BDG SYNTHESIS

Certificate of Analysis

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

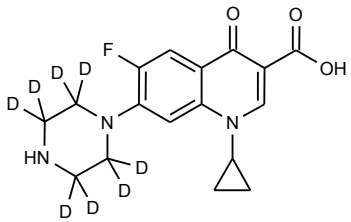
Barry Dent

Barry R. Dent, PhD, Director
15 November 2012

Name: **Ciprofloxacin-d_8**

CAS Number: 85721-33-1 (unlabelled)

Structure:



Molecular Weight: $C_{17}H_{10}D_8FN_3O_3$ = 339.39

Lot Number: BDG 5539

Appearance: Off-white powder

Corrected Purity: 99.1 % (HPLC) - 0.2 % (acetone) - 0.2 % (acetic acid) - 17.1 % (water) = 81.6 %

Isotopic Purity: Under 0.5 % d_0

Re-test Date: 15 November 2017

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: the small signals observed at the sites of deuteration are present at about 2.0 % of the intensity that would be expected for unlabelled material. This value is within the range specified by the manufacturer (CIL) for the labelled starting material that was used in the synthesis (98% atom D) and we conclude that H/D exchange has not occurred. The signals are most likely to arise from the presence of small amounts of d7 or d6 species. The absence of d0 species as a contributor to these signals was confirmed by HRMS (see below).
Residual Solvents: small amounts of acetone (0.2 % *w/w*) and acetic acid (0.2 % *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 (as opposed to two sharp singlets that would be expected for unlabelled material), indicating clean deuteration.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 340.1890. $C_{17}H_{11}D_8FN_3O_3$ $[M+H]^+$ requires *m/z* 340.1907. The deviation of 5.1 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 somewhat broadened, slightly tailing peak is observed (99.1 %). 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 48.67, H 4.63, D 3.89, N 9.93 %
$C_{17}H_{10}D_8FN_3O_3{\cdot}4.5H_2O$	Requires:	C 48.56, H 4.55, D 3.83, N 9.99 %
$C_{17}H_{10}D_8FN_3O_3$	Requires:	C 60.16, H 2.97, D 4.75, N 12.38 %

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 17.1 %
$C_{17}H_{10}D_8FN_3O_3{\cdot}4.5H_2O$	Requires:	H_2O 19.3 %

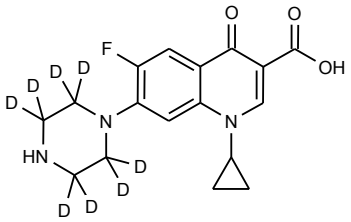
Of necessity, only a small sample could be used and only a single or duplicate analysis performed. We are unable to state what the errors in the reported water content are, but recommend that the result be used, as the best available, when determining corrected purity.

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

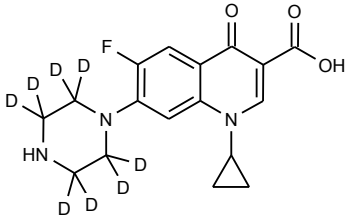
Proton NMR Spectrum of Ciprofloxacin-d_8 in DMSO-d_6 + DCl



Lot Number: BDG 5539

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Ciprofloxacin-d_8 in D_2O + DCl

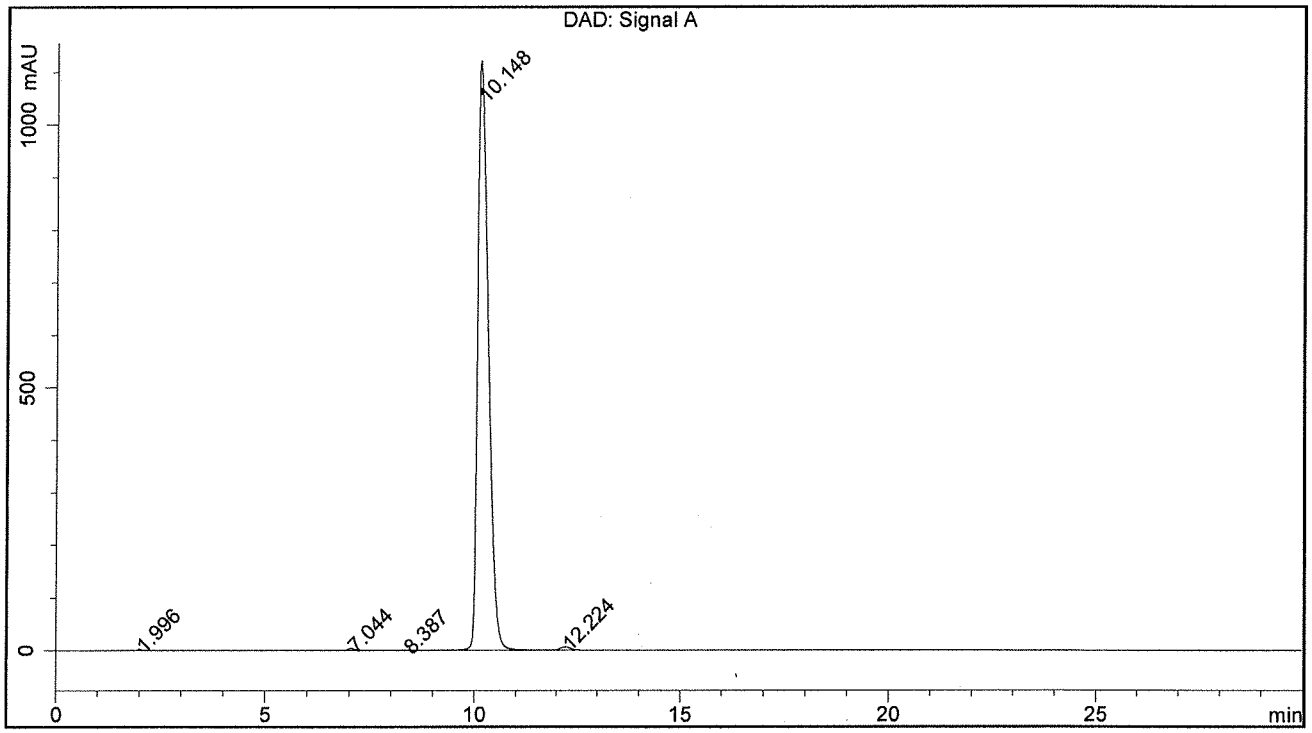


Lot Number: BDG 5539

BDG - Analysis of Ciprofloxacin-d8

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase : 87:13 25 mM Phosphoric Acid pH=3.0 (Triethylamine) : Acetonitrile
Flow Rate : 1.5 mL/min
Sample Solvent : Mobile phase
Column Temperature : 30C
Injection Volume : 10 uL
Detection : UV at 278 nm
Run Time: 30 mins

Sample Name	BDG 5539	**Instrument**	AnalyticalLC01
Acquisition	15/11/2012, 11:05:05	**Method (rev.)**	LC10125a (8)
Sequence	BDG_15Nov2012a	**Vial Position**	1
Operator	solvation010\cerityadmin	**Injection**	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	2.00 min	2.9367	16.9566	0.0834 min	0.083 %
2	7.04 min	3.8766	43.8501	0.1700 min	0.216 %
3	8.39 min	0.6898	8.7693	0.1883 min	0.043 %
4	10.15 min	1122.1545	20149.3226	0.2795 min	99.079 %
5	12.22 min	6.3975	117.7859	0.2788 min	0.579 %