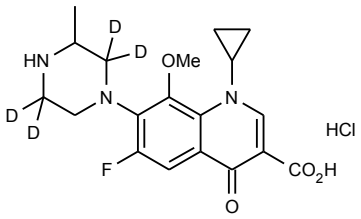
BDG SYNTHESIS

# 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 November 2006

| | |
|---|---|
| **Name:** | **Gatifloxacin-$d_4$ HCl** |
| **CAS Number:** | 112811-59-3 (unlabelled free base) |
| **Structure:** |  |
| **Molecular Weight:** | $C_{19}H_{18}D_4FN_3O_4 \cdot HCl = 415.88$ |
| **Lot Number:** | BDG 6610.1 |
| **Appearance:** | White, crystalline solid |
| **Corrected Purity:** | 100.0 % (HPLC) - 2.5 % (water) = 97.5 % |
| **Isotopic Purity:** | Under 0.5 % $d_0$ |
| **Re-test Date:** | 17 November 2011 |

**Storage and Handling:**

| | |
|---|---|
| Temperature: | ambient laboratory temperature; may be refrigerated. |
| 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 the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: no residual solvents 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 compared with the spectrum of unlabelled material, indicating clean deuteration.

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

Found *m/z* 380.1918. $C_{19}H_{19}D_4FN_3O_4$ $[M+H]^+$ requires *m/z* 380.1924. The deviation of 1.6 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 (100.0 %). 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 53.30, H 4.66, D 1.85, N 9.82 % |
| $C_{19}H_{18}D_4FN_3O_4 \cdot HCl \cdot 0.6H_2O$ | Requires: | C 53.48, H 4.77, D 1.89, N 9.85 %, $H_2O$ 2.53 % |
| $C_{19}H_{18}D_4FN_3O_4 \cdot HCl$ | Requires: | C 54.87, H 4.60, D 1.94, N 10.10 % |

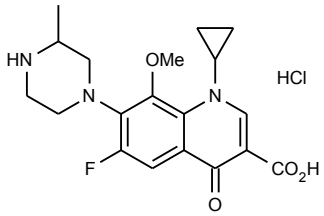
The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the "best-fit" hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend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

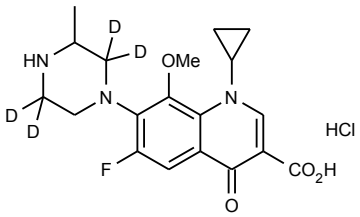
BDG SYNTHESIS

Proton NMR Spectrum of Gatifloxacin HCl (top) and Gatifloxacin-$d_4$ HCl (bottom) in DMSO-$d_6$ and $CF_3CO_2D$





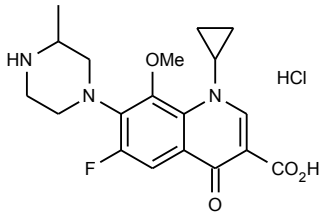


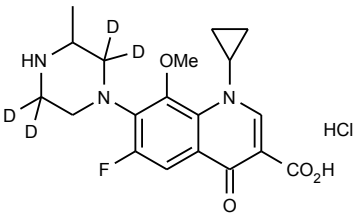



Lot Number: BDG 6610.1

BDG SYNTHESIS

# Carbon-13 NMR Spectrum of Gatifloxacin HCl (top) and Gatifloxacin-$d_4$ HCl (bottom) in DMSO-$d_6$ and $CF_3CO_2D$



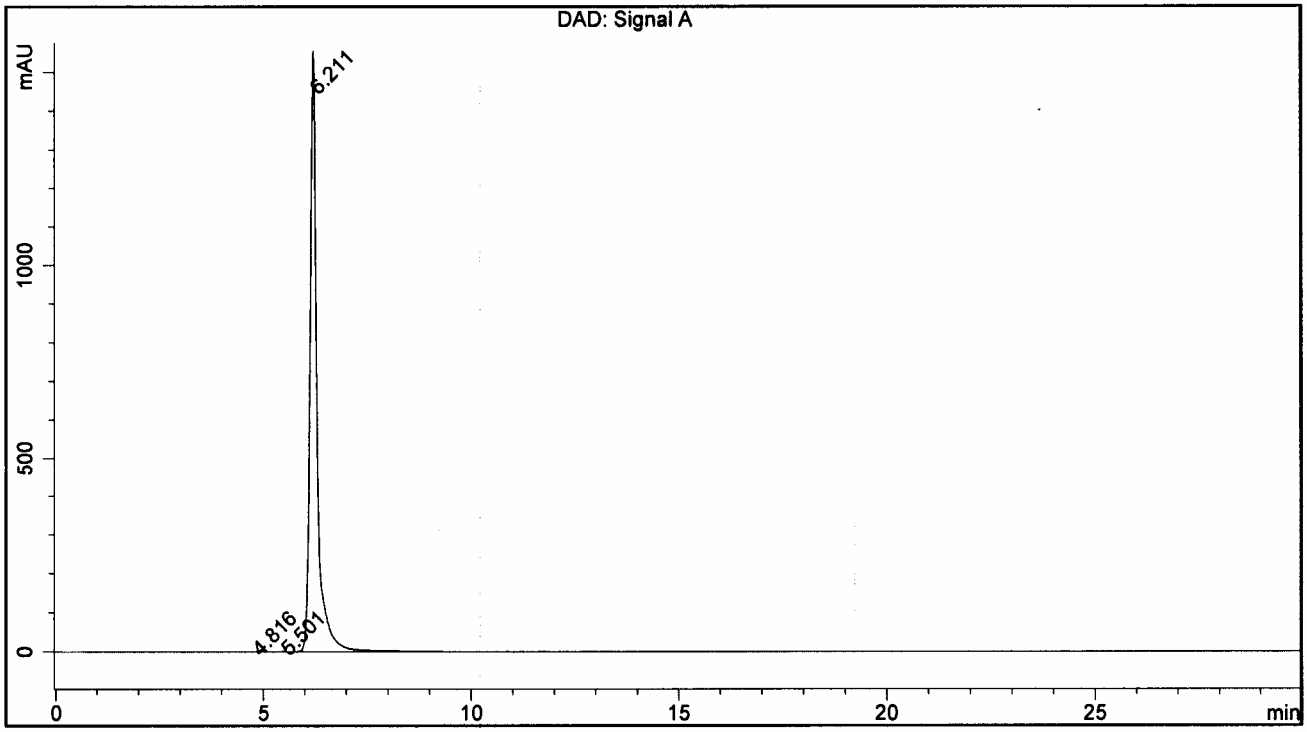




Lot Number: BDG 6610.1

BDG - Analysis of Gatifloxacin-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 10mM Sodium Dodecyl Sulphate 25mM Phosphoric Acid pH=3.0 : Acetonitrile
Flow Rate : 1.0 mL/min
Sample Solvent : Mobile Phase
Column Temperature : 20C
Injection Volume : 10 uL
Detection : UV at 296 nm

| Sample Name | BDG 6610.1 | Instrument | AnalyticalLC01 |
|---|---|---|---|
| Acquisition | 12/11/2006, 12:21:57 | Method (rev.) | LC10108a ( 3 ) |
| Sequence | BDG_12Nov2006a - Reprocessed | Vial Position | 1 |
| Operator | solvation010\cerityadmin | Injection | 2 of 2 |




**Area Percent Report**

| Peak# | RT | Peak Height | Peak Area | Width | Area % |
|---|---|---|---|---|---|
| 1 | 4.82 min | 0.2228 | 1.9766 | 0.1395 min | 0.011 % |
| 2 | 5.50 min | 0.4193 | 4.4554 | 0.1560 min | 0.025 % |
| 3 | 6.21 min | 1550.1986 | 18142.3205 | 0.1685 min | 99.965 % |