BDG SYNTHESIS

# Certificate of Analysis

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

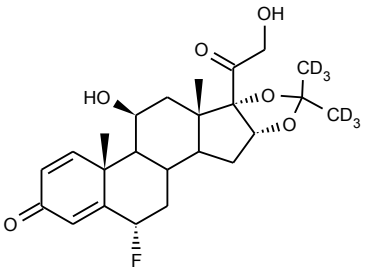
Barry Dent

Barry R. Dent, PhD, Director
17 September 2004

**Name:** **Flunisolide-$d_6$**

**CAS Number:** 3385-03-3 (unlabelled)

**Structure:**




**Molecular Weight:** $C_{24}H_{25}D_6FO_6$ = 440.53

**Lot Number:** BDG 3909.2

**Appearance:** White, crystalline solid

**Corrected Purity:** 97.9 % (HPLC) - 0.5 % (acetone) = 97.4 %

**Isotopic Purity:** Under 0.5 % $d_0$

**Re-test Date:** 17 September 2009

**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 absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a small amount of acetone (0.5 % *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, indicating clean deuteration.

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

Found *m/z* 440.2468. $C_{24}H_{25}D_6FO_6$ $[M]^+$ requires *m/z* 440.2481. The deviation of 3.0 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, symmetrical peak is observed (97.9 %). 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 65.56, H 5.71, D 2.74 % |
| $C_{24}H_{25}D_6FO_6$ | Requires: | C 65.43, H 5.72, D 2.74 % |

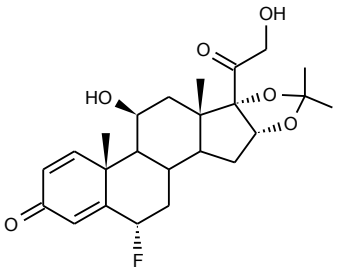
The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

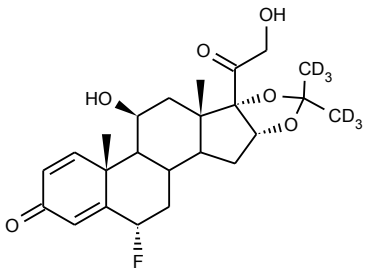
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

BDG SYNTHESIS

Proton NMR Spectrum of Flunisolide (top) and Flunisolide-$d_6$ (bottom) in DMSO-$d_6$

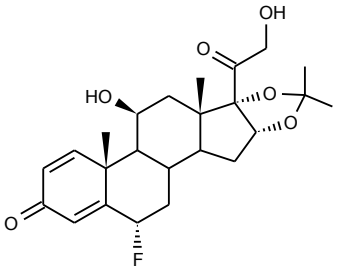


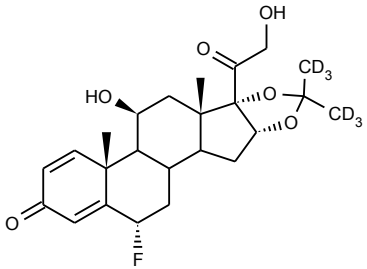




Lot Number: BDG 3909.2

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Flunisolide (top) and Flunisolide-$d_6$ (bottom) in DMSO-$d_6$




Lot Number: BDG 3909.2

Data File D:\HPCHEM\1\DATA\2004\SEP04\BDG09E\052-0302.D Sample Name: BDG3909.2

```
==========================================================================
Injection Date  : 9/17/04 3:12:43 PM          Seq. Line :   3
Sample Name     : BDG3909.2                    Location : Vial 52
Acq. Operator   : YRLman                          Inj :   2
                                              Inj Volume : 10 µl
Acq. Method     : N:\LC1100_2\1\METHODS\LC40186A.M
Last changed    : 9/17/04 2:08:29 PM by YRLman
Analysis Method : N:\LC1100_2\1\METHODS\LC40186A.M
Last changed    : 9/17/04 4:01:20 PM by YRLman
                  (modified after loading)

BDG - isocratic analysis of flunisolide on Luna C18, 5um, 250 x 4.6mm ID.  # LC40186
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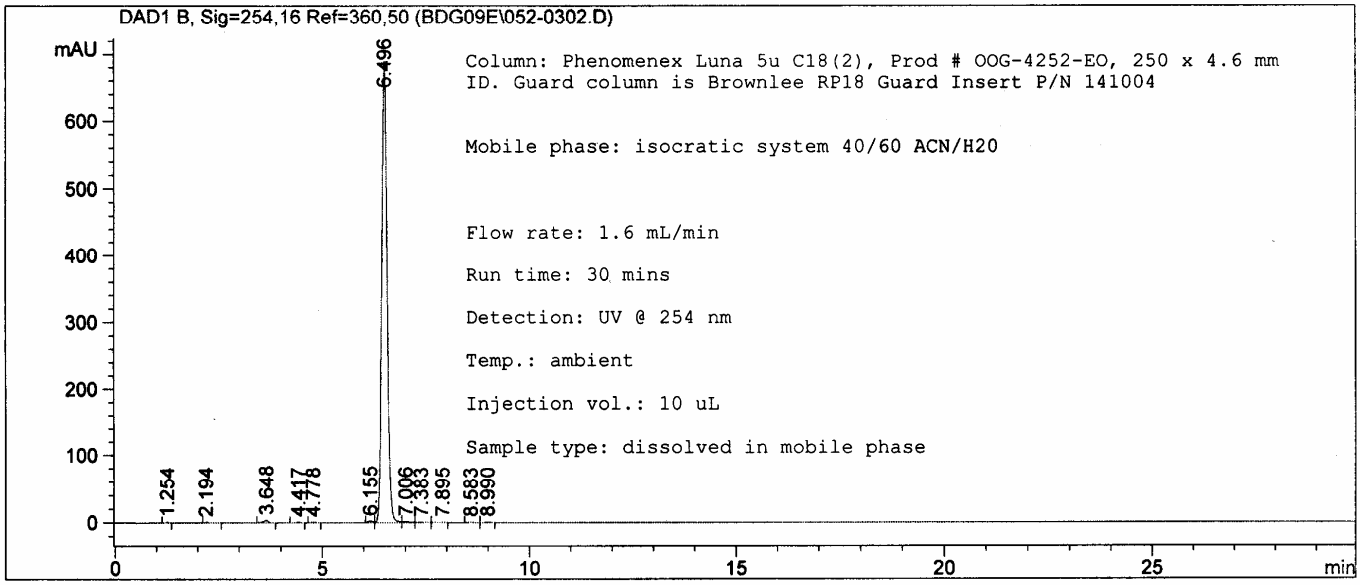


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==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 B, Sig=254,16 Ref=360,50
```

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 1.254 | MM | 0.0955 | 3.99583 | 6.97393e-1 | 0.0637 |
| 2 | 2.194 | MM | 0.1838 | 7.98259 | 7.23760e-1 | 0.1272 |
| 3 | 3.648 | BB | 0.1206 | 31.61217 | 3.74086 | 0.5037 |
| 4 | 4.417 | MM | 0.1447 | 8.36915 | 9.63828e-1 | 0.1333 |
| 5 | 4.778 | MM | 0.1421 | 6.90318 | 8.09515e-1 | 0.1100 |
| 6 | 6.155 | MF | 0.1378 | 22.21887 | 2.68744 | 0.3540 |
| 7 | 6.496 | FM | 0.1482 | 6145.77979 | 691.36298 | 97.9197 |
| 8 | 7.006 | FM | 0.2168 | 19.34131 | 1.48674 | 0.3082 |
| 9 | 7.383 | FM | 0.2920 | 10.36364 | 5.91595e-1 | 0.1651 |
| 10 | 7.895 | FM | 0.2512 | 8.60221 | 5.70776e-1 | 0.1371 |
| 11 | 8.583 | MF | 0.1929 | 4.77671 | 4.12664e-1 | 0.0761 |
| 12 | 8.990 | FM | 0.1915 | 6.39916 | 5.56923e-1 | 0.1020 |
| Totals : | | | | 6276.34461 | 704.60447 | |

```
 Results obtained with enhanced integrator!
==========================================================================
                        *** End of Report ***
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