

## BDG SYNTHESIS

### Certificate of Analysis

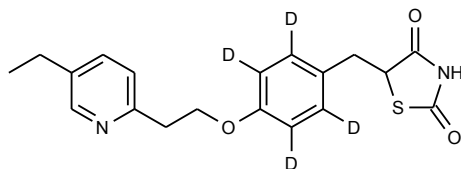
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

*Neil Beare*

Neil Beare, PhD, Director  
22 March 2016

**Name:** Pioglitazone-d<sub>4</sub>  
**CAS Number:** 111025-46-8 (unlabelled)

**Structure:**



**Molecular Weight:** C<sub>19</sub>H<sub>16</sub>D<sub>4</sub>N<sub>2</sub>O<sub>3</sub>S = 360.46  
**Lot Number:** BDG 6636.1  
**Appearance:** White, crystalline solid  
**Corrected Purity:** 98.4 % (HPLC) - 2.8 % (ethanol) = 95.6 %  
**Isotopic Purity:** Under 0.5 % d<sub>0</sub>  
**Re-test Date:** 22 March 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 sites of deuteration are greatly diminished, compared with what would be expected for unlabelled material, indicating clean deuteration.

Residual Solvents: a small amount of ethanol (2.8 % 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 what would be expected for unlabelled material, indicating clean deuteration.

### High-resolution Mass Spectrum (ESI+)

Found  $m/z$  361.1515.  $C_{19}H_{17}D_4N_2O_3S$   $[M+H]^+$  requires  $m/z$  361.1520. The deviation of 1.0 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 sharp, slightly tailing peak is observed (98.4 %). 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 63.05, H 4.81, D 2.49, N 7.47 % |
| $C_{19}H_{16}D_4N_2O_3S$ | Requires: | C 63.31, H 4.47, D 2.24, N 7.77 % |

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).

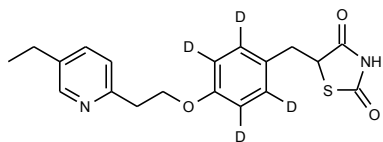
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

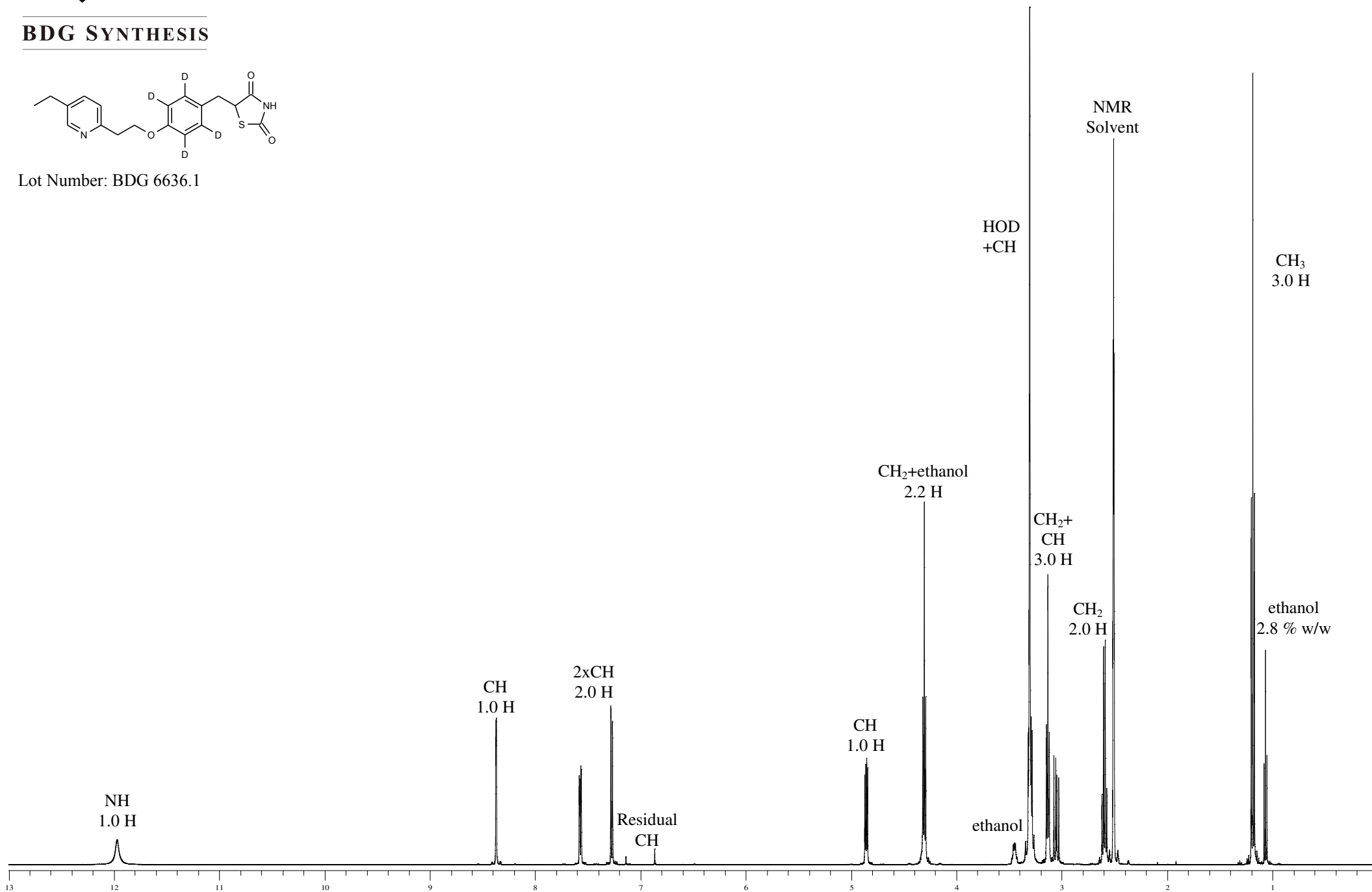


Proton NMR Spectrum of Pioglitazone-d<sub>4</sub> in DMSO-d<sub>6</sub>

**BDG SYNTHESIS**



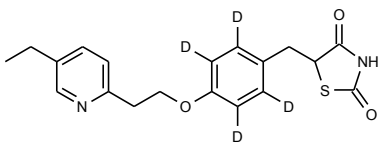
Lot Number: BDG 6636.1



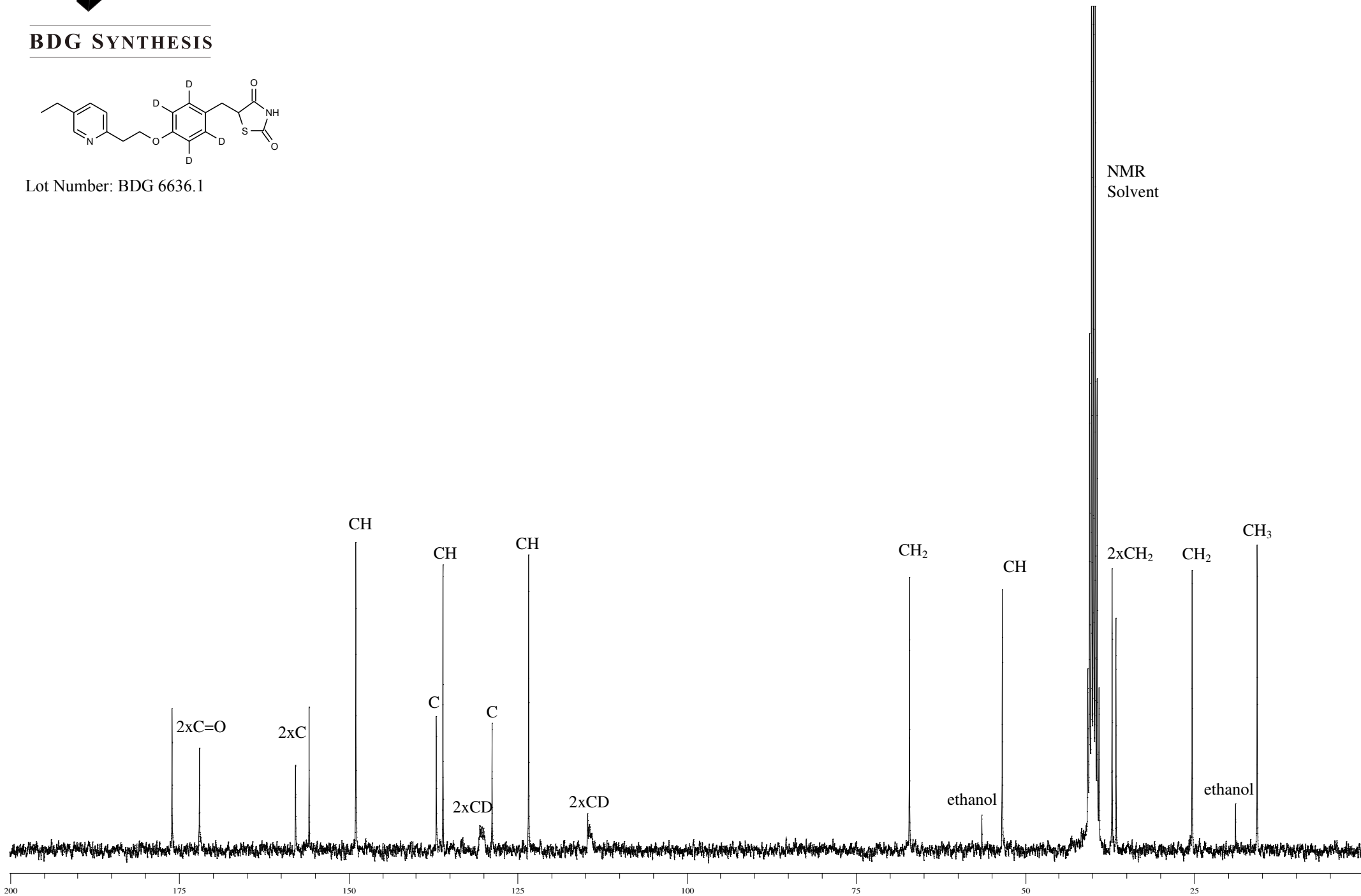


# Carbon-13 NMR Spectrum of Pioglitazone-d<sub>4</sub> in DMSO-d<sub>6</sub>

## BDG SYNTHESIS



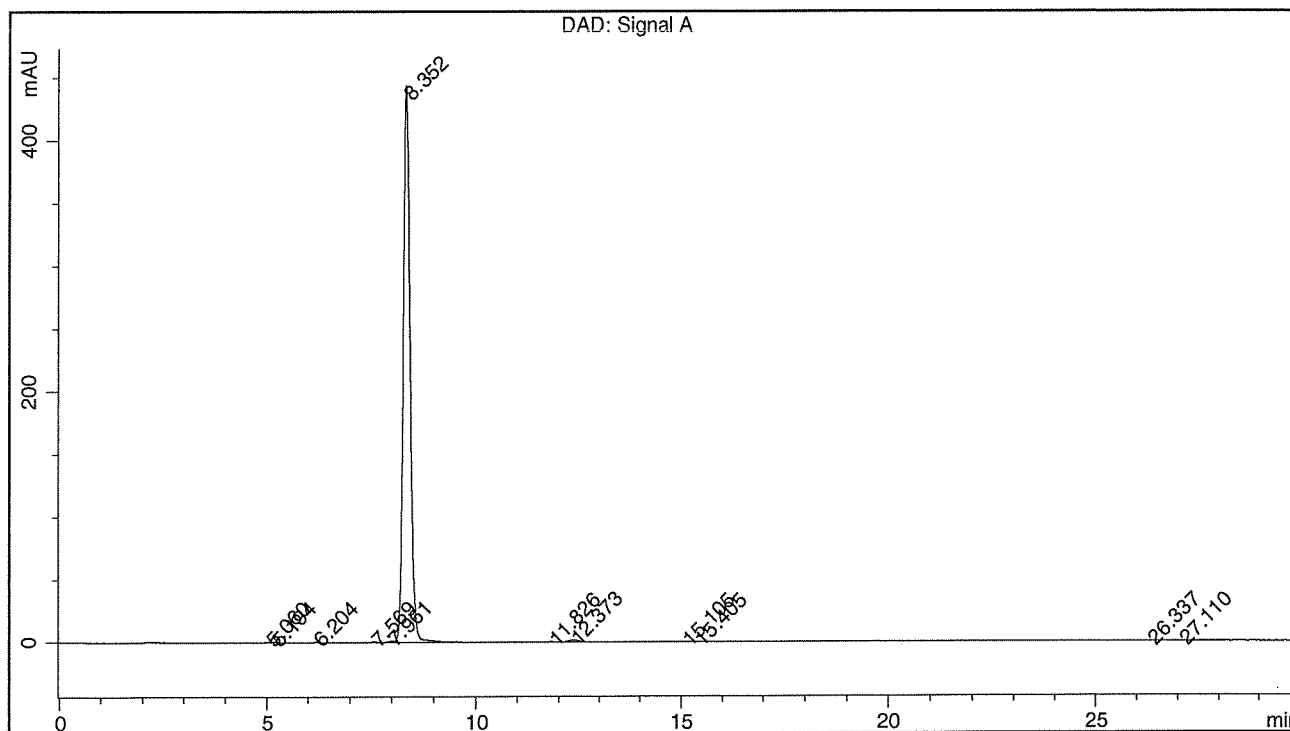
Lot Number: BDG 6636.1



BDG - Analysis of Pioglitazone-d4

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm  
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm  
 Mobile Phase : 50:50:2 100 mM Ammonium Acetate : Acetonitrile : Acetic Acid  
 Flow Rate : 1.0 mL/min . . . . Sample Solvent : 1:1 Mobile Phase : Acetonitrile  
 Column Temperature : 25 C . . . . Injection Volume : 10 uL . . . . Detection : UV at 269 nm

|                    |                              |                      |                |
|--------------------|------------------------------|----------------------|----------------|
| <b>Sample Name</b> | BDG 6636.1                   | <b>Instrument</b>    | AnalyticalLC01 |
| <b>Acquisition</b> | 22/03/2016, 12:54:57         | <b>Method (rev.)</b> | LC10132b ( 7)  |
| <b>Sequence</b>    | BDG_22Mar2016b - Reprocessed | <b>Vial Position</b> | 1              |
| <b>Operator</b>    | solvation010\cerityadmin     | <b>Injection</b>     | 1 of 1         |



Area Percent Report

| Peak# | RT        | Peak Height | Peak Area | Width      | Area %   |
|-------|-----------|-------------|-----------|------------|----------|
| 1     | 5.06 min  | 0.4521      | 3.7737    | 0.1294 min | 0.074 %  |
| 2     | 5.19 min  | 0.5665      | 5.7573    | 0.1426 min | 0.112 %  |
| 3     | 6.20 min  | 0.3348      | 4.3711    | 0.1843 min | 0.085 %  |
| 4     | 7.57 min  | 0.3143      | 3.4011    | 0.1582 min | 0.066 %  |
| 5     | 7.96 min  | 0.2368      | 1.3344    | 0.0857 min | 0.026 %  |
| 6     | 8.35 min  | 443.6943    | 5046.8271 | 0.1727 min | 98.425 % |
| 7     | 11.83 min | 0.2550      | 4.1403    | 0.2255 min | 0.081 %  |
| 8     | 12.37 min | 1.7129      | 30.9999   | 0.2671 min | 0.605 %  |
| 9     | 15.11 min | 0.1605      | 3.5726    | 0.2716 min | 0.070 %  |
| 10    | 15.41 min | 0.1464      | 2.5990    | 0.2310 min | 0.051 %  |
| 11    | 26.34 min | 0.2695      | 8.2775    | 0.3863 min | 0.161 %  |
| 12    | 27.11 min | 0.3033      | 12.5554   | 0.4950 min | 0.245 %  |