BDG SYNTHESIS

Certificate of Analysis

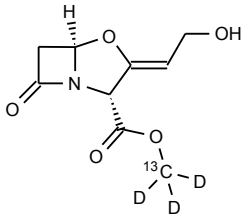
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare, PhD, Director
22 August 2016

Name: **Clavulanate Methyl Ester-^{13}C,d$_3$**

CAS Number: 57943-82-5 (unlabelled)

Structure:



Molecular Weight: $C_8{}^{13}CH_8D_3NO_5$ = 217.20

Lot Number: BDG 15533.3

Appearance: Pale yellow oil

Corrected Purity: 98.2 % (HPLC) - 0.9 % (ethyl acetate) = 97.3 %

Isotopic Purity: Under 0.5% M-4

Re-test Date: 22 August 2021

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.
Isotopic Labelling: signals at the site of deuteration are absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a small amount of ethyl acetate (0.9 % *w/w*) is observed.
Impurities: traces of unidentified impurities are seen in the baseline.

Carbon-13 NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.
Isotopic Labelling: the spectrum is of little value in determining isotopic purity, although the signal at the labelled site is massively enhanced and shows coupling to deuterium, as expected.

High-resolution Mass Spectrum (TOF MS ES+)

Found *m/z* 240.0752. $C_8{}^{13}CH_8D_3NNaO_5$ $[M+Na]^+$ requires *m/z* 240.0757. The deviation of 2.1 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for M-4 material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (98.2 %). 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 49.95, H 3.84, D 2.88, N 6.19 %
$C_8{}^{13}CH_8D_3NO_5$	Requires:	C 50.23, H 3.71, D 2.78, N 6.45 %

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.

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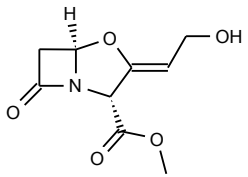
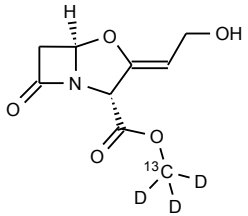
Proton NMR Spectrum of Clavulanate Methyl Ester (top) and Clavulanate Methyl Ester-$^{13}C,d_3$ (bottom) in DMSO-d_6





Lot Number: BDG 15533.3

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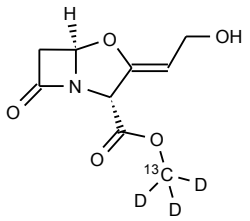
Carbon-13 NMR Spectrum of Clavulanate Methyl Ester-$^{13}C,d_3$ in DMSO-d_6



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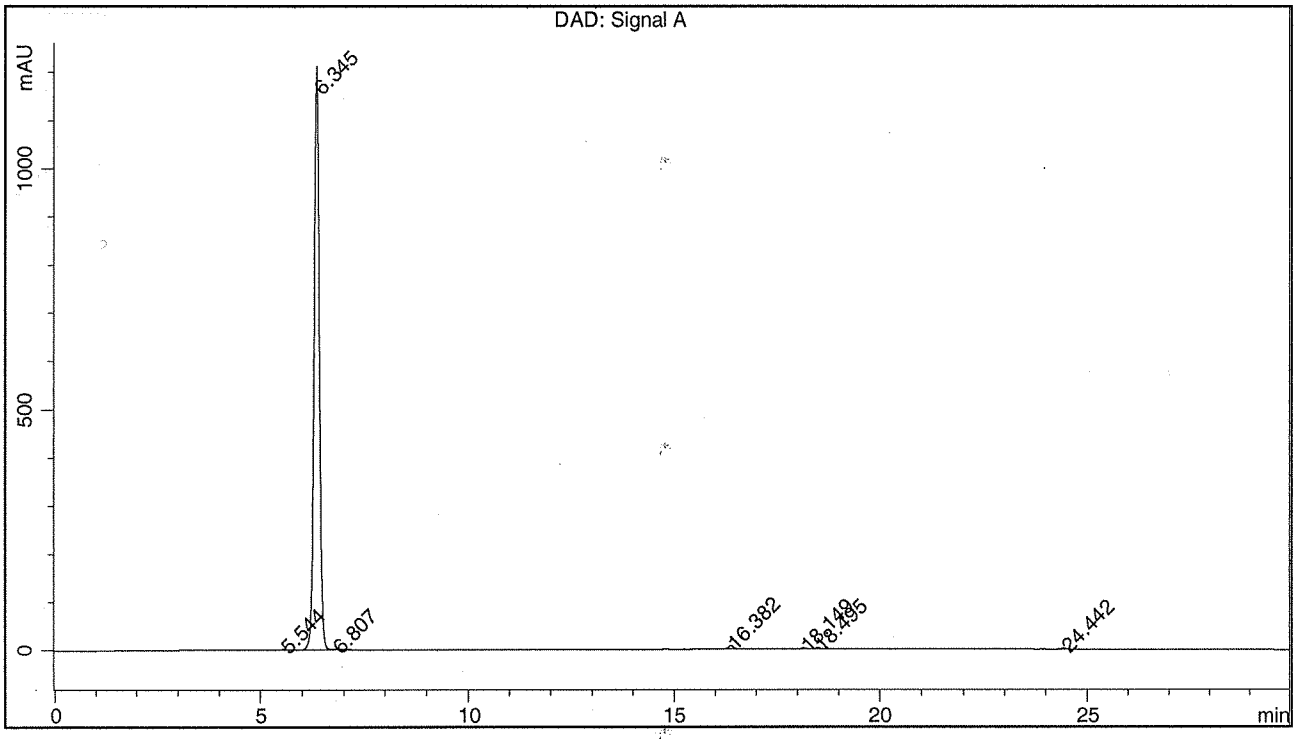
BDG - Analysis of Clavulanate Methyl Ester-13C,d3

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase A : 80:20 Water : Acetonitrile
Mobile Phase B : 50:50 Water : Acetonitrile
Gradient (A:B) : T0=100:0, T8=100:0, T18=0:100, T26=0:100, T27=100:0, T30=100:0
Column Temperature : 20 C . . . . . Flow Rate : 1.0 mL/min . . . . . Injection Volume : 10 uL
Sample Solvent : Initial Mobile Phase . . . . . Detection : UV 215 nm

Sample Name	BDG 15533.3	**Instrument**	AnalyticalLC01
Acquisition	22/08/2016, 12:21:40	**Method (rev.)**	LC10691a (5)
Sequence	BDG_22Aug2016c - Reprocessed	**Vial Position**	11
Operator	solvation010\cerityadmin	**Injection**	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	5.54 min	1.2252	10.5215	0.1362 min	0.098 %
2	6.34 min	1207.4681	10554.9521	0.1340 min	98.230 %
3	6.81 min	2.0370	54.2973	0.3350 min	0.505 %
4	16.38 min	6.9080	69.8657	0.1500 min	0.650 %
5	18.15 min	2.3576	16.0947	0.1076 min	0.150 %
6	18.50 min	2.9024	18.0225	0.1004 min	0.168 %
7	24.44 min	1.9158	21.4135	0.1704 min	0.199 %