



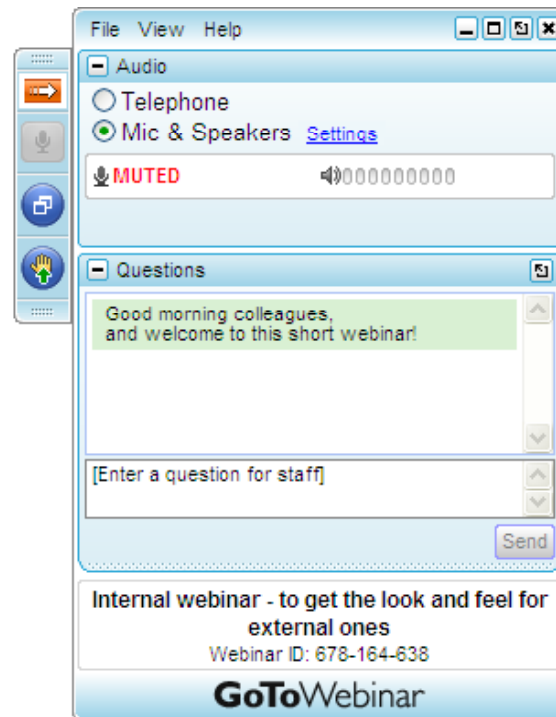
## Reference materials: Certificates of analysis



**Dr. Christian Zeine**  
(Basic concept by  
Dr. Julian Schwarz)

**Webinar Series 2013,**  
**May 23rd, 2013**

# Quick guide to the webinar tools



# Agenda

- **Definition for ‘Certificate of analysis’**
- **ISO 31: Certificate Content and Structure**
- **Certificates**
  - **Certified reference materials (CRMs)**
  - **Primary pharmaceutical reference standards**
  - **Secondary pharmaceutical reference standards**
  - **Impurity reference standards**
  - **Research materials**
- **Summary**

# What is a certificate (of analysis)?

**LGC** **ERM**

**CERTIFICATE OF ANALYSIS**  
**ERM<sup>®</sup> - CA010a**

**Hard Drinking Water UK - Metals**

Element	Number of test	Method	Value (mg/L)	Uncertainty (mg/L)
Aluminium	10	ICP-OES	0.05	±0.005
Barium	10	ICP-OES	0.05	±0.005
Bismuth	10	ICP-OES	0.05	±0.005
Boron	10	ICP-OES	0.05	±0.005
Calcium	10	ICP-OES	0.05	±0.005
Cadmium	10	ICP-MS	0.05	±0.005
Chromium	10	ICP-OES	0.05	±0.005
Copper	10	ICP-OES	0.05	±0.005
Iron	10	ICP-OES	0.05	±0.005
Lead	10	ICP-MS	0.05	±0.005
Manganese	10	ICP-OES	0.05	±0.005
Mercury	10	ICP-MS	0.05	±0.005
Nickel	10	ICP-OES	0.05	±0.005
Selenium	10	ICP-MS	0.05	±0.005
Silver	10	ICP-OES	0.05	±0.005
Sodium	10	ICP-OES	0.05	±0.005
Zinc	10	ICP-OES	0.05	±0.005

**NOTE**  
 European Reference Material (ERM<sup>®</sup> - CA010a) was originally certified as LGC6010 (Batch 02) (June 2002). It was produced and certified under the responsibility of LGC according to the principles set down in the Technical Guidelines for the European Reference Material<sup>®</sup>. The cooperation agreement between LGC and BIPM (BIPM Reference Material - Certificate of Analysis) is available on the website <http://www.bipm.org>.

**Accepted as an ERM<sup>®</sup> - Technique - Reference 010a**

**Signed:**  
 Dr John Bennett, UK Government Chemist  
 LGC Limited  
 Queens Road  
 Tadworth  
 Middlesex  
 TW20 2EX, UK

**USP** **CERTIFICATE of ANALYSIS**  
 USP REFERENCE STANDARDS-CERTIFIED REFERENCE MATERIAL

**USP Dextromethorphan Hydrobromide Certified Reference Material**

**LOT 80P118**

CN1CC[C@H]2C[C@@H](OC(=O)c3ccc(O)cc3)[C@@H](Br)C2

**Minimum Purity: 99.99%**  
**Maximum Weight: 0.0100g**  
**USP Reference: 800.014**

**Certified Property Value:** 1.0001 ± 0.0011 mg/mg

**Produced and Certified by:**  
 The United States Pharmacopeial Convention  
 12001 Twinbrook Parkway  
 Rockville, MD 20850  
 Telephone: 301-588-6000

**Stocking Number:** 120011

**Net Weight:** 500 mg

**Appearance:** White crystalline powder

**Smell:** None, faint

**Storage:** 20° to 25° (68° to 77° F)

**Instructions for Use:**  
 Do not dry. For quantitative USP-NF applications, determine the exact content gravimetrically, at the time of use, and use a calibration value of 1.0001 mg of dextromethorphan hydrobromide per mg of material on the certificate label. Store certificate tightly closed. Check certificate on the label for instructions on any other conditions on the certificate (USP-NF).

**Stability Reference:** The purity in the certified property value is expressed with an associated uncertainty (U) at 95% confidence interval and is dependent according to the method described in the USP (Section 2.6). The reported uncertainty is calculated as ±0.1%, giving the coverage factor k = 1.96, and is the combined uncertainty.

USP Dextromethorphan Hydrobromide, CRM Page 1 of 2

**LGC**

**CERTIFICATE**

Reference Substance

Lornoxicam Hydrobromide

Cc1ccc(O)c2c(c1)C(=O)N2C(=O)O

**Minimum Purity:** 99.99%  
**Maximum Weight:** 0.0100g  
**USP Reference:** 800.014

**Lot Number:** 99.021001  
**Expiration Date:** 211101  
**Long Term Storage:** 2 to 8 °C, 20%  
**Appearance:** white solid  
**Smell:** faint  
**Moisture:** 0.5%

**Release Date:** 2010-07-02

This certificate is valid for two years from the Release Date provided the packaging is stored under the recommended conditions.

**Product:** Lornoxicam Hydrobromide  
**Lot:** 99.021001  
**Release Date:** 2010-07-02

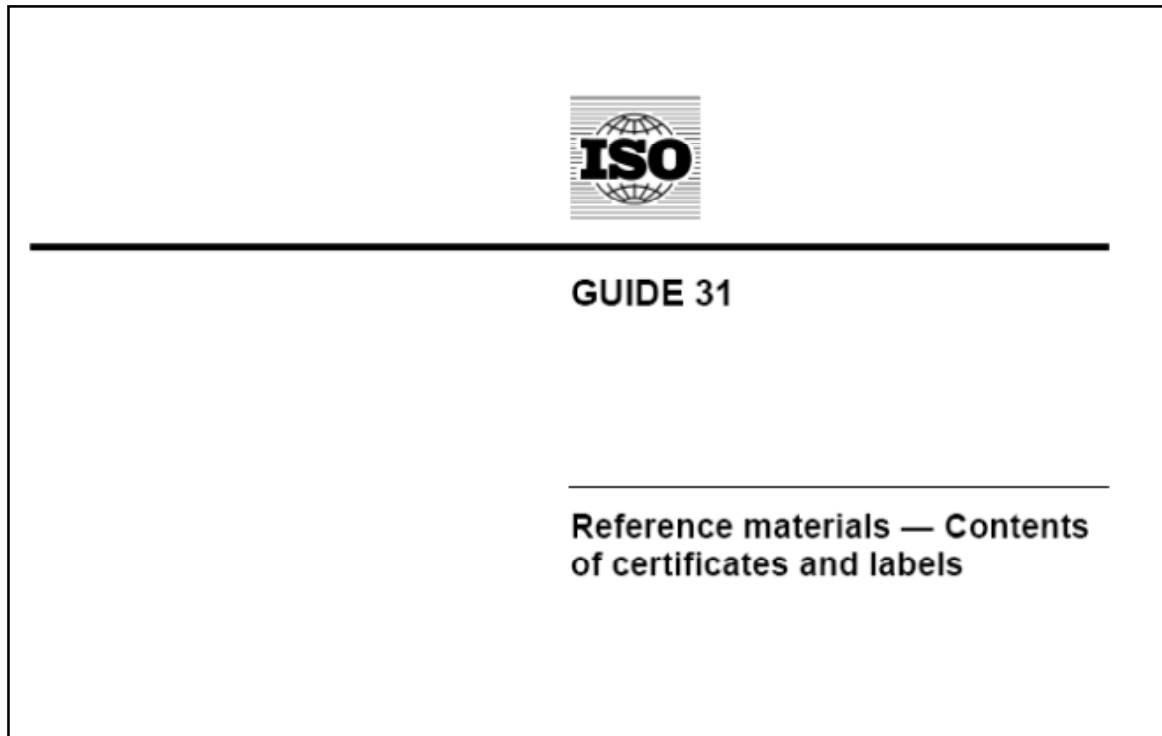
# Definition



- **ISO Guide 31:2000 defines certificates as:**  
“Document containing all the information which is essential to the use of a certified reference material”
- **Please also note:**  
“Without the certificate, the material (the CRM), however costly its production, is valueless.”  
  
“The certificate should not be parted from the CRM.”

# Definition

- **ISO Guide 31:2000**
  - **Defines content and structure of certificates**



**Currently under revision!**

# Content of a certificate

- **Possible content acc. to ISO Guide 31:2000**

- Name and address of certifying organisation
- Title of the document
- Name of the document
- Code and batch number
- Description – relevant information
- Intended use
- Instructions for use
- Hazardous situation
- Level of homogeneity
- **Certified property values and uncertainty**
- **Traceability**
- Date of certification
- Period of validity
- Stability, transportation and storage instructions
- Shelf life/expiry date
- ....

**CAN be included with a CRM!  
Not a MUST!**

# Agenda

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- ISO 31: Certificate Content and Structure
- **Certificates**
  - **Certified reference materials (CRMs)**
  - Primary pharmaceutical reference standards
  - Secondary pharmaceutical reference standards
  - Impurity reference standards
  - Research materials
- Summary



# Certificates – NIST CRM



National Institute of Standards & Technology

## Certificate of Analysis

Standard Reference Material® 917c

D-Glucose (Dextrose)

This Standard Reference Material (SRM) is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for glucose determinations employed in clinical analysis, and for routine critical evaluation of the daily working standards used in these procedures. A unit of this SRM consists of one bottle containing 50 g of crystalline D-glucose.

**Certified Purity and Uncertainty:** A NIST Certified Value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified chemical purity value of glucose was determined by measuring the mass fractions of impurities, including water, summing the impurities, and subtracting this sum from 100.0 %.

Certified Purity of D-Glucose as a Mass Fraction: 99.7% ± 0.3%

The uncertainty in the certified value is expressed as an expanded uncertainty,  $U$ , at the 95 % level of confidence and is calculated according to the method described in the ISO Guide [2,3]. The expanded uncertainty is calculated as  $U = k u_c$ , where  $u_c$  is intended to represent, at the level of one standard deviation, the uncertainty in measurement of the impurities. The coverage factor,  $k = 2$ , is determined from the Student's  $t$ -distribution corresponding to the appropriate degrees of freedom and a confidence level of 95 %.

### NOTICE AND WARNING TO USERS

SRM 917c IS INTENDED FOR IN VITRO DIAGNOSTIC USE ONLY.

### INSTRUCTIONS FOR USE

**Storage:** The SRM should be stored in its original bottle at temperatures between 20 °C and 25 °C. The bottle must be tightly re-capped after use and protected from heat, excessive moisture, and direct sunlight. Refrigeration in a desiccator is recommended for prolonged storage. However, the bottle and contents should be allowed to warm to room temperature before opening.

**Drying Instructions:** For laboratory environments where the relative humidity is below 75 %, there are no special drying requirements before use. For laboratory environments where the relative humidity is 75 % or above, the sample must be dried under vacuum at 60 °C for 24 hours before use. The surface of the material absorbs a significant amount of moisture when exposed to a relative humidity of approximately 75 %. Because the certified purity is based on a specific moisture content, any added moisture will lower the purity. NIST experience indicates that moisture gain is not a significant problem at a relative humidity of approximately 50 %.

**Instructions for Use as a Standard in Clinical Applications:** A 1 % (mass concentration) standard solution of glucose may be prepared by transferring 1.003 g (mass in air) of SRM 917c into a 100 mL volumetric flask, filling to approximately 100 mL with a 0.2 % (mass concentration) benzoic acid solution (a preservative), and swirling to dissolve. Adjust to volume with the 0.2 % benzoic acid solution. The benzoic acid should be ACS Reagent grade. The final glucose mass concentration of this solution is 10 mg/mL.

### SOURCE AND ANALYSIS

**Source:** The D-glucose used for this SRM was obtained from a commercial supplier.

**NIST Analysis for Purity:** The purity of SRM 917c was assessed by differential scanning calorimetry (DSC) and gas chromatography-mass spectrometry (GC-MS).

**Certified Purity and Uncertainty:** A NIST Certified Value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified chemical purity value of glucose was determined by measuring the mass fractions of impurities, including water, summing the impurities, and subtracting this sum from 100.0 %.

Certified Purity of D-Glucose as a Mass Fraction: 99.7% ± 0.3%

Gaithersburg, MD 20899  
Certificate Issue Date: 30 June 2009

Stephen A. Wise, Chief  
Analytical Chemistry Division

Robert L. Watters, Jr., Chief  
Measurement Services Division

SRM 917c

Page 1 of 2

Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at <http://physics.nist.gov/Pubs/>

[3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.K.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results From Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571-579 (2000).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.

SRM 917c

Page 2 of 2

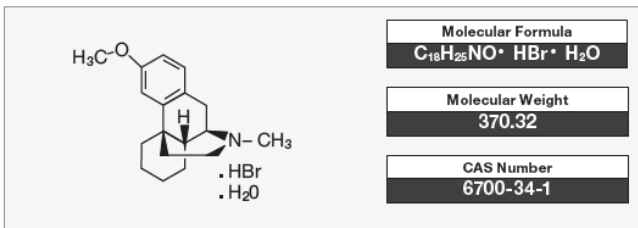
**Certification Details:** Three Laboratories evaluated three samples each of the candidate material in order to assign a value. The three samples were collected from the early, middle and late segments of the filling operation during the production of end use vials to assess homogeneity. The Mass Balance approach was used to assign a property value, i.e.  $[(100\% - \text{Total Chromatographic Impurities } \%) \div 100] \times [(100\% - \text{ROI}\%) \div 100]$ . Impurities were determined by USP HPLC (Dextromethorphan Hydrobromide) and Residue on Ignition, ROI <281> methods for this material, according to the *USP-NF* for Dextromethorphan Hydrobromide. All collaborating laboratories used a common protocol. The analysis of each detected impurity was treated as a separate experiment and the grand mean of means was used in uncertainty calculations.

Standards

ANALYSIS MATERIAL

USP Dextromethorphan Hydrobromide Certified Reference Material

LOT K0F118



**Certified Property Value:**  $1.000 \pm 0.001$  mg/mg

Produced and Certified by: The United States Pharmacopeial Convention  
12601 Twinbrook Parkway  
Rockville, MD 20850

measurement uncertainties specified, through 23 September 2013, provided the unopened RS is handled and stored in accordance with the instructions given in this certificate. Continued Suitability for Use studies will be conducted to confirm this period of validity.

**Certification Details:** Three Laboratories evaluated three samples each of the candidate material in order to assign a value. The three samples were collected from the early, middle and late segments of the filling operation during the production of end use vials to assess homogeneity. The Mass Balance approach was used to assign a property value, i.e.  $[(100\% - \text{Total Chromatographic Impurities } \%) \div 100] \times [(100\% - \text{ROI}\%) \div 100]$ . Impurities were determined by USP HPLC (Dextromethorphan Hydrobromide) and Residue on Ignition, ROI <281> methods for this material, according to the *USP-NF* for Dextromethorphan Hydrobromide. All collaborating laboratories used a common protocol. The analysis of each detected impurity was treated as a separate experiment and the grand mean of means was used in uncertainty calculations.

**Intended Use:**

This is an established USP Reference Standard. This certificate is valid only for the official uses of this particular Reference Standard found in the current version of the *USP-NF*. Its official applications are subject to change in the normal USP revision process.

**Non-Monograph Use:**

The suitability of this Reference Standard for use in non-compendial applications is solely the responsibility of the user.

[1] ISO; Guide to the Expression of Uncertainty in Measurement; ISBN 92-67-10188-9, 1st ed., International Organization for Standardization (ISO), Geneva, Switzerland (1993).

**Certified Property Value:  $1.000 \pm 0.001$  mg/mg**

**Appearance:** White crystalline powder

**Hazards:** Toxic, Irritant

*Frankenberg* *W Koch*

**Instructions for Use:**

Do not dry. For quantitative *USP-NF* applications, determine the water content titrimetrically at the time of use, and use a calculation value of 1.000 mg of dextromethorphan hydrobromide per mg of material on the anhydrous basis. Keep container tightly closed. Note: Instructions on the label take precedence over any other indication in the compendium (*USP-NF*).

# Certificates – USP CRM



- **Caution with uncertainty:**
  - Very small, looks good on a first glance
  - However, in this case: Uncertainty does not take into account water content, needs to be determined by user through Karl-Fischer-titration
  - Was raising discussions in the pharmaceutical community
    - Smaller than comparable materials from metrology institutes (see NIST CofA)
- **Traceability “hidden”, as with the NIST CofA as well**
  - Both materials only traceable to NIST resp. USP in house methods
  - Info on traceability can be more detailed with CRMs from other sources (e.g. some ERM materials)

# USP – Will there be further CRMs?



- **Pertaining discussion on CRMs inside USP:**  
„The USP Reference Standard project team seems to have mixed feeling whether or not the CRM would add value to current practices from Industry perspective.“  
(Comment at: <http://community.aapspharmaceutica.com>; Feb 2009)
- **No further CRMs for the USP-NF since more than three years**

# Agenda

- Definition for ‘Certificate of analysis’
- ISO 31: Certificate Content and Structure
- **Certificates**
  - Certified reference materials (CRMs)
  - **Primary pharmaceutical reference standards**
  - Secondary pharmaceutical reference standards
  - Impurity reference standards
  - Research materials
- Summary

# Primary pharmaceutical reference standards



- **Previous webinar: Recommended approach for primary RS (see also EP General text 5.12.)**
  - Usually purity of >95%
  - Full characterization and documentation of
    - Identity (with several qualitative techniques: NMR, MS, IR, UV/VIS, Elemental analysis, where appropriate X-ray structure analysis)
    - Purity (with HPLC => impurity profile)  
Identify peaks with area percentage >0.1%  
Maybe check relative response factor of impurity behind peak

... continued

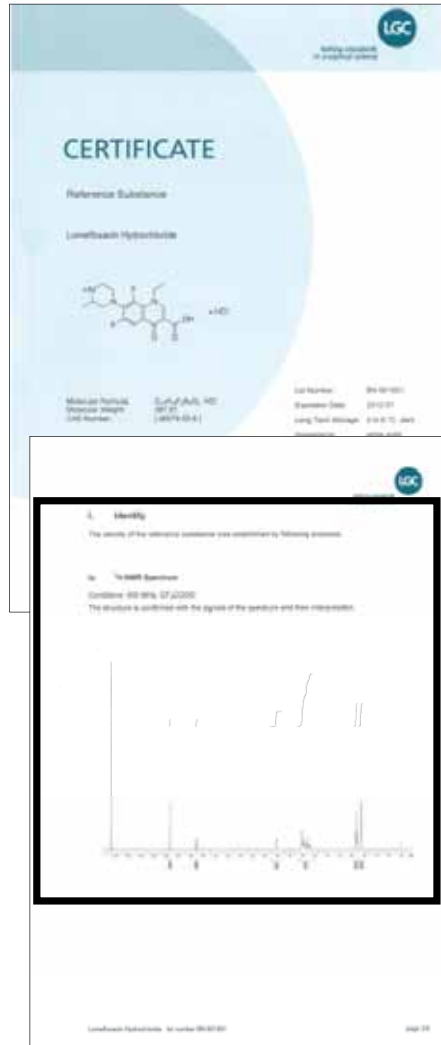
# Primary pharmaceutical reference standards

- **Recommended approach for primary RS (continued)**
  - Full characterization and documentation of
    - Residual solvents by GC-Headspace methods
    - Water content by Karl-Fischer titration
    - Loss on drying (sum of water and residual solvents)
    - Melting point (rough purity/identity information)
    - Sulphated Ash (inorganic impurities)
  - Assay, for primary RSs from purity calculation (with the results from all relevant examinations) plus at least one additional independent method (e.g. titration)





# Certificates pharmaceuti



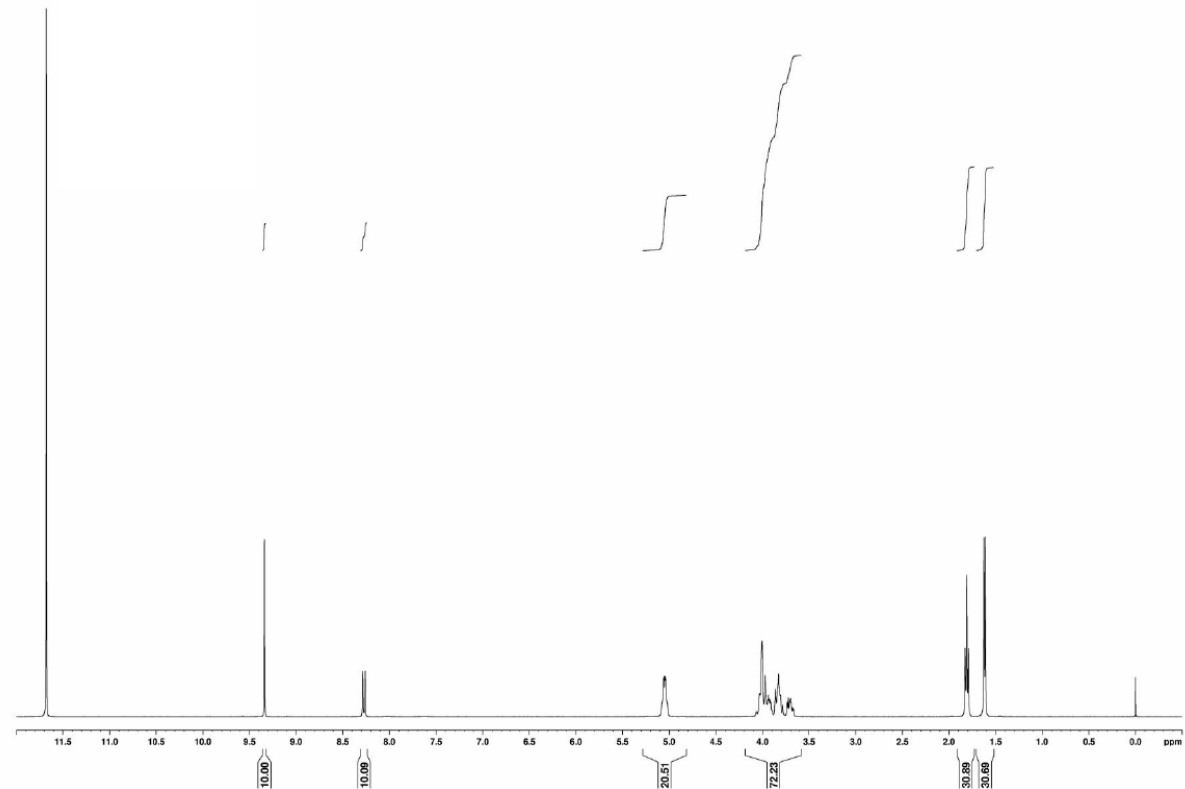
## I. Identity

The identity of the reference substance was established by following analyses.

### 1a. <sup>1</sup>H-NMR Spectrum

Conditions: 400 MHz, CF<sub>3</sub>COOD

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

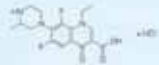


# Certificate pharmace

**LGC**  
Quality solutions  
for analytical science

**CERTIFICATE**

Reference Substance  
Lomefloxacin Hydrochloride

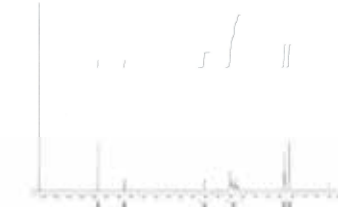


Molecular Formula:  $C_{19}H_{18}FN_4O_3$   
Empirical Weight: 387.37  
CAS Number: 146743-84-1

Lot Number: 06-001001  
Expiry Date: 03/12/21  
Using Test Method: 1.9.6.12, 2.0  
Issue Date: 06/06/2020

**4. Identify**  
The identity of the reference substance was established by following procedure:

by  $^{13}C$ -NMR Spectroscopy  
Conditions: 100 MHz,  $CF_3COOD$   
The structure is confirmed with the signals of the spectrum and their interpretation.

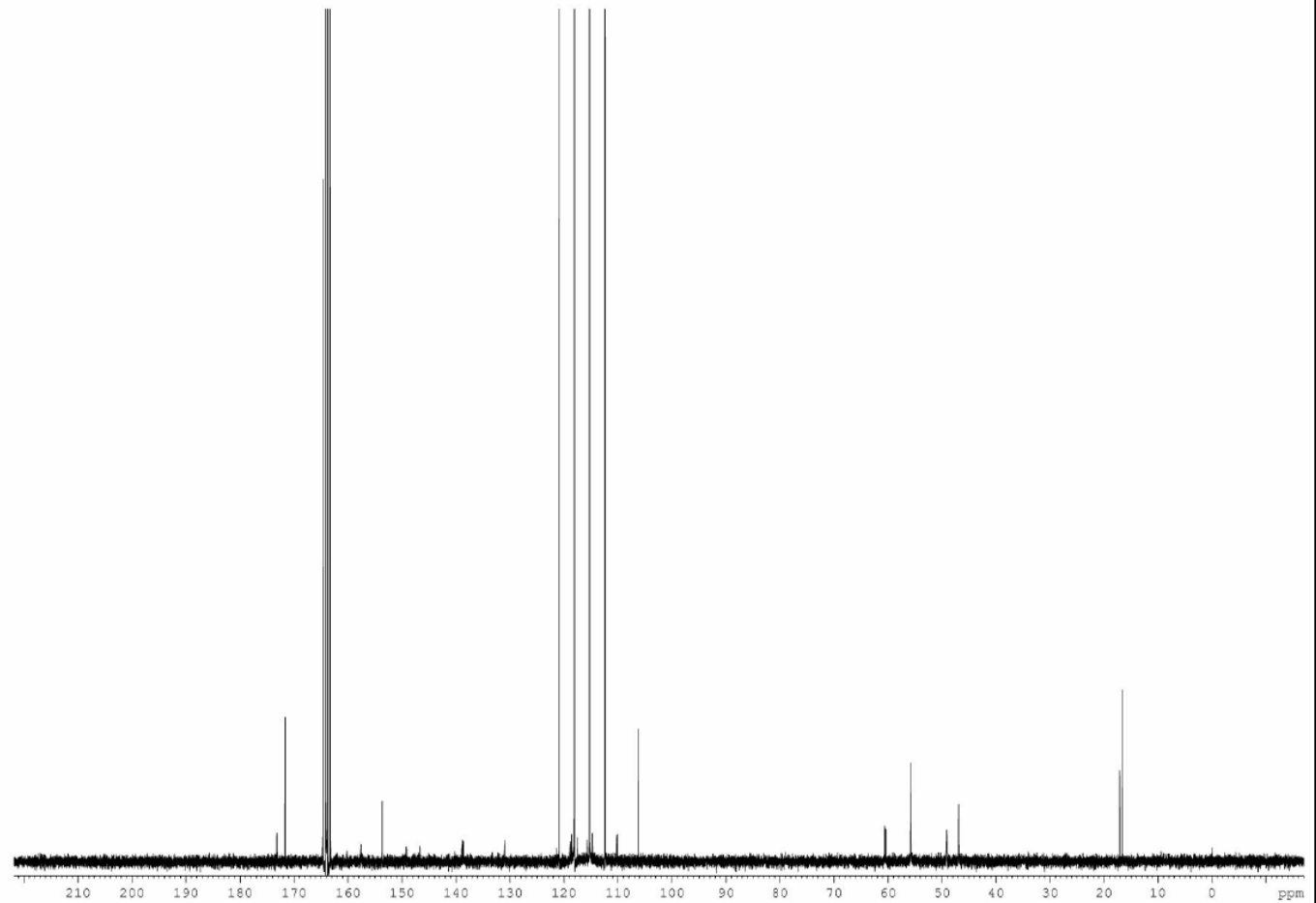


Lomefloxacin Hydrochloride, lot number 06-001001 page 1/1

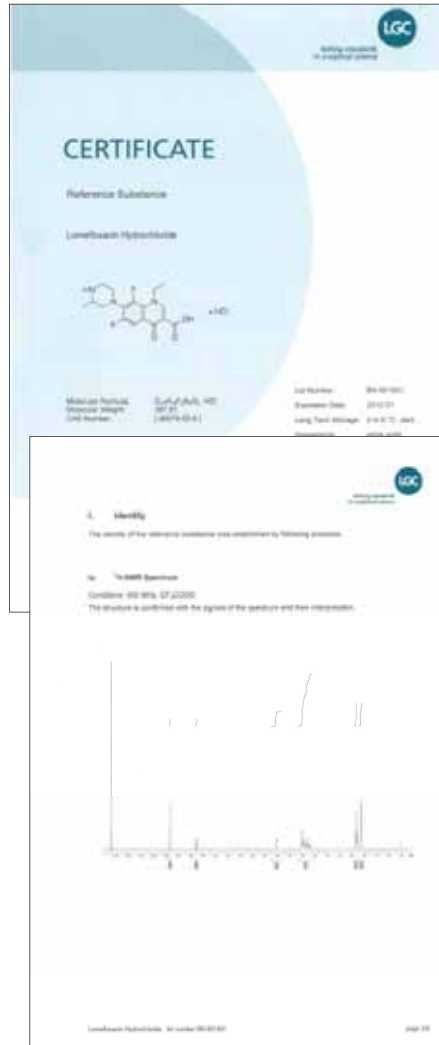
## Ib. $^{13}C$ -NMR Spectrum

Conditions: 100 MHz,  $CF_3COOD$

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

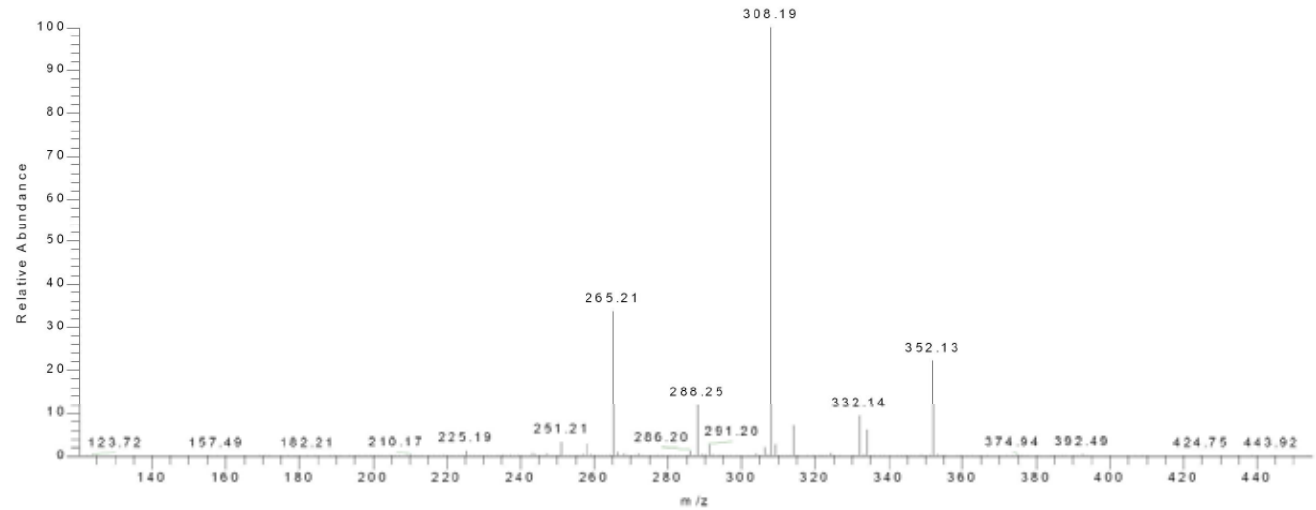


# Certificates pharmaceuti



## Ic. Mass Spectrum

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



m/z	fragments
352	$[ M - HCl + H ]^{\cdot+}$
308	$[ 352 - CO_2 ]^{\cdot+}$
265	$[ 308 - HF ]^{\cdot+}$

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

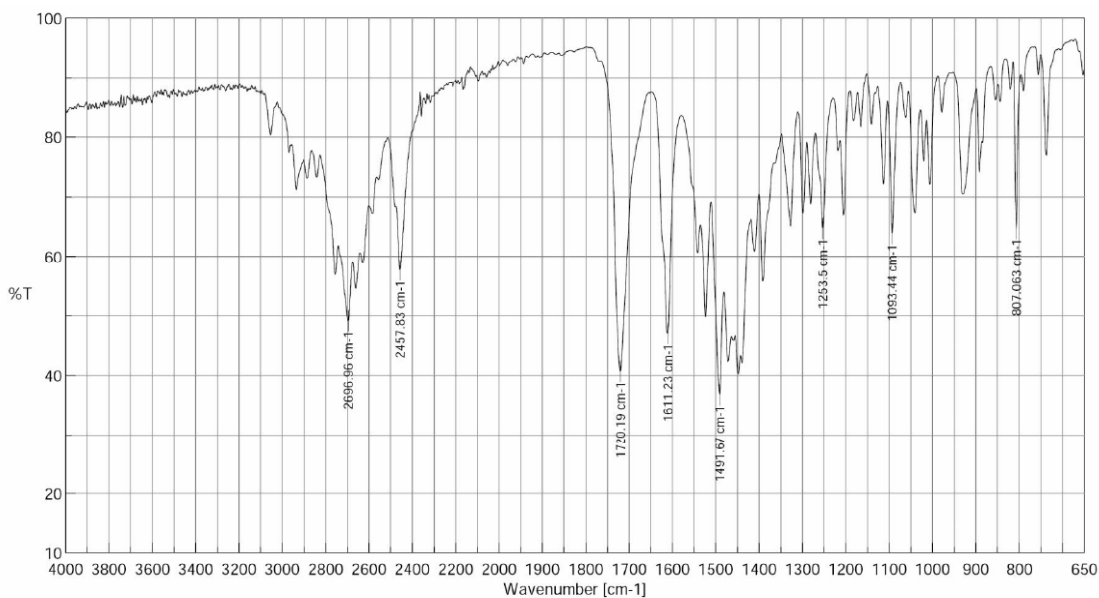
# Certificates – Primary pharmaceutical reference standards



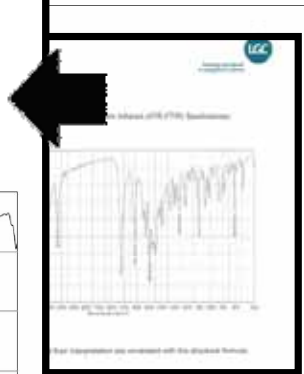
Excellence through measurement

## Id. 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.



**W. Water Content**  
 Method: Karl-Fischer Titration according to Ph. Eur. 2.2.14.12

**Results:**  
 Average: 0.19 %  
 Number of results: 10  
 Standard deviation: 0.01 %

**X. Loss on Drying**  
 Conditions: 100 °C for 160 min according to Ph. Eur. 2.2.14.10

**Results:**  
 Average: 0.28 %  
 Number of results: 10  
 Standard deviation: 0.001 %

**Y. Sublimed Ash**  
 Method: Ph. Eur. 2.2.22.4.10

**Results:**  
 Average: 0.24 %  
 Number of results: 10

**VI. Titration**  
 The assay of the substance substance was performed by titration.

**Conditions:**  
 Titrant: Sodium 2S, 1N  
 Titrant: 0.1 N NaOH  
 Indicator: 0.05 % Thymol Blue

**Results:**  
 Average: 99.94 %  
 Number of results: 10  
 Standard deviation: 0.08 %

**VII. Final Result**

**Pyridine**  
 Total impurities (PDC): 0.21 %  
 Water content: 0.19 %  
 Loss on drying: 0.28 %  
 Residue on ash: 0.24 %

**Assay:**  
 Assay (HPLC): 99.94 %  
 Assay (Titration): 99.94 %

The assay is in agreement with the assay stated in the certificate and is within the specified limits.

Release Date: 2019-07-02  
 Release Code: 2019-08-02

LGC Standards  
 Dr. Rainer Schirmer  
 Product Manager

# Certificates – Primary pharmaceutical reference standards



## II. Purity

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

### HPLC Conditions:

#### Column:

X Terra RP18  
5 µm, 150 x 3.9 mm

#### Conditions:

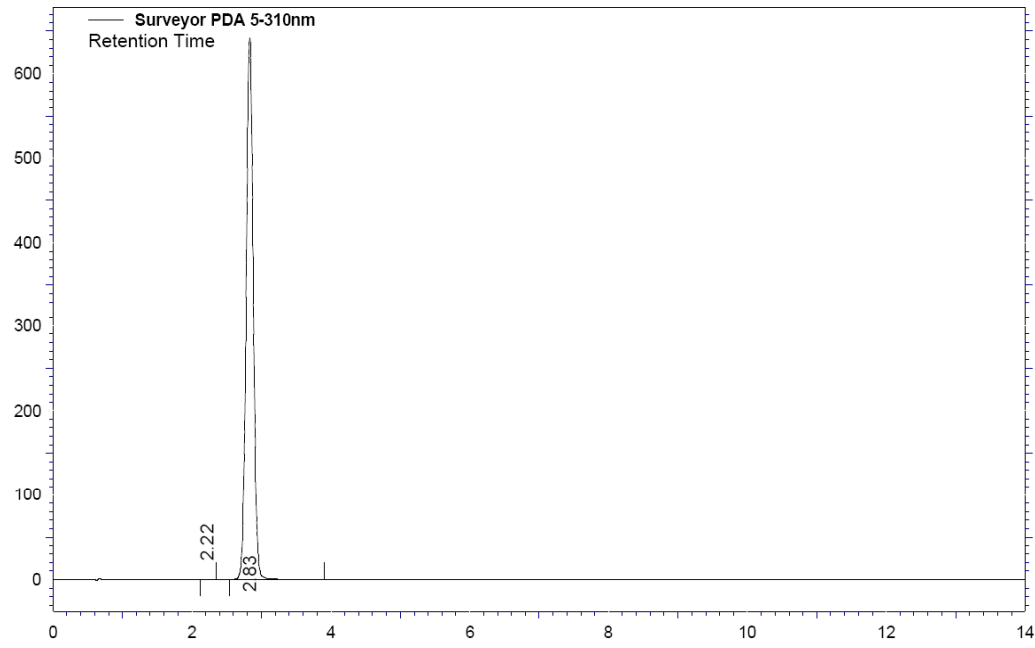
2.0 ml/min, 40 °C  
Water/Acetonitrile 80/20 (v/v); 0.54 %  
KH<sub>2</sub>PO<sub>4</sub>; 0.22 % octanesulfonic acid;  
adjust to pH 3.0

#### Detector:

DAD  
310 nm

#### Injector:

Auto  
2 µl; 0.5004 mg/ml in  
Water/Acetonitrile 50/50 (v/v)



Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	2.22	683	0.01
2	2.83	4562720	99.99
Totals		4563403	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 %.

**Results:**  
**Average** 99.99 %  
**Number of results** n=6  
**Standard deviation** 0.01 %

Standards



Background image showing several LGC Standards certificates. A black arrow points from the 'Area Percent Report' table to the 'Total' row of the first certificate, which is partially obscured by a black box.

### III. Water Content

Method: Karl Fischer titration according Ph Eur 5 (2.5.12)

#### Results:

Average	0.10 %
Number of results	n=3
Standard deviation	0.01 %

### IV. Loss on Drying

Conditions: 100 °C to 105 °C for 2 h according Ph Eur 5 (2.2.32)

#### Results:

Average	0.04 %
Number of results	n=3
Standard deviation	0.003 %

### V. Sulphated Ash

Method: Ph.Eur. 2002 (2.4.14)

#### Results:

Average	0.24 %
Number of results	n=3



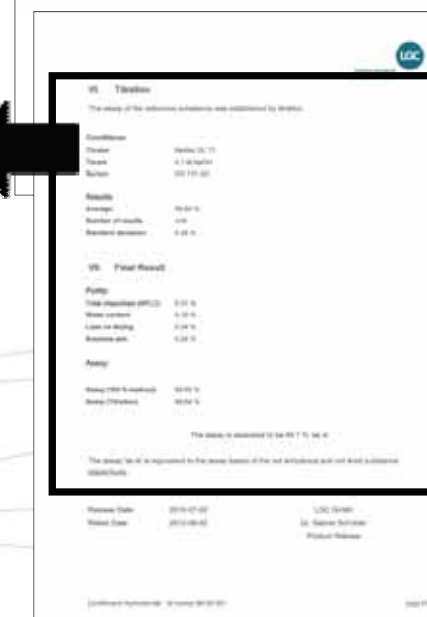
Excellence through measurement

The screenshot shows a laboratory report with three sections: III. Water Content, IV. Loss on Drying, and V. Sulphated Ash. Each section lists 'Results' with 'Average', 'Number of results', and 'Standard deviation'. A large black arrow points from the left towards the 'Water Content' section of the report. The report also includes a 'Final Result' section with a table of values and a 'Release Date' of 2019-07-02.

Cer  
pha



Excellence through measurement



## VI. Titration

The assay of the reference substance was established by titration.

### Conditions:

Titrator	Mettler DL 77
Titrant	0.1 M NaOH
Sensor	DG 111-SC

### Results

Average:	99.64 %
Number of results	n=6
Standard deviation	0.28 %

## VII. Final Result

### Purity:

Total impurities (HPLC)	0.01 %
Water content	0.10 %
Loss on drying	0.04 %
Sulphate ash	0.24 %

### Assay:

Assay (100 % method)	99.65 %
Assay (Titration)	99.64 %

The assay is assessed to be 99.7 % 'as is'

The assay 'as is' is equivalent to the assay based of the not anhydrous and not dried substance respectively.





# Agenda

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# Secondary pharmaceutical reference standards



- **FDA guidance from previous webinar**
  - ... A working standard (i.e. ***in-house(!)*** or secondary standard) is a standard that is *qualified against and used instead of the reference standard ...*
  - Desired qualifications
    - No requirement of full proof of structure  
Proof of identity (e.g. by IR or MS) against primary RS sufficient
    - Determination of assay of secondary RS against original primary RS





Setting standards  
in analytical science

#### ib. Mass Spectrum

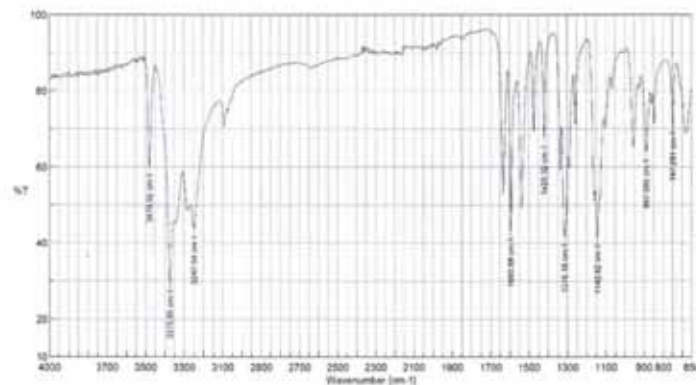
Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet

m/z	fragments
285	[M] <sup>+</sup>
248	[ (M - H) - HCl ] <sup>+</sup>
205	[ M - SO <sub>2</sub> NH <sub>2</sub> ] <sup>+</sup>

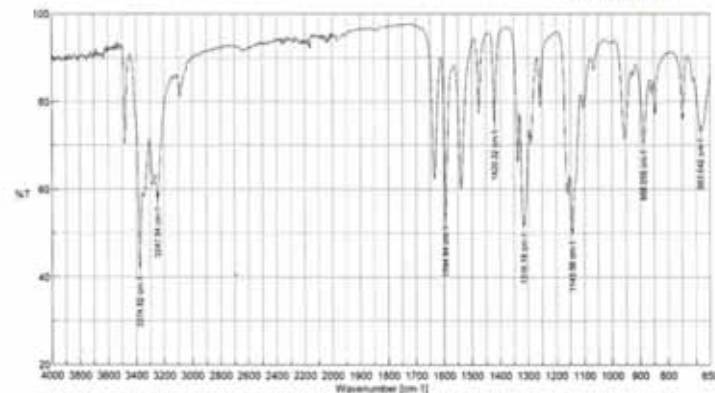
The signals of the mass spectrum and their interpretation are consistent with the structural formula.

#### ic. IR Spectrum

Method: Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy



Setting standards  
in analytical science



#### id. Melting Point

Result: 262 °C

#### ie. Elementary Analysis

Results of duplicate analysis:

	calculated	found
C	25.22 %	25.22 %
H	2.82 %	2.86 %
N	14.71 %	14.43 %

The signals of the IR spectrum and their interpretation are consistent with the spectrum of USP reference substance lot number I0F027 (spectrum of the USP RS is shown below):



Setting standards  
in analytical science

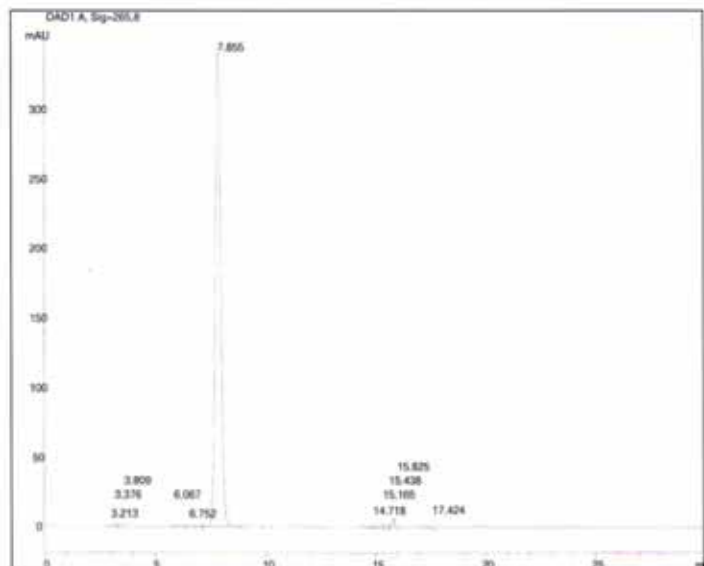
## II. ASSAY BY COMPARISON WITH USP REFERENCE STANDARD (HPLC)

Declared content of the USP RS (lot number 10F027):

Method: USP

### HPLC Conditions:

Column:	Conditions:	Detector:	Injector:
µBondapak C18	1.0 ml/min, 40 °C	DAD	Auto
10 µm, 300 x 3.9 mm	mob. Ph. A: 0.05M KH <sub>2</sub> PO <sub>4</sub> /Methanol 90/10 mob. Ph. B: Methanol	265 nm	5 µl; 0.6088 mg/ml in Methanol
	0 – 10 min mob. Ph. A /mob. Ph. B 100/0		
	10 – 12 min mob. Ph. A /mob. Ph. B to 80/20		
	12 – 14 min mob. Ph. A /mob. Ph. B 80/20		
	14 – 16 min mob. Ph. A /mob. Ph. to B 100/0		
	16 – 30 min mob. Ph. A /mob. Ph. B 100/0 (v/v)		



Benzothiadiazine Related Compound A (USP): 4-Amino-6-chloro-1,3-benzenedisulfonamide  
Lot Number 0011.01.08.04

LGC GmbH, Im Biotechnologiepark, TGZ II, D-14843 Luckenwalde, Germany

page 5/7



Setting standards  
in analytical science

results from the analysis

CRS	Area/conc	Sample	Area/conc
1	7881146.065	1	7661178.449
2	7891510.880	2	7778983.968
3	7867107.043	3	7822815.722
4	7878269.579	4	7700025.411
5	7894650.947	5	7811480.467
6	8001653.216	6	7838300.000
mean	7902389.62	mean	7768464.00
rsd	0.0063	rsd	0.0093

Multiple analysis runs based on multiple weighings were performed and the declared content of the USP reference substance was taken into consideration to assign an assay value to the secondary standard.

Result	98.3 %
Uncertainty	± 2.2 %

## III. CONCLUSION

The reference substance was analysed by <sup>1</sup>H-NMR, MS, IR, melting point and elementary analysis. All methods proved the identity of the reference substance.

The <sup>1</sup>H-NMR spectrum and the IR spectrum was checked against USP reference substance lot number 10F027.

## IV. FINAL RESULT

### Assay:

Comparison with USP reference standard 98.3 % ± 2.2 %

The result is obtained by the division of the areas CRS/sample.

The expanded uncertainty is assessed by the combination of the standard deviations with a coverage factor k = 2.

Benzothiadiazine Related Compound A (USP): 4-Amino-6-chloro-1,3-benzenedisulfonamide  
Lot Number 0011.01.08.04

LGC GmbH, Im Biotechnologiepark, TGZ II, D-14843 Luckenwalde, Germany

page 6/7

# Agenda

- Definition for ‘Certificate of analysis’
- ISO 31: Certificate Content and Structure
- **Certificates**
  - Certified reference materials (CRMs)
  - Primary pharmaceutical reference standards
  - Secondary pharmaceutical reference standards
  - **Impurity reference standards**
  - Research materials
- Summary

# CofA: Impurity RS



Excellence through measurement

**CERTIFICATE**  
Reference Substance  
4-Methoxy-2-(2-methoxy-5-methylphenyl)-2-methylpropanoic acid  
1-Cleas (Cleasmax, Sulphate B-Cleas)

**I. Identity**  
The identity of the reference substance was established by following procedure:  
**1a. <sup>13</sup>C-NMR Spectrum**  
 Conditions: 400 MHz, 25000 Hz  
 The substance is confirmed with the signals of the spectrum and their assignment:

**II. IR Spectrum**  
Method: Interfered Transmittance Fourier Transform Infrared (FT-IR) Spectrometry  
The signals of the IR spectrum and their assignment are compared with the reference literature:

**III. Purity**  
The purity of the reference substance was assessed by high performance liquid chromatography (HPLC):

**HPLC Conditions**

Parameter	Equipment	Reagents	Apparatus
Injection	1	1	1
Injection Volume (µL)	1	1	1
Flow Rate (mL/min)	1	1	1
Column	1	1	1
Mobile Phase	1	1	1
Detection	1	1	1
Wavelength (nm)	1	1	1
Temperature (°C)	1	1	1

**IV. Mass Spectrum**  
Method: A.2.01.01.01: Acquisition Temperature: 200 °C, Apert 400  
The signals of the mass spectrum and their assignment are compared with the reference literature:

**V. Final Result**  
The purity is assessed by the following procedure:  
 The assay for it is conducted by the assay based on the chromatography and related substance impurities:  
 Assay Date: 2012-08-08  
 LGC Group  
 Dr. Stefan Günther  
 Product Manager

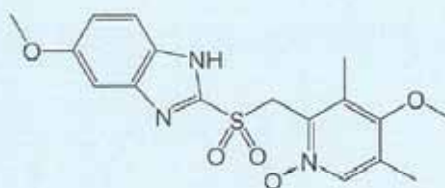
# CofA: Impurity RS



## CERTIFICATE

### Reference Substance

4-Methoxy-2-[[[(5-methoxy-1H-benzimidazol-2-yl)sulphonyl]methyl]-3,5-dimethylpyridine 1-Oxide (Omeprazole Sulphone N-Oxide)

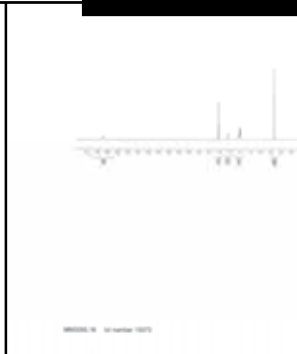


Molecular Formula:  $C_{17}H_{19}N_3O_6S$   
Molecular Weight: 377.42  
CAS Number: [ 158812-85-2 ]

Catalogue Number: MM0095.16  
Lot Number: 15573  
Long-term Storage: 2 to 8 °C, dark  
Appearance: white solid  
Melting Point: 185 °C (dec.)  
Assay 'as is': 99.2 %

Date of shipment:

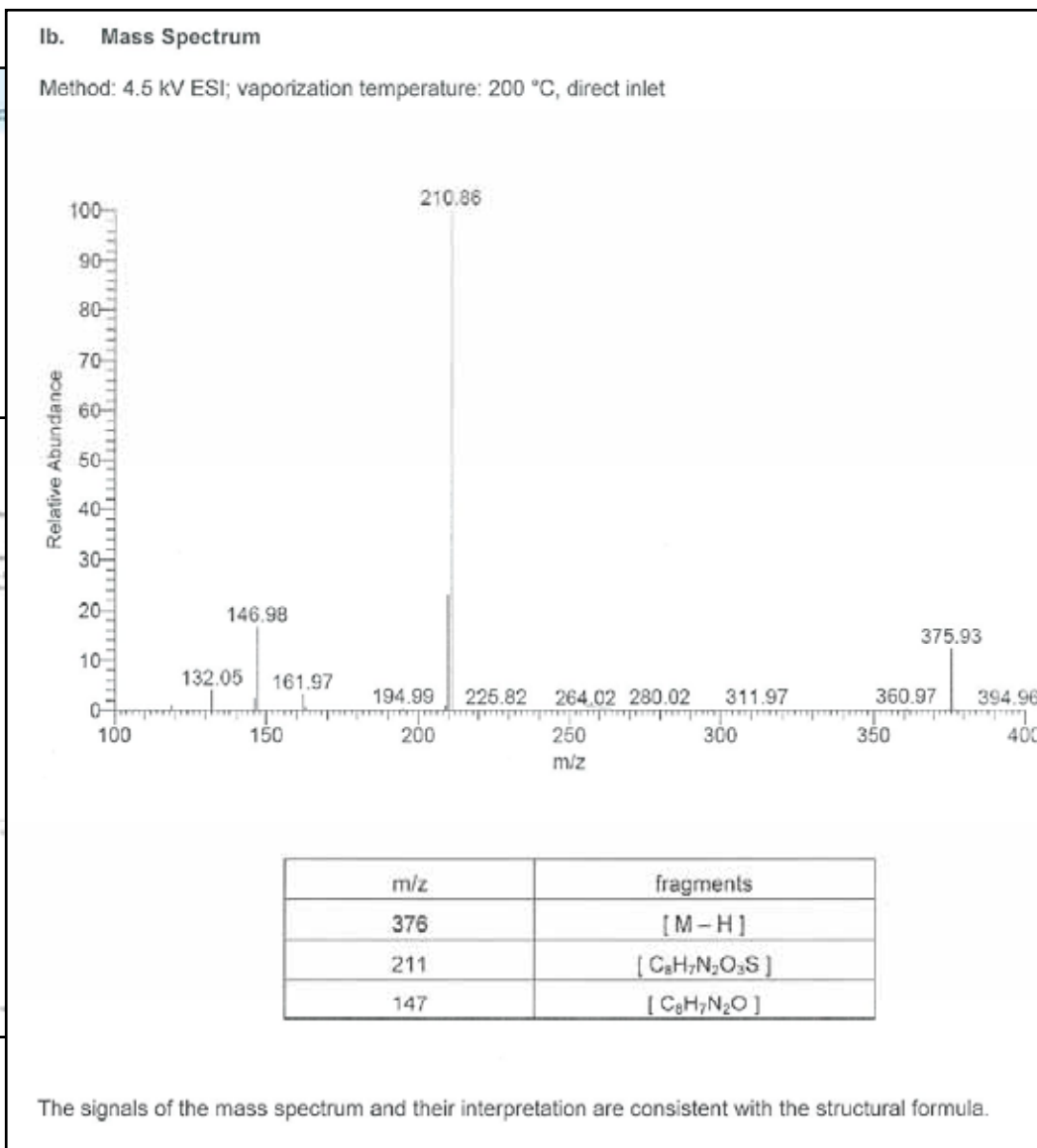
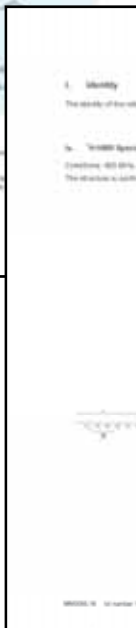
This certificate is valid for two years from the date of shipment provided the substance is stored under the recommended conditions.







# CofA: Impurity RS

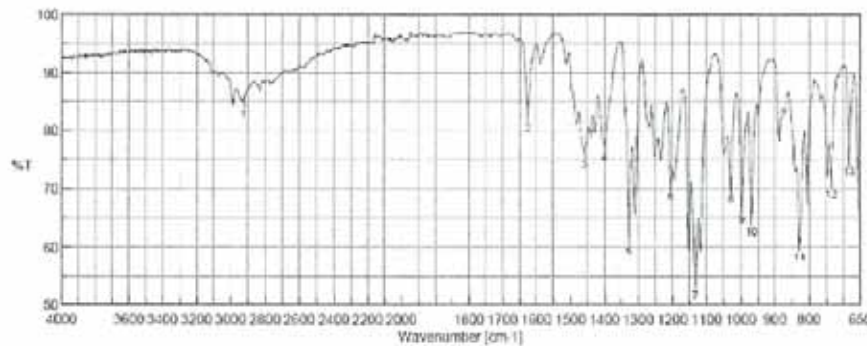


The signals of the mass spectrum and their interpretation are consistent with the structural formula.

Co

**Ic. IR Spectrum**

Method: Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy



No.	Position	Intensity
1	2955.48	64.6026
2	1624.73	62.3126
3	1458.00	76.1450
4	1400.07	77.3327
5	1326.29	61.3715
6	1204.33	70.5593
7	1132.01	53.7549
8	1028.34	70.1758
9	996.050	66.711
10	959.055	64.5746
11	828.277	60.3420
12	736.71	71.0276
13	682.677	74.981

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

**II. Purity**

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

**HPLC Conditions:**

Column:	Conditions:	Detector:	Injector:
Hypersil Gold (C18)	1.0 ml/min, 40 °C	DAD	Auto
5 µm, 150 x 4.6 mm	0 – 15 min Water/Acetonitrile 80/20	220 nm	5 µl; 0.0654 mg/ml in
	15 – 20 min Water/Acetonitrile to 60/40		Water/Acetonitrile 50/50 (v/v)
	20 – 25 min Water/Acetonitrile 60/40		
	25 – 30 min Water/Acetonitrile to 80/20		
	30 – 35 min Water/Acetonitrile 80/20 (v/v);		
	0.1 % H <sub>3</sub> PO <sub>4</sub>		



**II. Water Content**

Method: Karl-Fischer Reagent

**Results**

Sample	0.00 %
Water content	0.00 %
Residual moisture	0.00 %

**IV. Residual Solvents**

Method: GC-MS

Result: 0.00 % Chloroform  
0.00 % Acetone

**V. Final Result**

Total impurities (PHE-01)	0.00 %
Water content	0.00 %
Residual solvents	0.00 %
Heavy metal (PHE-01)	0.00 %

The purity of substance is 99.99 % (w/w).

The assay for it is according to the assay based on the monograph and related substance impurities.

Prepared Date: 2012-08-08

LGC-0008

*[Signature]*  
Dr. Sabine Günther  
Product Manager

The release of this report is subject to the terms and conditions of the contract.

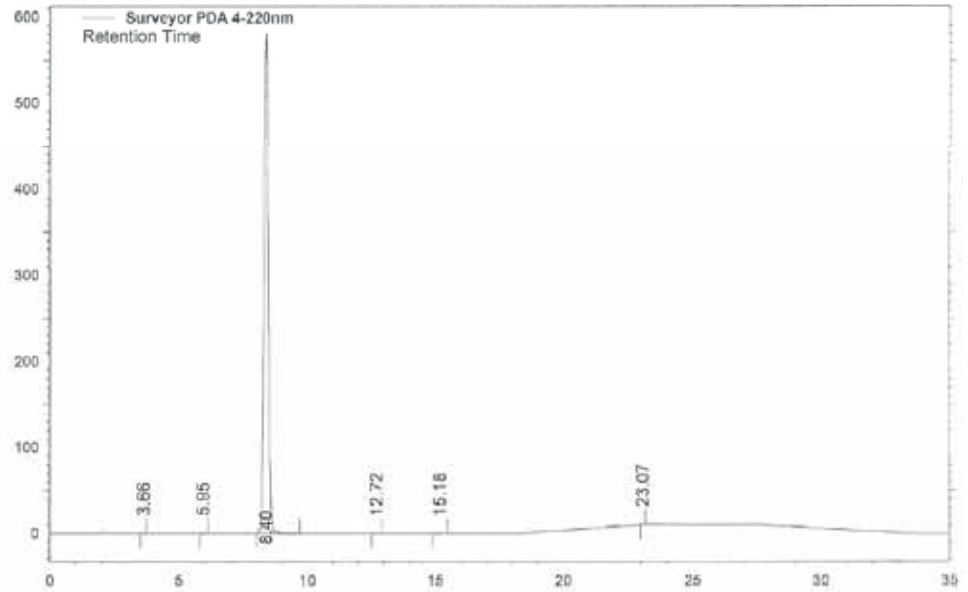
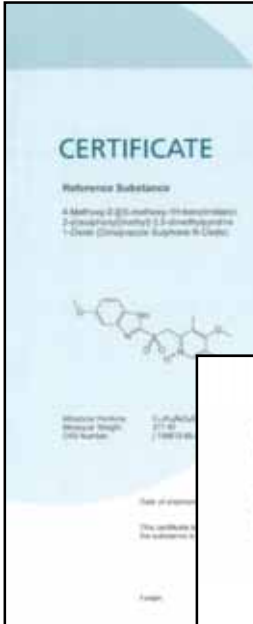
Product No.: 123456789

Issue 01, 2012-08-08

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Cof



Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	3.66	1422	0.02
2	5.95	2481	0.04
3	8.40	6745401	99.85
4	12.72	1480	0.02
5	15.18	3383	0.05
6	23.07	1693	0.03
Totals		6755860	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 %.

**Results:**  
 Average 99.85 %  
 Number of results n=3  
 Standard deviation 0.01 %

### III. Water Content

Method: Karl Fischer titration

#### Results:

Average	0.20 %
Number of results	n=3
Standard deviation	0.01 %

### IV. Residual Solvents

Method: <sup>1</sup>H-NMR

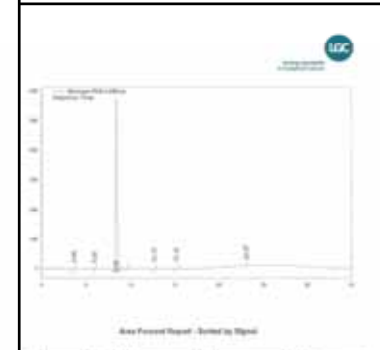
Result: 0.46 % Chloroform  
0.04 % *n*-Hexane

### V. Final Result

Total impurities (HPLC)	0.15 %
Water content	0.20 %
Residual solvents	0.50 %
Assay (100 % method) <sup>1</sup>	99.15 %

The assay is assessed to be 99.2 % 'as is'

The assay 'as is' is equivalent to the assay based on the not anhydrous and not dried substance respectively.



Retention Time	Abundance
1.00	0.00
2.00	0.00
3.00	0.00
4.00	0.00
5.00	0.00
6.00	0.00
7.00	0.00
8.00	0.00
9.00	0.00
10.00	100.00
11.00	0.00
12.00	0.00
13.00	0.00
14.00	0.00
15.00	0.00
16.00	0.00
17.00	0.00
18.00	0.00
19.00	0.00
20.00	0.00

Retention Time	Abundance
1.00	0.00
2.00	0.00
3.00	0.00
4.00	0.00
5.00	0.00
6.00	0.00
7.00	0.00
8.00	0.00
9.00	0.00
10.00	100.00
11.00	0.00
12.00	0.00
13.00	0.00
14.00	0.00
15.00	0.00
16.00	0.00
17.00	0.00
18.00	0.00
19.00	0.00
20.00	0.00

<sup>1</sup> The calculation of the 100 % method follows the formula:

$$\text{Assay (\%)} = (100 \% - \text{KF} - \text{RES}) * \frac{\text{Purity HPLC (\%)}}{100 \%}$$

Water (KF) and Residual solvents (RES) are considered as absolute contributions, HPLC purity is considered as relative contribution.

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  - Secondary pharmaceutical reference standards
  - Impurity reference standards
  - **Research materials**
- Summary

# CofA: Research material



Excellence through measurement

## 1. Identification

CAS Number:

467-02-7

Catalogue Number:

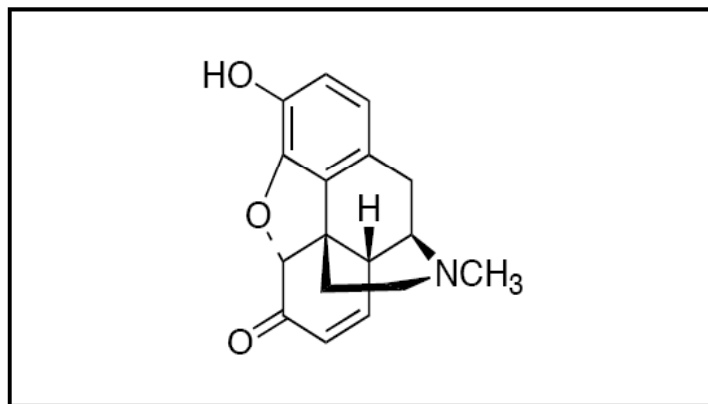
Product:

Morphinone

Synonyms:

(5 $\alpha$ )-7,8-Didehydro-4,5-epoxy-3-hydroxy-17-methylmorphinan-6-one;

Structure:



Molecular Formula:

$C_{17}H_{17}NO_3$

Molecular Weight:

283.32

Source of Product:

Synthetic

# CofA: Research material



Excellence through measurement

## 2. Analytical Information

**Lot Number:**

**Melting Point:**

140-145°C (dec.)

**Boiling Point:**

N/A

**Atmosphere:**

Air

**Appearance of Product:**

Light Yellow Solid

**Solubility**

Methanol

**Method for Determining Identity:**

<sup>1</sup>H NMR (CD<sub>3</sub>OD) Spectroscopic and Mass Spectrometric Analysis

**Stability**

Not determined

**Purity:**

94.5% by HPLC

**Long Term Storage Condition:**

Controlled Substance, -20°C Freezer

**Additional Information:**

TLC Conditions: SiO<sub>2</sub>; Dichloromethane : Methanol : Ammonium Hydroxide = 9 : 1 : 0.05; Visualized with UV and AMCS; Single spot, R<sub>f</sub>=0.6.

<sup>1</sup>H NMR and Mass spectra conform to structure.



# Agenda

- **Definition for ‘Certificate of analysis’**
- **ISO 31: Certificate Content and Structure**
- **Certificates**
  - **Certified reference materials (CRMs)**
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- **Summary**

# Summary

- **Certificates**
  - ... are important documents relevant for the correct use of a certified reference material
    - Or a standard, or a research material
- **Content of certificate**
  - ... is relevant for the corresponding use
    - i.e. a research material with poor purity/assay information not suitable for quantitative purposes like determination of API's assay figures or impurity levels
      - For both applications high risks of overestimation of analyte

# Closing remarks



- PDF of presentation downloadable from our website <http://pharma.lgcstandards.com/>
  - When on website, look under ‘Events’
  - PDF is approx. 5 MB
- This webinar last one of our Spring series of webinars
  - We will repeat it in Autumn 2013 for Europe and Asia
    - The last webinars had more than 100 registrants, so we are quite happy about the success
  - We will also present the highlights soon for our American customers

# Thank you for your attention!



## Questions?

[christian.zeine@lgcstandards.com](mailto:christian.zeine@lgcstandards.com)