BDG SYNTHESIS

Certificate of Analysis

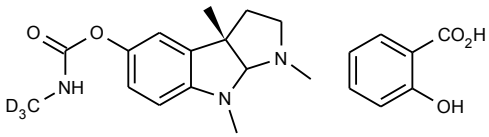
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare, PhD, Director
11 September 2014

Name: **Physostigmine-d_3 Salicylate**

CAS Number: 57-64-7 (unlabelled)

Structure:



Molecular Weight: $C_{15}H_{18}D_3N_3O_2 \cdot C_7H_6O_3$ = 416.49

Lot Number: BDG 2855.3

Appearance: Off-white powder

Purity By HPLC: 99.6 %

Isotopic Purity: Under 0.5 % d_0

Re-test Date: 11 September 2019

Storage and Handling:

Temperature:	refrigerate for prolonged storage; may be handled and shipped at ambient temperature.
Humidity:	may be hygroscopic; store desiccated; recommended to determine water content periodically.
Light:	store in an amber vial and protect from bright light.
Caution:	only experienced laboratory personnel should handle the material. Solutions of this material should be used immediately.

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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 site of deuteration are absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a trace (under 0.1 % *w/w*) of diethyl ether 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.
Isotopic Labelling: signals at the site 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* 279.1896. $C_{15}H_{19}D_3N_3O_2$ $[M+H]^+$ requires *m/z* 279.1900. 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 sharp, symmetrical peak is observed (99.6 %). The peak at 6.4 minutes is salicyclic acid. 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 63.26, H 5.75, D 1.44, N 10.08 %
$C_{15}H_{18}D_3N_3O_2 \cdot C_7H_6O_3$	Requires:	C 63.44, H 5.81, D 1.45, N 10.09 %

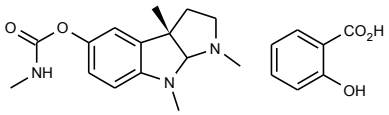
The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

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.

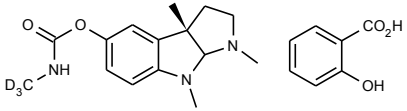
The re-test date is assigned from experience gained with the material in the laboratory and/or on storage. It is not possible to perform formal storage studies because of the small amount of material available.

BDG SYNTHESIS

Proton NMR Spectrum of Physostigmine Salicylate (top) and Physostigmine-d_3 Salicylate (bottom) in Methanol-d_4



NCH_3
3.0 H



Lot Number: BDG 2855.3

CH_3
3.1 H

$2xNCH_3$
6.0 H

NMR
Solvent
+NCH

CH+
HOD

↓

NCH
1.1 H

CH_2
2.0 H

CH
1.0 H

CH
1.0 H

5xCH
4.9 H

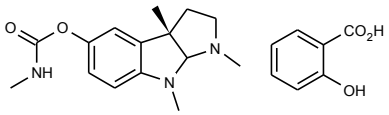
diethyl
ether

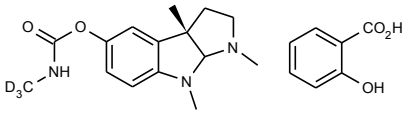
diethyl
ether

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Physostigmine Salicylate (top) and Physostigmine-d_3 Salicylate (bottom) in Methanol-d_4



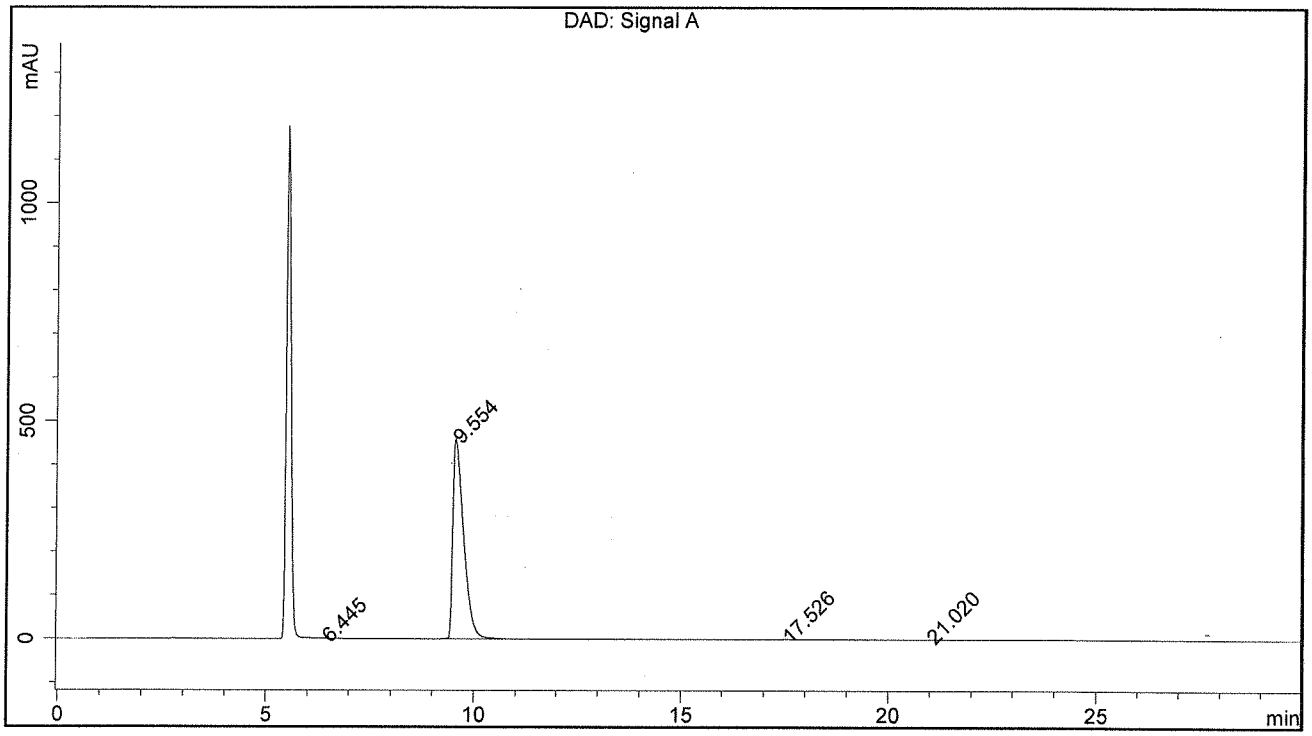


Lot Number: BDG 2855.3

BDG - Analysis of Physostigmine-d3 Salicylate

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase : 80:20 0.1M Ammonium Acetate pH 6.0: Acetonitrile-
Flow Rate : 1.0 mL/min
Sample Solvent : 80/20 H2O/CH3CN
Injection Volume : 10 uL
Detection: UV 305 nm

Sample Name	BDG 2855.3	Instrument	AnalyticalLC01
Acquisition	11/09/2014, 12:44:43	Method (rev.)	LC10628a (9)
Sequence	BDG_11Sep2014g - Reprocessed	Vial Position	12
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	6.44 min	0.7534	10.4880	0.2024 min	0.124 %
2	9.55 min	458.5754	8393.3209	0.2775 min	99.607 %
3	17.53 min	0.3694	9.5240	0.3208 min	0.113 %
4	21.02 min	0.4045	13.0699	0.4130 min	0.155 %