BDG SYNTHESIS

Certificate of Analysis

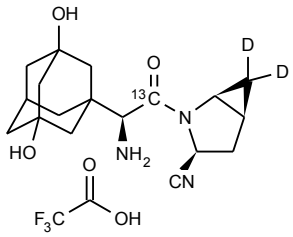
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare, PhD, Director
30 April 2016

Name: **5-Hydroxysaxagliptin-^{13}C,d$_2$ TFA Salt**

CAS Number: 841302-24-7 (unlabelled, free base)

Structure:



Molecular Weight: $C_{17}{}^{13}CH_{23}D_2N_3O_3 \cdot C_2HF_3O_2$ = 448.44

Lot Number: BDG 5999.2

Appearance: White, crystalline solid

Corrected Purity: 98.2 % (HPLC) - 2.4 % (diethyl ether) - 4.2 % (water) = 91.6 %

Isotopic Purity: Under 0.5% M-3

Re-test Date: 30 April 2021

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:	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 site of deuteration are absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a small amount of diethyl ether (2.4 % *w/w*) and a trace (under 0.1 % *w/w*) of acetone are 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. Signals for the trifluoroacetate moiety are not observed.
Isotopic Labelling: the signal at the site of deuteration is absent compared with the spectrum of unlabelled material, indicating clean deuteration and the signal at the ^{13}C labelled site is massively enhanced as expected.
Impurities: signals for diethyl ether (a crystallisation solvent) are also observed.

High-resolution Mass Spectrum (TOF MS ES+)

Found *m/z* 335.2133. $C_{17}{}^{13}CH_{24}D_2N_3O_3$ $[M+H]^+$ requires *m/z* 335.2133. The deviation of 0.0 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for M-3 material was seen (detection limit about 0.5 %).

HPLC

A somewhat broadened, 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 51.96, H 5.73, D 0.96, N 8.43 %
$C_{17}{}^{13}CH_{23}D_2N_3O_3 \cdot C_2HF_3O_2 \cdot 1.1H_2O$	Requires:	C 51.51, H 5.64, D 0.86, N 8.97 %, H_2O 4.23 %
$C_{17}{}^{13}CH_{23}D_2N_3O_3 \cdot C_2HF_3O_2$	Requires:	C 53.79, H 5.39, D 0.90, N 9.37 %

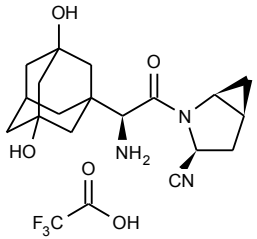
The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the "best-fit" hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend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

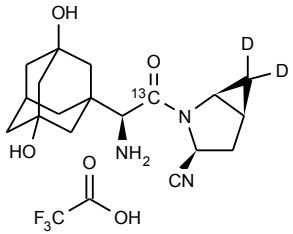
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 5-Hydroxysaxagliptin TFA Salt (top) and 5-Hydroxysaxagliptin-$^{13}C,d_2$ TFA Salt (bottom) in Methanol-d_4

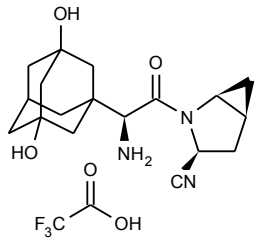


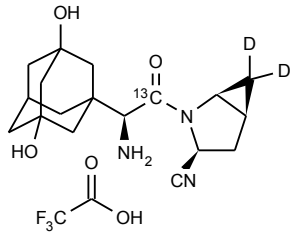


Lot Number: BDG 5999.2

BDG SYNTHESIS

Carbon-13 NMR Spectrum of 5-Hydroxysaxagliptin TFA Salt (top) and 5-Hydroxysaxagliptin-$^{13}C,d_2$ TFA Salt (bottom) in Methanol-d_4



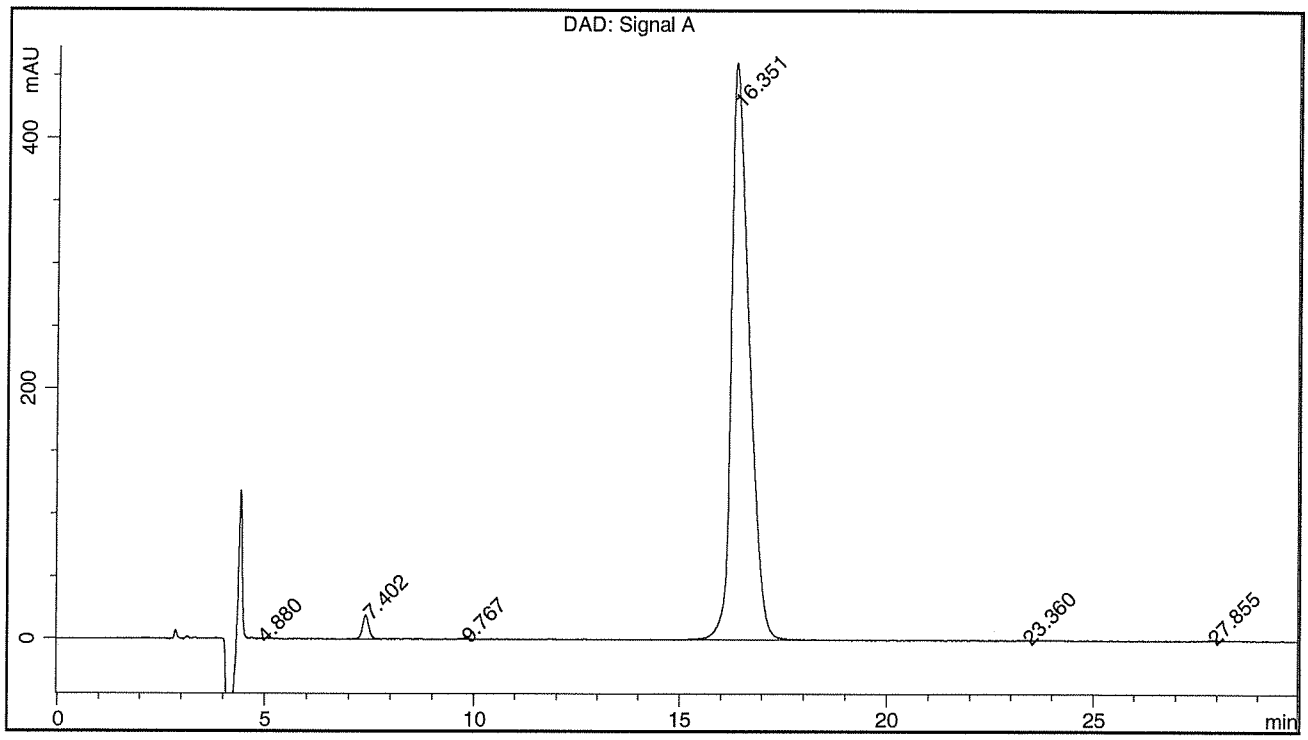


Lot Number: BDG 5999.2

BDG - Analysis of 5-Hydroxysaxagliptin-13C,d2 TFA Salt

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase : 92:8:0.1 Water : Acetonitrile : Trifluoroacetic Acid
Flow Rate : 1.0 mL/min
Sample Solvent : Mobile Phase
Column Temperature : 20 C
Injection Volume : 10 uL
Detection : UV at 212 nm

Sample Name	BDG 5999.2	Instrument	AnalyticalLC01
Acquisition	30/04/2016, 12:08:49	Method (rev.)	LC10431j (8)
Sequence	BDG_30Apr2016b - Reprocessed	Vial Position	12
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	4.88 min	0.4324	2.5237	0.0921 min	0.018 %
2	7.40 min	19.0695	207.9167	0.1712 min	1.468 %
3	9.77 min	0.8886	15.1514	0.2349 min	0.107 %
4	16.35 min	461.1361	13903.2103	0.4437 min	98.178 %
5	23.36 min	0.5448	13.0474	0.2994 min	0.092 %
6	27.86 min	0.6052	19.3865	0.4057 min	0.137 %