

New Certified Reference Materials for Chromatography

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sigma-aldrich.com/analytical

Use of Certified Reference Materials (CRMs)

Quality Assurance

- Ensuring Accuracy of the Mean and Precision
- Ensuring an Analytical Method provides correct Results

Traceability of results to acknowledged standards

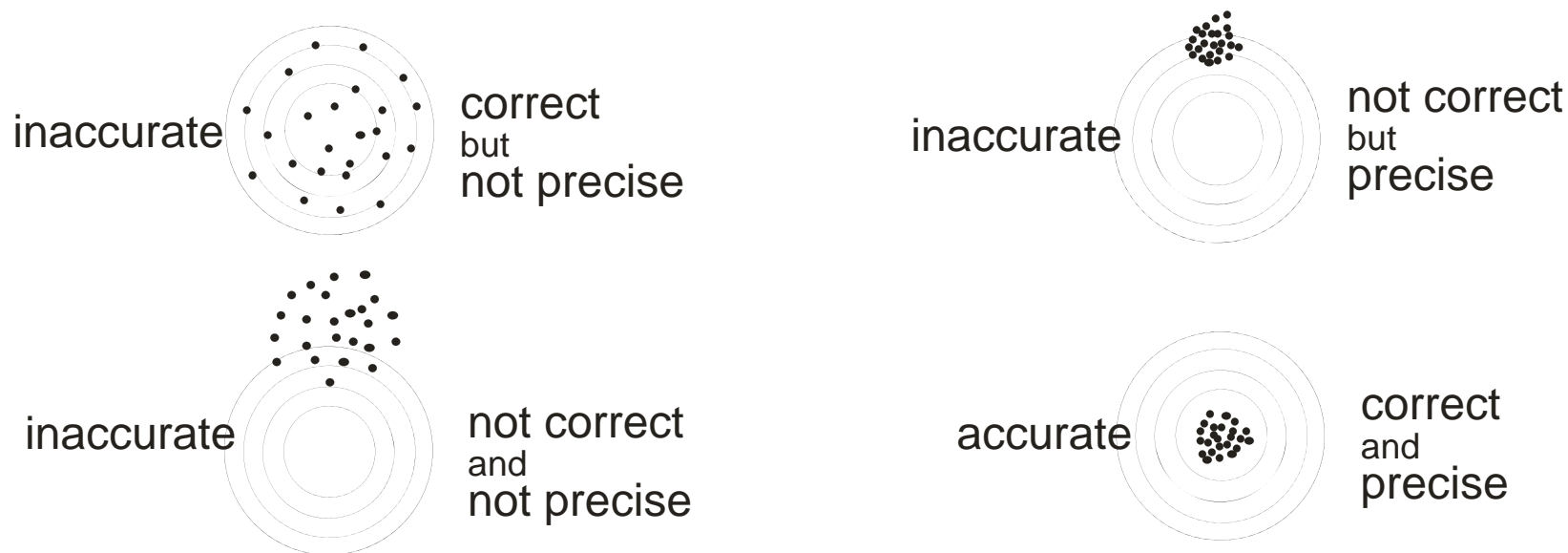
Calibration

Validation of Analytical Methods

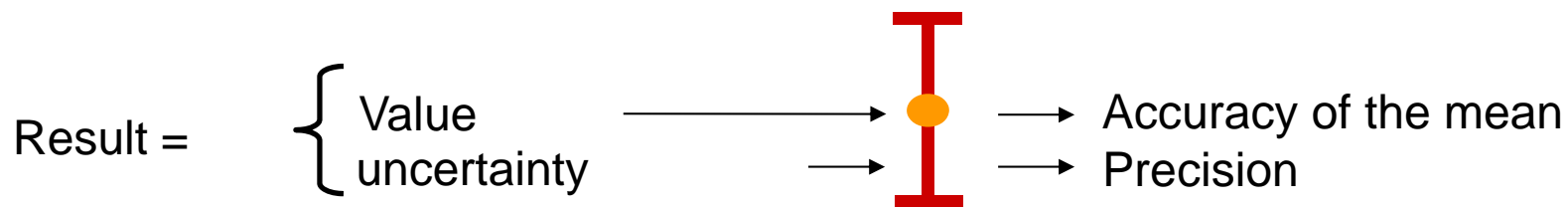
Interlaboratory Trials

ISO 17025 accredited labs have to use CRMs on regular base

Accuracy, Accuracy of the Mean & Precision



Accuracy = accuracy of the mean + precision



Accuracy of the mean: systematic errors
 Precision: accidental errors (by random)
 Accuracy: overall deviation

Definition „Certified Reference Material“

Reference material, accompanied by a **certificate**, one or more of whose **property values are certified**, by a **procedure** which **establishes its traceability** to an accurate realization of the unit in which the property values are expressed, and for which each certified value is **accompanied by an uncertainty** to a **stated level of confidence**.

VIM:1995, ISO guide 30/31

National Metrological Institutes (NMIs)

Providing acknowledged standards, examples for NMIs are:

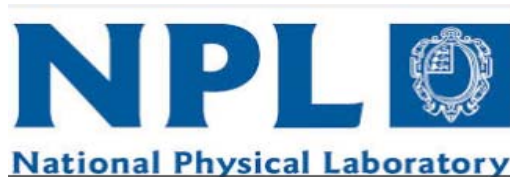
- USA: National Institute of Standards and Technology (NIST)



- Germany: Federal Institute for Materials Research and Testing (BAM)



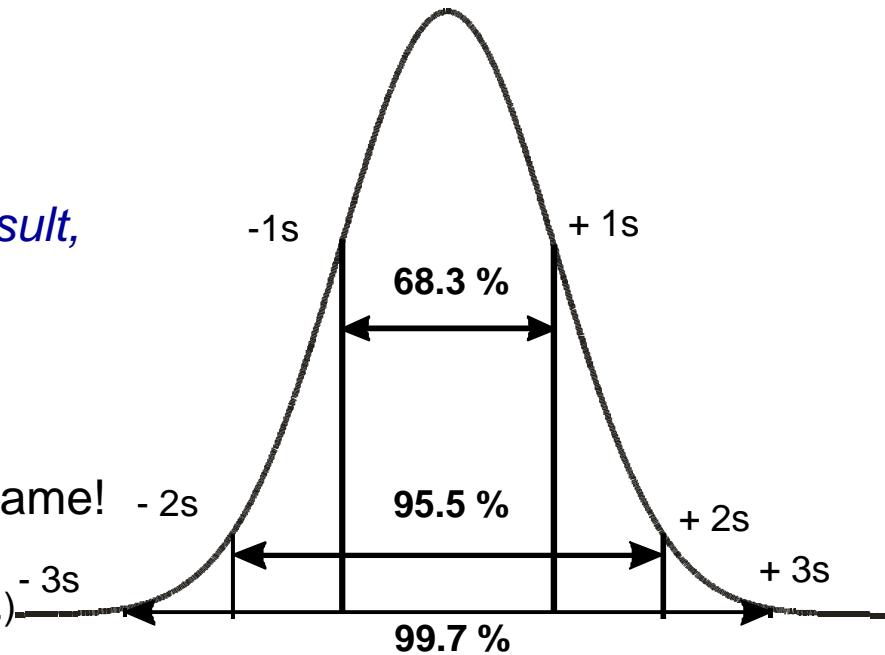
- UK National Physics Laboratory



Uncertainty

Definition: *part of the measurement/result, describes the variation of the values*

Mistake and Uncertainty are not the same!
Mistake = can not be determined
(dictionary: mistake, oversight, accidental slip, ...)



Typical uncertainties in chemical analysis:

Simple sample, metrological level:	0.05-0.4%
Simple sample (no Matrix), normaler level	0.5-1.5%
Analysis in Matrix	2-50%

Problem: *Users prefer low uncertainties*

Consequence is: many CRM manufacturers calculate (or estimate) the uncertainty too optimistic (ignoring relevant shares).

Traceability

ISO 17025 Section 5.6: Measurement traceability 5.6.3 Reference standards and reference materials

Definition: property of a value to be related by an unbroken chain of comparison measurements to a reasonable normative.

(VIM 6:10 – ref: BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML, International Vocabulary of Basic and Metrological Terms in Metrology, 3rd edition, ISO, Geneva, 1993)

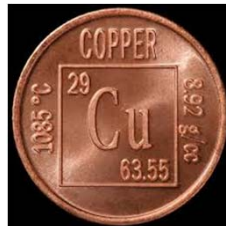
The uncertainty of comparison measurement $Y_3 > Y_2 > Y_1$ all uncertainties of all comparison measurements are greater than the uncertainty of the reference.

The length of this “traceability chain” is not limited, but: the uncertainty is increasing!

$X_3 \% \text{ Cu} \pm Y_3 \%$



$X_2 \% \text{ Cu} \pm Y_2 \%$



$X \% \text{ Cu} \pm Y \%$



ISO 17025 + ISO Guide 34 Accreditation

ISO 17025: „General Requirements for the Competence of **Calibration and Testing Laboratories**“

- Many labs do have 17025 Accreditation
- Important: Traceability, uncertainty, education, infrastructure
- Accreditation is bound to a lab and scope

ISO Guide 34: „General Requirements for the Competence of **Reference Material Producers**“

- Only few labs do have ISO Guide 34 Accreditation
- Important: Traceability, uncertainty, homogeneity, stability, shelf life
- Accreditation is bound to a lab and scope

ISO 34 + 17025: “Gold Standard“ Accreditation for **Reference Material manufacturers**

- Only very few labs do have both
- Acknowledgement of competence and reliability

Produced in double accredited
laboratory fulfilling
**ISO/IEC 17025 and
ISO Guide 34**

ISO/IEC 17025



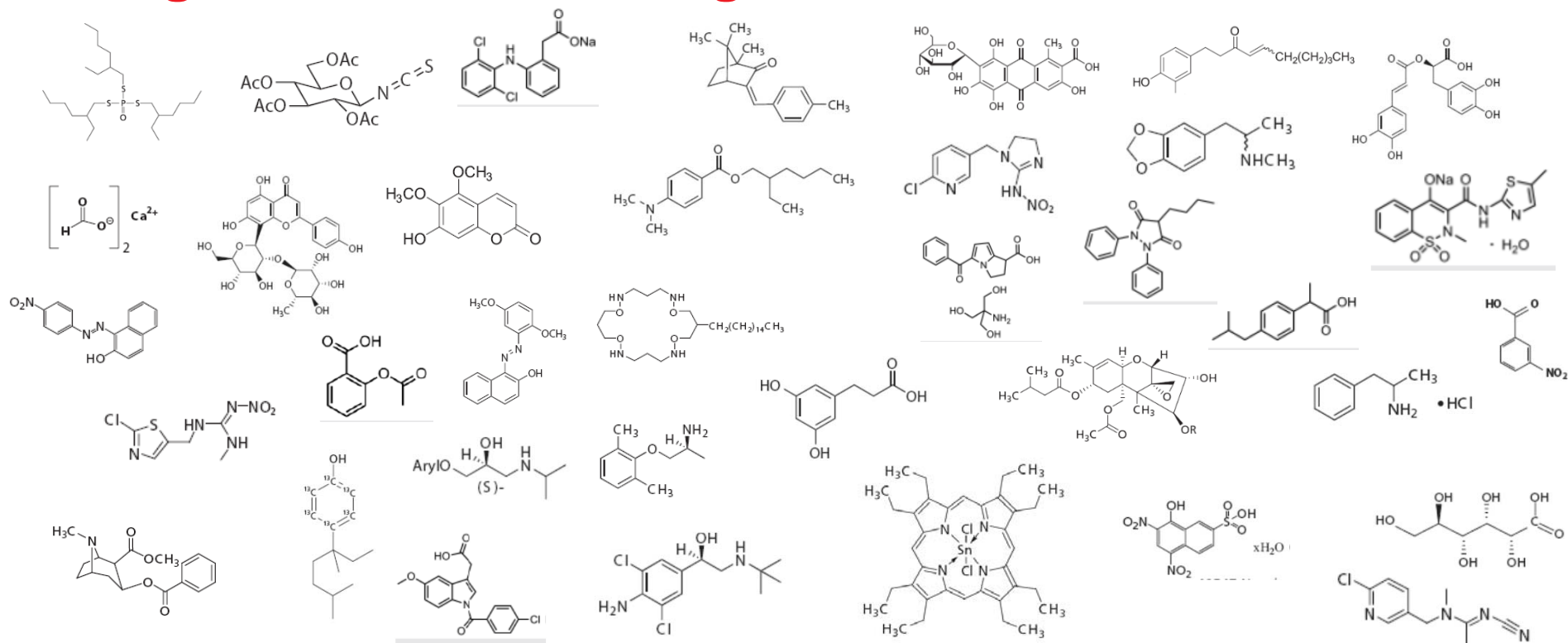
STS 490

ISO Guide 34 und
ISO/IEC 17025



SRMS 001

Organic CRMs: Challenges



- Numberless organic compounds
- Only few international standards available
- **Traceability?**

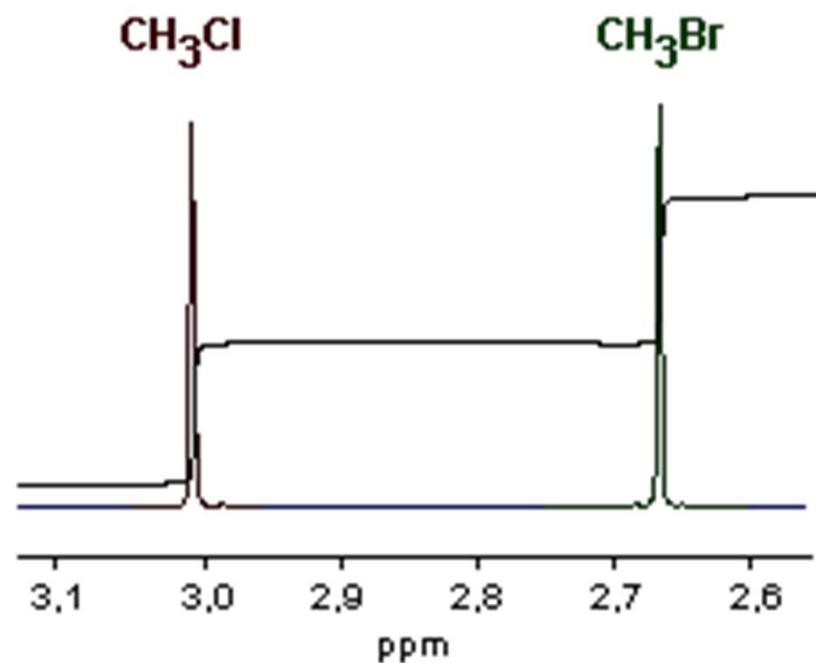
Alternative approach: qNMR

Quantitative NMR (qNMR) is a Relative Primary Method

Signal intensity is:

- proportional to the number of protons
- independent of chemical structure (chemical shift is impacted)

NMR spectrum of an equimolar mixture of CH₃Cl and CH₃Br



Advantages of Quantitative NMR

Traceability independent of chemical identity

Low Uncertainties

- Expanded uncertainties between 0.1% and 0.5%

Non-destructive

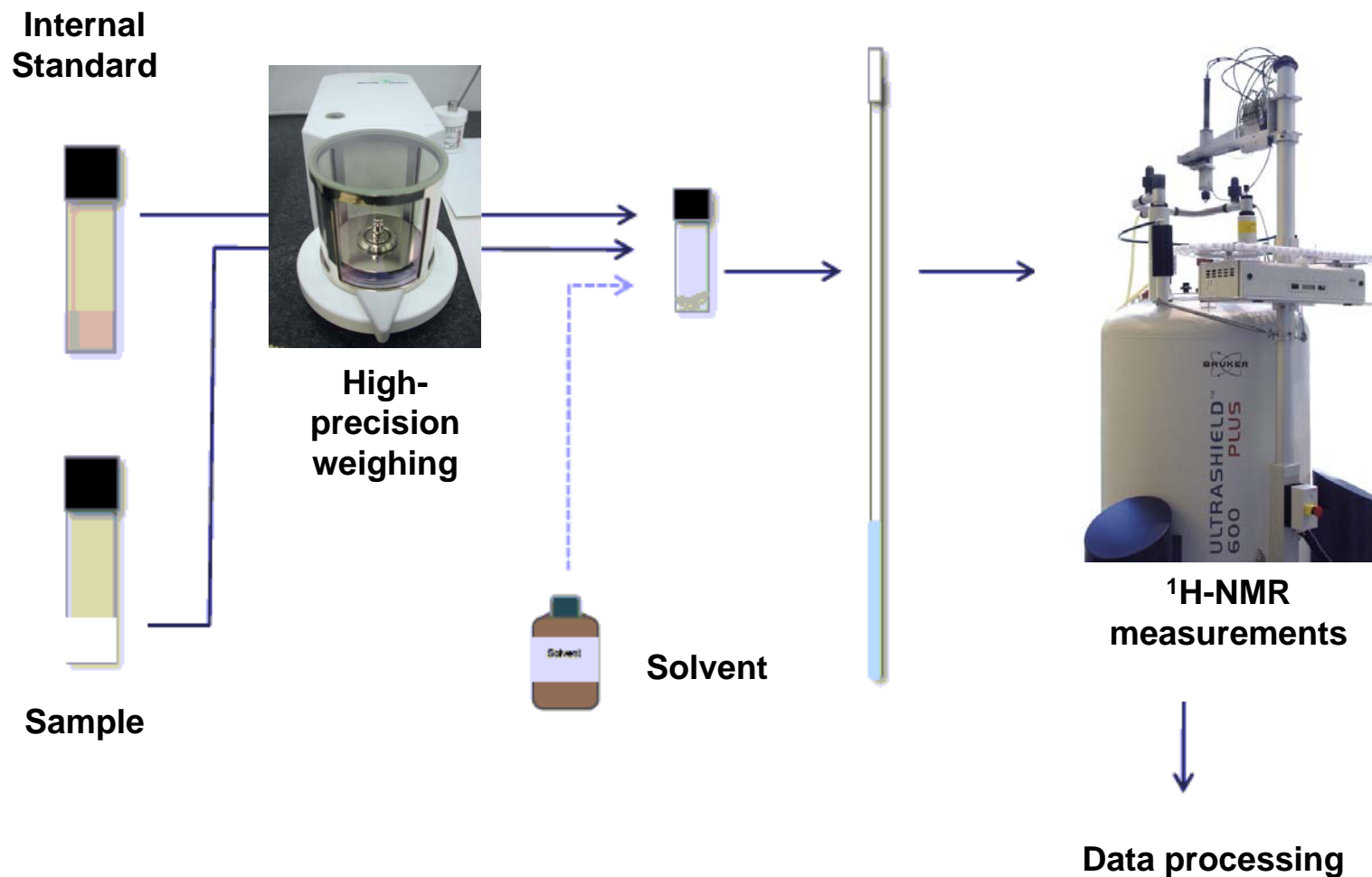
Structural information on target compound

Information on impurities

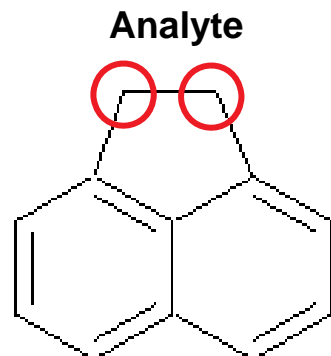
Jancke H. NMR als primäre Methode. Nachrichten aus Chemie, Technik und Laboratorium, 1998, 46, 722

Jancke, H. NMR Spectroscopy as a Primary Analytical Method, Document 98/02 to the 4th Session of the CCQM, Sèvres 1998

Quantitative NMR (qNMR): Procedure



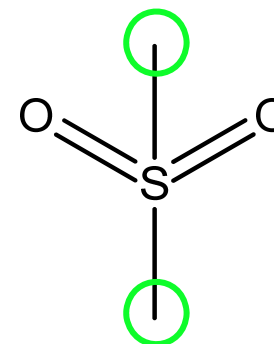
Content Assignment by qNMR



Acenaphthene

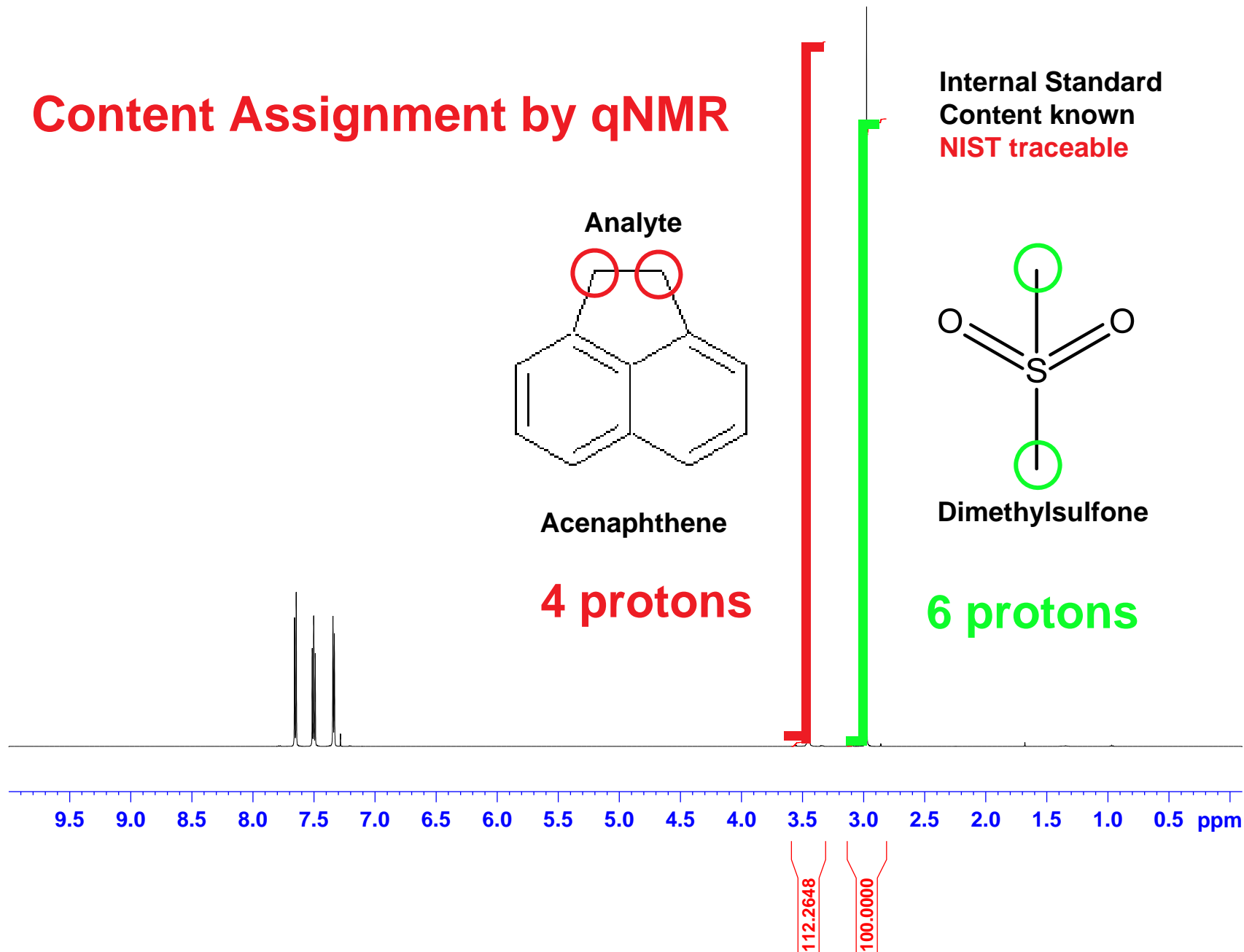
4 protons

Internal Standard
Content known
NIST traceable



Dimethylsulfoxide

6 protons



Content Assignment by qNMR

Internal standard and the reference standard are weighed together into one NMR tube (with solvent)

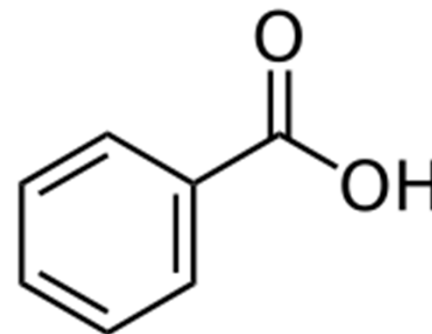
Intensities of appropriate signals of the reference standard (I_x) and the internal standard (I_{Std}) are used for calculation

Content P_x can be calculated by the following equation:

$$P_x = \frac{I_x}{I_{Std}} \cdot \frac{N_{Std}}{N_x} \cdot \frac{M_x}{M_{Std}} \cdot \frac{m_{Std}}{m_x} P_{Std}$$

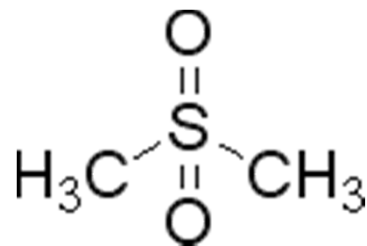
- Number of nuclei contributing to the resonance (N_x , N_{Std})
- Molar masses (M_x , M_{Std})
- Initial weight (m_x , m_{Std}) and purity of internal standard (P_{Std})

Traceability to NIST Standard Reference Materials



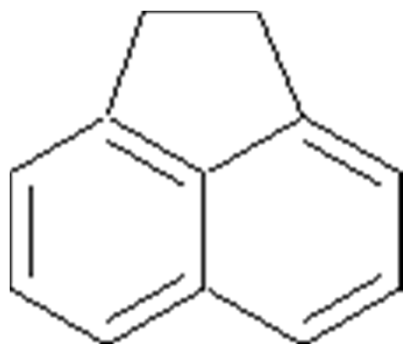
Benzoic acid
NIST SRM

qNMR



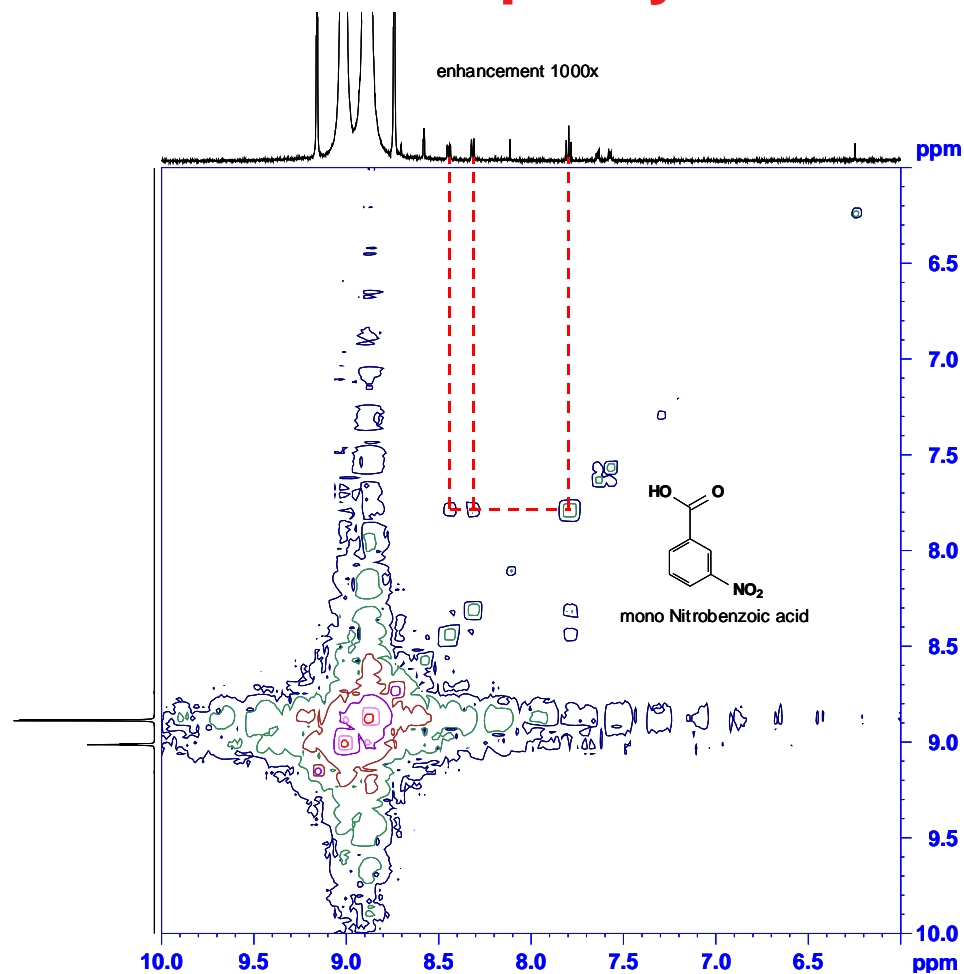
Dimethylsulfone (IS)
Fluka 41837

qNMR



Acenaphthene
Fluka 05426

Signal allocation and purity: 2D-NMR: H-H COSY



2D-COSY of 3,5-dinitrobenzoic acid. Signals of impurity 3-nitrobenzoic acid detectable. Easy detection at concentration below 0,1%

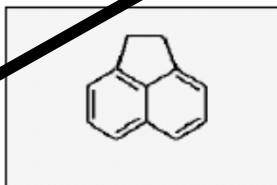
Traceability ^[2]: NIST SRM 350b (Benzoic acid)

Certificate

Produced by double accreditation
laboratory fulfilling
ISO/IEC 17025 and
ISO Guide 34

This certificate is designed in accordance with ISO Guide 31 ^[1].

Substance: **Acenaphthene**
Product no.: 05426
Lot no.: BCB01028V
Formula: C₁₂H₁₀
Molecular mass: 154.21
Traceability ^[2]: NIST SRM 350b (Benzoic acid)
Certificate issue date: October 19, 2010
Expiry: Oct/2012



Certified value and uncertainty according to ISO Guide 35 ^[3] and Eurachem/CITAC Guide ^[4]		
Substance	Certified value	Expanded uncertainty $U = k \cdot u_c$
Acenaphthene	98.5 % (g/g)	0.3 % (g/g)

Minimum sample: 50 mg is recommended as the minimal sample amount. If less material is used, it is recommended to increase the certified uncertainty by a factor of two for half of sample and a factor of four for one fourth mg of sample.

Drying instruction: This material does not require drying before use.

Intended use: Use this CRM as calibrant for chromatography or any other analytical technique.

Storage and handling: The CRM should be stored in the original bottle at room-temperature (20-25°C). After use the bottle should be tightly closed and protected from excessive moisture and light.

CRM operations: *A. Rück*
A. Rück, Ph.D.

Certification body: *Klaus Dieter Schmitt*
K.-D. Schmitt, Ph.D.



SRMS 001
ISO Guide 34



STS 490
ISO/IEC 17025



16368-02
ISO 9001

Certified value

98.5 % (g/g)

Expanded uncertainty, $U = k \cdot u_c$ ($k = 2$)

0.3 % (g/g)

Organic *Trace*CERT: CRMs for Chromatography

Acids and Esters

Aldehydes and Aldehyde Derivatives

Amines and Amides (e.g. acryl amide)

Amino Acids

Antibiotics / Drugs (including veterinary drugs e.g. antibiotics)

FA / FAME

Natural Products

Pesticides

Phthalates (plasticizers)

Polyaromatic Hydrocarbons (PAHs)

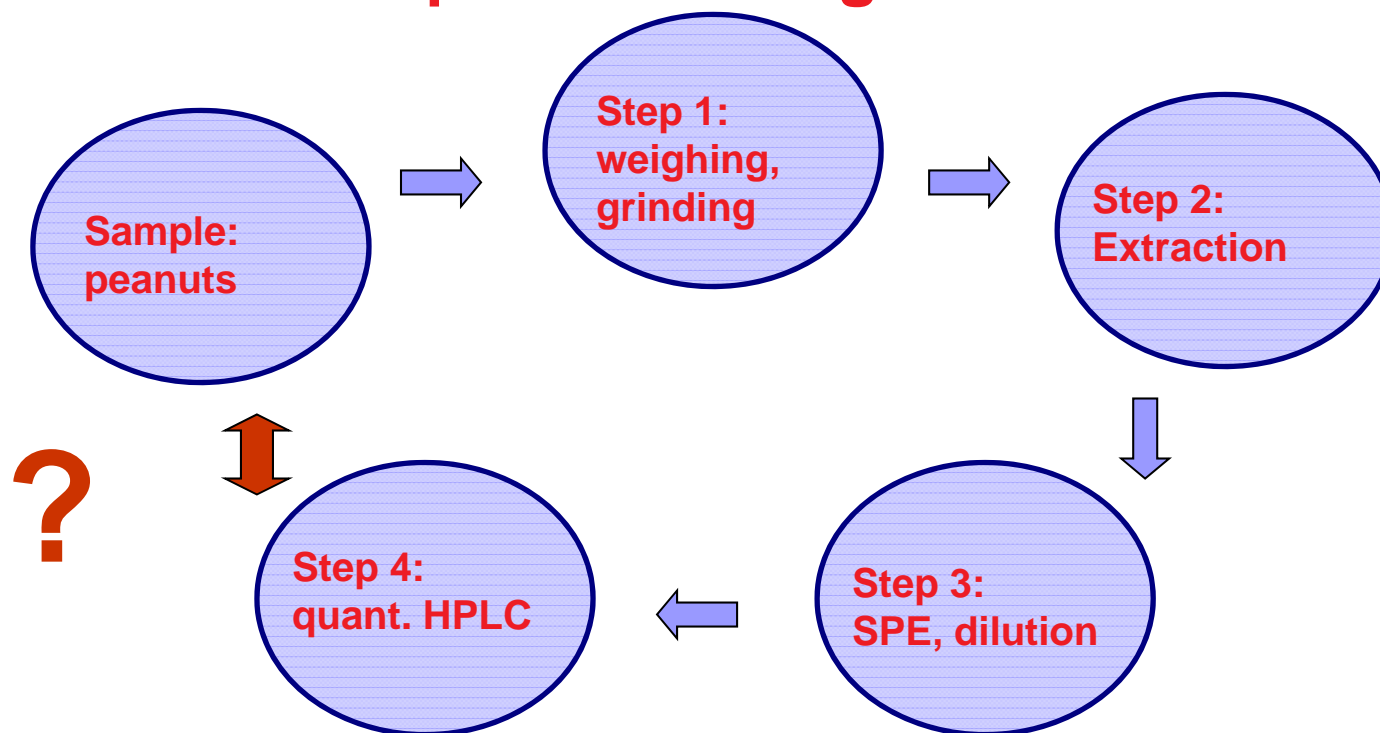
SVHC (semi-volatile hydrocarbons)

Difference Matrix CRM ↔ Pure Substance CRM

Matrix CRM ⇒ certified impurities

Pure Substance CRM ⇒ certified content

Aflatoxin B1 in peanuts: usage of CRMs



Non-problematic:
Problematic:

Weighing, dilution, quant. HPLC
Grinding, Extraction, SPE

How to connect the HPLC result with the whole analytical method?
Neat compound CRM (Aflatoxin B1) only for the verification of the accuracy of the mean of step 4 (ONLY of step 4)

Usage of Matrix CRMs



Spiking with labelled Standard: assumption, that the labelled substance behaves like a non-labelled and that interaction with matrix is the same.

Matrix CRM: shows absence of interferences, loss of the sample, systematic errors. Shows completeness of extraction & purification.

BCR-264 Defatted Peanut Meal Aflatoxin B1, high level

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European Reference Materials

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Flash News

2006
Certified reference materials for detection of potato event EH92-527-1

June 29, 2006

ERM® Partners

irm
 Institute for Reference Materials and Measurements

Welcome to the European Reference Materials site

Three major European reference materials producers have combined forces to produce a brand new standard in reference materials to ensure reliability and comparability of the results of chemical analysis.

European Reference Materials are certified materials, which undergo uncompromising peer evaluation and offer highest quality and reliability.

They are a major tool for improving the confidence in, and the mutual recognition of test results and certificates in a global market. CRMs comply with high metrological requirements, ensuring traceability of measurements results, and are the end-point of the traceability chain, thus being primary standards in chemistry.

**European Reference Materials:
 a new brand for Certified Reference Materials**

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COMMUNITY BUREAU OF REFERENCE - BCR

CERTIFIED REFERENCE MATERIAL
BCR-264

CERTIFICATE OF ANALYSIS

AFLATOXIN B ₁ IN DEFATTED PEANUT MEAL		
Mass fraction Aflatoxin B ₁ ¹⁾		Number of accepted sets of data p
Certified value ²⁾	Uncertainty ³⁾	
206 µg/kg	13 µg/kg	11
1) Expressed on material as supplied. 2) This value is the unweighted mean of p accepted mean values, independently obtained by p sets of results. 3) The uncertainty is taken as the half width of the 95 % confidence interval of the mean defined in (2). It does not include any uncertainty on the value for the molar absorption coefficient of aflatoxin B ₁ in chloroform which has been adopted for this certification (see § 7.2 of the report).		
When the reference material is used to assess the performance of a method, the user should refer to the recommendations laid down in the last chapter (instructions for use) of the certification report.		

DESCRIPTION OF THE MATERIAL

The material is a finely ground defatted peanut meal. It is supplied in units of about 150 g in sachets sealed under vacuum.

The minimum recommended sample intake is 20 g.

INSTRUCTIONS FOR USE

Samples should be stored unopened at - 18 °C. To avoid water condensation they should be allowed to warm to room temperature (e.g. overnight) before opening. The contents should be thoroughly mixed before sub-samples are taken. After opening, the material should be used on the same day.

Brussels, June 1993

 BCR
 for certified true copy

PARTICIPATING LABORATORIES

- Institut für Angewandte Botanik, Hamburg (D)
- Istituto Superiore di Sanità, Roma (I)
- Leatherhead Food R.A., Leatherhead, Surrey (UK)
- LUFA, Kiel (D)
- R.I.V.M., Bilthoven (NL)
- RHM Research and Engineering Ltd., High Wycombe (UK)
- RIKILT, Wageningen (NL)
- Royal Veterinary and Agricultural University, Copenhagen (DK)
- State Laboratory, Dublin (IRL)
- Technische Universität München, Bayerische Hauptversuchsanstalt für Landwirtschaft, Freising (D)
- TNO Biotechnology and Chemistry Institute, Zeist (NL)

ANALYTICAL METHODS USED

The methods used for certification involved instrumental determination by high performance liquid chromatography using a variety of separating and detection conditions and two-dimensional TLC. The methods also varied in their initial extraction and clean-up procedures. Details of the methods used are given in the certification report.

LEGAL NOTICE

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NOTE

A detailed technical report on the analysis procedure and the treatment of the analytical data is supplied with each sample.

Certificate

Commission of the European Communities
Community Bureau of Reference

bcr information

REFERENCE MATERIALS

THE CERTIFICATION OF THE AFLATOXIN B₁ MASS FRACTION OF THREE PEANUT MEAL AND TWO COMPOUND FEED REFERENCE MATERIALS

(CRMs 262, 263, 264, 375 & 376)

H.P. VAN EGMOND⁽¹⁾, G. WOOD⁽²⁾, S. PATEL⁽³⁾, L. TUINSTR⁽⁴⁾,
A. BOENKE⁽⁵⁾, B. SCHURER⁽⁶⁾, P.J. WAGSTAFFE⁽⁷⁾

⁽¹⁾ RIVM
Bilthoven
The Netherlands

⁽²⁾ Leatherhead Food Research Association
Leatherhead
United Kingdom

⁽³⁾ RHM Research and Engineering Ltd
High Wycombe
Bucks
United Kingdom

⁽⁴⁾ RIKILT
Wageningen
The Netherlands

⁽⁵⁾ Commission of the European Communities
Community Bureau of Reference
200 rue de la Loi
B-1049 Brussels

Contract Nos 5394/1/5/227/90/9-BCR-NL (10) & 2721/1/5/227/87/5-BCR-NL (10)

FINAL REPORT

Directorate-General
Science, Research and Development

1994

EUR 15286 EN

Certification Report

Length: here 90 pages

Index of content: 4 pages

Content: feasibility study in terms of homogeneity, stability, results, statistical evaluation, Certification ...

Why: the whole process has got to be traceable

Matrix CRMs for Environmental Analysis

RTC is world leader in the production and certification of soil and sediment CRMs

Cooperation with US EPA

Available CRMs:

- Soil Matrix CRMs (Metals/Inorganic and Organic & Hydrocarbon)
- Soil, Sludge and Sediment Matrix CRMs - Trace Element
- Underground Storage Tank Hydrocarbon Calibration CRMs
- Water Pollution Matrix (Metals/Inorganic and Organic & Hydrocarbon)
- And many more ...

Matrix CRMs for Environmental Analysis

Water CRMs

Matrix	Metals and Inorganics	Organics	Physical Properties
Water Supply (WS)	>35 products	>28 products	>18 products
Water Pollution (WP)	>29 products	>48 products	>20 products
Sea water	>3 products	>3 products	>5 products

Soil CRMs (Generic soils with known levels of analytes)

	Metals and Inorganics	Organics	Physical Properties
Soil / Solid waste	>13 products	>49 products	>7 products


Matrix CRMs (Soil with known sand, silt, clay and organic matter composition, and analytes)

	Metals and Inorganics	Organics	Physical Properties
Sandy Clay	✓	✓	✓
Loamy Sand	✓	✓	✓
Sandy Loam	✓	✓	✓
Sand	✓	✓	✓
Clay	✓	✓	✓
Sediment	✓	✓	—
Soil	✓	✓	—
Sandy Clay Loam	—	✓	—
Silty Clay	✓	✓	—
Loamy Clay	✓	✓	—
Clay Loam	✓	✓	—
Silty Loam	✓	✓	—
Loam	✓	✓	—
Clean Soil	✓	✓	✓
Other Matrices*	✓	✓	—

*Other matrices include Fly Ash, (sewage) Sludge, Dusts and Paints. NB Paints are dry powders, and primarily certified for lead

Matrix CRMs for Environmental Analysis - CoA

Certificate of Analysis
CERTIFIED REFERENCE MATERIAL

Dioxins/Furans - Clay Loam
Number: CRMS81-10G
Lot: 019661
Solvent (Matrix): Clay Loam
Hazard: Irritant
Storage & Handling: Store at room temperature.
Expiration Date: See Sample Label
Certification Date: December 04, 2012
Certified By:  Christopher Rucinski - QA Director

Analyte	Units	Certified ^{1,4} Value	k ²	Stand. Deviat
1,2,3,4,6,7,8-Hpocd	pg/g	170 ± 6.82	2.05	15.3
1,2,3,4,7,8,9-Hpocdf	pg/g	355 ± 10.7	2.05	23.8
1,2,3,4,6,7,8-Hpocdd	pg/g	670 ± 31.2	2.05	72.7
Total Heptachlorodibenzo-p-dioxin (Total HPCDD)	pg/g	672 ± 32.0	2.05	72.9
Total Heptachlorodibenzofuran (Total HPCDF)	pg/g	526 ± 15.7	2.05	34.0
1,2,3,4,7,8-Hxocdd	pg/g	51.7 ± 2.35	2.05	5.47
1,2,3,6,7,8-Hxocdd	pg/g	509 ± 25.4	2.05	59.4
1,2,3,7,8,9-Hxocdd	pg/g	846 ± 46.3	2.05	109
Hxocdd, total	pg/g	1,390 ± 69.7	2.05	159
1,2,3,4,7,8-Hxocdf	pg/g	719 ± 32.0	2.05	74.2
1,2,3,6,7,8-Hxocdf	pg/g	682 ± 32.7	2.05	76.3
1,2,3,7,8,9-Hxocdf	pg/g	302 ± 11.3	2.05	25.9
2,3,4,6,7,8-Hxocdf	pg/g	564 ± 23.3	2.05	53.8
Total Hexachlorodibenzofuran (Total HxCDF)	pg/g	2,260 ± 92.0	2.05	207
1,2,3,4,6,7,8,9-OCDF	pg/g	190 ± 9.84	2.05	22.9
1,2,3,4,6,7,8,9-OCDD	pg/g	516 ± 29.3	2.05	69.3
1,2,3,7,8-Peocdd	pg/g	880 ± 30.3	2.05	89.3
1,2,3,7,8-Peocdf	pg/g	794 ± 30.8	2.05	70.7
2,3,4,7,8-Peocdf	pg/g	700 ± 24.5	2.05	55.9
Peocdf, total	pg/g	1,510 ± 47.9	2.05	108
Peocdd, total	pg/g	878 ± 31.6	2.05	71.1
TCDD, total	pg/g	108 ± 4.30	2.05	9.80

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Additional Information



Description
The sample size provided is 10 g of soil. The soil has been sterilized to minimize degradation of the sample. The sample has been sized to 100 mesh. Recommended storage condition is 4°C. The sample has been intentionally prepared with an apparent headspace.

Storage
The sample should be stored at 4°C. It has been determined to be stable for the duration of the shelf life of the product. After sub-sampling replace cap securely and store remaining sample at 4°C. The shelf life of the product was determined by historic stability of similar CRMs. The expiry popularity upon successful stability testing by a 17025 accredited laboratory. Stability and shelf life after opening must be determined by the user, taking into account as follows:

Preparation Instructions
Mix well prior to use.
For Method 8280A, an eight gram sample size contains sufficient concentrations of analyte quantitative limits will be realized.
For Method 8290, a one gram sample will contain concentrations of analytes such that levels realized. This holds true though levels of detection will be elevated owing to the reduced sample size.


Scope and Application
The Dioxins and Furans in Soil Certified Reference Material (CRM) consists of a single poly of soil, fortified with dioxins and furans. Being a natural matrix waste sample the analyte is representative, etc. as is typical for similar matrices received by the laboratory for analysis. Analytes that are listed on the enclosed Certificate of Analysis. The sample has been analyzed round-robin to meet the requirements specified by the ISO Guides 34 and 35, and ISO 17025.

Evaluation of Results
The Reference Value, 95% confidence interval (CI) for the Reference Value and 95% PI are obtained by the methods identified in the 'Scope and Application' section of this Certificate from the beginning to the end of the bottling sequence and sent for analysis by an independent round-robin was used to calculate reference values by the USEPA EMSL-CINN's computer. The generated BIWEIGHT mean, BIWEIGHT standard deviation and BIWEIGHT standard Confidence Interval (CI) for the mean and the 95% Prediction Interval (PI). For normally distributed data the classical calculation method used to generate a 95% CI. For non-Gaussian data sets, BIWEIGHT data are also used to calculate a 95% PI. The 95% PI compares well to a 95% normally distributed data, the BIWEIGHT 95% PI typically represents approximately a ±2 BIWEIGHT mean. Again, the BIWEIGHT method is more robust than classical methods of Laboratories performing the same analytical procedures on a sample whose values have 1 that the true mean, as determined by the method, is within the 95% CI window. Laboratories 95% PI window 19 out of 20 analyses. Laboratories should use the PI as guidance for lab Additional information on the program may be obtained by referring to the reference or by Additionally contact RTC for additional guidance - 1(307)742-5452 - support@rti-corp.com

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Certificate of Analysis
CERTIFIED REFERENCE MATERIAL

Dioxins/Furans - Clay Loam
Number: CRMS81-10G
Lot: 019661
Solvent (Matrix): Clay Loam
Hazard: Irritant
Storage & Handling: Store at room temperature.
Expiration Date: See Sample Label
Certification Date: December 04, 2012
Certified By:  Christopher Rucinski - QA Director

Health and Safety Information
All RTC Certified Reference Materials are intended only for professional use by properly trained laboratory personnel. This CRM has been reviewed for both health and safety and shipping risks. It is classified as non hazardous and is not classified as hazardous goods for shipping by road, sea or air transport.
A full International MSDS as a downloadable pdf file is available at www.rti-corp.com

1 Certified values are the robust statistical mean when prepared according to instructions from an interlaboratory study and internal rigorous testing.
2 The standard deviation is the robust statistical standard deviation from the round robin interlaboratory study.
3 Expanded Uncertainty (U_{exp}) - All uncertainty values in this document expressed as ± value are expanded uncertainties.
4 k: Coverage factor derived from a t-distribution table, based on the degrees of freedom of the data set. Confidence Interval = 95%
5 TRACEABILITY: The standard was manufactured under an ISO 17025 certified quality system. The balance used to weigh raw materials is accurate to ±0.0001g and calibrated regularly using mass standards traceable to NIST. All dilutions were performed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.

HOMOGENEITY ASSESSMENT: Between-bottle homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled over the course of the bottling operation. Samples were taken in the following manner: the units produced in the bottling operation were divided into three chronological groups, those from the Early third, the Middle third, and the Late third (Groups). A pre-determined number of sample units were then randomly selected from each group. A subset of each group was then randomly selected for chemical analysis. The results of the chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA).

UNCERTAINTY STATEMENT: Uncertainty values in this document are expressed as Expanded Uncertainty (U_{exp}) corresponding to the 95% confidence interval. U_{exp} is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a t-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

THIS PRODUCT WAS DESIGNED, PRODUCED AND VERIFIED FOR ACCURACY AND STABILITY IN ACCORDANCE WITH ISO 17025 (ACIass Cert AR-1467) and ISO GUIDE 34 (ACIass Cert AR-1470).
MSDS reports for components comprising greater than 1.0% of the solution or 0.1% for components known to be carcinogens are available upon request.
Manufactured and certified by Sigma-Aldrich RTC, Inc.

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Matrix CRMs for Environmental Analysis - CoA

Certified Value with Uncertainty and Confidence Interval

Additional Information

- Description
- Storage
- Preparation Instructions
- Scope and Application
- Evaluation of Results

Traceability

Homogeneity Assessment

Uncertainty Assessment

Summary

New Method for Content Assignment of Organic Compounds by qNMR

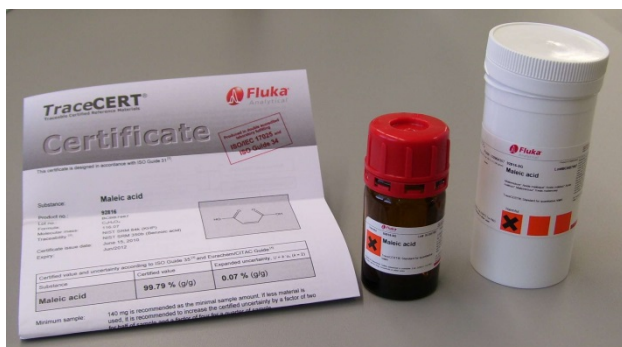
- Traceability independent of chemical identity
- Low Uncertainties
- Non-destructive
- Structural information and information on impurities

Generation of new CRMs for Chromatography

- Quality Assurance
 - Ensuring Accuracy of the Mean and Precision
 - Ensuring an Analytical Method provides correct Results

Differentiation between pure substance CRMs/matrix CRMs

Thank you!



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