

## BDG SYNTHESIS

### Certificate of Analysis

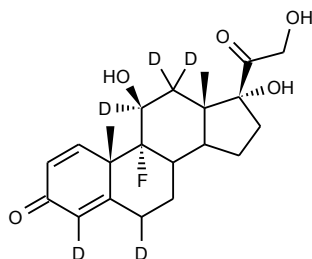
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

*Neil Beare*

Neil Beare, PhD, Director  
18 April 2016

**Name:** Isoflupredone-d<sub>5</sub>  
**CAS Number:** 338-95-4 (unlabelled)

**Structure:**



**Molecular Weight:** C<sub>21</sub>H<sub>22</sub>D<sub>5</sub>FO<sub>5</sub> = 383.47  
**Lot Number:** BDG 16670.4  
**Appearance:** White, crystalline solid  
**Corrected Purity:** 99.1 % (HPLC) - 0.3 % (methanol) - 2.3 % (water) = 96.5 %  
**Isotopic Purity:** Under 0.5 % d<sub>0</sub>  
**Re-test Date:** 18 April 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. Avoid strongly alkaline solutions as H/D exchange may occur.

## 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 sites of deuteration are greatly diminished compared with the spectrum of unlabelled material as expected.

Residual Solvents: a small amount of methanol (0.3 % w/w) 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 sites of deuteration have collapsed to small multiplets compared with the spectrum of unlabelled material as expected.

### High-resolution Mass Spectrum (TOF MS ES+)

Found  $m/z$  406.2055.  $C_{21}H_{22}D_5FNaO_5$   $[M+Na]^+$  requires  $m/z$  406.2054. The deviation of 0.2 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 %). Minor signals are seen for  $d_1$ - $d_4$  material.

### HPLC

A sharp, symmetrical 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 64.16, H 5.90, D 2.56 %
$C_{21}H_{22}D_5FO_5 \cdot 0.5H_2O$	Requires:	C 64.27, H 5.91, D 2.57 %, $H_2O$ 2.30 %
$C_{21}H_{22}D_5FO_5$	Requires:	C 65.78, H 5.78, D 2.63 %

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.

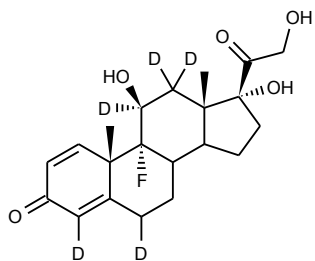
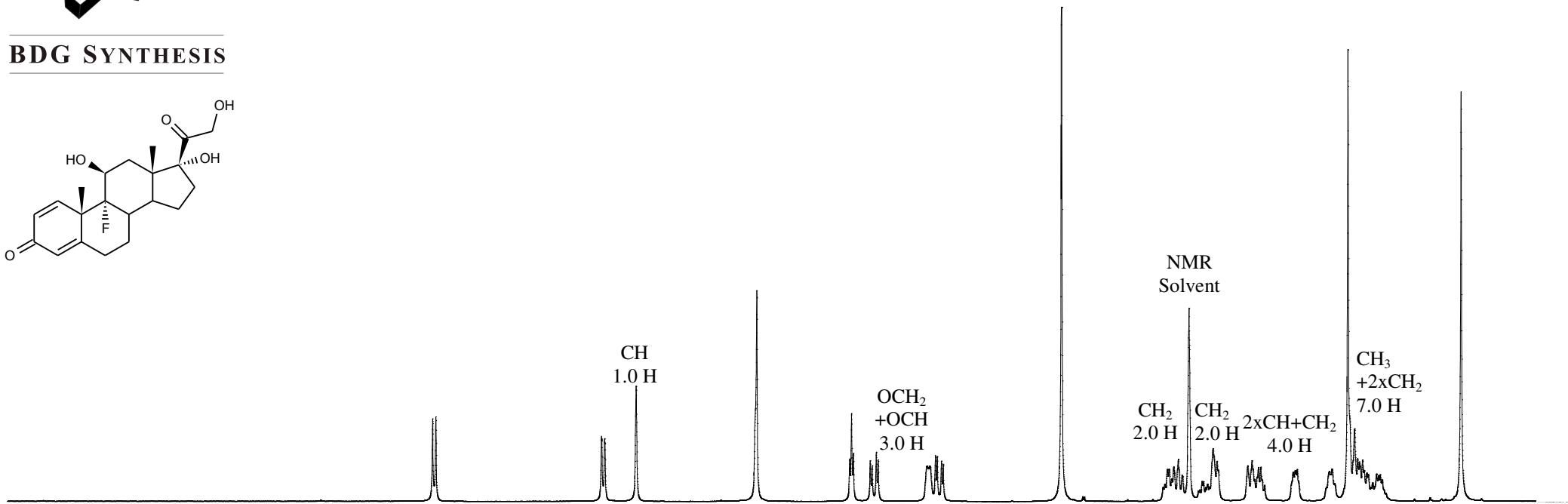
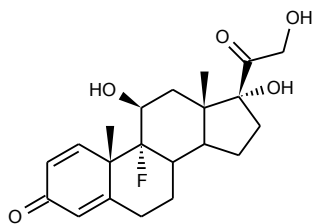
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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.

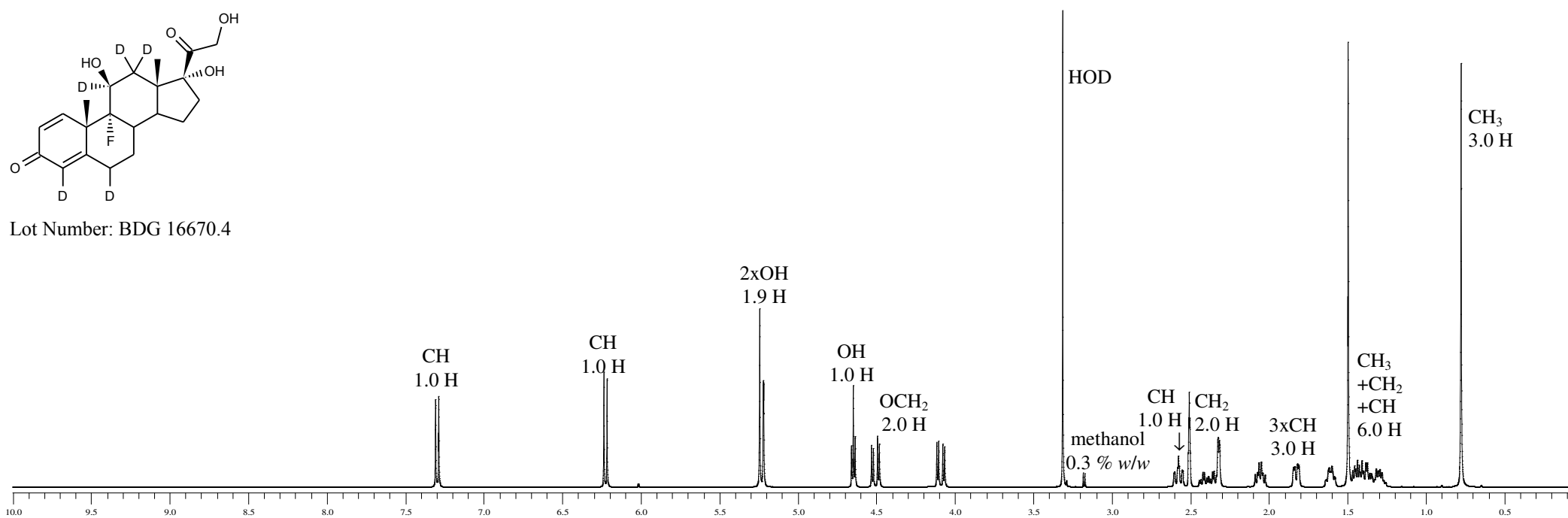


Proton NMR Spectrum of Isoflupredone (top) and Isoflupredone-d<sub>5</sub> (bottom) in DMSO-d<sub>6</sub>

**BDG SYNTHESIS**



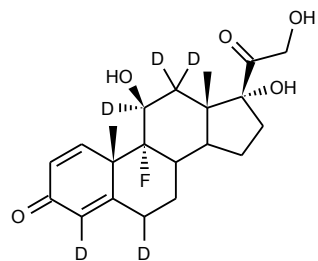
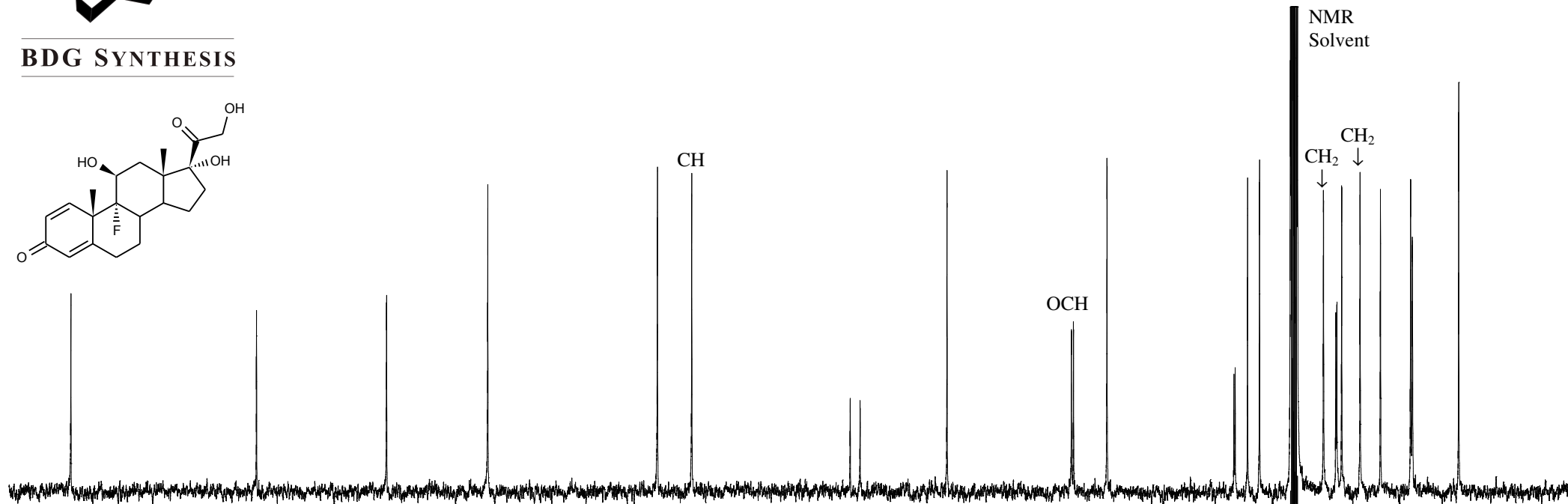
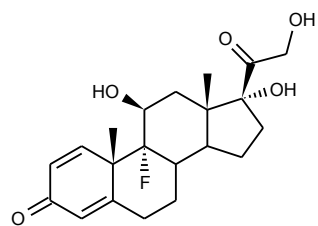
Lot Number: BDG 16670.4



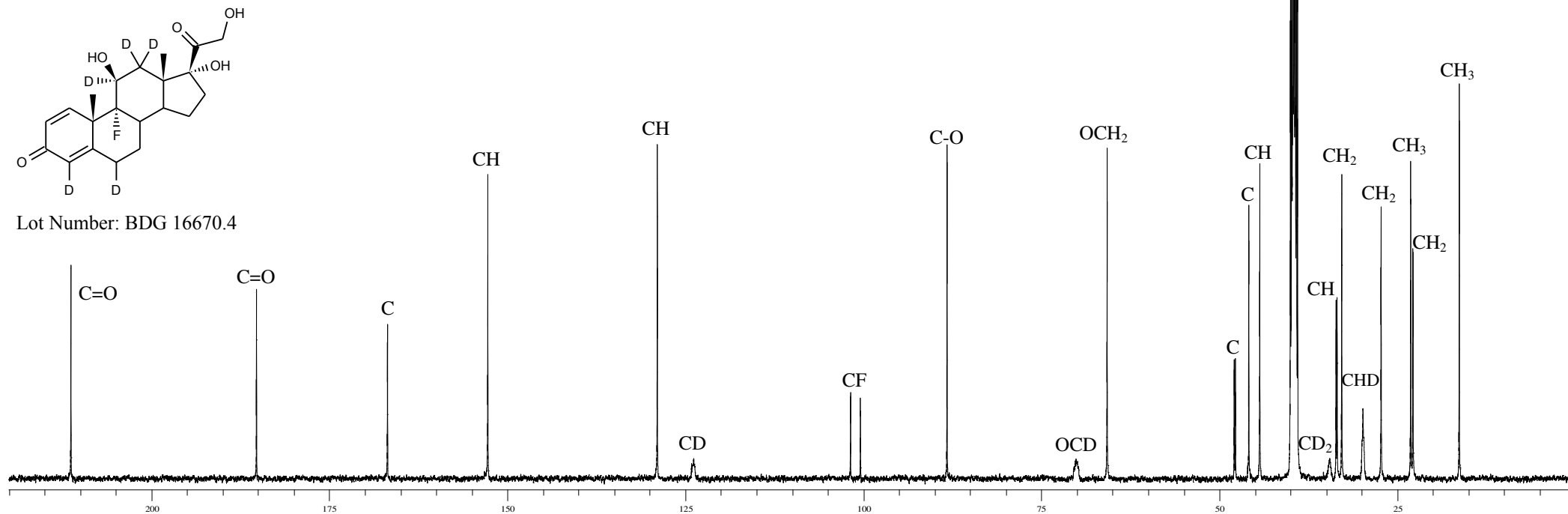


Carbon-13 NMR Spectrum of Isoflupredone (top) and Isoflupredone-d<sub>5</sub> (bottom) in DMSO-d<sub>6</sub>

**BDG SYNTHESIS**



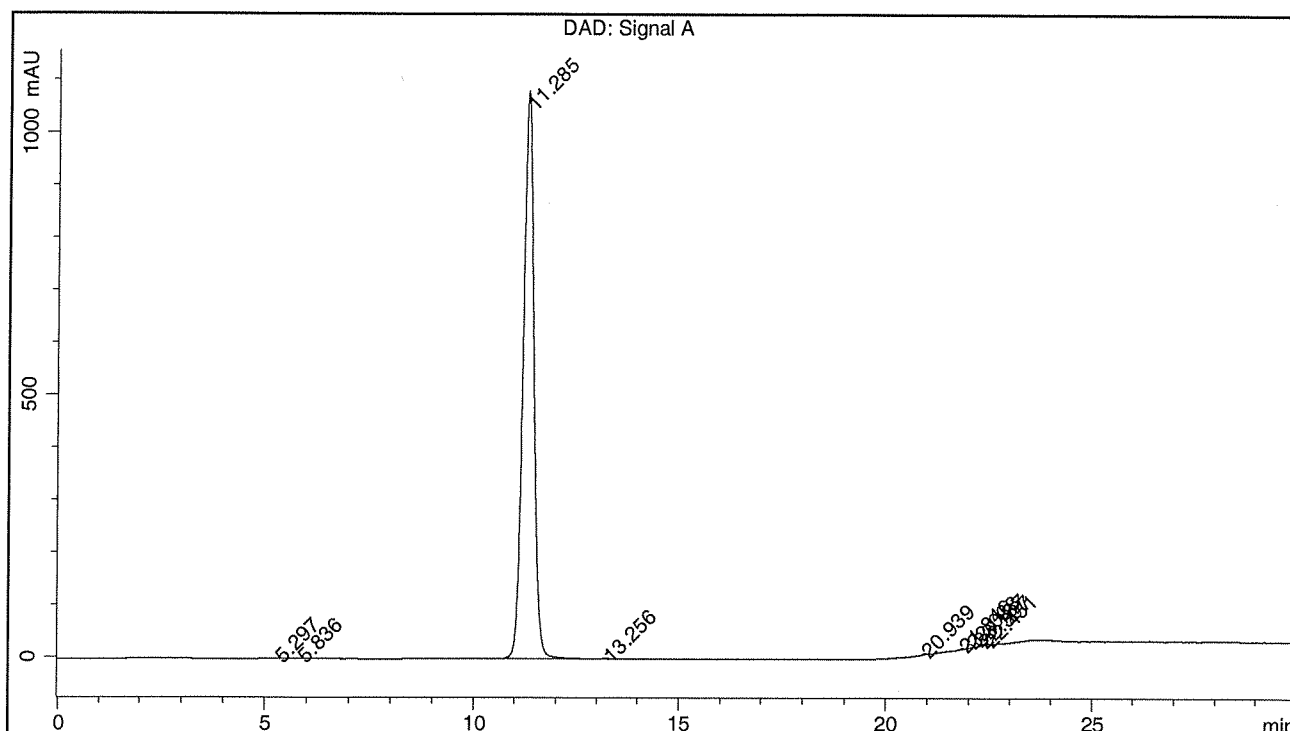
Lot Number: BDG 16670.4



BDG - Analysis of Isoflupredone-d5

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm  
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm  
 Mobile Phase A : 45:55 Water : Methanol  
 Mobile Phase C : Methanol  
 Gradient ( A:C ) : T0=100:0, T15=100:0, T20=0:100, T26=0:100, T27=100:0, T30=100:0  
 Flow Rate : 1.0 mL/min . . . . Column Temperature : 30 C . . . . Injection Volume : 10 uL  
 Sample Solvent : 30:70 Water : Methanol . . . . Detection : UV 242 nm

<b>Sample Name</b>	BDG 16670.4	<b>Instrument</b>	AnalyticalLC01
<b>Acquisition</b>	18/04/2016, 10:37:39	<b>Method (rev.)</b>	LC10674b ( 17)
<b>Sequence</b>	BDG_18Apr2016a - Reprocessed	<b>Vial Position</b>	71
<b>Operator</b>	solvation010\cerityadmin	<b>Injection</b>	1 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	5.30 min	0.3268	4.7801	0.1806 min	0.024 %
2	5.84 min	0.3615	5.5505	0.1931 min	0.028 %
3	11.28 min	1081.9602	19491.2314	0.2782 min	99.142 %
4	13.26 min	0.6358	15.4177	0.3009 min	0.078 %
5	20.94 min	2.9454	15.1674	0.0821 min	0.077 %
6	21.85 min	2.0714	10.8518	0.0791 min	0.055 %
7	22.03 min	7.4600	49.4309	0.0973 min	0.251 %
8	22.19 min	7.7537	41.7592	0.0808 min	0.212 %
9	22.31 min	3.1718	14.5658	0.0716 min	0.074 %
10	22.45 min	2.5219	11.1797	0.0717 min	0.057 %