Phosphate + 10 mM Sodium Heptanesulphonate pH=3.0 (H3PO4)
Mobile Phase B : Acetonitrile
Gradient (A:B) : T0=70:30; T20=30:70, T25=30:70, T30=70:30, T35=70:30
Flow Rate : 1.0 mL/min . . . . . Sample Solvent : Mobile Phase . . . . . Column Temperature : 20C
Injection Volume : 10 uL . . . . . Detection : UV at 258 nm

Sample Name	BDG 6047.5	Instrument	AnalyticalLC01
Acquisition	30/08/2015, 15:42:01	Method (rev.)	LC10273b (7)
Sequence	BDG_30Aug2015b - Reprocessed	Vial Position	52
Operator	solvation010\cerityadmin	Injection	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	4.05 min	0.2153	2.0292	0.1260 min	0.022 %
2	9.42 min	0.2318	4.3262	0.2397 min	0.048 %
3	9.95 min	0.1783	2.2834	0.1694 min	0.025 %
4	11.21 min	0.5700	3.8666	0.1031 min	0.043 %
5	12.44 min	1228.0503	8914.9764	0.1127 min	98.583 %
6	12.89 min	2.3461	22.4185	0.1335 min	0.248 %
7	13.26 min	2.3175	13.3251	0.0891 min	0.147 %
8	13.39 min	3.2745	22.1822	0.1030 min	0.245 %
9	13.55 min	3.1914	21.4600	0.1004 min	0.237 %
10	13.70 min	2.9116	22.0188	0.1122 min	0.243 %
11	14.42 min	0.4672	3.5727	0.1112 min	0.040 %
12	15.46 min	0.9087	8.0601	0.1275 min	0.089 %
13	21.40 min	0.2350	2.5757	0.1438 min	0.028 %