BDG SYNTHESIS

Certificate of Analysis

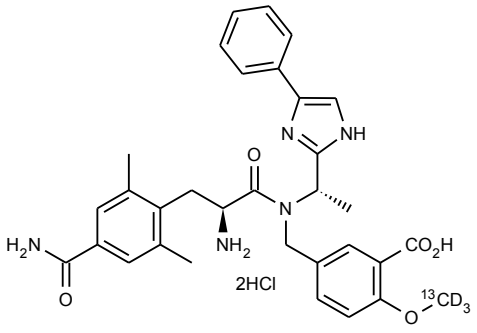
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare, PhD, Director
11 March 2015

Name: **Eluxadoline-^{13}C,d$_3$ Dihydrochloride**

CAS Number: 864825-13-8 (unlabelled)

Structure:



Molecular Weight: $C_{31}{}^{13}CH_{32}D_3N_5O_5 \cdot 2HCl = 646.58$

Lot Number: BDG 15192.3

Appearance: Off-white, crystalline solid

Corrected Purity: 98.1 % (HPLC) - 7.7 % (water) = 90.4 %

Isotopic Purity: Under 0.5% M-4

Re-test Date: 11 March 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.

Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available. The complexity of the spectrum indicates two conformers of the product are present in solution.
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: 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. The compexity of the spectrum indicates two conformers of the product are present in solution.
Isotopic Labelling: the spectrum is of little value in determining isotopic purity as the signal at the labelled site is massively enhanced, as expected.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 574.2930. $C_{31}{}^{13}CH_{33}D_3N_5O_5$ $[M+H]^+$ requires *m/z* 574.2938. The deviation of 1.4 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 somewhat broadened, tailing peak is observed (98.1 %). 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 54.83, N 9.96 %
$C_{31}{}^{13}CH_{32}D_3N_5O_5 \cdot 2HCl \cdot 3.0H_2O$	Requires:	C 55.00, N 10.00 %, H_2O 7.71 %
$C_{31}{}^{13}CH_{32}D_3N_5O_5 \cdot 2HCl$	Requires:	C 59.59, N 10.83 %

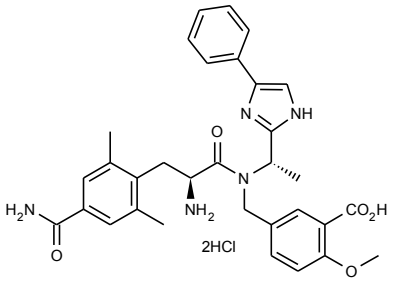
The elemental analyses fall substantially outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the “best-fit” hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend that the “best-fit” water content be used when determining corrected purity.

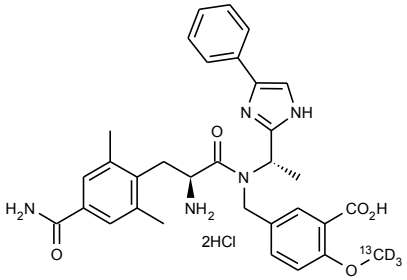
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 Eluxadoline Dihydrochloride (top) and Eluxadoline-$^{13}C,d_3$ Dihydrochloride (bottom) in Methanol-d_4

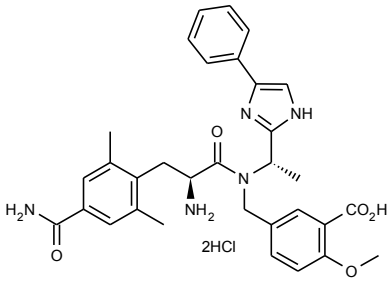


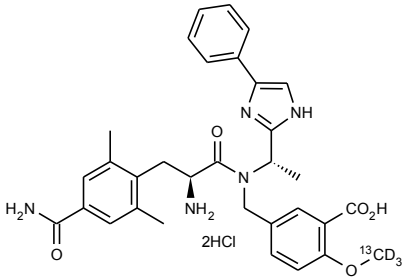


Lot Number: BDG 15192.3

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Eluxadoline Dihydrochloride (top) and Eluxadoline-$^{13}C,d_3$ Dihydrochloride (bottom) in Methanol-d_4





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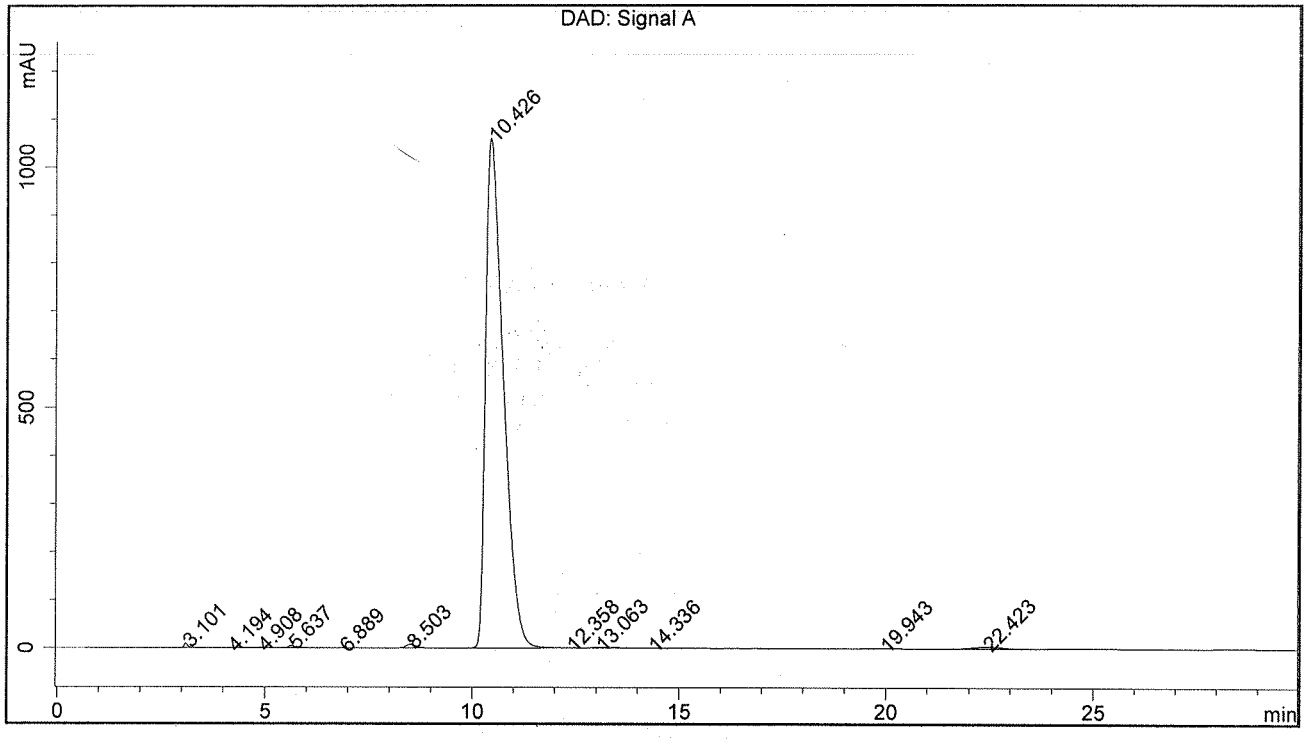
BDG - Analysis of Eluxadoline-13C,d3 Dihydrochloride

Column : Phenomenex Luna C18(2) 5 um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase : 83:17:0.05 Water : Acetonitrile : Trifluoroacetic Acid
Flow Rate : 1.0 mL/min . . . . . Column Temperature : 20 C . . . . . Detection: UV 240 nm
Sample Solvent : 80:20 Water : Acetonitrile . . . . . Injection Volume : 10 uL

Sample Name	BDG 15192.3	**Instrument**	AnalyticalLC01
Acquisition	11/03/2015, 17:49:19	**Method (rev.)**	LC10646b (2)
Sequence	BDG_11Mar2015a	**Vial Position**	1
Operator	solvation010\cerityadmin	**Injection**	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	3.10 min	9.5437	58.3648	0.0953 min	0.180 %
2	4.19 min	0.2731	10.8625	0.5080 min	0.033 %
3	4.91 min	1.1067	14.5712	0.1856 min	0.045 %
4	5.64 min	5.0213	55.4638	0.1609 min	0.171 %
5	6.89 min	0.2510	3.2769	0.1963 min	0.010 %
6	8.50 min	7.3955	112.7642	0.2299 min	0.347 %
7	10.43 min	1059.6473	31858.4973	0.4508 min	98.074 %
8	12.36 min	0.6151	13.3951	0.3141 min	0.041 %
9	13.06 min	1.5597	79.4117	0.6700 min	0.244 %
10	14.34 min	0.2825	4.2340	0.1989 min	0.013 %
11	19.94 min	0.9485	52.4302	0.6849 min	0.161 %
12	22.42 min	4.1730	220.8906	0.7135 min	0.680 %