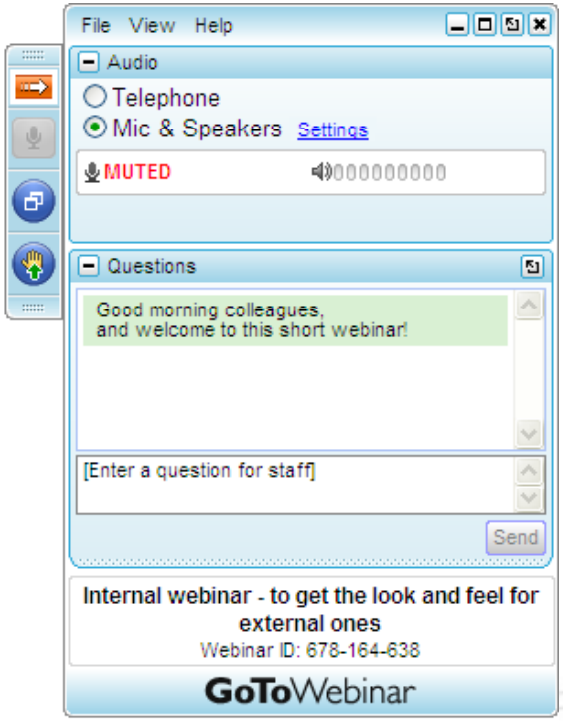
Reference materials: Certificates of analysis

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

Webinar Series 2013,

May 23rd, 2013

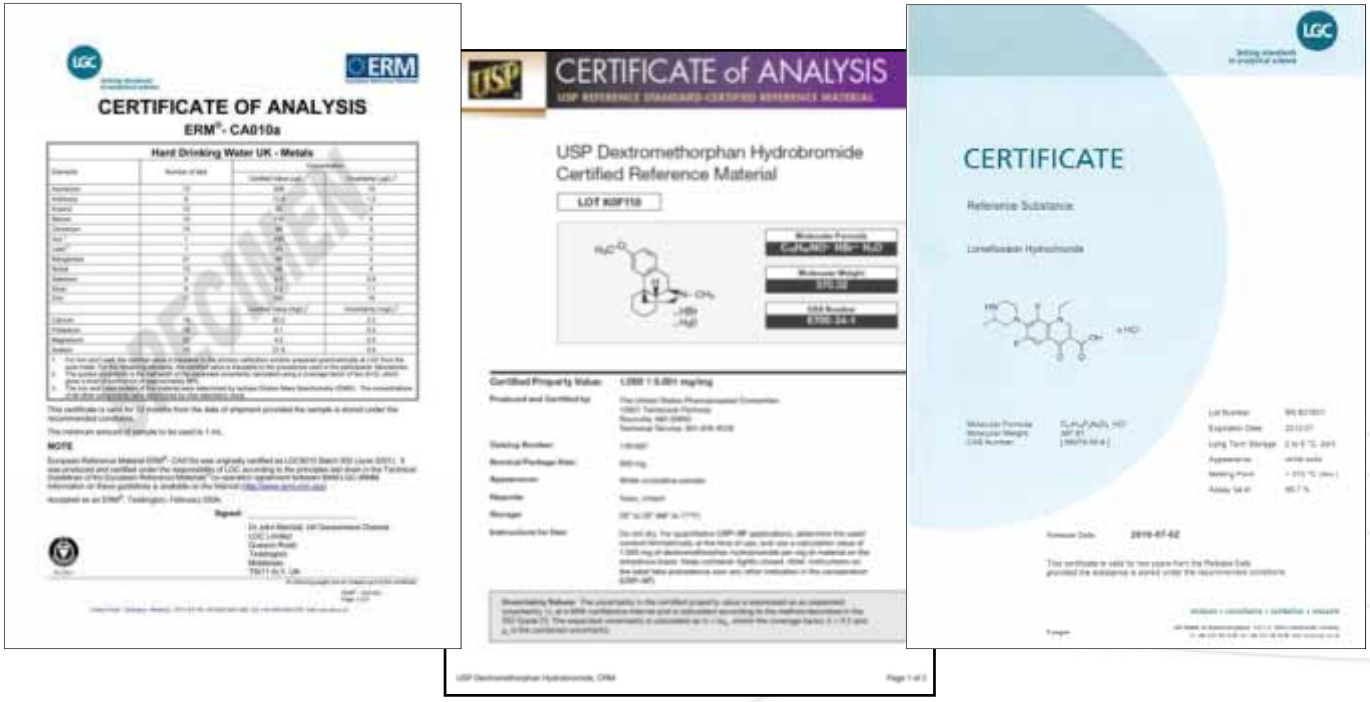
Quick guide to the webinar tools



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)?



Definition

- **ISO Guide 31:2000 defines certificates as:**

  “Document containing all the information which is essential to the use of a certified reference material”

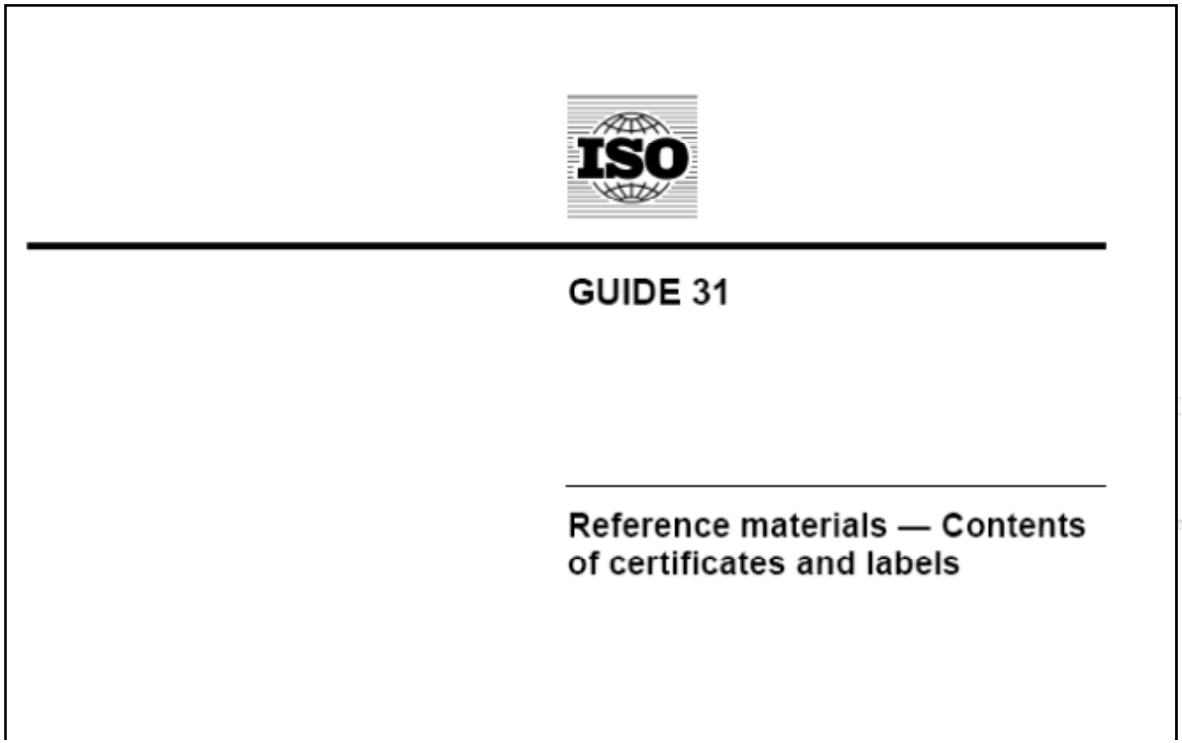
- **Please also note:**

  “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

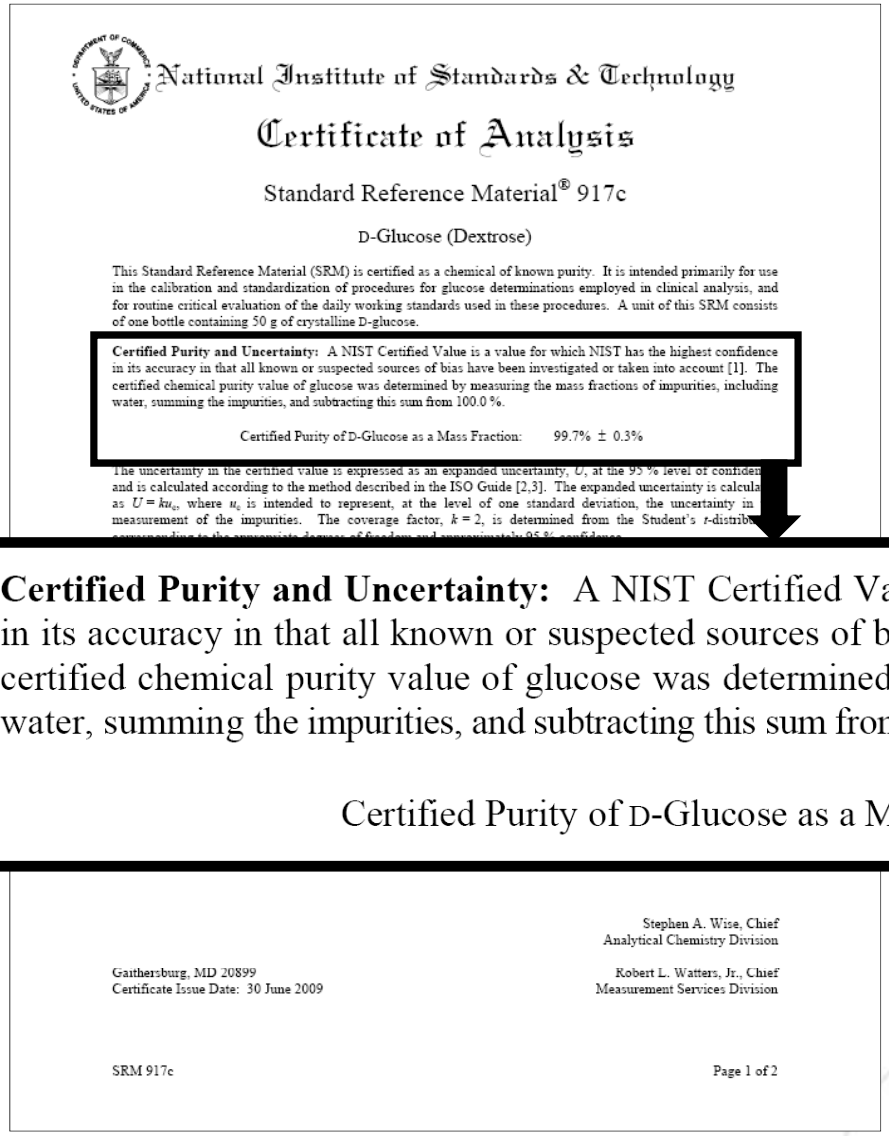
- **Possible content acc. to ISO Guide 31:2000**
  - Name and address of certifying organisation
  - Title of the document
  - Name of the document
  - Code and batch number
  - Description – relevant information
  - Intended use
  - Instructions for use
  - Hazardous situation
  - Level of homogeneity
  - Certified property values and uncertainty
  - Traceability
  - Date of certification
  - Period of validity
  - Stability, transportation and storage instructions
  - Shelf life/expiry date
  - ….

CAN be inclu-ded with a CRM! Not a MUST!

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**

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 $U = ku_c$, 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 approximate degrees of freedom and approximately 95 % confidence.

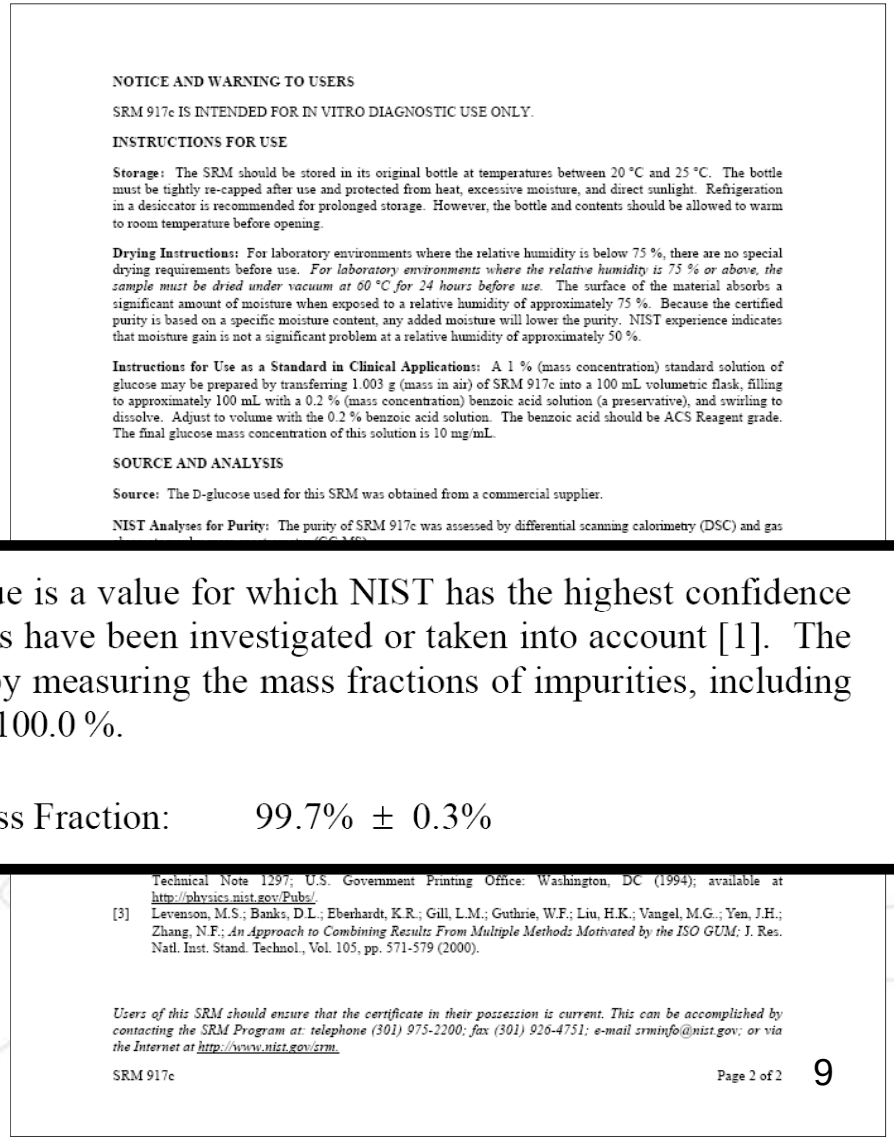
Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 30 June 2009

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

SRM 917c

Page 1 of 2



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 Analyses for Purity: The purity of SRM 917c was assessed by differential scanning calorimetry (DSC) and gas

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%

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; 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% - Total Chromatographic Impurities %) ÷ 100] *[(100%- ROI%) ÷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.

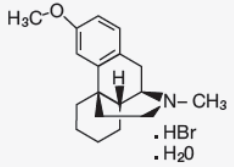
ndards

YSIS

ATERIAL

USP Dextromethorphan Hydrobromide
Certified Reference Material

LOT K0F118



Molecular Formula	Molecular Weight	CAS Number
$C_{18}H_{25}NO \cdot HBr \cdot H_2O$	370.32	6700-34-1

Certified Property Value: 1.000 ± 0.001 mg/mg

Produced and Certified by: The United States Pharmacopeial Convention
12601 Twinbrook Parkway

Certified Property Value: 1.000 ± 0.001 mg/mg

Appearance: White crystalline powder

Hazards: Toxic, Irritant

within the 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% - Total Chromatographic Impurities %) ÷ 100] *[(100%- ROI%) ÷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).

P Reference Standards Expert Committee, in accordance with their rules and procedures, Reference Standard lot is suitable to assess compliance with the monograph standards for critical characteristics of this lot are usually determined independently in three or more P, government, academic, and industrial collaborators.

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 traceabilitiy 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

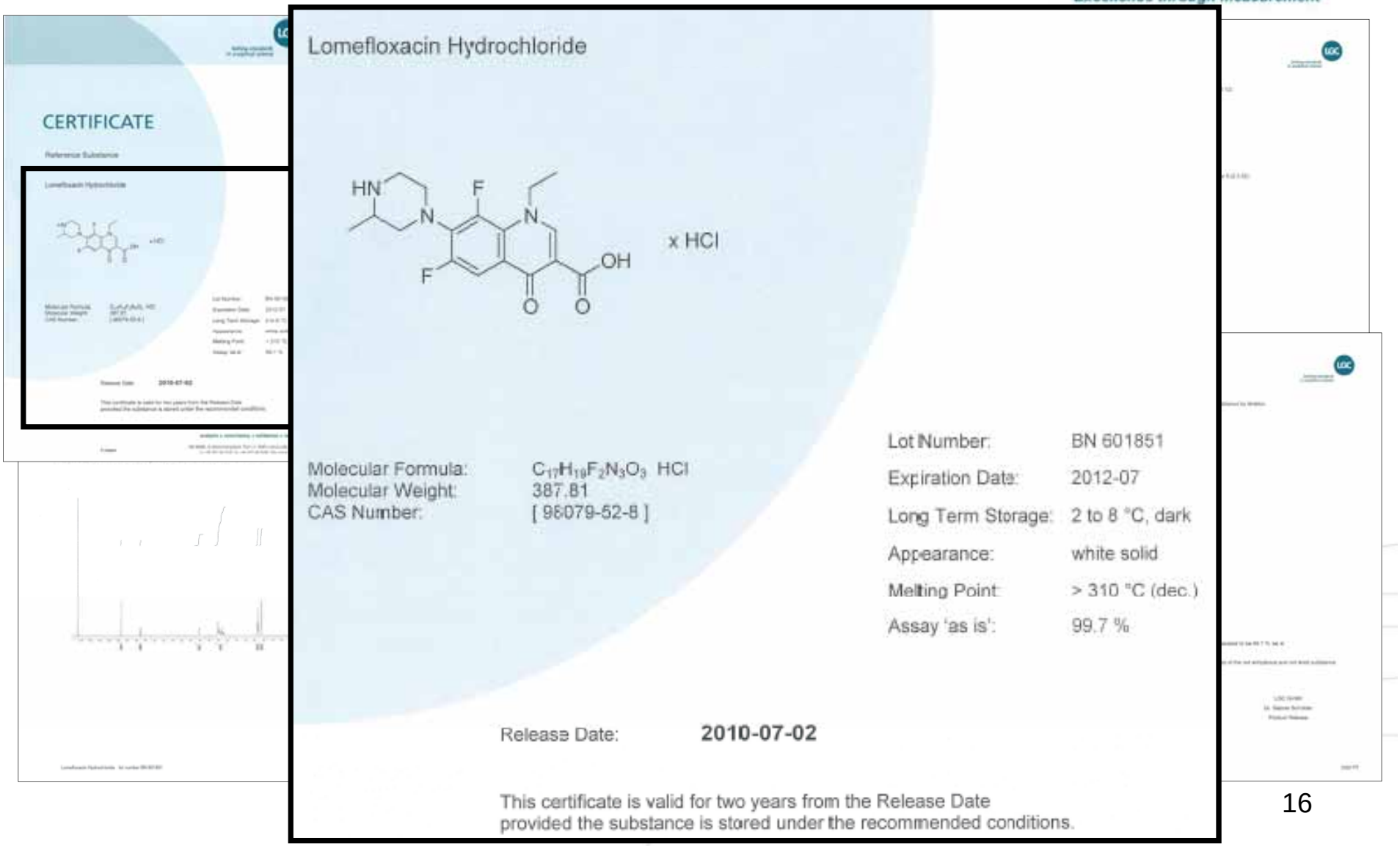
Primary pharmaceutical reference standards

- **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)

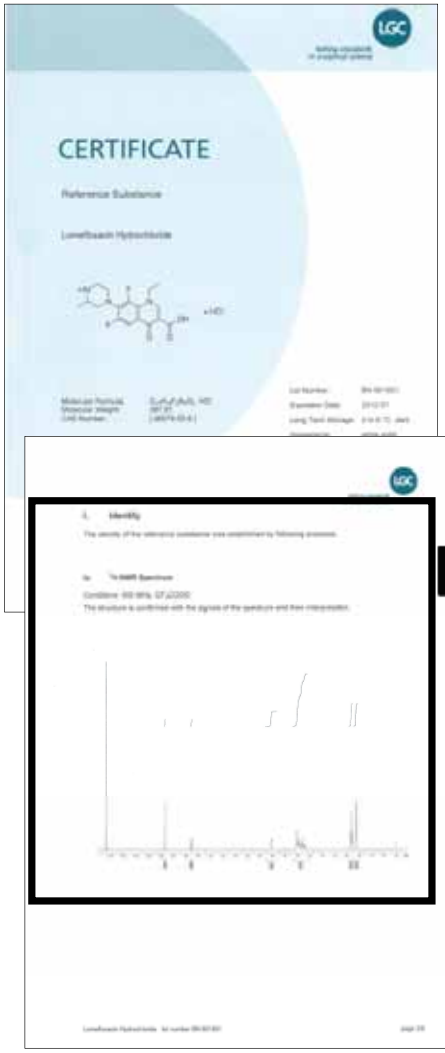
    - Assay, for primary RSs from purity calculation (with the results from all relevant examinations) plus at least one additional independent method (e.g. titration)

Certificates – Primary pharmaceutical reference standards

LGC Standards
Excellence through measurement



Certificates pharmaceuti



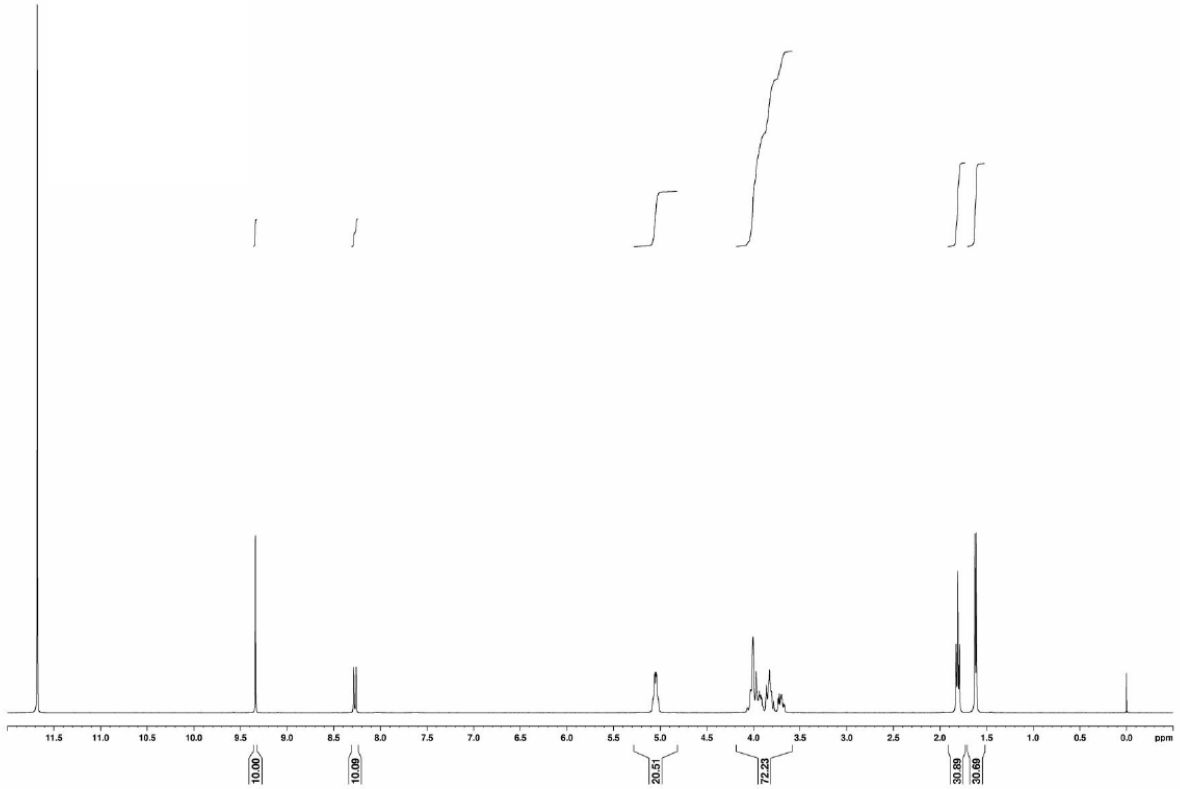
I. Identity

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

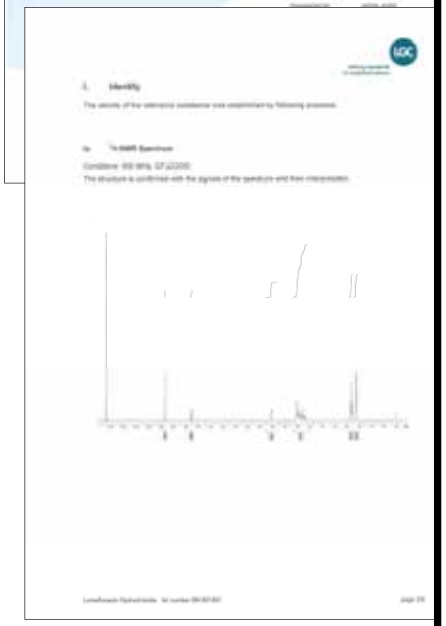
Ia. ^{1}H-NMR Spectrum

Conditions: 400 MHz, CF_3COOD

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



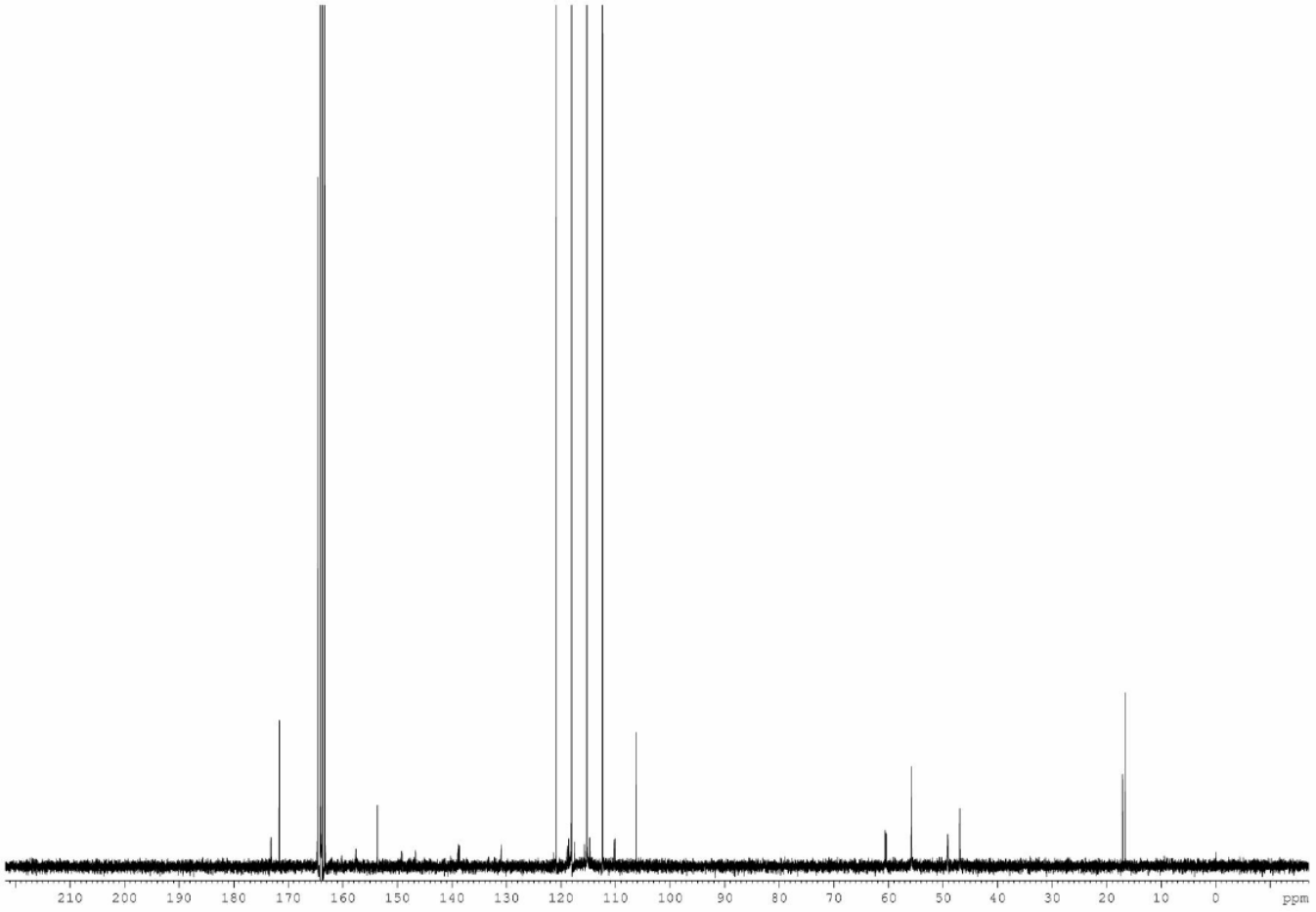
Certificat
pharmace



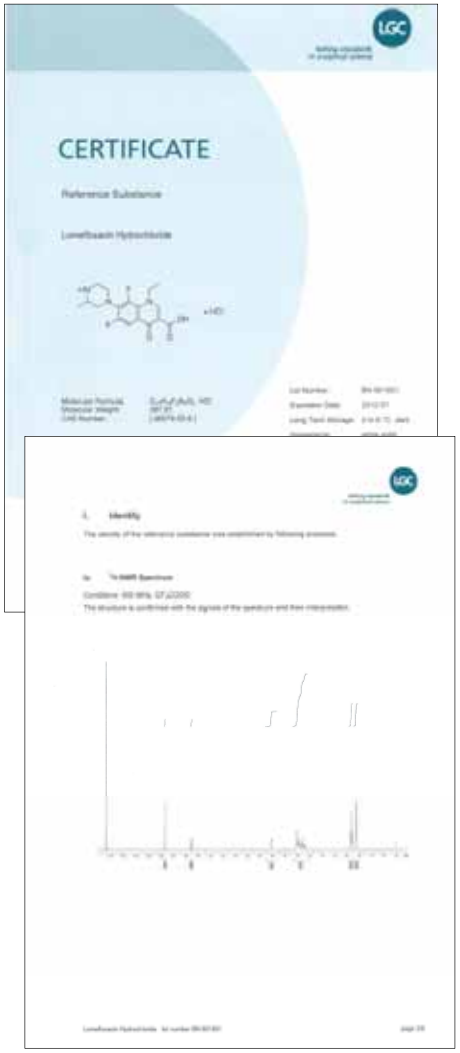
Ib. ^{13}**C-NMR Spectrum**

Conditions: 100 MHz, CF_3COOD

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

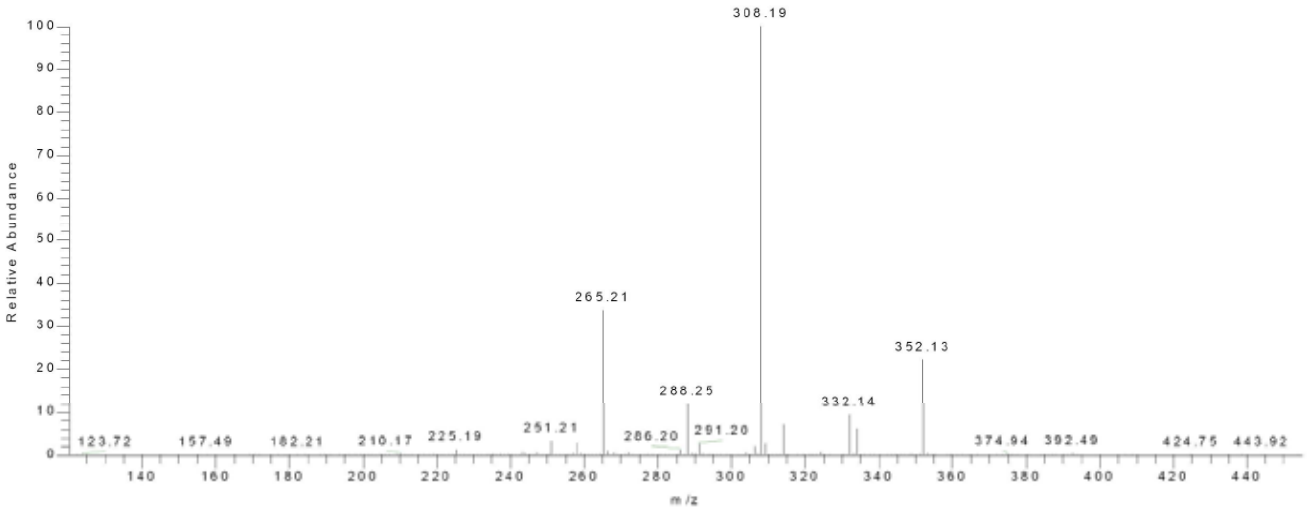


Certificates pharmaceuti



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	$[308 - HF]^{\cdot +}$

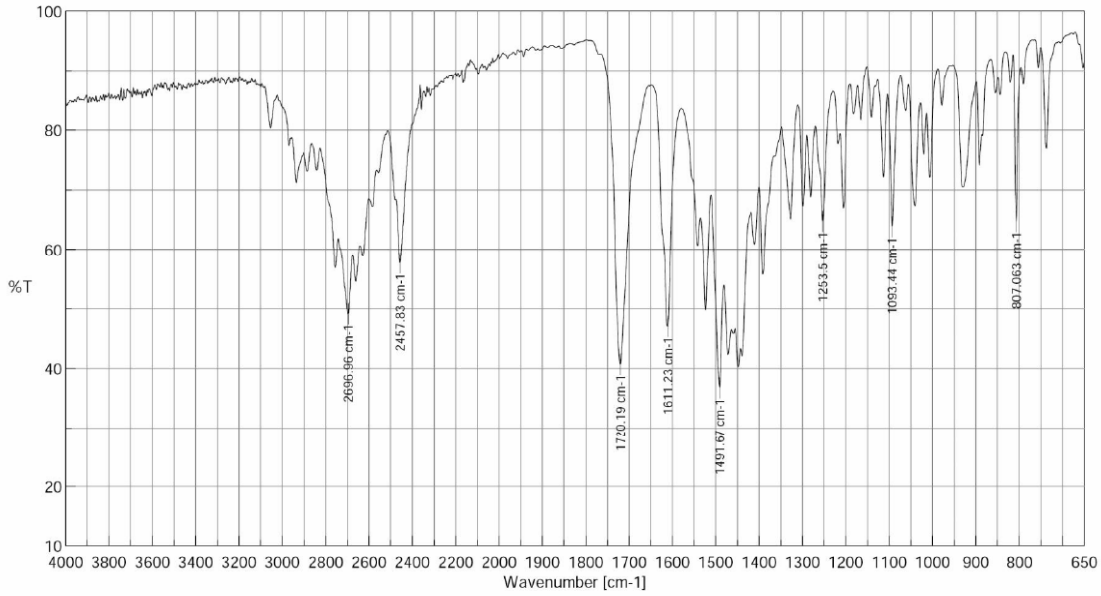
The signals of the MS spectrum and their interpretation are consistent with the structural formula.

Certificates – Primary pharmaceutical reference standards

LGC 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.

Certificates – Primary pharmaceutical reference standards

LGC Standards
Excellence through measurement

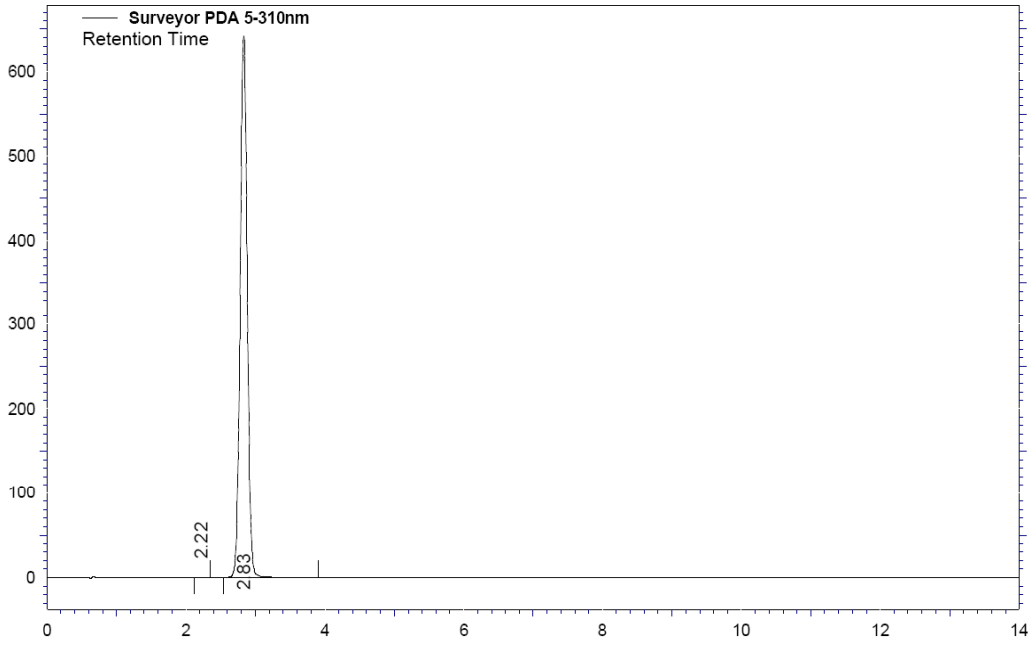


II. Purity

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

HPLC Conditions:

Column:	**Conditions:**	**Detector:**	**Injector:**
X Terra RP18	2.0 ml/min, 40 °C	DAD	Auto
5 µm, 150 x 3.9 mm	Water/Acetonitrile 80/20 (v/v); 0.54 % KH_2PO_4; 0.22 % octanesulfonic acid; adjust to pH 3.0	310 nm	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 %

dards

LGC Standards

Excellence through measurement

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

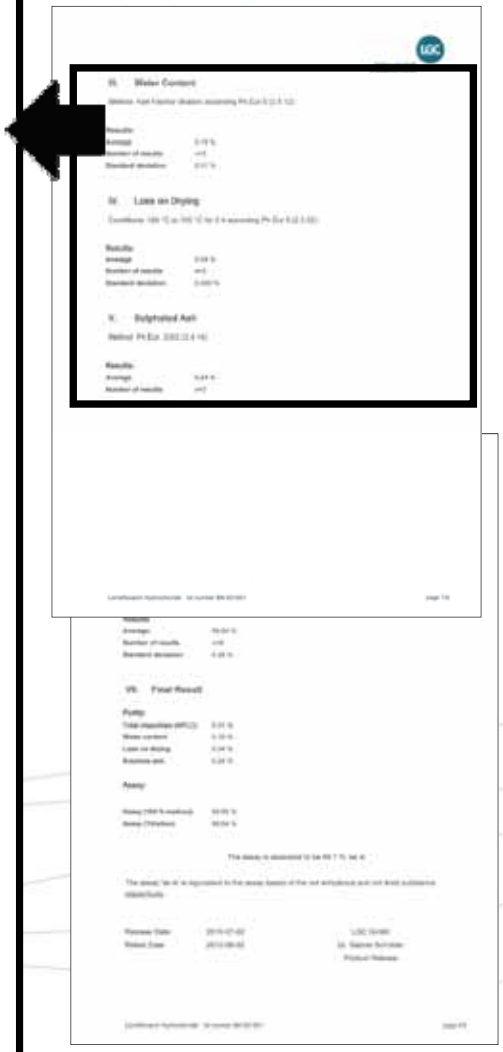
Method: Ph.Eur. 2002 (2.4.14)

Results:

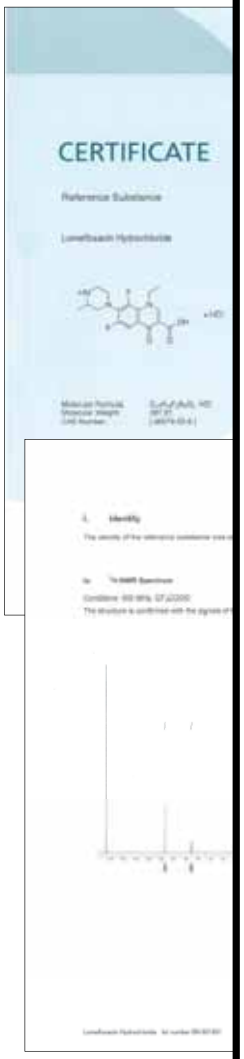
Average	0.24 %
Number of results	n=3

LGC Standards

Excellence through measurement



Cer
pha



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.

LGC Standards

Excellence through measurement

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**

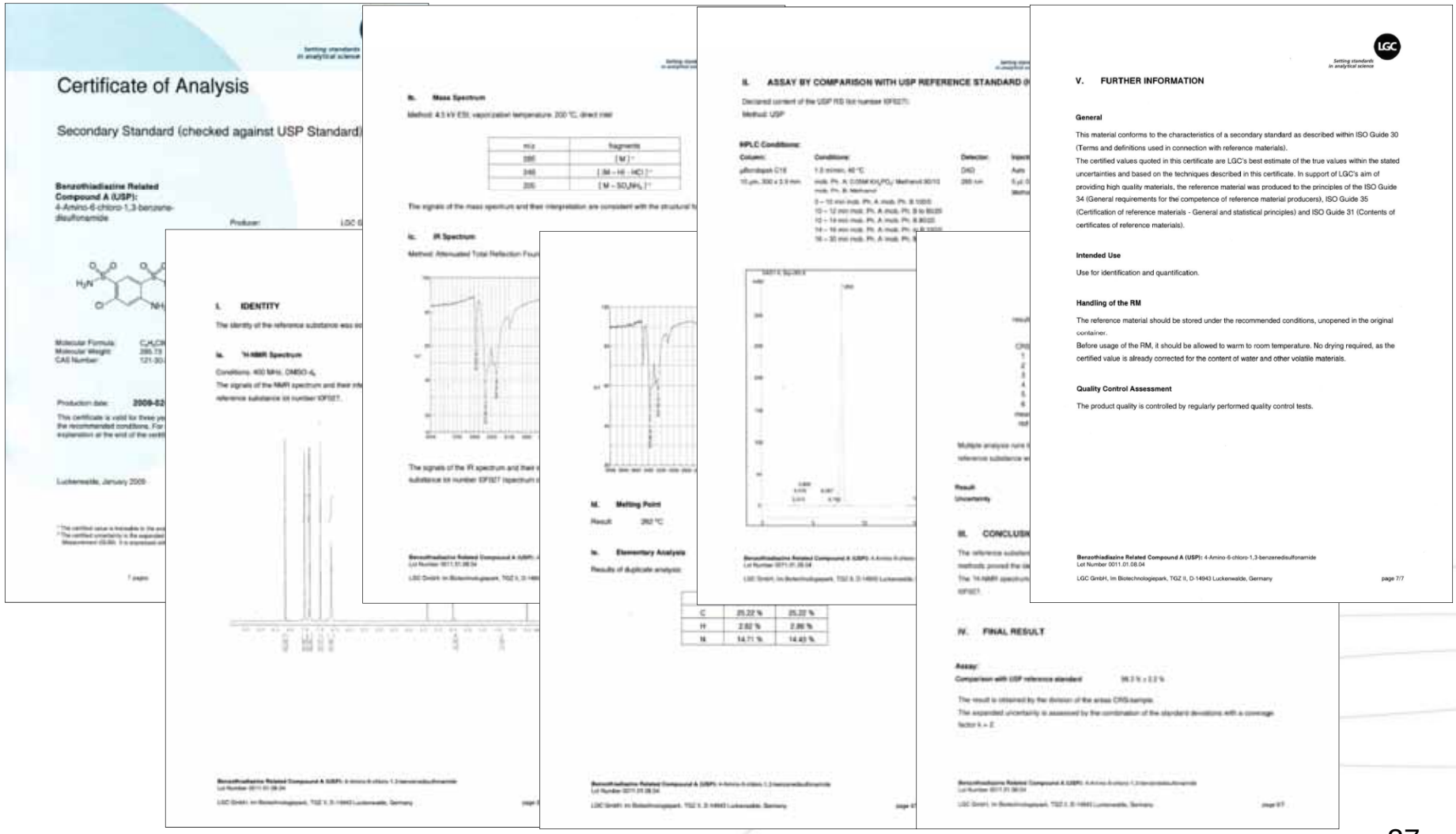
Secondary pharmaceutical reference standards

- **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

Secondary pharmaceutical reference standards – Certificates

LGC Standards

Excellence through measurement



Setting standards in analytical science

Ib. Mass Spectrum

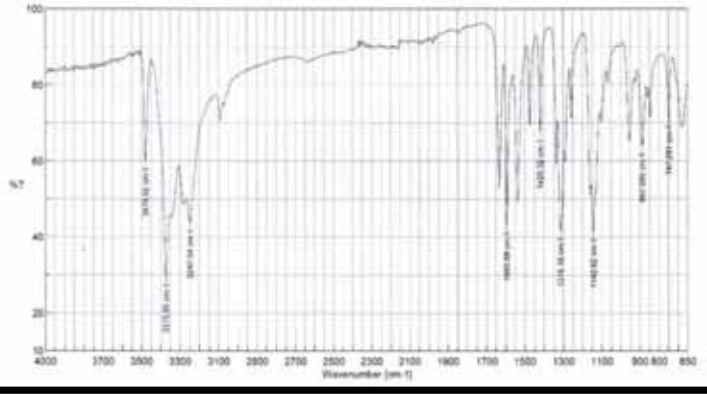
Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet

m/z	fragments
285	$[M]^{+\cdot}$
248	$[(M - H) - HCl]^{+\cdot}$
205	$[M - SO_2NH_2]^{+\cdot}$

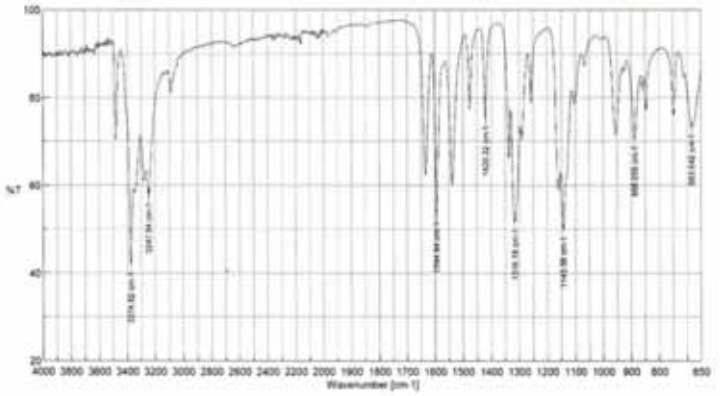
The signals of the mass spectrum and their interpretation are consistent with the structural formula.

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):

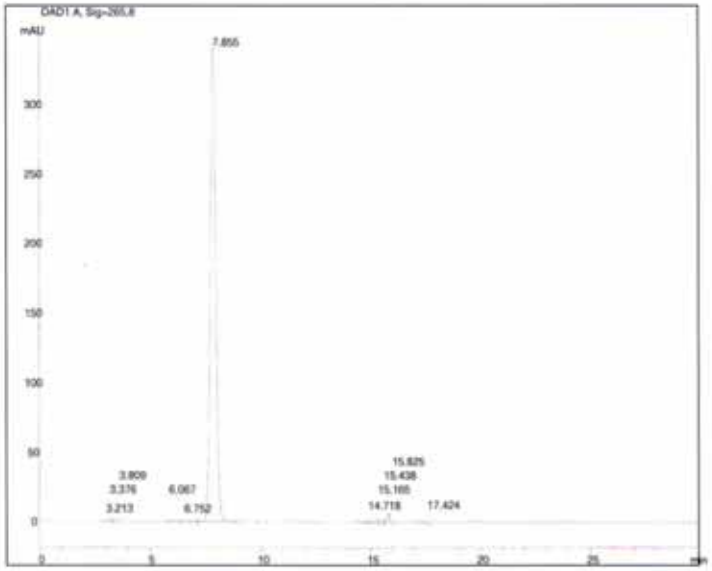
II. ASSAY BY COMPARISON WITH USP REFERENCE STANDARD (HPLC)

Declared content of the USP RS (lot number I0F027):

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_2PO_4/ Methanol 90/10 mob. Ph. B: 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)	265 nm	5 µl; 0.6088 mg/ml in Methanol



results from the analysis

CRS	Area/conc
1	7881146.065
2	7891510.880
3	7867107.043
4	7878269.579
5	7894650.947
6	8001653.216
mean	7902389.62
rsd	0.0063

Sample	Area/conc
1	7661178.449
2	7776983.968
3	7822815.722
4	7700025.411
5	7811480.467
6	7838300.000
mean	7768464.00
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 ^{1}H-NMR, MS, IR, melting point and elementary analysis. All methods proved the identity of the reference substance.
The ^{1}H-NMR spectrum and the IR spectrum was checked against USP reference substance lot number I0F027.

IV. FINAL RESULT

Assay:

Comparison with USP reference standard 98.3 % ± 2.2 %

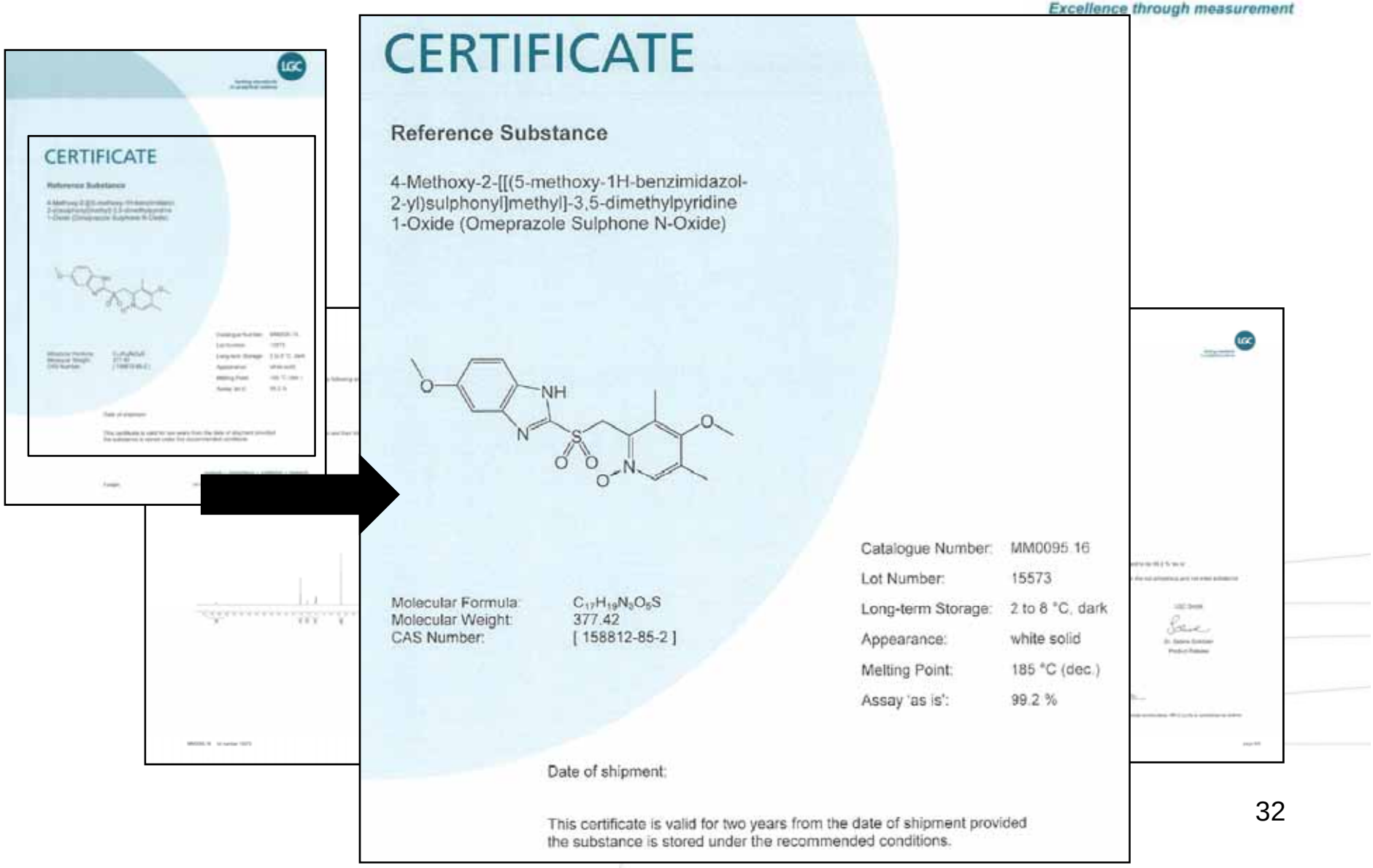
The result is obtained by the division of the areas CRS/sample.
The expanded uncertainty is assessed by the combination of the standard deviations with a coverage factor k = 2.

Agenda

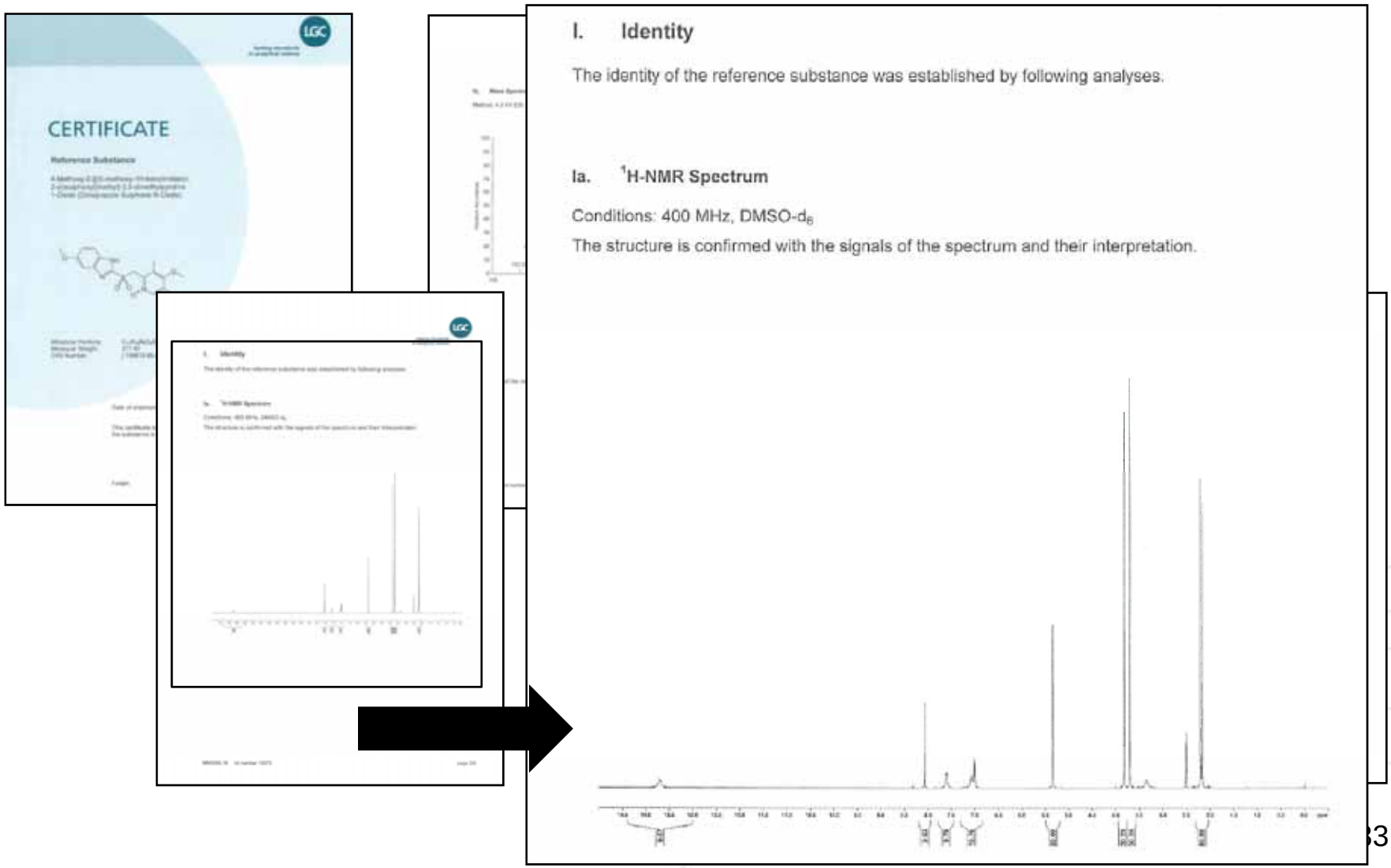
- **Definition for ‘Certificate of analysis’**
- **ISO 31: Certificate Content and Structure**
- **Certificates**
  - **Certified reference materials (CRMs)**
  - **Primary pharmaceutical reference standards**
  - **Secondary pharmaceutical reference standards**
  - **Impurity reference standards**
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

CofA: Impurity RS

CofA: Impurity RS



CofA: Impurity RS



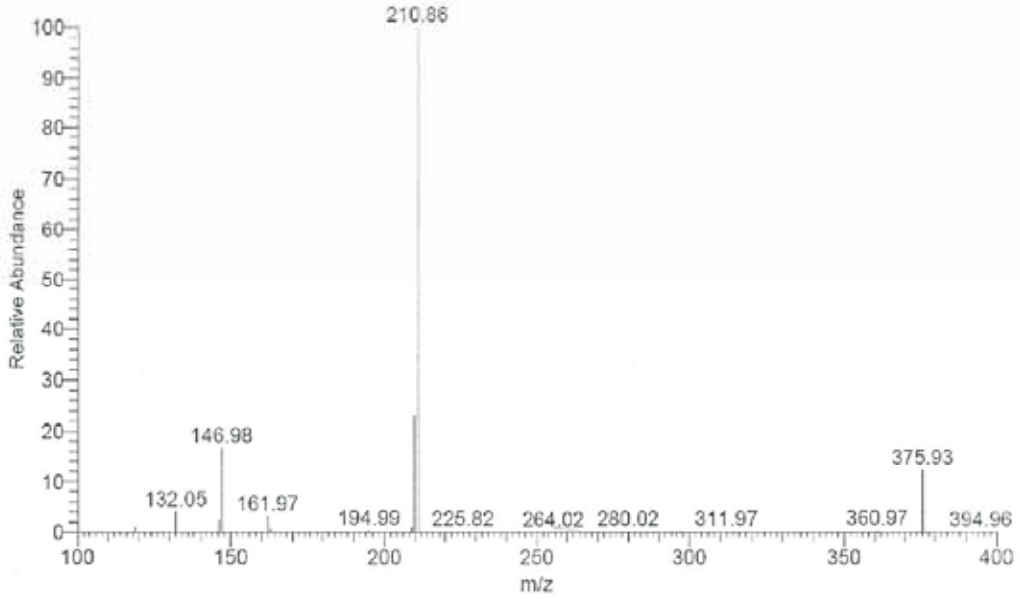
CofA: Impurity RS

LGC Standards
Excellence through measurement

CERTIFICATE

Ib. Mass Spectrum

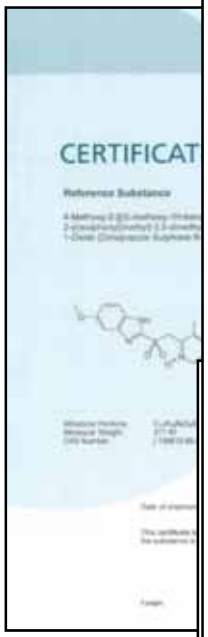
Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet



m/z	fragments
376	[M – H]
211	[$C_8H_7N_2O_3S$]
147	[$C_8H_7N_2O$]

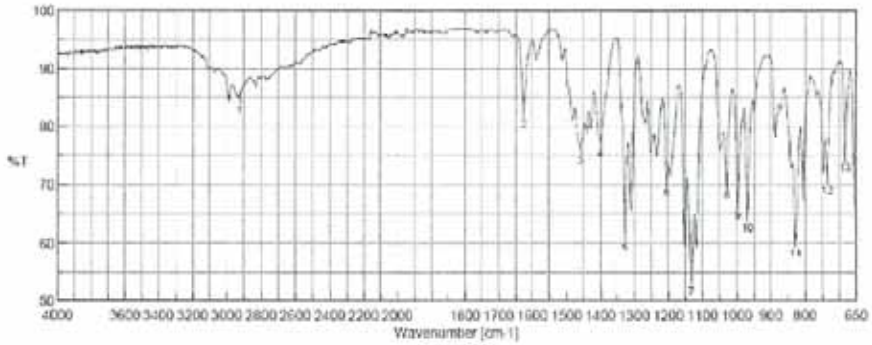
The signals of the mass spectrum and their interpretation are consistent with the structural formula.

Co



Ic. IR Spectrum

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



Result of Peak Picking

No.	Position	Intensity
1	2925.48	64.8026
2	1824.73	82.8126
3	1458.00	76.1453
4	1400.07	77.3327
5	1326.79	61.9716
6	1204.33	70.5593
7	1132.01	53.7540
8	1028.84	70.1759
9	936.053	66.711
10	869.056	64.5746
11	828.277	60.3426
12	735.71	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).

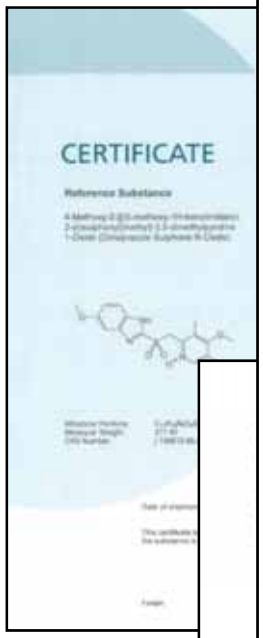
HPLC Conditions:

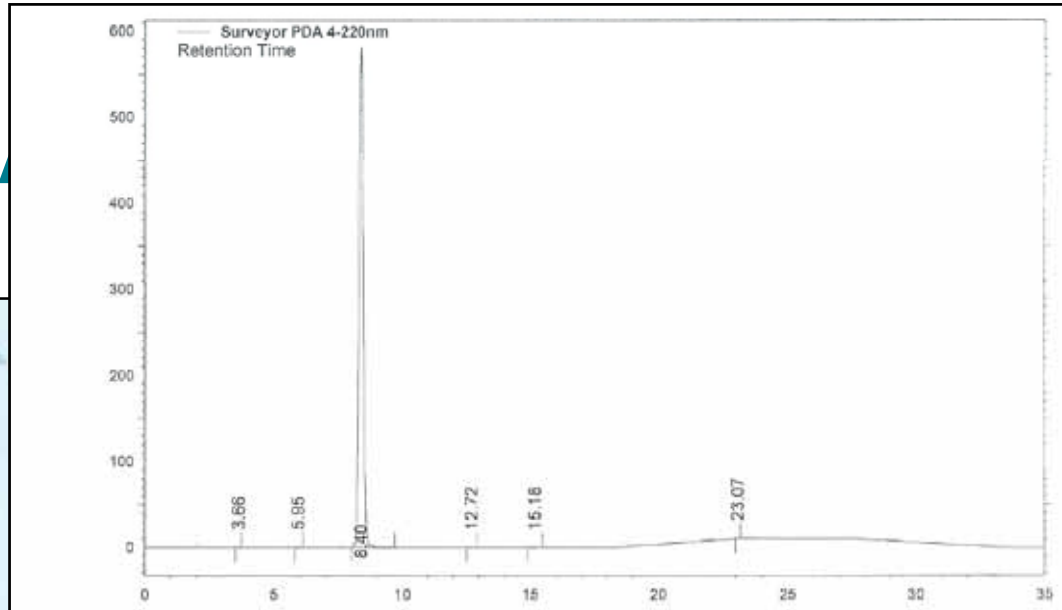
Column:	Conditions:	Detector:	Injector:
Hypersil Gold (C18)	1.0 ml/min, 40 °C	DAD	Auto
5 µm, 150 x 4.6 mm	0 – 15 min Water/Acetonitrile 80/20 15 – 20 min Water/Acetonitrile to 60/40 20 – 25 min Water/Acetonitrile 60/40 25 – 30 min Water/Acetonitrile to 80/20 30 – 35 min Water/Acetonitrile 80/20 (v/v); 0.1 % H_3PO_4	220 nm	5 µl; 0.0654 mg/ml in Water/Acetonitrile 50/50 (v/v)

Cof

LGC Standards

Excellence through measurement





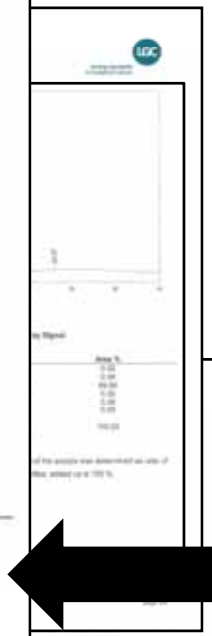
Area Percent Report - Sorted by Signal

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: ^{1}H-NMR

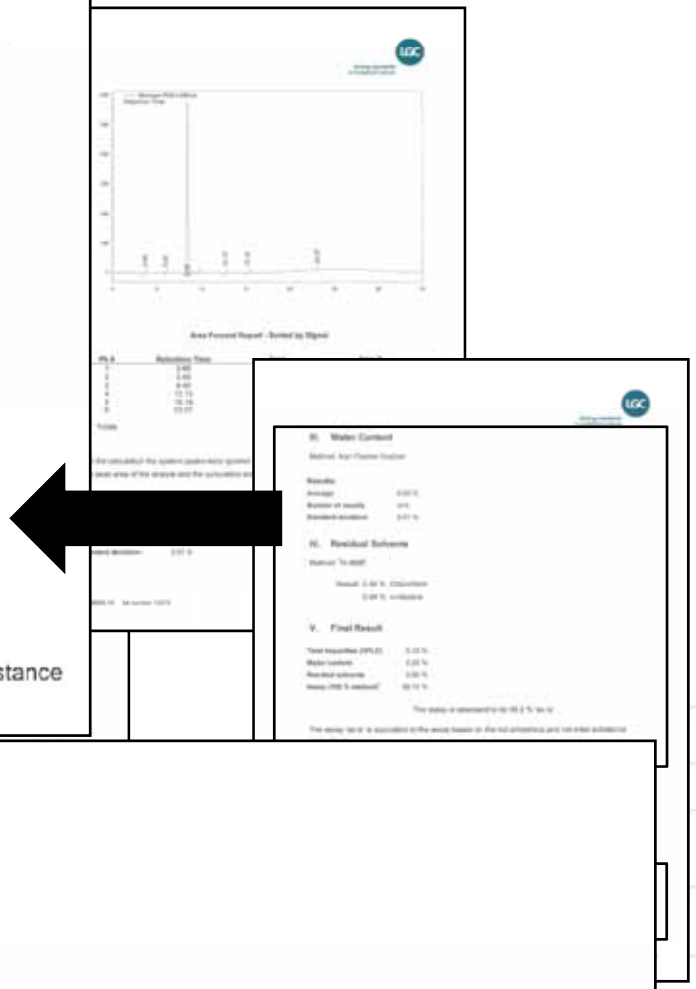
Result: 0.46 % Chloroform
0.04 % *n*-Hexane

V. Final Result

Total impurities (HPLC)	0.15 %
Water content	0.20 %
Residual solvents	0.50 %
Assay (100 % method)[1]	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.



[1] 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.

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: Research material

LGC Standards
Excellence through measurement

1. Identification

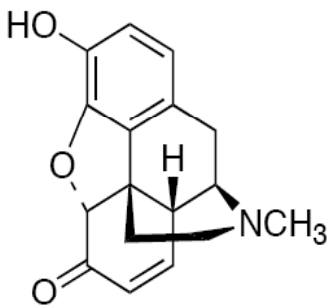
CAS Number:
467-02-7

Catalogue Number:

Product:
Morphinone

Synonyms:
(5α)-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

LGC Standards
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:
^{1}H NMR (CD_3OD) 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_2; Dichloromethane : Methanol : Ammonium Hydroxide = 9 : 1 : 0.05; Visualized with UV and AMCS; Single spot, Rf=0.6.
^{1}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)**
  - **Primary pharmaceutical reference standards**
  - **Secondary pharmaceutical reference standards**
  - **Impurity reference standards**
  - **Research materials**
- **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