

Certificate of Analysis

Reference Material - Primary Standard

Product Name: Hydroxymethylcelecoxib (4-[5-[4-(Hydroxymethyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide) 1.0 mg/ml in Methanol

Catalogue Number: LGCAMP0344.02-01

Lot Number: 10668

CAS Number: 170571-00-3

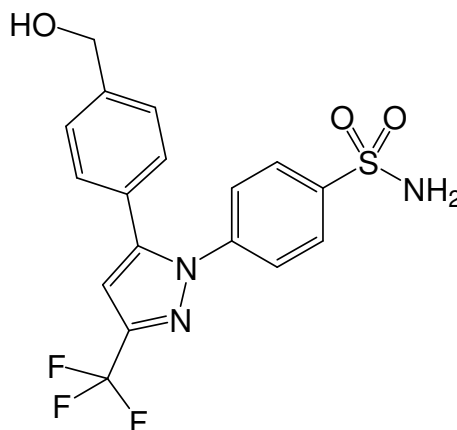
Molecular Formula: C₁₇H₁₄F₃N₃O₃S

Molecular Weight: 397.37

Solvent: Methanol

Volume per Ampoule: Not less than 1 ml ¹

Long-term Storage: 2 to 8 °C, dark



Expiry Date: November-2015


Intended Use: The primary aim of this material is for identification, calibration and quantification.

Component	Concentration ("as is")	Uncertainty
see product name	1.000 mg/ml ²	U = 0.005 mg/ml ³
Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the about 95 % level of confidence using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the purity, material density and balance and weighing technique. Concentration based on material weighings and material purity factor (assay of the neat material).		

The solution's concentration and homogeneity are verified by independent method.

LGC certifies that this standard meets the specification stated in this certificate and warrants this product to meet the stated acceptance criteria through the retest date when stored unopened as recommended. Product should be used shortly after opening to avoid concentration changes due to evaporation. Warranty does not apply to ampoules stored after opening.

Release Date:
Luckenwalde, May 2012

Signed: 
Dr. Sabine Schröder
Unit for Reference Materials

¹ Ampoules are overfilled to ensure a minimum 1 ml volume fill. We advise laboratories to use measured volumes of this standard solution before diluting to the desired concentration.

² The value is based on the results of analytical techniques, which calibration and verification was carried out with standards traceable to SI-units. The value is expressed on an "as is" basis. The concentration with its uncertainty is valid in the range between 19 °C and 25 °C.

The identity is verified by data from international scientific literature.

Gravimetrically prepared using qualified balances calibrated annually by accredited calibration service. Calibration verification performed daily prior to use utilizing weights traceable to SI via other mass standards.

³ The uncertainty "U" is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM). It is corresponding to a level of confidence of about 95 %. Standard uncertainties are indicated with "u".

Verification of Concentration and Homogeneity			
Lot Number	Verified Concentration (mg/ml)		% RSD - Homogeneity
	Result	Acceptance Criteria	Result Acceptance Criteria
10668	1.002	± 3 %	0.830 ≤ 3 %
Concentration verified by HPLC			

Solution Standard Assay Parameters		External Calibration (100 % amount)	
Analysis Method	HPLC		
Column:	60 RP Select B, 5 µm, 125 x 4 mm	Number of Measurements:	6
Injector:	Auto; 2 µl; 0.9999 mg/ml in Methanol		
Flow:	1.0 ml/min, 40 °C	Detector:	250 nm
Conditions:	mob. Phase A: Water + 0.1 % H ₃ PO ₄ , mob. Phase B: Acetonitrile + 0.1 % H ₃ PO ₄ 0-6 min A/B 65/35, 6-9 min A/B to 30/70, 9-11 min A/B to 65/35, 11-19 min A/B 65/35 (v/v)		

Neat Material Data		
Product Name:	Hydroxymethylcelecoxib (4-[5-[4-(Hydroxymethyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide)	
CAS Number:	170571-00-3	
Molecular Formula:	C ₁₇ H ₁₄ F ₃ N ₃ O ₃ S	
Molecular Weight:	397.37	
Compound Lot:	1320	
Test	Method	Result
Melting Point (°C)*	SOP 06-010	153 °C
¹ H-NMR Spectrum*	SOP 06-053	conform / complies to structure
IR Spectrum*	SOP 06-036	conform / complies to structure
Mass Spectrum (ESI)*	SOP 06-022	conform / complies to structure
Assay by carbon titration (as is)*	Elementary Analysis	98.63 %
The expanded uncertainty according to the assay is U = 0.44 % (about 95 % level of confidence using a coverage factor of k = 2).		

*: Validated method performed by ISO/IEC 17025 accredited testing lab

The assay of the neat material is verified by the 100 % method using HPLC, corrected with water (KFT) and residual solvents.

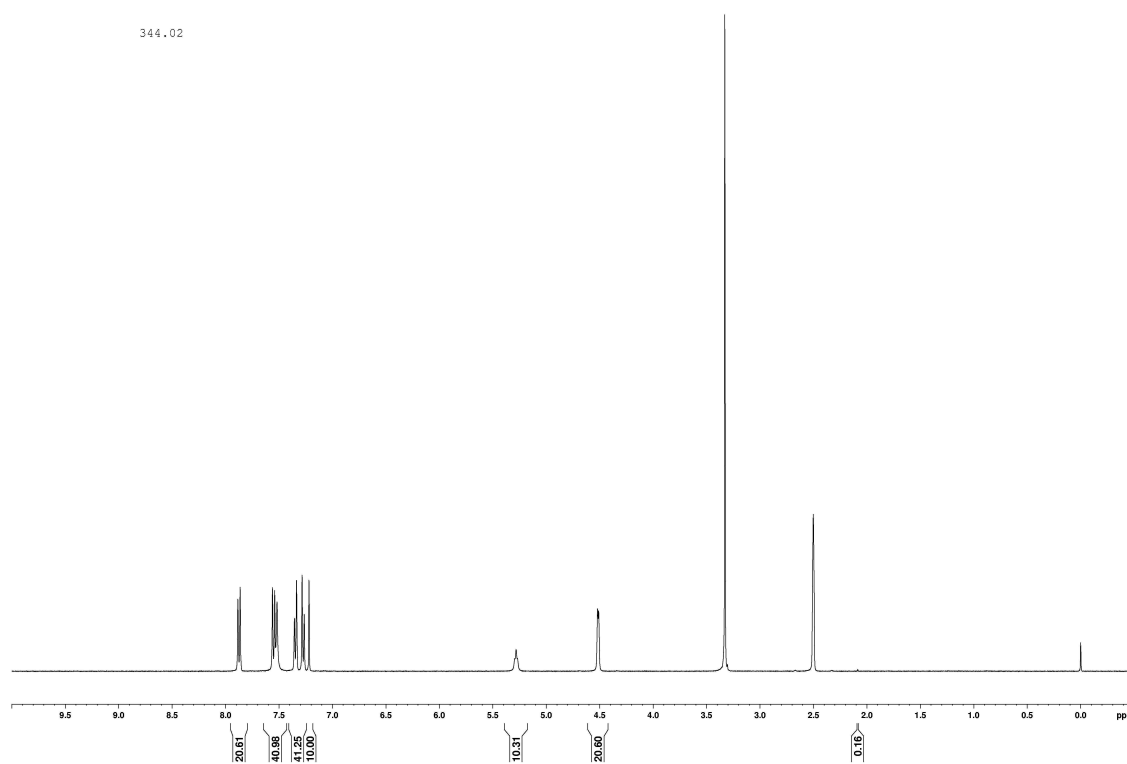
I. Identity

The identity of the reference substance (neat material) was established by the following analyses.

Ia. ¹H-NMR Spectrum

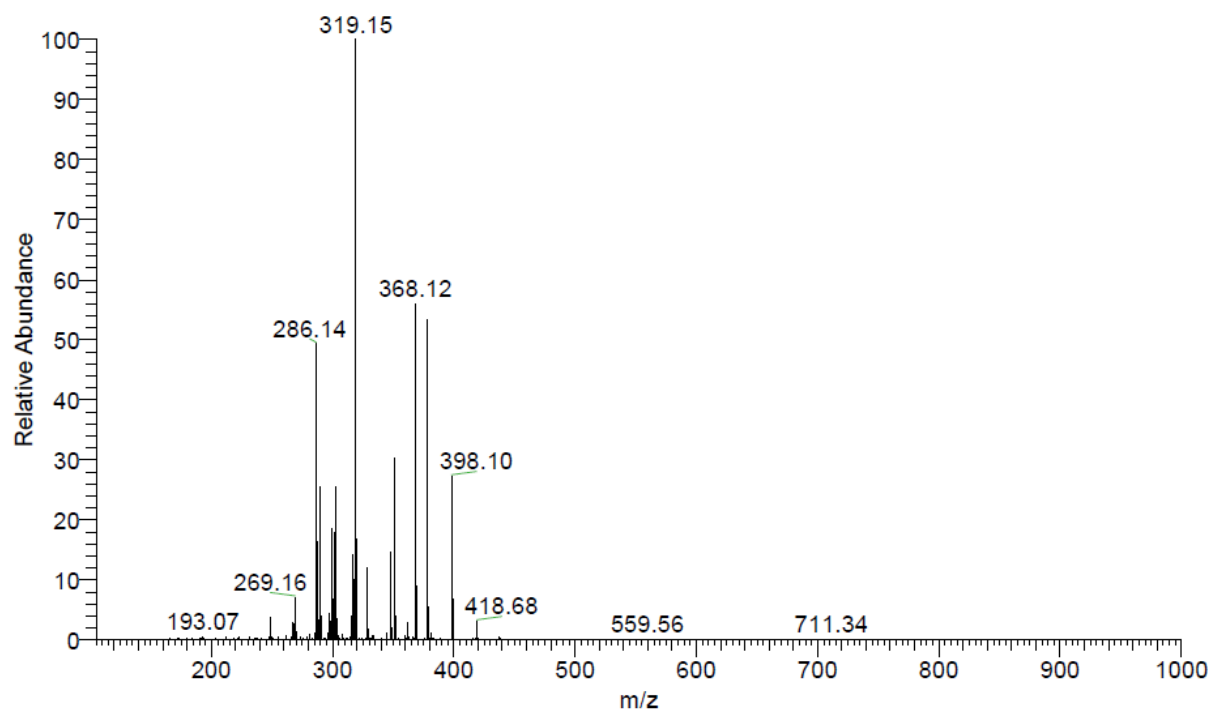
Conditions: 400 MHz, DMSO-d₆

The structure is confirmed with the signals of the spectrum and their interpretation.



1b. Mass Spectrum

Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet

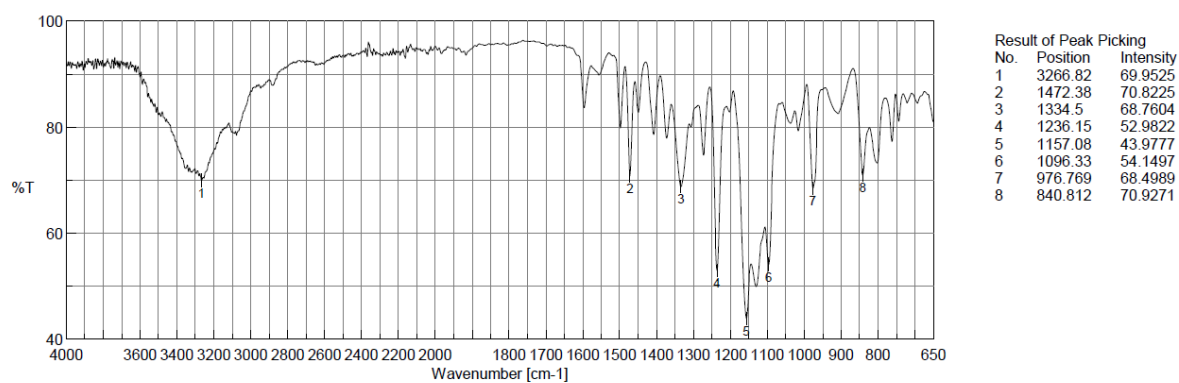


m/z	fragments
398.10	[MH]
368.12	[MH – CH ₂ O]
319.15	[MH – HNO ₂ S]
286.14	[MH – OH – C ₂ F ₃ N]

The signals of the mass spectrum and their interpretation are consistent with the structural formula.

lc. IR Spectrum

Method: attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy



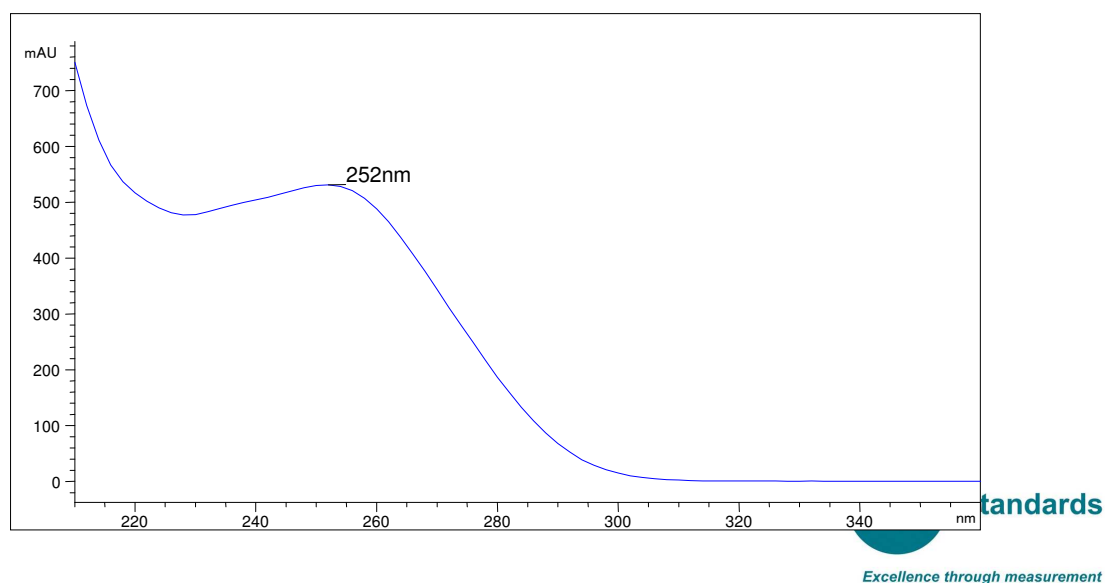
The signals of the IR spectrum and their interpretation are consistent with the structural formula.

Id. Melting Point

153 °C

le. UV Spectrum

Method: HPLC (DAD-detection)



II. Assay by Elementary Analysis (Carbon Titration – neat material)

Method: percentage carbon found in relation to percentage carbon as calculated for molecular formula

Results:

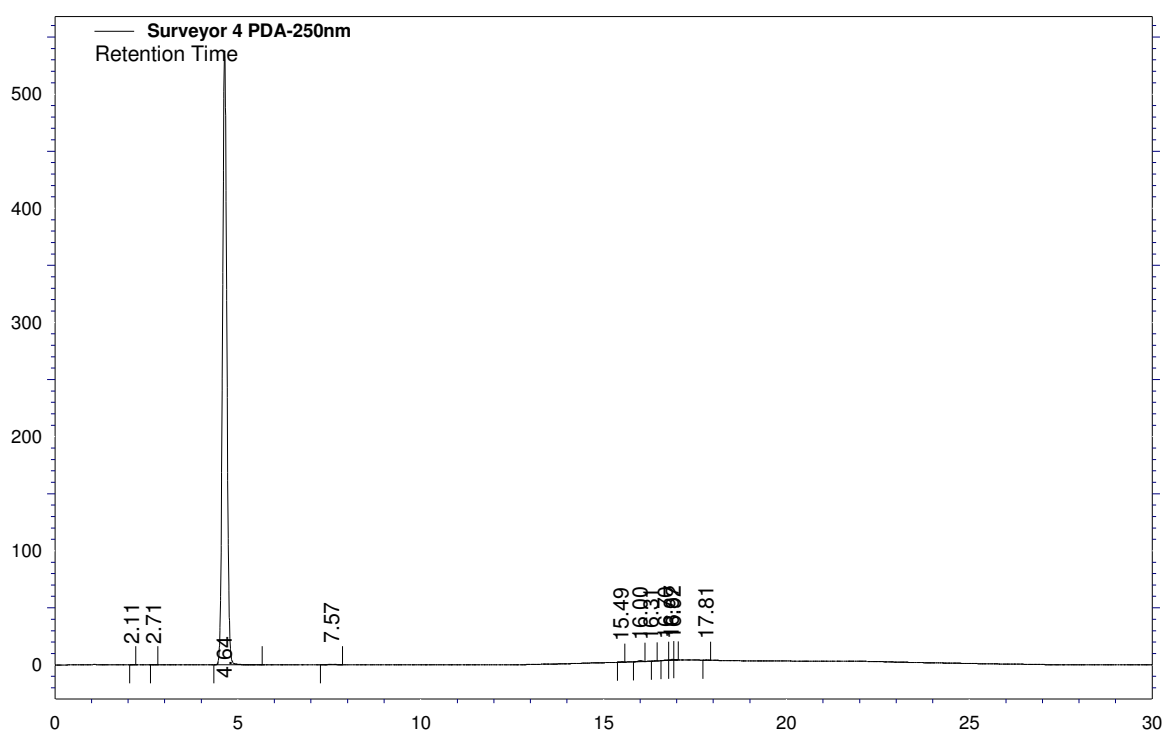
Arithmetic mean (n=3) 98.63 % (mass fraction)

Uncertainty U 0.44 %

III. Purity

IIIa. High Performance Liquid Chromatography (HPLC)

The purity of the reference substance (neat material) was analysed by high performance liquid chromatography (HPLC).



Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	2.11	691	0.02
2	2.71	426	0.01
3	4.64	4380882	99.53
4	7.57	6565	0.15
5	15.49	1109	0.03
6	16.00	4801	0.11
7	16.31	258	0.01
8	16.70	936	0.02
9	16.87	3216	0.07
10	16.92	946	0.02
11	17.81	1668	0.04
Totals		4401498	100.00

For the calculation the system peaks were ignored. The content of the analyte was determined as ratio of the peak area of the analyte and the cumulative areas of the purities, added up to 100 %.

HPLC Conditions:

Column:	Conditions:	Detector:	Injector:
RP 60 Select B	1.0 ml/min, 40 °C	DAD	Auto
5 µm, 125 x 4 mm	0-10 min Water/Acetonitrile 60/40 10-15 min Water/Acetonitrile to 30/70 15-20 min Water/Acetonitrile 30/70 20-25 min Water/Acetonitrile to 60/40 25-30 min Water/Acetonitrile 60/40 (v/v); 0.1 % H ₃ PO ₄	250 nm	4 µl; 0.0834 mg/ml in Water/Acetonitrile 50/50 (v/v)

Results:

Arithmetic mean (n=6) 99.53 %

IIIb. Water Content

Method: coulometric Karl Fischer titration

Results:

Arithmetic mean (n=3) 0.81 % (mass fraction)

IIIc. Residual Solvents

Method: ^1H -NMR

No significant amounts of residual solvents were detected ($< 0.05\%$).

IV. Stability and Homogeneity

Accelerated stability studies indicate no significant instability. The given validity period is based on this data. This is backed up by historical data over the range of several years for the neat substance.

Homogeneity assured by validated process of preparation (incl. ampoulation), verified by homogeneity testing (HPLC).

V. Further Information

General

For laboratory use only. Not suitable for human or animal consumption.

This material conforms to the characteristics of a primary standard as described within ISO Guide 30 (Terms and definitions used in connection with reference materials).

The certified values quoted in this certificate are LGC's best estimate of the true values within the stated uncertainties and based on the techniques described in this certificate.

Handling of the RM

Before usage of the RM, it should be allowed to warm to room temperature. The concentration with its uncertainty is guaranteed in the range between $19\text{ }^{\circ}\text{C}$ and $25\text{ }^{\circ}\text{C}$. The uncertainty accounts for the temperature-dependent density in this range.

Quality Control Assessment

The product quality is controlled by regularly performed quality control tests (retests).

Revision	Date	Reason for Revision
00	May 2012	Release of the Lot – initial version
01	November 2012	Copyright added