CCQM-K179
Polar analyte in solid organic material:
Mass fraction of oxytetracycline hydrochloride in solid organic material

Key Comparison
Track C

Draft B Report
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SUMMARY

The CCQM-K179 comparison, undertaken with a parallel pilot study CCQM-P224, was coordinated by the BIPM and UME on behalf of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) Working Group on Organic Analysis (OAWG). It was undertaken for National Measurement Institutes (NMIs) and Designated Institutes (DIs) which provide measurement services in organic analysis under the CIPM Mutual Recognition Arrangement (MRA) and was designated a Track C comparison within the OAWG implementation of the CCQM Strategy for Programme Development 2021-2030.[1]

The ability to assign the mass fraction content of the primary component in a solid organic material that an NMI makes available as a pure substance Reference Material or that is used by an NMI in-house as a Primary Reference Material is a critical technical competency for the provision of SI-traceable quantitative measurement results in organic analysis. The purity property value assigned to the Primary Reference Material in a measurement hierarchy anchors the calibration chain for all results linked to that material.

Participation in the narrow-scope, Track C purity comparison organized by the OAWG allows an NMI/DI to demonstrate that their procedure for the mass fraction assignment of salt-forming analytes and its associated uncertainty are fit for purpose for their intended application. Evidence of successful participation in formal, relevant international comparisons is required under the CIPM Mutual Recognition Arrangement (MRA) to support calibration and measurement capability (CMC) claims made by NMIs and DIs.

Fourteen NMIs in addition to the BIPM, submitted results in CCQM-K179 (one additional laboratory submitted results to the pilot study). Participants were required to assign the mass fraction of oxytetracycline hydrochloride salt (OTC HCl), standardized to the value expected at 50% relative humidity, present in a solid material containing the oxytetracycline hydrochloride salt as the principal component.

Seven participants assigned their final value for the comparison through the combination of values obtained by independent mass balance and qNMR methods, seven participants reported a result from a mass balance method only and one participant reported a value based on a qNMR method only.

Successful participation in CCQM-K179 demonstrates capabilities for assigning the mass fraction of organic compounds in the form of salt materials, with molar mass in the range of 75 g/mol to 500 g/mol, having high polarity (pKow > -2), including compounds presenting significant hygroscopicity, in an organic solid material.

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INTRODUCTION

Evidence of successful participation in formal, relevant international comparisons is required to establish measurement capability claims (CMCs) made by NMIs and Designated Institutes (DIs) with active programmes in organic analysis. In April 2019, the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) approved the Key Comparison (KC) CCQM-K179 on high polarity pure organics present in the form of salt materials. CCQM-K179 was designed to assess participants' capabilities for the mass fraction value assignment of high purity organic substances containing a salt-forming, polar analyte ($pK_{OW} > -2$) having a molar mass in the range 75-500 g/mol as the primary component. It is a component of the overall OAWG strategy of Track C key comparisons that serve to underpin and benchmark NMI capabilities for the provision of primary calibration services for organic analysis.

Oxytetracycline is a member of the tetracyclines group of broad-spectrum antibiotic compounds, widely used in veterinary medicine, that have a common basic structure. Because of concerns with the potential health risk to the consumer of long-term exposure to low levels of these compounds, monitoring programs for the presence of tetracycline residues in the environment and in food of animal origin including meat, fish, milk, eggs and honey are in place in many countries.[2] These activities, which improve food safety and reduce the potential for technical trade barriers in this area, need to be supported by a sound reference measurement infrastructure for tetracycline analysis.

This comparison compliments CCQM-K148.b, run in parallel on the same material but targeting oxytetracycline free base as the measurand, and CCQM-K148.a, which examined the measurement of a non-polar organic analyte present as the primary component in a high-purity organic material. In addition, the current CCQM-K179 comparison material poses a genuine challenge due to its highly hygroscopic nature. The comparison protocol distributed to participants included specific instructions on handling and reporting of purity values at standardized conditions of relative humidity.

The following sections of this report document the timeline of CCQM-K179, the measurands, study material, participants, results, and the measurement capability claims that participation in CCQM-K179 can support. The Appendices reproduce the official communication materials and summaries of information about the results provided by the participants.

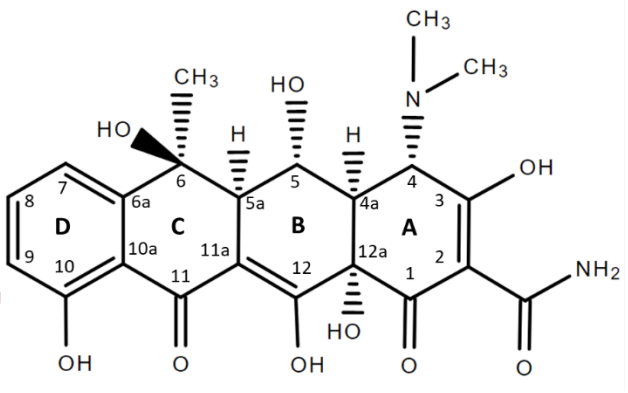
TIMELINE

Table 1. Comparison timeline

Date	Action
April 2019	Proposed to CCQM
June 2022	Draft protocol presented to OAWG as potential Track C Key Comparison
October 2022	OAWG authorized CCQM-K179 as a Track C Key Comparison; protocol approved
October 2022	Call for participation to OAWG members
October 2022 - March 2023	Study samples shipped to participants. The range in shipping times reflects delays from shipping and customs.
15 June 2023	Results due to coordinating laboratory
August 2023	Draft A.1 report sent to participants
October 2023	Draft A report presented to OAWG
October 2024	Draft B report distributed to OAWG
TBD	Final report approved by OAWG

MEASURAND

The comparison requires the assignment of the mass fraction, reported in mg/g, of oxytetracycline hydrochloride salt (OTC.HCl) in a unit of the comparison material under standardized conditions of relative humidity. Fig. 1 below displays the molecular structure of the OTC.HCl salt (4*S* epimer).



Oxytetracycline hydrochloride (OTC.HCl)

Molar mass = 496.9 g/mol; pK_{OW} ~ 0.5

Fig 1 Structure and conventional numbering of oxytetracycline hydrochloride

STUDY MATERIALS

The comparison material was produced by TÜBITAK-UME. A bulk source material of OTC.HCl in the form of a fine yellow crystalline powder was homogenized in a 3D mixer and kept in a vacuumed container until filling to minimize moisture uptake. About 0.5 g of the material were filled into each vial of the comparison batch using an automatic filling machine.

Each participant received as a minimum two vials of the comparison material, each containing a minimum of 500 mg of OTC.HCl. Participants who planned to use multiple independent methods to contribute to their final property value assignment (e.g. a mass balance procedure and a separate qNMR procedure) were allowed to request an additional vial. The recommended minimum sample intake for analysis was 10 mg. The comparison samples were provided in amber glass vials sealed with PTFE-lined screw-caps. Measurement results were to be reported on the material as received without additional treatment but taking into account the hygroscopicity correction described in the comparison protocol.

Homogeneity Assessment of Study Material

The homogeneity of the batch was tested using an LC-UV method for the content of OTC and the main structurally related impurities (SRIs). An oven-transfer, coulometric Karl Fisher titration was used for determination of water content and ion chromatography for chloride ion content. The uncertainty contribution due to inhomogeneity of the assigned values was evaluated by ANOVA. Ten vials were selected at regular intervals from the filling sequence to ensure that the results would indicate any trend in the filling process. Each vial was analyzed in a random order to ensure any trends in the bottling process were separated from possible trends resulting from the analytical sequence.

The results obtained indicated no statistically significant difference in the within- and between-vial levels of the mass fraction of each component in the material. The upper limit for the uncertainty contribution due to inhomogeneity in all cases was sufficiently small as to be unlikely to influence the effective comparison of participant results. A summary of the observed within- and between-sample variability for the major components is shown in Table 2.

Table 2. Homogeneity assessment for the main component OTC, the main structurally related impurity, water and chloride in the comparison material.

ANOVA Estimate	**OTC**	**Imp A**	**H_2O**	**Cl^-**
Between-unit CV (%)	0.36%	0.77%	0.64%	0.87%
Within-unit CV (%)	0.83%	1.10%	1.03%	1.44%
Upper limit of relative uncertainty contribution due to inhomogeneity	0.27%	0.43%	0.37%	0.47%

Probability of falsely rejecting the hypothesis that all samples have the same concentration	< 5%	< 5%	< 5%	< 5%

A plot of the normalized mass fraction for each analyte obtained for the homogeneity assessment is plotted by filling sequence in Fig. 2. The normalized values of repeat measurements from three aliquots taken from each individual vial are plotted.

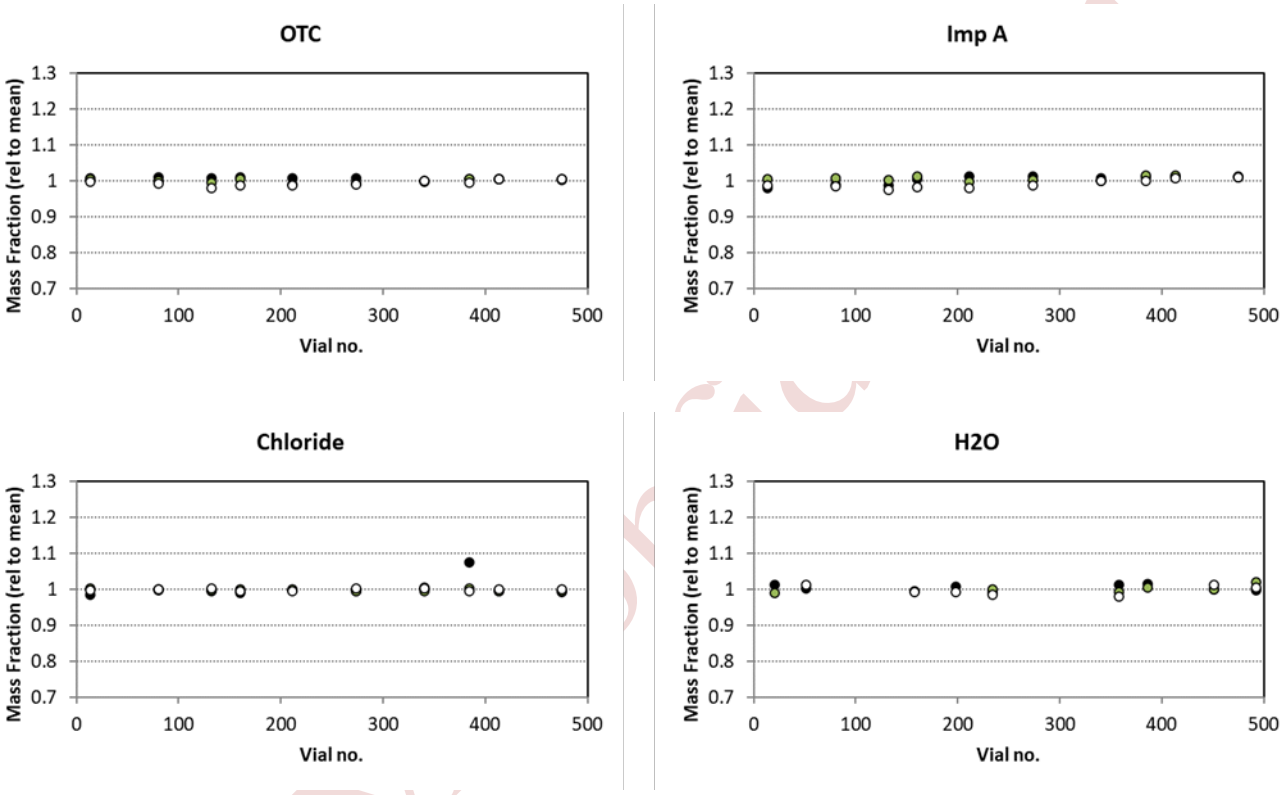


Fig. 2 Homogeneity evaluation for OTC, the major structurally related impurity A, water and chloride in the comparison material.

Stability Assessment of Study Material

An isochronous stability study was undertaken for OTC, structurally related impurities, water and chloride on storage at 4 °C, 22 °C and 40 °C in the dark. The analytical methods used were the same as in the homogeneity study. The material is sufficiently stable, within the proposed time scale of the comparison, when stored at 4 °C or 22 °C. OTC and some impurities were not stable at 40 °C. Precautions were taken to monitor if the comparison material is exposed to temperature above 25 °C during shipment and if this occurs replacement material will be provided.

The mass fractions of OTC and chloride relative to the mean value of reference samples stored at -20 °C are shown in Fig. 3 for samples stored at 4 °C and 22 °C during the stability study period. The plot displays the normalized results of duplicate analysis of samples prepared from two units of CCQM-K179. The upper and lower dashed lines indicate the uncertainty of the regression line, which reflects the analytical method variance in the absence of a significant instability trend.

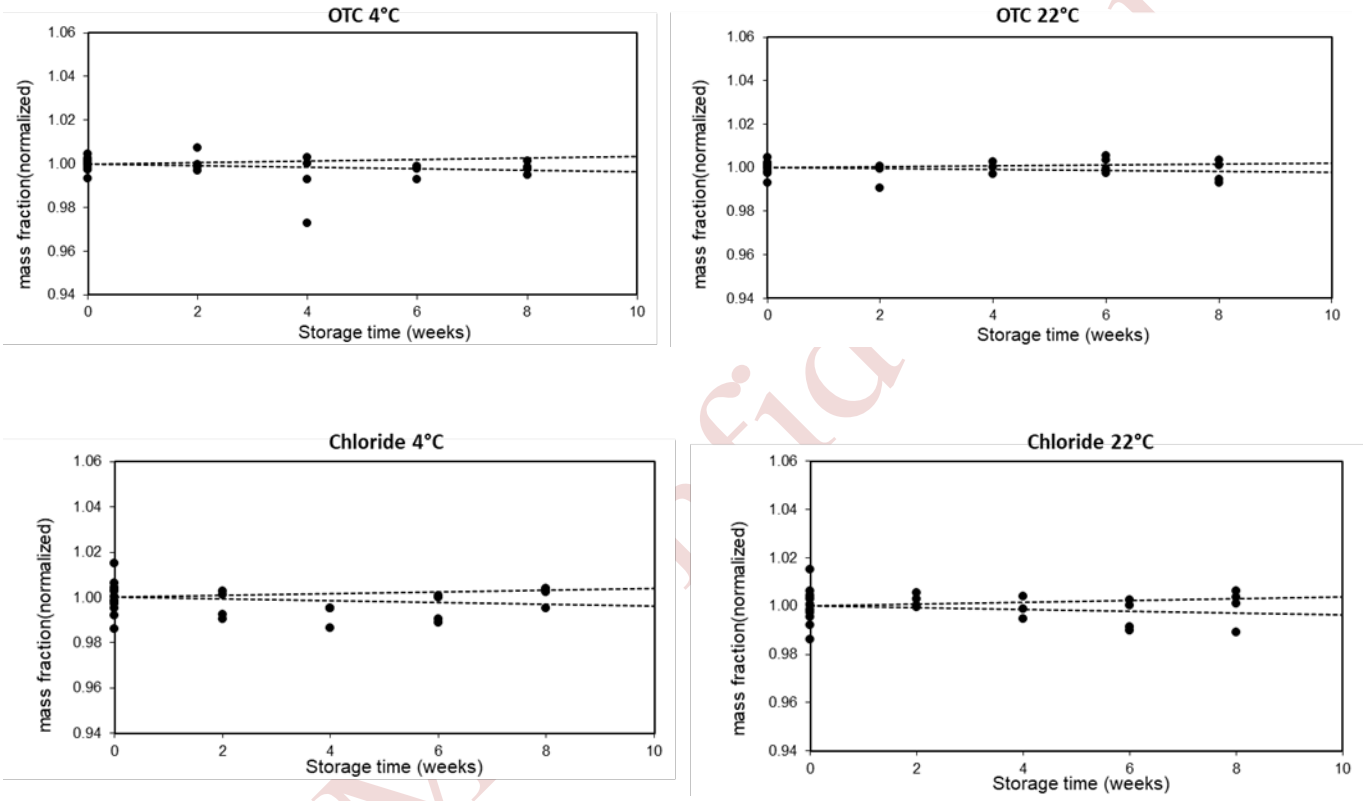


Fig. 3 Stability evaluation of OTC and chloride content in samples stored at 4 °C and 22 °C for 8 weeks.

PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION

The call for participation was distributed in October 2022 with the intent to distribute samples in November 2022, receive results in March 2023 (subsequently postponed to May and eventually June 2023), and discuss results at the online OAWG meeting in October 2023. See Table 1 for study timeline. Appendix A reproduces the call for participation and study protocol.

Fifteen institutes registered to participate in the key comparison and one institute, BVL, registered to participate in the parallel pilot study CCQM-P224 (Table 3). The results of the pilot study are not discussed in this report.

Table 3: Institutions Registered for CCQM-K179

NMI or DI	Code	Country	Contact
Bureau International des Poids et Mesures	BIPM	France	Gustavo Martos, Steven Westwood
EXHM/GCSL-EIM	EXHM	Greece	Ilias Kakoulides
Government Laboratory, Hong Kong, China	GLHK	Hong Kong, China	Wai-hong FUNG, Chun-wai TSE, Jasmine Po-kwan LAU
Health Sciences Authority, Chemical Metrology Laboratory	HSA	Singapore	Pui Sze Cheow, Tang Lin Teo
National Institute of Metrology, Quality and Technology	INMETRO	Brazil	Eliane Cristina Pires do Rego, Wagner Wollinger
Department of Chemistry Malaysia	KIMIA	Malaysia	SHIMA HASHIM
Korea Research Institute of Standards and Science	KRISS	Korea	Sunyoung Lee, Ki Hwan Choi
National Institute of Metrology, China	NIM China	China	Fuhai SU, Qinghe ZHANG
National Measurement Institute, Australia	NMIA	Australia	Stephen Davies
National Metrology Institute of Japan	NMIJ	Japan	Yoshitaka Shimizu
National Metrology Institute of South Africa	NMISA	South Africa	Désirée Prevoo-Franzsen
TUBITAK Ulusal Metroloji Enstitusu (UME)	UME	Turkey	Mine Bilsel
D.I. Mendeleev All-Russian Institute for Metrology	VNIIM	Russia	Anatoliy Krylov, Alena Mikheeva
National Institute of Metrology	NMIT	Thailand	Sornkrit Marbumrung, Ponhatai Kankaew
National Metrology Laboratory of the Philippines	NMLPhil	Philippines	Alleni T. Junsay

Either two or three units of the comparison material were shipped by the coordinating laboratory to each participant. The number of vials provided depended on whether the participants used a single purity assignment method or the combination of multiple approaches. Participants returned a form acknowledging receipt of the samples, advising the comparison coordinator if any obvious damage had occurred to the vials during shipping, and noting whether a monitoring strip inside the container indicated exposure to a temperature in excess of 25 °C during the shipping process. Problems were reported in shipment of the comparison material due to exposure to excessive temperature by HSA, GLHK, NIMT and KIMIA. One participant, KRISS, requested additional samples due to the malfunctioning of their refrigerator, which resulted in the initial samples being exposed to temperatures above 25°C. Replacement units were shipped to all the participants concerned.

Participants were required to report their estimate of the mass fraction of oxytetracycline as the hydrochloride salt present in the material in mg/g, standardized to the value expected at 50% relative humidity (RH). The result should be based on combined values obtained by the measurement of multiple aliquots from at least one of the vials supplied. Participants were also required to verify the accuracy of their RH measurements and those who used a mass balance procedure were required to report the combined mass fraction assignment (estimated if measured at RH = 50%) and associated uncertainty for the each of the contributing sub-classes of impurity: total structurally related organic impurities, water, chloride, residual solvent and total non-volatiles/inorganics content.

A copy of the text in the format of the Excel spreadsheet provided to participants to submit their results is reproduced in Appendix C.

RESULTS

Participants were requested to report a single estimate of the mass fraction (in mg/g) of OTC.HCl in the comparison material, standardized to the value expected at 50% RH. In addition to the quantitative results, participants were instructed to describe their analytical methods, approach to uncertainty estimation, and the core competencies they felt were demonstrated in this study. Appendices B, C, and D reproduce the relevant registration, reporting and core competency forms, respectively.

Participants using a mass balance procedure were required to report the combined mass fraction assignment and associated uncertainty for the assigned sub-classes of impurity: total structurally related organic impurities, water, chloride, residual solvent and total non-volatiles/inorganics content. In addition, participants were encouraged but not required to identify and provide mass fraction estimates for all significant individual impurity components quantified in the comparison sample.

CCQM-K179 results were submitted by all 15 institutions that received samples. Seven participants assigned their final value for the comparison through the combination of values obtained by independent mass balance and qNMR methods, seven participants reported a result from a mass balance method only and one participant reported a value based on a qNMR method only.

Calibration Materials Used by Participants

Participants established the metrological traceability of their results using certified reference materials (CRMs) with stated traceability and/or commercially available high purity materials for which they determined the purity. Table 4 lists the CRMs that were reported by the participants that performed the value assignment of the main component using qNMR methods.

Table 4. CRMs and high-purity materials used as source of traceability for OTC qNMR measurements in CCQM-K179.

CRM	Provider	Used by	In-house purity assignment of CRM
QNMR010 (Maleic acid)	NMIA	HSA, NMIA	
Maleic acid CRM	Inmetro	Inmetro	
TraceCERT Maleic acid	Merck	EXHM	EXHM (qNMR)
HRM-1012A (Acesulfame potassium)	HSA	HSA	
CRM 4601 (3,5-Bis(trifluoro methyl) benzoic acid)	NMIJ	HSA, NMIJ, GLHK	
NIST PS1 (Benzoic acid)	NIST	HSA, UME, KRISS	
TraceCERT 1,2,4,5-Tetrachloro-3-nitrobenzene	Merck	NMISA, Inmetro, NIMT	NMISA, Inmetro (qNMR)
CRM GBW 06120 (Ethylparaben)	NIM	NIM	

Traceability of qNMR measurements was achieved through the use of appropriate standard materials, either produced or value assigned in-house by NMIs/DIs having demonstrated relevant capabilities in previous CCQM Track A Key comparisons. However, NIMT directly used the certified value of a commercial standard from Merck.

Participants using a mass balance approach employed a variety of CRMs, commercial standards and other materials value-assigned in-house as calibrators for the different techniques used to

quantify all the impurity sub-classes: total structurally related organic impurities, water, chloride, residual solvent and total non-volatiles/inorganics.

Participant Results for OTC.HCl content in CCQM-K179

The different approaches used by participants for the mass fraction assignment of OTC.HCl were as follows:

- Mass balance as the sole method: EXHM*, KIMIA, NMLPhil, VNIIM, BIPM, KRISS*, NMIA* (*used qNMR as confirmation method only).
- qNMR corrected by independent impurity measurements: NMISA
- Combination of mass balance and qNMR (uncorrected by independent impurity measurements): HSA, NMIJ, NIM, GLHK, UME, and NIMT.
- Combination of mass balance and qNMR (corrected by independent impurity measurements): INMETRO

Table 5. CCQM-K179 results for the mass fraction assignment of OTC.HCl and the individual reported values from mass balance (MB) and qNMR methods employed by participants.

NMI	CCQM.K179 (mg/g)	u(w) (mg/g)	U_{95}(w) (mg/g)	MB (mg/g)	qNMR (mg/g)
HSA	838.8	7.3	14.6	848.9	828.7
BIPM	851.4	3.9	7.9	851.4	
NMIJ	853.5	3.6	7.2	858.2	848.7
NIM	854.5	5.2	10.4	858.03	851.05
NMIA	856	4	8	856	860
NMISA	859	7.1	14	840	859
INMETRO	859.9	3.6	7.2	862.8	856.9
GLHK	860.3	4.4	8.9	865.1	856.5
EXHM	860.64	5.04	10.08	860.64	860.06
KRISS	867.4	6.7	13.1	867.4	876.6
UME	879.5	8.4	16.7	878.8	880.1
NMLPhil	890.7	5.2	10.4	890.7	
KIMIA	897	4.95	9.9	897	
NIMT	908.2	24.35	48.7	906.29	910
VNIIM	919.3	1.5	3	919.3	

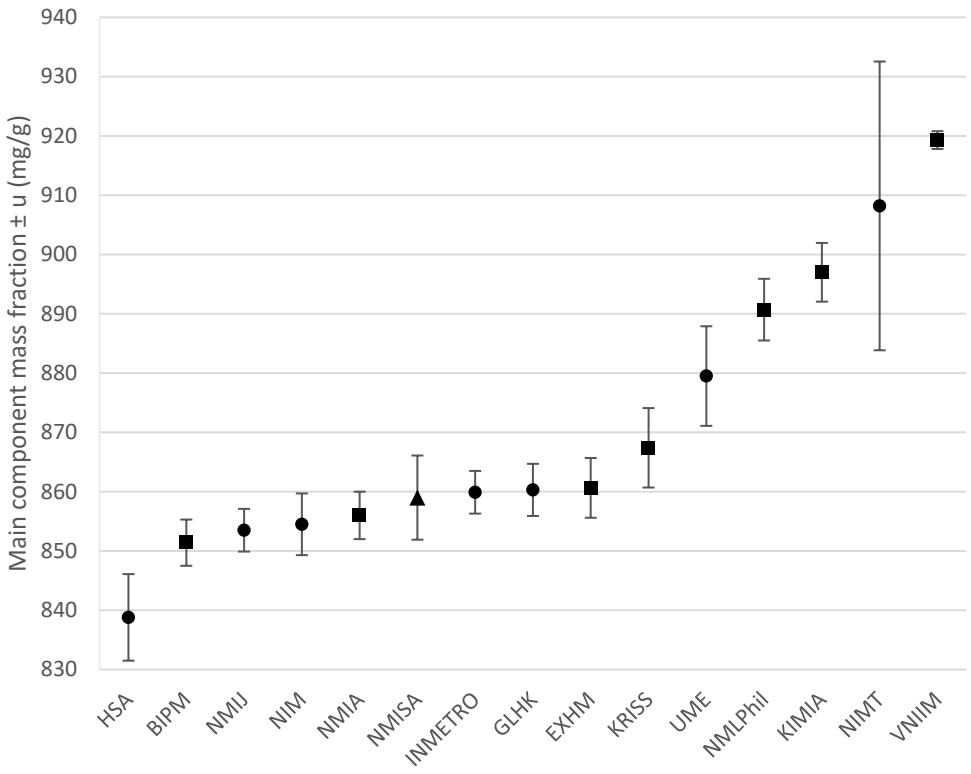


Fig. 4 CCQM-K179 reported results for the mass fraction assignment of OTC.HCl. The squares, triangles and circles indicate the assignment methods mass balance, qNMR or the combination of both, respectively.

Structurally related Impurity (SRI) content

Methods based on LC-UV were the predominant approach used to analyze the material for structurally related impurity content. Other methods used included LC-CAD and LC-MS for impurity identity determination or confirmation. A summary of the chromatographic methods and conditions used per participant is given in Appendix E. Several participants reported instability of impurities under the studied conditions, which included different solvents for sample dissolution. A more detailed discussion of the structurally related impurities in the comparison material can be found in the CCQM-K148.b report.[3]

Table 6. CCQM-K179 results in the mass fraction assignment of structurally related organic impurities. Participants marked with * reported the structurally related impurities as free base forms so the SRI hydrochloride salt content was estimated assuming 1:1 stoichiometry and using the impurities molar masses or the OTC molar mass for unidentified impurities.

	Reported		SRI.HCl salt	
NMI	**w (mg/g)**	**u(w) (mg/g)**	**w (mg/g)**	**u(w) (mg/g)**
VNIIM*	17.75	0.93	19.24	1.0
GLHK	32	2.5	32	2.5
INMETRO	33.4	2.4	33.4	2.4
NIMT*	31.23	0.71	33.70	0.77
EXHM	34.44	2.62	34.44	2.62
NMIJ	34.56	2.8	34.56	2.8
NMIA	36.5	0.7	36.5	0.7
KIMIA*	33.96	2.67	36.65	2.88
KRISS	38.1	0.7	38.1	0.7
BIPM*	35.4	1.2	38.2	1.3
HSA	44.3	8.6	44.3	8.6
NIM	48.38	2.1	48.38	2.1
NMLPhil*	46.8	4.8	50.5	5.2
UME*	47.6	0.4	51.4	0.4
NMISA*	62	5.6	67	6.0

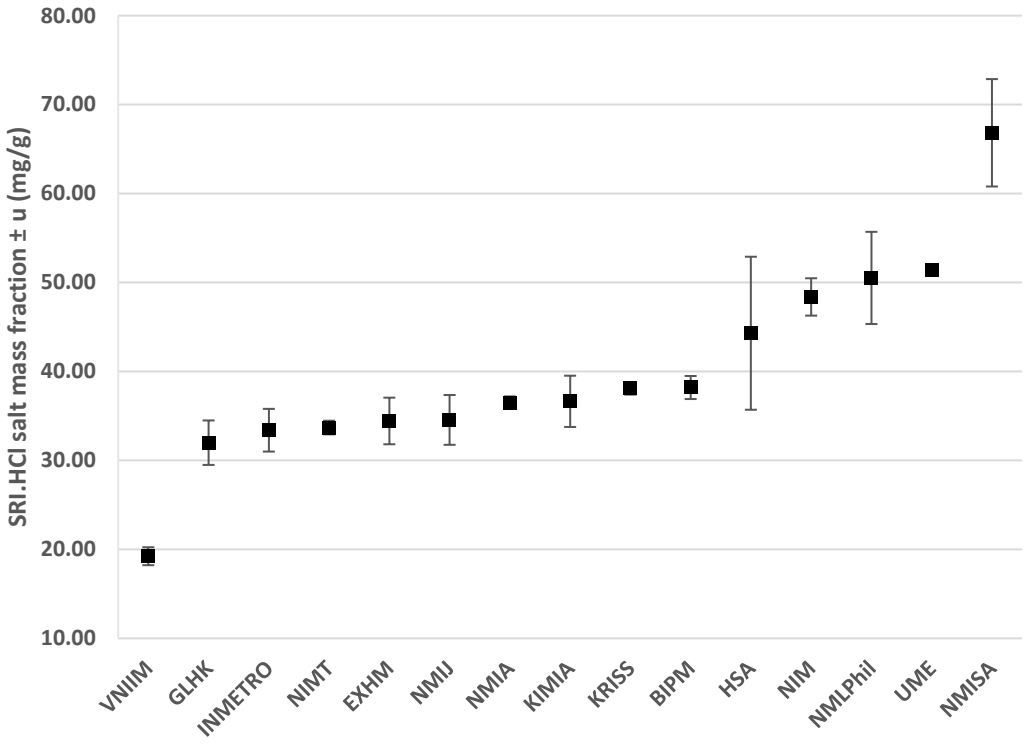


Fig. 5 *CCQM-K179 results for the mass fraction assignment of structurally related organic impurities in the form of HCl salts.*

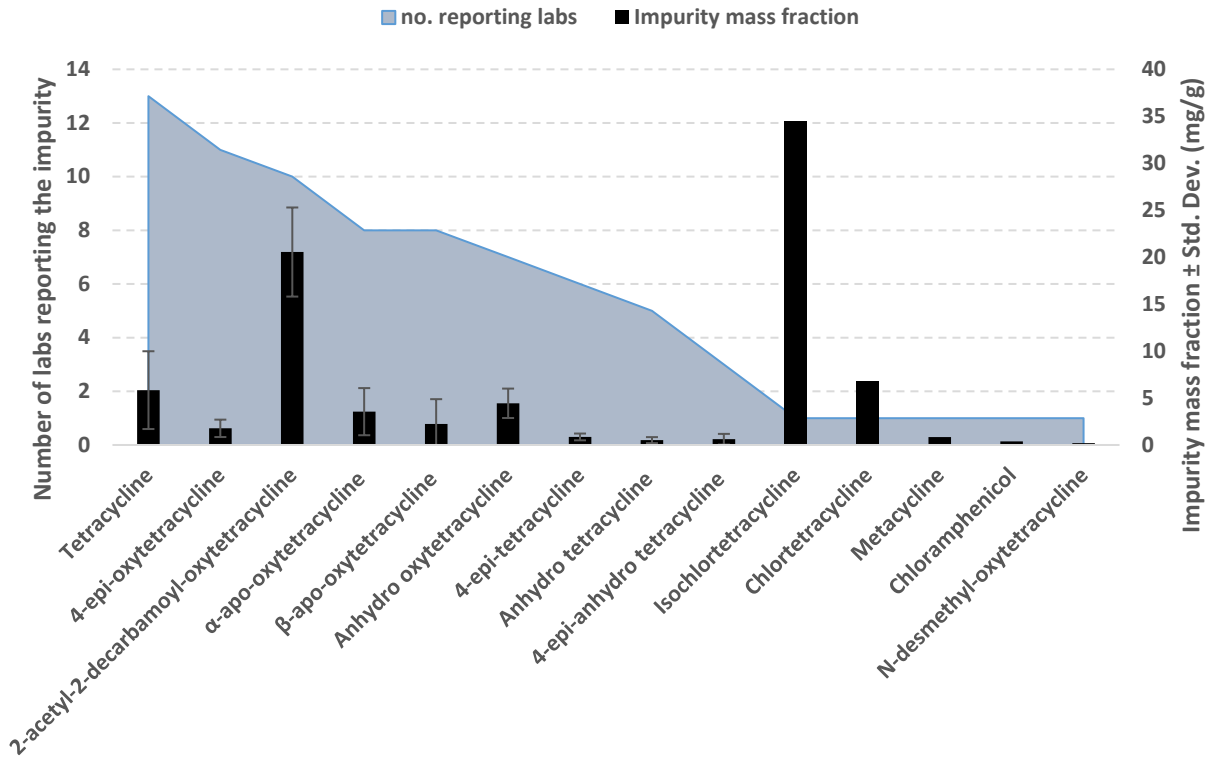


Fig. 6 *Mass fraction values of reported structurally related impurities in CCQM-K179 material ranked by the number of laboratories that identified each impurity. Some participants reported related impurities as free base whereas others reported them as hydrochloride salts.*

Water content

All participants used coulometric Karl Fischer titration, either after introduction of the sample directly into the titration cell or through transfer of the water content into the titration cell from an oven-heated aliquot of the comparison material using a flow of dry gas. A few participants used a TGA confirmatory technique.

Table 7. CCQM-K179 results for the water content assignment at standardized conditions of 50% RH and values obtained under laboratory's conditions of relative humidity.

NMI	w (mg/g) (50% RH)	u(w) (mg/g)	w (mg/g) (Lab RH)	Lab RH (%)
HSA	106.4	4	106.8	46-57
BIPM	110.3	3.7	110.3	51.6
NMIJ	107.04	2.71	106.95	49-50
NIM	89.9	4.09	89	47.2
NMIA	107.5	4	107.3	54
NMISA	97.5	2.26	95.9	45
INMETRO	101.8	1.2	104.6	58

GLHK	102.8	6		49-52
EXHM	105.33	0.66	100.28	44
KRISS	78.1	1.8	74.8	40-43
UME	73.4	0.5	74.8	54
NMLPhil	58	1.91	58.2	37-39
KIMIA	69.42	4.02	69.59	57
NIMT	59.07	16.64	62.16	59
VNIIM	62.34	1.21	62.34	50

To report values estimated at standardized conditions of 50% RH (Fig. 7), participants were asked to correct their mass fraction assignments using the equation provided in the comparison protocol (Appendix A). Overall, the relative magnitude of the correction for the water content assignment applied by the participants was smaller than 5%, which led to very small differences between the values assigned at laboratories' RHs and the reported ones at 50% RH (Table 7).

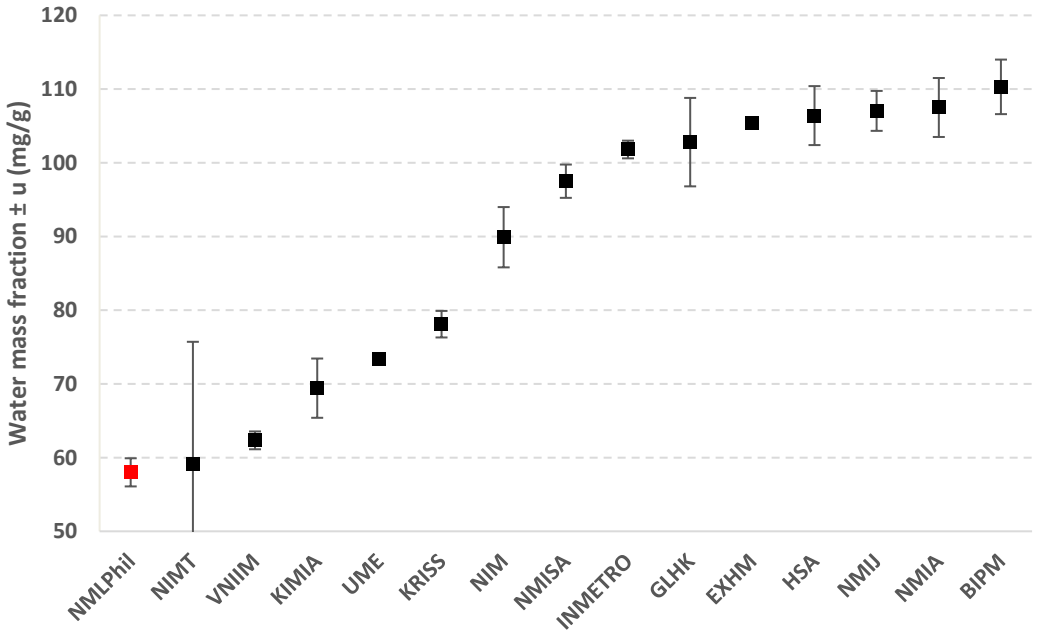


Fig. 7 CCQM-K179 reported results for the mass fraction assignment of water content at 50% RH. The dots in red correspond to measurements obtained under mean RH humidity conditions below the recommended 42% value.

Chloride content

Ion chromatography was predominantly used to analyze the material for chloride ion content. Other methods used included ICP-MS, X-ray fluorescence and CE-UV. A summary of the methods and conditions used per participant are given in Appendix E. Most participants reported total Cl^- ion content, regardless of whether it was associated to OTC (therefore part of the measurand), associated to the SRIs or residual. Alternatively, BIPM reported Cl^- impurity content (not part of OTC.HCl) and NIM and INMETRO reported residual Cl^- (neither part of OTC.HCl nor the SRI.HCl).

For comparison purposes, Cl^- impurity mass fraction, defined as the fraction of chloride that is not part of the measurand OTC.HCl, was calculated for all participants. This was done by firstly calculating the OTC-associated Cl^- content via multiplication of the participant's reported OTC.HCl mass fraction by the mass ratio of Cl^- to OTC.HCl (i.e. $w_{OTC.HCl}$ x 35.45/496.90), and secondly, subtracting it from the total Cl^- reported value. In the case of NIM, INMETRO and UME, the total Cl^- content value was taken from their CCQM-K148.b submitted results. Table 8 depicts reported Cl^-, Cl^- impurity content and residual Cl^-, i.e. subtracting Cl^-(OTC) and Cl^-(SRI) from total Cl^-. The Cl^- impurity content calculation for NIMT, NMISA and UME resulted in negative values, indicating that the reported chloride value was lower than the expected content based on equimolar OTC : HCl amounts. Cl^- impurity content for all other participants was plotted in Fig. 8.

Table 8. Mass fraction values and standard uncertainties of CCQM-K179 reported Cl^- (w_{Cl}) and calculated impurity (w_{Cl_imp}) and residual (w_{Cl_res}) Cl^-. *n.r.*: not reported.

NMI	w_{Cl} (mg/g)	$u(w_{Cl})$ (mg/g)	w_{Cl_imp} (mg/g)	$u(w_{Cl\_imp})$ (mg/g)	w_{Cl_res} (mg/g)	$u(w_{Cl\_res})$ mg/g
NIMT	61.66	4.89	-3.13	0.08	-5.5	5.2
NMISA	58.5	1.5	-2.78	0.02	-7.6	1.6
EXHM	62.4	1.36	1.00	0.01	-1.5	1.4
NMIJ	63.07	0.07	2.18	0.01	-0.3	0.3
BIPM	2.67	0.50	2.67	0.50	0.0	0.5
KRISS	64.6	0.4	2.72	0.03	0.0	0.6
NMIA	64	5.5	2.93	0.02	0.3	5.5
GLHK	64.7	2.2	3.32	0.02	1.0	2.2
NIM	2.62	0.028	4.25	0.79	0.7	0.8
HSA	64.4	2.3	4.56	0.04	1.4	2.4
NMLPhil	68.6	3	5.05	0.04	1.4	3.0
INMETRO	1.8	1.1	6.30	1.13	3.9	1.1
VNIIM	72.86	2.22	7.27	0.01	5.9	2.2
KIMIA	72.33	2.42	8.33	0.05	5.7	2.5
UME	n.r.	n.r.	-1.4	1.0	-5.1	1.0

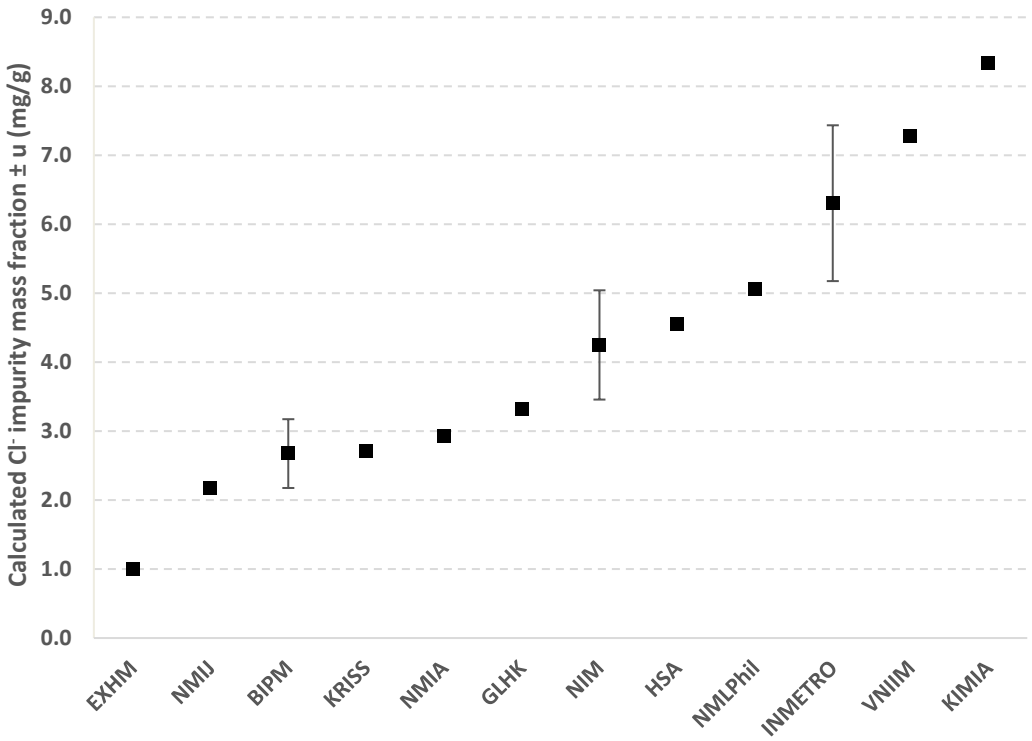


Fig. 8 CCQM-K179 calculated chloride impurity content that is not part of the measurand. NIMT, NMISA and UME calculated values were -3.1, -2.8 and -1.4 mg/g and are not represented.

Since all participants except NMLPhil participated in the parallel comparison CCQM-K148.b for the determination of the free base OTC content in the same OTC.HCl material, the OTC.HCl mass fraction could be calculated based on CCQM-K148.b results and the equimolarity between the free base OTC and HCl. This second set of values was compared to the CCQM-K179 values in Fig. 9.

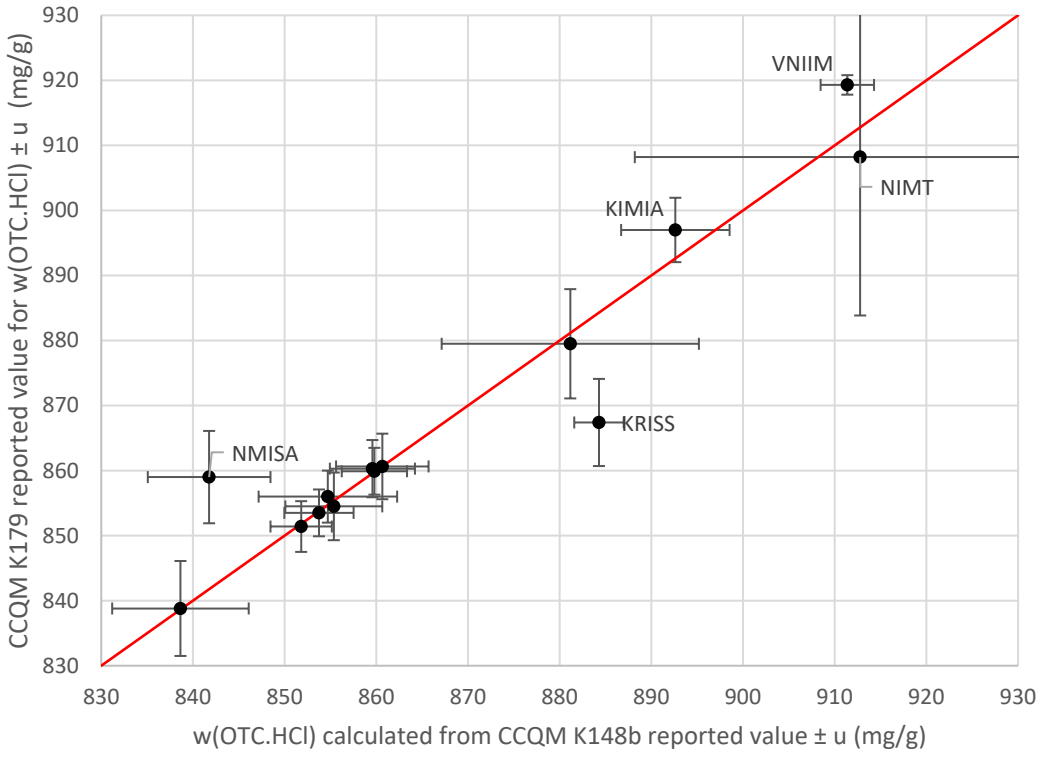


Fig. 9 Comparison between OTC.HCl mass fraction values reported in CCQM-K179 and calculated from participant's results for OTC free base in CCQM-K148.b. The red line indicates agreement between both sets of values.

Volatile organics content

Ten participants provided information on the volatile organic content of CCQM-K179 material. Five participants reported no evidence for the presence of residual solvent above their method detection limits. The results reported by participants with their associated standard uncertainties (k = 1) are listed in Table 9.

Only two participants reported levels above 1 mg/g of this class of impurity. An overview of methods used by each participant to assign and verify total VOC content is provided in Appendix E.

Table 9. CCQM-K179 results for the mass fraction assignment of volatile organic content.

NMI	w (mg/g)	u(w) (mg/g)
HSA	0.024	0.66
NMISA	0.47	0.087
BIPM	0	0.1
NMIJ	0	0.35

NMIA	0	0
NIM	0.89	0.02
GLHK	0.021	1
INMETRO	0.2290	0.0094
EXHM	0	0.01
UME	0.17	0.001
KRISS	0.1	1.6
KIMIA	1.8	1.2
VNIIM	0.56	0.007
NIMT	0	1.44
NMLPhil	4.22	1.94

Non-volatiles / inorganics content

Five participants reported levels above 1 mg/g for this class of impurity. However, it is noted that hydrogen ion content, if considered an inorganic impurity present in equimolar amounts to chloride, would represent between 1.7 and 2 mg/g according to chloride results reported by participants. EXHM included total chloride ion content under this impurity class, even though most chloride constitutes a part of the measurand. An overview of methods used by each participant to assign and verify non-volatiles / inorganic content is provided in Appendix E.

Table 10. CCQM-K179 results for the mass fraction assignment of non-volatiles / inorganics content. *EXHM reported value included total chloride content.

NMI	w (mg/g)	u(w) (mg/g)
HSA	0	1.44
BIPM	2.95	0.53
NMIJ	0.16	0.1
NIM-C	0.18	0.009
NMIA	0	1.2
NMISA	<1	0.005
INMETRO	1.8	1.1
GLHK	0.017	1
EXHM*	64.16	1.40
KRISS	0.1	0.7
UME	0	0
NMLPhil	5.43	0.39
KIMIA	0.25	1.44
NIMT	5.42	0.41
VNIIM	< 0.04	0.02

KEY COMPARISON REFERENCE VALUE (KCRV)

The key comparison reference value was based on the KCRV of the parallel key comparison CCQM-K148.b conducted on the same material.[3] The KCRV for the OTC free base content determined in CCQM-K148.b was multiplied by the ratio of the molar masses of the OTC hydrochloride salt to the OTC free base:

$$KCRV_{K179} = KCRV_{K148.b} \cdot \frac{M_{OTC.HCl}}{M_{OTC}} = (792.0 \pm 5.2)\ mg \cdot g^{-1} \cdot \frac{496.900}{460.439}$$
$$= (854.7 \pm 5.7)\ mg \cdot g^{-1} (k = 1)$$

Eq.1

The CCQM-K148.b KCRV was based on the mass balance approach, which required estimating the mass fraction of each impurity type in the material based on selected participants data. The CCQM-K179 results dataset is a subset of CCQM-K148.b except for the results of NML-Phil, who did not participate in the latter. This laboratory reported mass fraction results for water, chloride, the tetracycline impurity, inorganics and volatiles. However, their reported water content (58 ± 1.9 mg/g, k=1) was significantly lower than the reference value of 104.1 ± 1.2 mg/g, which indicated insufficient equilibration of the comparison samples with ambient humidity prior to weighing. The equilibration issue was highlighted during technical discussions with participants and could bias the determination of the other impurity compounds if not appropriately considered. Therefore, NML-Phil results were not included in the calculation of the KCRV, rendering the CCQM-K148.b results as a valid and more comprehensive dataset for the KCRV calculation in CCQM-K179 according to equation 1. The mass fraction reference values of the individual impurity types in the comparison material are based on the CCQM-K148.b study report[3] in Table 11. The table includes the content of HCl associated to SRIs, which was calculated assuming 1:1 stoichiometry and the OTC molar mass as an approximate estimate of the molar mass of SRIs. The calculated mass balance value of 854.3 ± 5.2 mg/g agreed with the KCRV (Eq. 1).

Table 11. *Individual reference values (RV) estimated for all impurity types in the CCQM-K179 comparison material.*

Impurity	**RV (mg/g)**	**u (mg/g)**	**Estimate**
H_2O	104.1	1.2	HB-Gauss
HCl_imp	3.0	0.4	Based on SRI
SRI	38.3	5.0	Rect. distr
Inorg-{Cl}	0.1	0.0	HB-Gauss
Volatile Org	0.2	0.1	HB-Gauss
Mass Balance	**854.3**	**5.2**	**1000-Σi**

Equation 1 assumes that HCl is associated with OTC following a stoichiometry 1:1. This is consistent with Fig. 10, which shows how the sum of the contents of chloride associated to OTC

and to the structurally related impurities agrees with the total chloride content measured by participants.

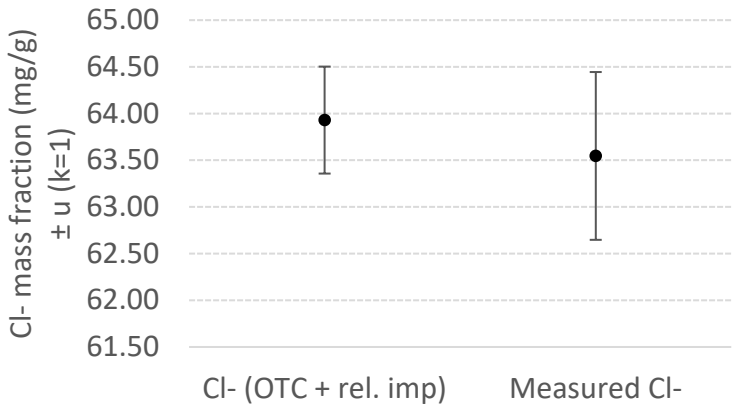


Fig. 10 *Chloride content as measured by participants (HB-gaussian estimate) or as content sum of chloride associated to OTC and structurally related impurities. Error bars correspond to standard uncertainties.*

The results of participants in CCQM-K179 are plotted in Fig. 11 against the KCRV.

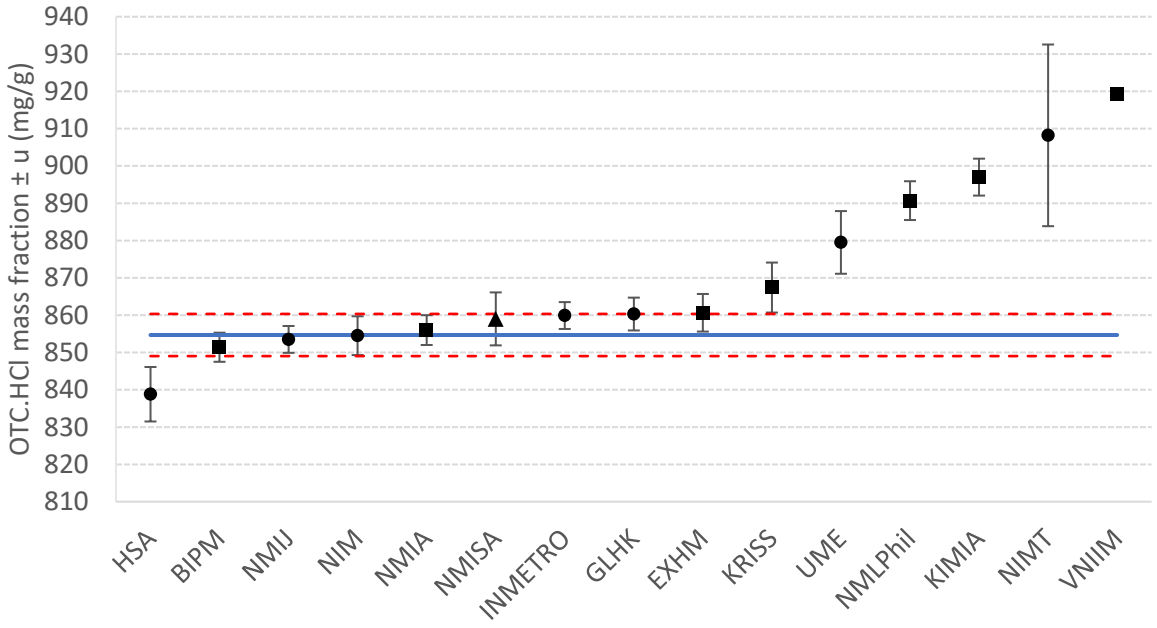


Fig. 11 *Participants reported values for CCQM-K179 against the KCRV plotted as a horizontal blue line with its standard uncertainty interval depicted as dotted red lines. The squares, triangles and circles indicate the assignment methods mass balance, qNMR or the combination of both, respectively. Error bars are reported standard uncertainties.*

DEGREES OF EQUIVALENCE (DoE)

The degrees of equivalence were calculated for each participant reported value in CCQM-K179 based on the KCRV and its uncertainty (Fig. 12 and Table 12). A participant result is compatible with the KCRV when the DoE U_{95} (expanded uncertainty at a 95% level of confidence) of the result exceeds the absolute value of the DoE.

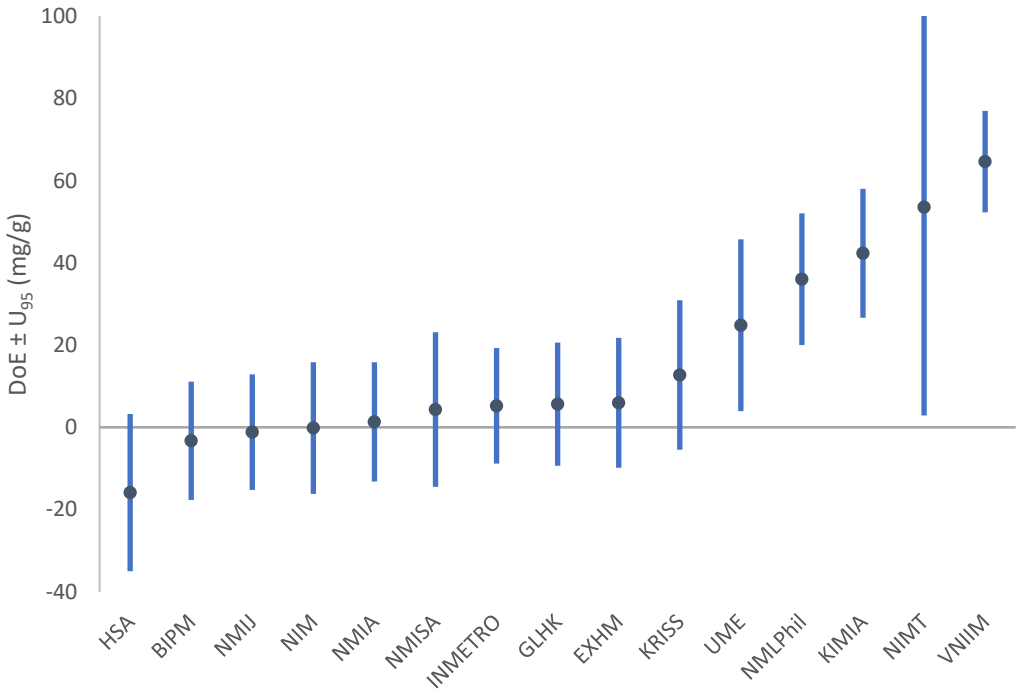


Fig. 12 *Degrees of equivalence and expanded uncertainties of CCQM-K179 results reported by participants.*

Table 12. *Degrees of equivalence and expanded uncertainties for CCQM-K179 results expressed in mg/g and relative to the KCRV.*

Participant	DoE (mg/g)	DoE (%)	DoE U_{95} (mg/g)	DoE U_{95} (%)
HSA	-15.9	-1.9	18.5	2.2
BIPM	-3.3	-0.4	13.7	1.6
NMIJ	-1.2	-0.1	13.4	1.6
NIM	-0.2	0.0	15.4	1.8
NMIA	1.3	0.2	13.9	1.6
NMISA	4.3	0.5	18.2	2.1
INMETRO	5.2	0.6	13.4	1.6
GLHK	5.6	0.7	14.3	1.7
EXHM	6.0	0.7	15.1	1.8
KRISS	12.7	1.5	17.5	2.1

UME	24.8	2.9	20.3	2.4
NMLPhil	36.0	4.2	15.4	1.8
KIMIA	42.3	5.0	15.0	1.8
NIMT	53.5	6.3	50.0	5.8
VNIIM	64.6	7.6	11.7	1.4

The degrees of equivalence for the additional impurity measurands reported by participants are shown in Table 13. Further details about the calculated impurity reference values can be found in the report of the parallel CCQM-K148.b comparison.[3]

Table 13. *Degrees of equivalence and expanded uncertainties of CCQM-K179 results for the mass fraction assignment of OTC.HCl and the three major impurity subclasses. Results that agree or disagree with the corresponding KCRV are indicated in green or red, respectively.*

Measurand →	**OTC.HCl**		**H_2O**		**Cl^-**		**SRI**	
Participant ↓	**DoE (mg/g)**	**DoE U_{95} (mg/g)**	**DoE (mg/g)**	**DoE U_{95} (mg/g)**	**DoE (mg/g)**	**DoE U_{95} (mg/g)**	**DoE (mg/g)**	**DoE U_{95} (mg/g)**
HSA	-15.9	18.5	2.3	9.8	0.9	6.6	2.7	15.3
NMISA	4.3	18.2	-6.6	7.7	-5.0	5.7	23.7	15.0
BIPM	-3.3	13.7	6.2	9.6	0.0	5.0	-2.9	10.3
NMIJ	-1.2	13.4	2.9	8.1	-0.5	4.9	-6.2	11.3
NMIA	1.3	13.9	3.4	9.9	0.5	11.6	-4.5	10.1
NIM	-0.2	15.4	-14.2	10.0	1.6	5.1	8.9	10.8
GLHK	5.6	14.3	-1.3	13.3	1.2	6.5	-8.7	11.0
INMETRO	5.2	13.4	-2.3	6.7	4.1	5.3	-7.4	11.0
EXHM	6.0	15.1	1.2	6.5	-1.1	5.5	-5.3	11.2
UME	24.8	20.3	-30.7	6.2	-2.2	5.2	9.3	10.1
KRISS	12.7	17.5	-26.0	7.0	1.1	5.0	-2.4	10.1
KIMIA	42.3	15.0	-34.7	9.9	8.8	6.7	-4.3	11.4
VNIIM	64.6	11.7	-41.8	6.6	9.3	6.3	-20.6	10.2
NIMT	53.5	50.0	-45.1	33.5	-1.9	10.6	-7.1	10.1
NMLPhil	36.0	15.4	-46.1	7.2	5.1	7.6	8.5	13.9

USE OF CCQM-K179 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K179 demonstrates the measurement capabilities in determining the mass fraction of organic compounds in the form of salt materials, with molar mass in the range of 75 g/mol to 500 g/mol, having high polarity (pKow > -2), including compounds presenting significant hygroscopicity, in an organic solid material.

Depending on the characterization procedure applied, the participants demonstrated capabilities for organic purity assignment by a mass balance or qNMR approach or by the combination of results obtained using both methods.

Core Competency Statements and CMC support

Appendix G lists the tables containing the Core Competencies claimed by the participants in CCQM-K179. The information in these tables was provided by the participants. Details of the analytical methods used by each participant in this study are provided in Appendix E.

Five out of fifteen participants reported values for the mass fraction of oxytetracycline hydrochloride in the comparison material that did not agree with the KCRV (Fig. 12). NIMT, NML-Phil, VNIIM, UME, NIM, KRISS and KIMIA underestimated the water content (Table 13) due to insufficient sample equilibration at ambient humidity, resulting in higher OTC.HCl purity values for these laboratories, except NIM and KRISS. NIM water underestimation was sufficiently small to be counterbalanced by the slightly higher reported SRI content while KRISS agreement with the KCRV could be due to their specific purity calculation method that effectively ignored water content and only considered Cl^- and SRI measurements (Appendix F). In fact, this calculation may also explain the disagreement between CCQM-K148.b mass balance-based results and CCQM-K179 results observed for KRISS in Fig. 9.

NMISA overestimated the related impurity content in the comparison material in relation to the consensus value (62 ± 5.6 mg/g vs. 38.3 ± 5 mg/g, k=1). The laboratory identified the impurities isochlortetracycline and chlortetracycline at 34.4 mg/g and 6.8 mg/g, respectively, neither of which was observed by any other participant. However, their OTC.HCl mass fraction value was consistent with the KCRV, likely because their reported qNMR result was not corrected for the major impurity outlier isochlortetracycline as quantified by LC-UV.

Eleven laboratories used qNMR, either as confirmatory method, standalone method or in combination with mass balance (Table 5). The resonance signals mostly used for quantification were those in the aromatic region induced by protons H-7, H-8 and H-9 (Appendix E). Signals at

3.8 ppm (H-5) and 1.6-1.8 ppm (C-CH3) were also used by a few participants. The signal at 4.3 ppm (H-4) was described by some participants as unsuitable for quantification due to hydrogen-deuterium exchange with the solvent. However, HSA recognized the potential lability of the H-4 proton and controlled the analysis conditions performing NMR analysis with 1-2 hours after sample dissolution. Their results obtained using H-4 were cross-checked with those quantified using the methyl protons in 0.01N DCl in D_2O and found to be comparable.

CONCLUSIONS

The reported values from the fifteen CCQM-K179 participants for the OTC hydrochloride salt mass fraction agreed within ca. 8 %.

Water content values presented the highest variability, seemingly reflecting the challenge of measuring significantly hygroscopic materials. Hygroscopicity did not only appear to affect mass balance results, but also qNMR results as sample preparation required special attention, e.g., sufficient equilibration. The equation provided in the protocol to standardize mass determinations to the values expected at 50 % relative humidity had little impact on the results since most laboratories worked under relative humidity conditions close to the reference value of 50 %.

A consistent set of nine structurally related impurities were identified by two or more participants, with one predominant impurity identified by eight participants as 2-acetyl-2-decarbamoyl-oxytetracycline. A good agreement on the chloride content (± 15 mg/g) and negligeable amounts of volatiles and inorganics were found by the participants.

The reporting of chloride ion content presented some disparity, with a few participants reporting residual chloride, i.e. the counterion of structurally related impurities, and most participants reporting total chloride, regardless of whether it was the counterion of oxytetracycline (therefore part of the measurand) or that of the related impurities. Nonetheless, residual chloride could be calculated for all participants and was found at levels ranging from 1 to 8 mg/g, with a consensus value around 3 mg/g consistent with all related impurities being present as hydrochloride salts.

Participants in CCQM-K179 demonstrated and benchmarked their ability to assign the mass fraction content of a polar and significantly hygroscopic solid organic compound, in the form of a salt material, present as the primary component in an organic material. Results from five participants were not consistent with the KCRV within the combined 95% expanded uncertainty range of the unilateral degree of equivalence due mainly to the underestimation of the water content in the material.

ACKNOWLEDGEMENTS

The study coordinators thank the participating laboratories for the extensive and comprehensive investigations undertaken to characterize the comparison material and their co-operation in checking the data and providing the information reported in this study in a timely manner.

REFERENCES

(1) *CCQM Working Group on Organic Analysis: Strategy 2021-2030*.

(2) Granados-Chinchilla, F.; Rodríguez, C. Tetracyclines in Food and Feedingstuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. *Journal of Analytical Methods in Chemistry* **2017**, *2017*, 1–24. https://doi.org/10.1155/2017/1315497.

(3) *CCQM-K148.b. Mass Fraction of Oxytetracycline in Solid Organic Material*.; 2024.

APPENDIX A: Call for Participation and comparison protocol

CCQM-K179
Polar analyte in solid organic material:
Mass fraction of oxytetracycline hydrochloride salt

Key Comparison
Track C

Study Protocol
[October 2022]

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INTRODUCTION

Oxytetracycline (OTC) is a member of the tetracyclines group of broad-spectrum antibiotic compounds, widely used in veterinary medicine, that have a common basic structure. Because of concerns with the potential health risk to the consumer of long-term exposure to low levels of these compounds, monitoring programs for the presence of tetracycline residues in food of animal origin including meat, fish, milk, eggs and honey are in place in many countries.[1] These activities, which reduce the potential for trade barriers in this area, need to be supported by a sound reference measurement infrastructure for tetracycline analysis.

This comparison underpins core competencies of National Metrology Institutes (NMIs) for the mass fraction value assignment of high purity organic substances containing a salt-forming analyte as the primary component (molar mass between 75-500 g/mol). Evidence of successful participation in formal, relevant international comparisons is required to establish measurement capability claims (CMCs) made by NMIs and Designated Institutes (DIs). with active programmes in organic analysis.

Food safety continues to be a priority sector of the OAWG for the 2021-2030 period. The WG strategy document [2] includes pure organics that are salt materials as a proposed area for Track C comparisons. CCQM-K179 Track C key comparison is to be conducted in 2022 on the value assignment of the mass fraction content of oxytetracycline hydrochloride salt. This comparison is run in parallel with CCQM-K148.b (mass fraction assignment of oxytetracycline free base) on the same material.

TIMELINE

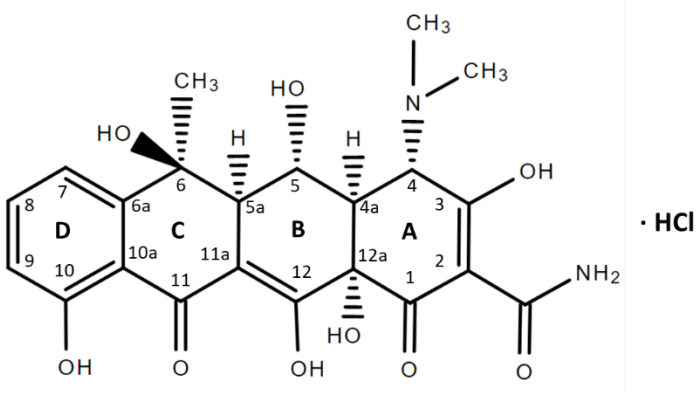
Table 1 lists the timeline for the proposed study.

Table 1:

Date	**Action**
April 2021	Sample Preparation
January 2022	Homogeneity and Stability Testing completed
October 2022	Call for participation to OAWG members
November 2022	Sample Distribution completed
March 2023	Deadline for Submission of Results
April 2023	Preliminary Discussion of Results

MEASURANDS

The comparison will require the assignment of the mass fraction, reported in mg/g, of the oxytetracycline hydrochloride (OTC.HCl) salt in a unit of the comparison material under standardized conditions of relative humidity. Fig. 1 below displays the molecular structure of the oxytetracycline hydrochloride salt (4-*S* epimer).



Oxytetracycline hydrochloride salt (OTC.HCl)

Molar mass = 496.89 g/mol; pK_{OW} ~ 0.5

Fig. 1: Structure and conventional numbering of oxytetracycline hydrochloride

STUDY MATERIAL

The comparison material was produced by TÜBITAK-UME. A bulk source material of OTC.HCl in the form of a fine yellow crystalline powder was homogenized in a 3D mixer and kept in a vacuumed container until filling to minimize moisture uptake. About 0.5 g of the material were filled into each of the glass vials that constitute the comparison batch using an automatic filling machine.

Each participant will receive as a minimum two vials of the comparison material, each containing a minimum of 500 mg of OTC.HCl. Participants who plan to use multiple independent methods to contribute to their final property value assignment (e.g. a mass balance procedure and a separate qNMR procedure) can request an additional vial. The comparison samples will be provided in amber glass vials fitted with PTFE-lined, screw-caps. They should be placed in storage at 4°C in the dark upon receipt.

Vials should be equilibrated to the laboratory's ambient temperature prior to opening. The material is hygroscopic. Prior to any gravimetric operations and sampling of the bulk material the vial must be allowed to equilibrate at the laboratory ambient relative humidity (preferably maintained in the range 42-80%). Measurement results are to be reported on the material as received without additional treatment and taking into account the hygroscopicity correction described below.

Recommended Minimum Sample Amount

A minimum sample amount for analysis of 10 mg is recommended to reduce to a negligeable level the potential for an influence due to between-vial inhomogeneity on the determination of the major component.

Hygroscopicity correction – IMPORTANT!

OTC.HCl has been demonstrated to be significantly hygroscopic. Fig. 2 shows the reversible sorption/desorption of water from a sample of the material as a function of relative humidity (RH) and time. The figure also shows a model for the relationship between the observed mass at equilibrium at a specific RH in the range RH 40% - RH 80%. This corresponds to a relative increase of mass of a sample of the comparison material due solely to water sorption by approximately 0.4% for every 10% increase in the ambient RH (within the range RH 40% to RH 80%).

A vial used as a source of material for measurements should be equilibrated to the laboratory's ambient conditions of temperature and relative humidity (RH) prior to opening. The relative humidity in a laboratory where gravimetric or water content measurements of the material are undertaken should be maintained as far as possible in the range RH 42% - RH 80%.

Weighing protocol and correction for relative humidity

As a result of the hygroscopicity of the material, a given mass will contain a varying amount of water as a function of the ambient humidity when the sample mass was determined. It will not be feasible for each participant laboratory to operate under identical conditions of RH. As a result, in order to obtain a valid comparison of results between participants, it will be necessary to correct all mass determinations to the value expected for that sample at an agreed reference RH and to use this standardized value in all subsequent calculations.

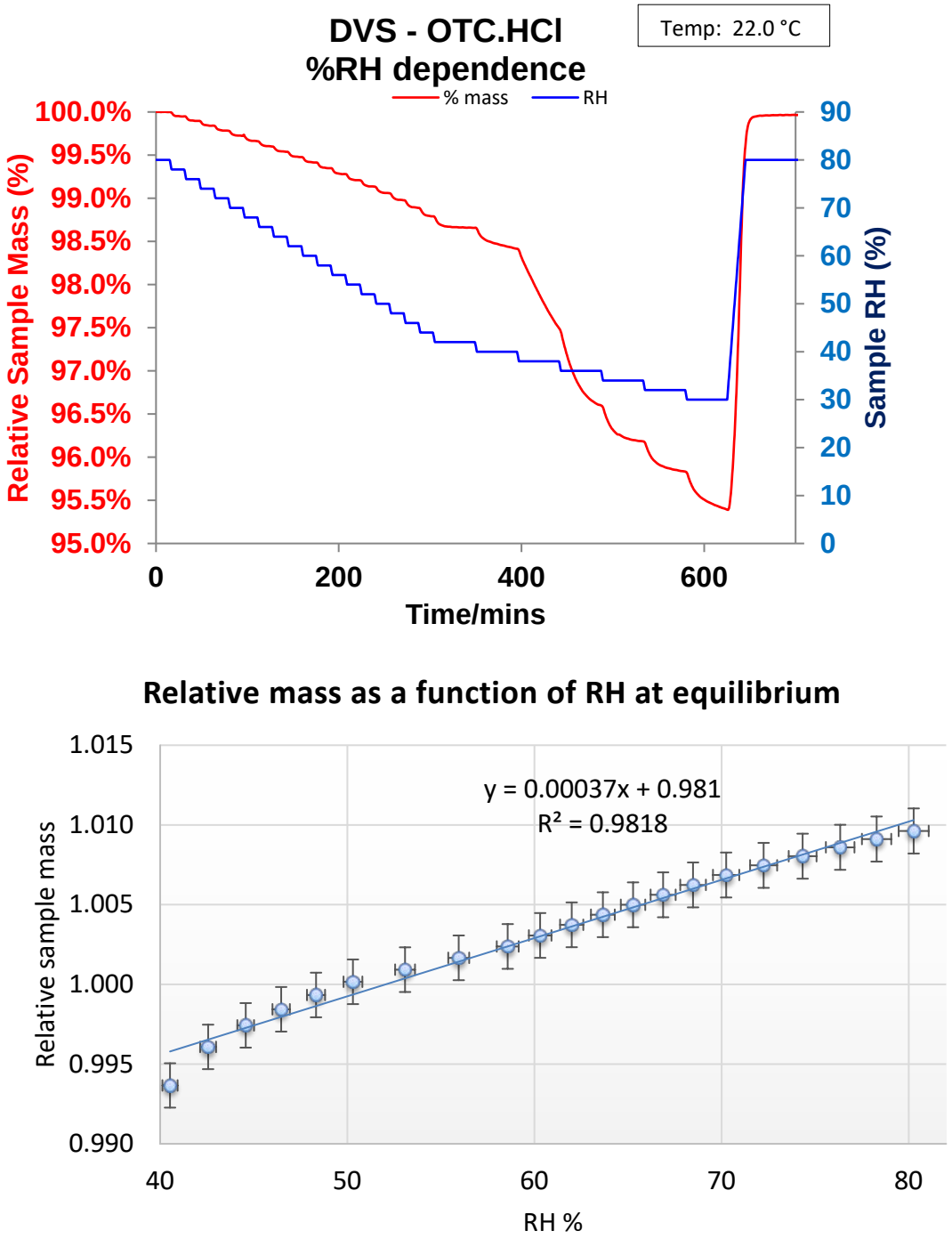


Fig. 2 Water sorption (% mass change) as a function of time and %RH for oxytetracycline HCl salt (Top) and the calculated linear regression function modelling the relationship between the sample mass at equilibrium and the %RH (Bottom).

The environmental relative humidity (RHx) at which each weighing was undertaken must be monitored and recorded. Each aliquot needs to equilibrate at the ambient RHx before placing it in

a balance pan in order to achieve a stable weighing value. In our experience the time required to reach equilibration varies depending on the size of the aliquot and it may take more than 60 min.

The observed mass of sample (m_{RH_X}) recorded at the ambient RH_X shall be normalized to the expected mass of the same sample at RH 50 % ($m_{RH_{50}}$) using the equation:

$$m_{RH_{50}} = \frac{m_{RH_X}}{1 + F(RH_X - 50)} \qquad Eq.1$$

Where F = 0.00037 and u(F) = 0.00003

For the calculation, RH_X is the numerical value of the environmental relative humidity when expressed as a percentage. The application of the equation is appropriate within the 42% RH - 80% RH range. Outside these limits assignments of m_{RH50} become less accurate. Participants are advised to verify the accuracy of their relative humidity measurements.

The standardized value, $m_{RH_{50}}$, must be used for subsequent calculations (mass balance, qNMR).

Example of Mass Standardization for Hygroscopicity

A sample of the material is weighed to a constant final mass of 11.80 mg in a laboratory where RH_X is 42%. In this case RH_X = 42 and:

m_{RH_X} = 11.80 mg

$$m_{RH_{50}} = \frac{11.80}{1 + 0.00037(42 - 50)} = 11.83\ mg$$

i) For calculations of OTC.HCl content by qNMR, structurally related impurities, chloride ion, etc (i.e. all measurements <u>other</u> than water content), the standardized value for m_{RH50} of 11.83 mg should be used as the mass of the sample for subsequent calculations.

ii) For assignment of water content a more careful correction is required. For example:
   a. the sample of total mass 11.80 mg of CCQM-K179 at RH 42% has an observed mass fraction content of water of 30.0 mg/g.*
   b. the amount of water in 11.80 mg of CCQM-K1179 with mass fraction content 30.0 mg/g at RH 42% corresponds to (11.80*0.030) mg or 0.354 mg
   c. absolute water content estimated for the sample if measured at RH 50% equals 0.354 mg (content at RH 42%) adjusted for the value of the difference between m_{RH42} and m_{RH50} of (11.83 - 11.80) mg or +0.030 mg
   d. absolute water content of the sample at RH 50% is 0.384 mg (0.354 + 0.030) mg
   e. final reported value for mass fraction water content of CCQM-K179 based on this sample, corrected to RH 50%, is 32.5 mg/g (= 0.384/11.83)

* Please note that the reported value for water content of the CCQM-K179 material used in the example above is purely hypothetical and must not be regarded in any way as an indication of the true water content of the material.

Homogeneity Assessment of Study Material

The homogeneity of the batch was tested using an LC-UV method for the content of OTC and main structurally related impurities. An oven-transfer, coulometric Karl Fisher titration was used for water content and ion chromatography for chloride ion content. The uncertainty contribution due to inhomogeneity of the assigned values was evaluated by ANOVA. Ten vials were selected at regular intervals from the filling sequence to ensure that the results would indicate any trend in the filling process. Each vial was analyzed in a random order to ensure any trends in the bottling process were separated from possible trends resulting from the analytical sequence.

The results obtained indicated no statistically significant difference in the within- and between-vial levels of the mass fraction of each component in the material. The upper limit for the uncertainty contribution due to inhomogeneity in all cases was sufficiently small as to be unlikely to influence the effective comparison of participant results. A summary of the observed within- and between-sample variability for the major components is shown in Table 2:

Table 2. Homogeneity assessment for OTC, chloride, major structurally related impurity and water in the comparison material.

ANOVA Estimate	**OTC**	**Imp A**	**H_2O**	**Cl^-**
Between-unit CV (%)	0.36%	0.77%	0.64%	0.87%
Within-unit CV (%)	0.83%	1.10%	1.03%	1.44%
Upper limit of relative uncertainty contribution due to inhomogeneity	0.27%	0.43%	0.37%	0.47%
Probability of falsely rejecting the hypothesis that all samples have the same concentration	< 5%	< 5%	< 5%	< 5%

A plot of the normalized mass fraction for each analyte obtained for the homogeneity assessment is plotted by filling sequence in Fig. 3. The normalized values of repeat measurements from three aliquots taken from each individual vial are plotted.

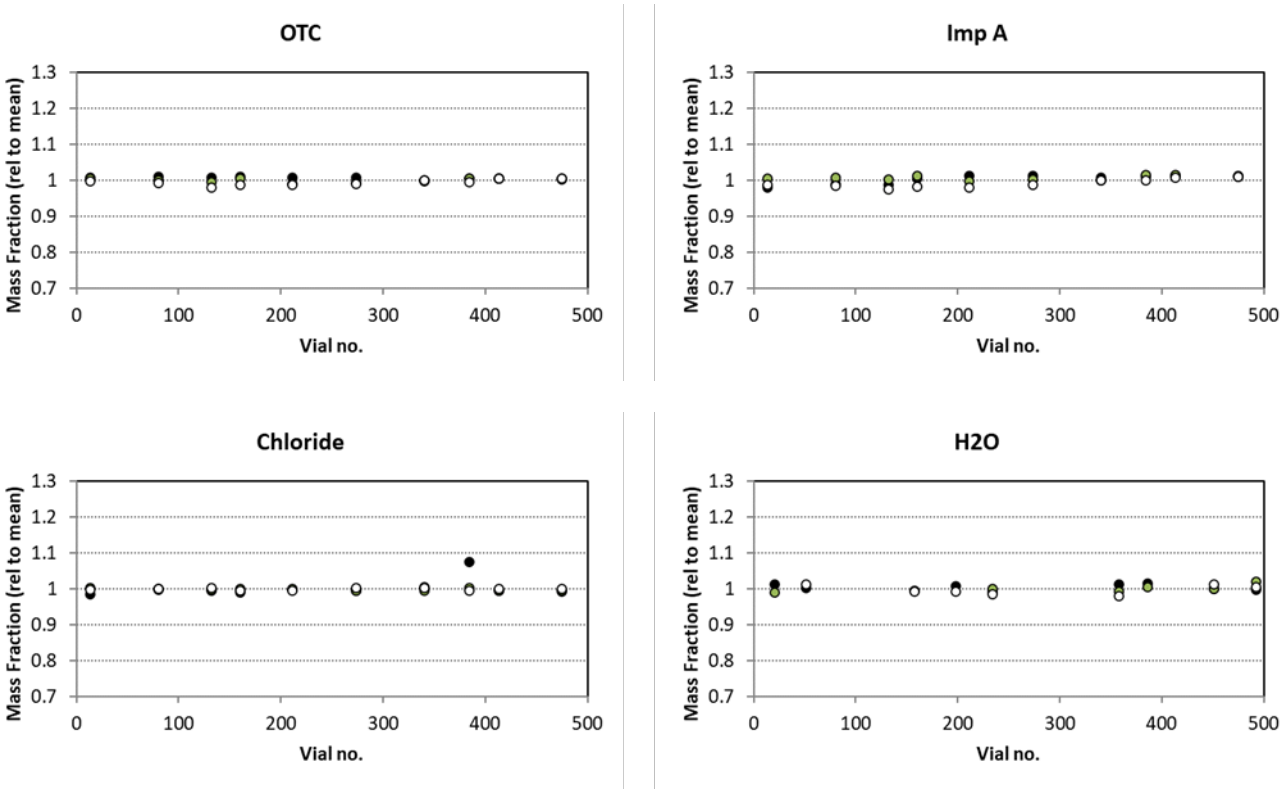


Fig. 3 Homogeneity evaluation for OTC, chloride, major structurally related impurity A and water in the comparison material.

Stability Assessment of Study Material

An isochronous stability study was undertaken for OTC, structurally related impurities, water and chloride on storage at 4 °C, 22 °C and 40 °C in the dark. The analytical methods used were the same as in the homogeneity study. The material is sufficiently stable, within the proposed time scale of the comparison, when stored at 4 °C or 22 °C. OTC and some impurities were not stable at 40 °C. Precautions will be taken to monitor if the comparison material is exposed to temperature above 30 °C during shipment and if this occurs replacement material will be provided.

The mass fractions of OTC and chloride relative to the mean value of reference samples stored at -20 °C are shown in Fig. 4 for samples stored at 4 °C and 22 °C during the stability study period. The plot displays the normalized results of duplicate analysis of samples prepared from two units of CCQM-K179. The upper and lower dashed lines indicate the uncertainty of the regression line, which reflects the analytical method variance in the absence of a significant instability trend.

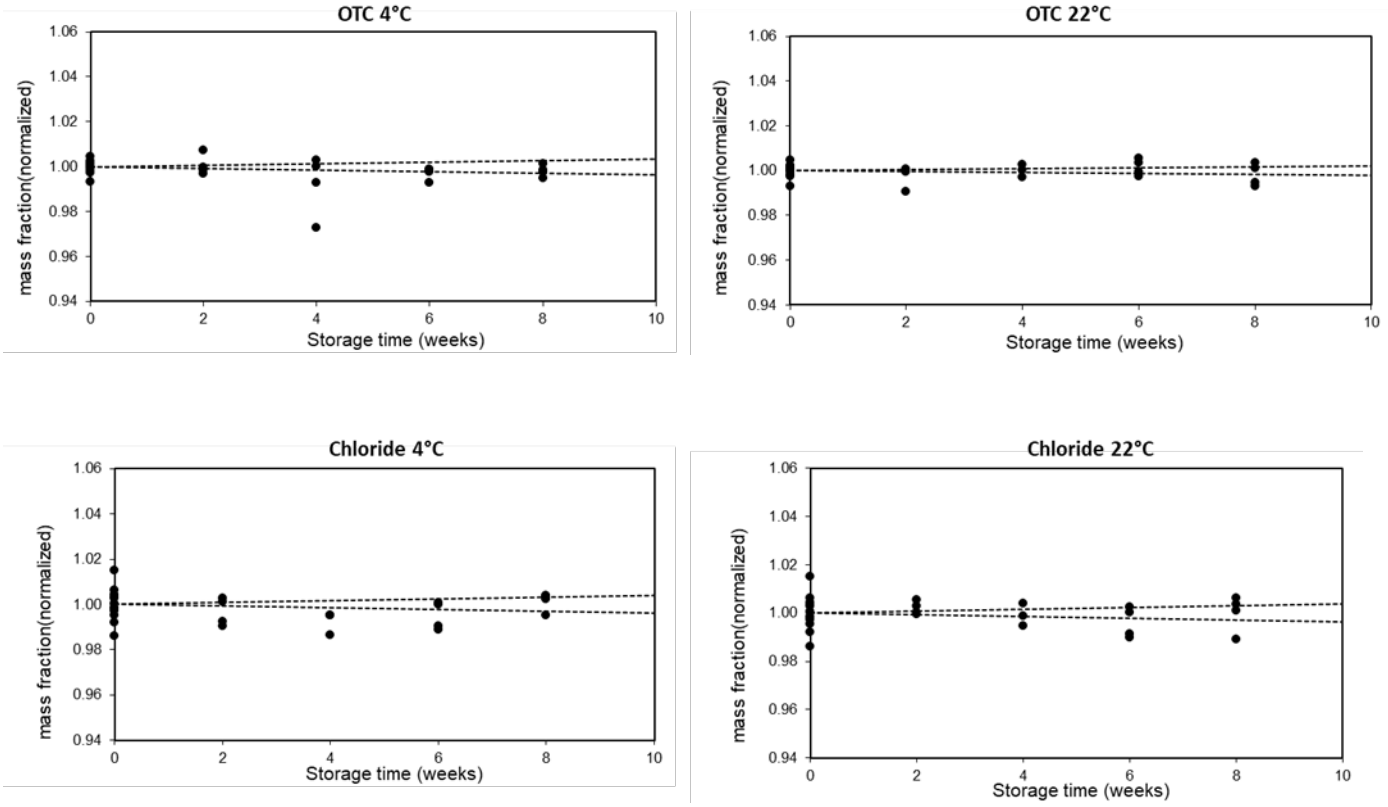


Fig. 4 Stability evaluation of OTC and Chloride content in samples stored at 4 °C and 22 °C for 8 weeks.

INSTRUCTIONS AND SAMPLE DISTRIBUTION

Participants are requested to notify the comparison coordinator of specific requirements for shipment documentation required to facilitate customs clearance into their country and to liaise with the coordinating laboratory during the delivery process.

Participants will be notified by the coordinating laboratory in advance of the shipment of the materials and will be given details of the carrier used for the shipment.

Participants will be asked to return a form acknowledging receipt of the samples, to advise the comparison coordinator of any damage to the vials during shipping, and to indicate based on a monitoring strip included with the shipment whether the shipping container had been exposed to a temperature in excess of 30 °C during the transport process.

RESULTS

Participants are required to report their estimate of the mass fraction of OTC.HCl as the salt content present in the material in mg/g, standardised to the value expected at 50% RH. The result should be based on combined values obtained by the measurement of multiple aliquots from at least one of the vials supplied. Participants are also required to verify the accuracy of their relative humidity measurements.

There is no restriction on the use of methods to obtain data to assign the mass fraction content of OTC.HCl in the comparison material, but only one overall result can be submitted by each participant.

In addition to the quantitative results, participants will be instructed to describe their analytical methods, approach to uncertainty estimation, and the Core Competencies they felt were demonstrated in this study.

An electronic data submission form will be supplied. The draft result reporting spreadsheet is attached to this protocol (Annex A).

The following information **shall** be included in the result reporting form:

- Laboratory information;
- Names of staff for inclusion as contributing authors in the Final Report of the comparison;
- Temperature and relative humidity in area(s) where gravimetric operations are performed and water content measurements are undertaken;
- Primary Component giving the mass fraction content of OTC.HCl salt (in mg/g) estimated if measured at RH = 50% with the combined standard uncertainty and the expanded uncertainty at a 95% confidence range;
- Measurement equation and uncertainty budget for the OTC.HCl assignment.

Participants using a mass balance approach as either the sole or a contributing method to their overall value assignment **shall** in addition report the Secondary Component (Impurity) levels in the material by providing assigned values and the associated standard uncertainty for each secondary component estimated if measured at RH = 50% contributing to the assignment of the mass fraction and standard uncertainty of OTC.HCl. This table shall include assignments for some or all of:

- total structurally related impurities
- water
- residual organic solvent
- chloride ion
- other non-volatiles/inorganics

It is noted that, due to the hygroscopicity of oxytetracycline salt, reporting the value adjusted for measurement at RH = 50% is particularly important for the value of the water content.

A representative chromatogram from analysis of a sample solution shall also be provided where HPLC-based methods are used to evaluate the structurally related impurity content.

Participants **may** provide further information supporting a claim for a generic water content measurement competency linked to the results obtained for this material (for those institutes wishing to make CMC claims for water content).

Participants using a qNMR approach as a contributing method to their final value assignment **shall** provide information on the:

- deuterated solvent(s) used;
- standard(s) (internal or external)
  - name and source
  - purity and associated uncertainty (in mg/g)
  - basis for the traceability of the purity of the standard(s);
- balance for gravimetric sample preparation:
  - make, model and resolution
  - repeatability (standard deviation [SD] of at least ten repeat determinations of a tared reference mass [m])
  - minimum sample weight (mass for which 2*SD/m < 0.1%)

Participants using an approach other than mass balance or qNMR as either their sole or as a contributing method to their final value assignment shall also provide a brief outline of the procedure and all critical method parameters.

When a participant combines the results of two or more independent methods to obtain the final value reported for the comparison, the individual results for each method shall be reported. A compilation of all such contributing results, including their degree of equivalence with the KCRV, will be included in an Annex to the Final Report.

USE OF RESULTS FROM CCQM-K179 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K179 will demonstrate the measurement capability for determining the mass fraction of organic compounds in the form of salt materials, with molar mass in the range 75 g/mol to 500 g/mol and having high polarity ($pK_{ow} > -2$), including compounds presenting significant hygroscopicity. If specifically requested, a CMC competency can also be

claimed to be demonstrated for the assignment of water content present at similar levels in comparable polar, hygroscopic organic solids.

Core Competency Statements and CMC support

The template for the potential Core Competency claims arising from successful participation in CCQM-K179 is provided in Annex B below.

REFERENCES

[1] Granados-Chinchilla F, Rodríguez C. Tetracyclines in Food and Feeding stuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. J Anal Methods Chem. 2017;2017:1315497. doi: 10.1155/2017/1315497

[2] CCQM Working group on Organic Analysis: Strategy 2021-2030

Bureau International des Poids et Mesures

APPENDIX B: Registration form

CCQM-K148.b/P187.b & CCQM-K179/P224
Mass fraction of oxytetracycline base (OTC) and oxytetracycline hydrochloride salt (OTC.HCl) in a solid organic material

REQUEST TO REGISTER TO PARTICIPATE IN:

- ☐ **CCQM-K148.b Track A (mass fraction of OTC)**
- ☐ **CCQM-K179 Track C (mass fraction of OTC.HCl)**
- ☐ **CCQM-P187.b (mass fraction of OTC)**
- ☐ **CCQM-P224 (mass fraction of OTC.HCl)**

(Participation in the CCQM-148.b and CCQM-179 comparisons is only permitted for National Metrology Institutes or Designated Institutes recognized under the CIPM MRA)

ORGANIZATION / DEPARTMENT / LABORATORY

[Organization Name]

CONTACT PERSON FOR THE COMPARISON

[Contact person for comparison]

E-MAIL, TELEPHONE

[Contact details]

ADDRESS FOR SHIPMENT OF SAMPLES

[Address details]

CONTACT PERSON FOR SAMPLE DELIVERY (if different)

[Contact details]

E-MAIL, TELEPHONE

[Contact details]

Date _____

Complete and return to gustavo.martos@bipm.org **before October 30, 2022**

APPENDIX C: Reporting Form

CCQM-K179 CCQM-P224 Reporting Form 1.4
Participant identification

CCQM-K179 / CCQM-P224
Mass fraction of Oxytetracycline.HCl **in high purity material**

Data Submission Form

Please complete all pages of the reporting form and submit it by email before March 1, 2023 to: gustavo.martos@bipm.org

Avoid formulas in the fill-in cells (marked in yellow). Mathematical expressions can be inserted using the "Symbols" button in the "Insert" submenu.

Registered comparison participation:	**CCQM-K179**	**CCQM-P224** (delete as appropriate)
Reporting Date		
Institute		
Submitted by (name)		
E-mail address		
Contributing authors for acknowledgement in Final Report:		

CCQM-K179 CCQM-P224 Reporting Form 1.4
Comparison Results

RESULTS

a. Mass Fraction assignment - main component

Measurand	Mass Fraction (mg/g)	Combined Standard Uncertainty (mg/g)	Coverage Factor (k)	Expanded Uncertainty (mg/g)
Oxytetracycline HCl (corrected to RH 50%)				

b. Mass Fraction assignments - impurity components [required for participants using a mass balance procedure, optional otherwise]

Measurand	Mass Fraction (mg/g)	Combined Standard Uncertainty (mg/g)	Coverage Factor (k)	Expanded Uncertainty (mg/g)
Total related structure impurities				
Water content (observed at local RH)				
Water content (corrected to RH 50%)				
Chloride ion				
Total non-volatiles and inorganics				
Volatile organics content				

c. Mass Fraction assignments - individual impurity components [optional]

Measurand	Mass Fraction (mg/g)	Combined Standard Uncertainty (mg/g)	Coverage Factor (k)	Expanded Uncertainty (mg/g)
Impurity 1				
Impurity 2				
Impurity 3				
Impurity 4				
[additional entries as required]				

d. Environmental conditions

Measurement	Temperature (°C)	Relative Humidity (%)
Gravimetric operations		
Water content measurements		

Results 2/10

CCQM-K179 CCQM-P224 Reporting Form 1.4
Analytical Method for Mass Balance procedure

Information about the procedures used

[NB - To complete your entry, please insert additional rows as necessary]

1. Related substance impurity content

Analytical instrumentation used (e.g., LC, GC, GC-MS, etc.)	
Sample amount per analysis (approximate)	mg
Number of samples analyzed	
Sample derivatization (if used)	
Sample preparation (solvent, concentration)	
Chromatographic Columns used (type and manufacturer)	
Chromatographic conditions (e.g., GC temperature program, LC mobile phase and gradient, injection size, numder of samples analyzed, number of replicates per sample)	

CCQM-K179 CCQM-P224 Reporting Form 1.4
Analytical Method for Mass Balance procedure

Assignment method (e.g., relative response, external calibration, internal standard, IDMS)	
Reference standards used (if applicable) (Please specify the compounds, source and role)	
UV wavelength(s) monitored in LC-UV (if applicable)	
SIM/MRM(s) monitored in MS (if applicable)	
Assessment of response factors (as applicable) (Please describe assumptions or investigations into the relative response factors of impurities to the main component. If no information is provided, a 1:1 response factor will be assumed)	
Any other information	

[NB - To complete your entry, please insert additional rows as necessary]

Mass balance method 4/10

CCQM-K179 CCQM-P224 Reporting Form 1.4
Analytical Method for Mass Balance procedure

2.Water content

Sample amount per analysis (approximate)	mg
Number of samples analyzed	
Instrumentation (e.g., coulometric Karl Fischer titration, TGA)	
Analytical conditions	

3. Residual solvent content

Sample amount per analysis (approximate)	mg
Number of samples analyzed	
Instrumentation (e.g., headspace GC, NMR, etc)	
Analytical conditions	

4. Combined non-volatile content

Sample amount per analysis	mg
Number of samples analyzed	
Instrumentation (e.g., TGA, EA, ICP-MS)	
Analytical conditions	

Mass balance method 5/10

CCQM-K179 CCQM-P224 Reporting Form 1.4
Analytical Method for qNMR procedure

Information about the qNMR procedure(s) used

[NB - To complete your entry, please insert additional rows as necessary]

Solvent(s) used	
qNMR procedure (eg. internal standard, external standard, etc.)	
Name and source of standard(s)	
Purity and uncertainty of standard(s)	
Traceability source	

Gravimetry

Type of balance (make, model and resolution)	
Balance repeatability	(µg)
Minimum weight	(mg)

Sample preparation

Smallest mass of analyte	(mg)
Smallest mass of standard	(mg)
Number of independent samples prepared	

CCQM-K179 CCQM-P224 Reporting Form 1.4
Analytical Method for qNMR procedure

Number of replicate analyses per sample	

qNMR parameters

Spectrometer	
Experimental parameters	
Processing software	
Integration parameters	
Lineshape (FWHM of solvent peak)	

Signal/Noise

Standard peak	
Analyte peak	

CCQM-K179 CCQM-P224 Reporting Form 1.4
Analytical Method for qNMR procedure

Other approaches (e.g. CRAFT, QM full spin analysis, etc.)	

[NB - To complete your entry, please insert additional rows as necessary]

CCQM-K179 CCQM-P224 Reporting Form 1.4
Other Analytical Methods

Analytical instrumentation used	
Sample amount per analysis (approximate)	mg
Number of samples analyzed	
Sample preparation	

[NB - To complete your entry, please insert additional rows as necessary]

CCQM-K179 CCQM-P224 Reporting Form 1.4
Value assignment and MU Budget

Contributing results for Oxytetracycline HCl in CCQM-K179 / CCQM-P224

Mass balance result (if used)		mg/g
qNMR result (if used)		mg/g
Other results (if used)		mg/g
Final reported result (as entered in "Results" Worksheet)		mg/g

Measurement equation
Describe both:
1. Measurement equation for individual methods

2. Measurement equation for combination of values if results of two or more methods were combined for the assignment

Uncertainty budget
(please include breakdown of the budget, describing major individual uncertainty contributions and how they were combined)

[NB - To complete your entry, please insert additional rows as necessary]

APPENDIX D: Core Competency Table

CCQM-K179	*NMI*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification		*Summary of methods used to establish the qualitative identity (e.g., comparison with independent sample, mass spec., NMR, other)*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179		*Indicate method(s) used to quantify mass fraction of OTC in the material*
Oxytetracycline.HCl content (mg/g)		*Reported comparison result (± $U_{95\%}$)*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity		*Indicate method(s) used to quantify mass fraction of structurally related impurities in the material*
Total structurally related impurity (mg/g)		*Reported comparison result (± $U_{95\%}$)*
Assignment of water content		*Indicate method(s) used to quantify mass fraction water content in the material*
Category of water content assignment*		*Select from list below* the applicable category of general water content assignment competency*
Water content (mg/g)		*Reported comparison result (± $U_{95\%}$)*
Assignment of residual solvent content		*Indicate method(s) used to quantify mass fraction residual solvent content in the material*
Total residual solvent (mg/g)		*Reported comparison result (± $U_{95\%}$)*
Assignment of inorganic content		*Indicate method(s) used to quantify mass fraction total non-volatile content in the material*
Total non-volatiles (mg/g)		*Reported comparison result (± $U_{95\%}$)*

General Instructions:

- Replace "*NMI*" with the acronym for your institution in the first cell of the middle column
- Place a tick, cross or N/A (not applicable) in each middle column cell as appropriate for each competency
- In each right hand column cell replace the blue text with the relevant information for your comparison result

* To be completed by NMIs intending or anticipating to make CMC claims for the assignment of water content in solid organic materials. Choose one of the following categories:

- polar organic solid, water content < 20 mg/g
- polar organic solid, water content > 20 mg/g

APPENDIX E: Summary of participants' analytical information

Methods in brackets used as supporting evidence, not for reporting.

Participant	SRI[1]	Water[2]	Chloride[3]	VOC	Inorganic	OTC.HCl - qNMR[4]
HSA	LC-UV 275 nm, RR (LC-UV 254, LC-MS/MS)	KFT-DA (KFT-OT)	IC (TQ-ICP-MS)	qNMR (GC-MS)	TGA, ICP-MS	MA, AceK, BA, BTFMBA (4.3 ppm)
NMISA	LC-UV 272 nm, DC	KFT-OT 125°C	IC	GC-MS	TGA	TCNB (6.9, 7.1 ppm)
BIPM	LC-UV 275 nm, DC	KFT-OT 170°C	IC	qNMR	IC	
NMIJ	LC-UV 270 nm, LC-CAD, DC (LC-hrMS)	KFT-OT 120°C	IC	GC-FID	TGA, IC	BTFMBA (1.8 ppm)
NMIA	LC-UV 254 nm, RRF (LC-UV 270 nm)	KFT-DA	IC	GC-MS, NMR	TGA (qNMR, EA)	MA (6.7-7.8 ppm)
NIM	LC-UV 270 nm, DC	KFT-DA	IC	GC-FID (GC-MS)	ICP-MS	Ethylparaben (1.8 ppm)
GLHK	LC-UV 270 nm, RR (LC-hrMS)	KFT-OT 160°C	ICP-MS	qNMR	TGA, ICP-MS	BTFMBA (3.8 ppm)
INMETRO	LC-UV 270 nm, RRF (LC-MS/MS)	KFT-DA	XRF	TGA, qNMR (GC-MS)	ICP-OES, ICP-MS	MA, TCNB (6.7-7.8 ppm)
EXHM	LC-UV (CAD) 254 nm, RRF (LC-MS)	KFT-OT 140°C , KFT-DA	IC	GC-MS, GC-FID	ICP-MS	MA (3.8 ppm)
NMLPhil	LC-UV 270 nm, RR	KFT-OT 160°C	IC	TGA	TGA	
UME	LC-UV 275 nm, RR	KFT-OT 160°C	IC	GC-FID (NMR)		BA (7.0 ppm)
KRISS	LC-UV 270, 355 nm, RR (355 nm) (LC-MS)	KFT-OT 150°C	IC	GC-MS	TGA	BA (6.8-7 ppm)
KIMIA	LC UV 270, 288, 355 nm, RR	KFT-DA (TGA)	IC	GC-FID (GC-MS, TGA)	TGA	
VNIIM	LC-UV 254 nm, DC, RRF	KFT-OT 150°C	CE-UV 374 nm	GC-FID (GC-MS)	TGA	
NIMT	LC-UV 355 nm, RR	KFT-OT 160°C		TGA		TCNB (6.8-7.7 ppm)

Notes:

1) *Assignment methods: RR (relative response); RRF (relative response with estimation of response factors), DC (direct calibration), SA (Standard addition).*
2) *Karl Fischer titration (KFT) with direct sample addition (DA) or oven transfer (OT) at specified temperature.*
3) *Ion chromatography (IC), Inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence (XRF), Capillary electrophoresis with UV detection (CE-UV) at specified wavelength.*
4) *Internal standard(s) used (chemical shift of integrated oxytetracycline signal used for quantification).*

APPENDIX F: Summary of measurement equations and uncertainty budgets

Participant: HSA

Measurement equation for mass balance approach:

Approach 1: Mass fraction of oxytetracycline HCl (mg/g) was calculated using the equation below:

$m_{MB\ (HCl)\_1} = (1000 - I_{RSI}) \times (1000 - F_{Others})/1000$ *(1)*

where,
I_{RSI} is the mass fraction (mg/g) of total structurally related impurities determined by HPLC-DAD;

F_{Others} is the sum of mass fraction (mg/g) of other impurities.

$I_{RSI} = I_{LC\text{-}DAD} + I_{NR} + I_{ND}$ *(2)*

where,
$I_{LC\text{-}DAD}$ is the mass fraction (mg/g) of total structurally related impurities detected by HPLC-DAD;
I_{NR} is the mass fraction (mg/g) of non-resolved organic impurities in HPLC-DAD (has a value of zero but has an associated uncertainty estimated from LOQ);

I_{ND} is the mass fraction (mg/g) of non-detected organic impurities in HPLC-DAD (has a value of zero but has an associated uncertainty estimated from LOD).

$F_{Others} = F_{VO} + F_W + F_{IR}$ *(3)*

where,
F_{VO} is the mass fraction (mg/g) of residual organic solvent;
F_W is the mass fraction (mg/g) of water;
F_{IR} is the mass fraction (mg/g) of total non-volatiles/inorganics.

Approach 2: Mass fraction of oxytetracycline HCl (mg/g) was calculated using equation below:

$m_{MB\ (HCl)\_2} = m_{MB(base)} \times MW_{oxytetracycline\ HCl}/MW_{oxytetracycline\ base}$ (4)

where,
$m_{MB\ (base)}$ is the mass fraction (mg/g) of oxytetracycline free base (obtained from mass balance approach as reported in CCQM-K148.b)
$MW_{oxytetracycline\ base}$ is the molecular weight of oxytetracycline *free base*
$MW_{oxytetracycline\ HCl}$ is the molecular weight of oxytetracycline HCl

The final mass fraction of oxytetracycline HCl (mg/g) using mass balance is obtained from the arithmetic mean of the two results, i.e. $m_{MB\ (HCl)\_1}$ and $m_{MB\ (HCl)\_2}$. (5)

Measurement equation for qNMR approach:
Mass fraction of oxytetracycline HCl (mg/g) was calculated using the equation below:

$m_{qNMR} = P_{ISTD} \times (I_X / I_{ISTD}) \times (n_{ISTD} / n_X) \times (M_X / M_{ISTD}) \times (m_{ISTD} / m_X)$ *(6)*

where,

Appendix F: Summary of measurement equations and uncertainty budgets

P_{ISTD}: mass fraction of internal standard (mg/g)
I_X: integral area of quantification peak of analyte
I_{ISTD}: integral area of quantification peak of internal standard
n_{ISTD}: number of protons of the quantification peak of internal standard
n_X: number of protons of the quantification peak of analyte
M_X: molecular weight of analyte (oxytetracycline HCl) (g/mol)
M_{ISTD} molecular weight of internal standard (g/mol)
m_{ISTD} mass of internal standard (g)
m_X: mass of study sample (g)

$$m_X = m_{RH_{50}} = \frac{m_{RH_X}}{1 + 0.00037(RH_X - 50)} \qquad (7)$$

The final mass fraction of oxytetracycline HCl (mg/g) using qNMR is obtained from the arithmetic mean of the four results, i.e. using acesulfame potassium as ISTD in 0.01 N DCl D2O, using maleic acid as ISTD in 0.01 N DCl D2O, using benzoic acid as ISTD in CD3OD and using 3,5 bis(trifluoromethyl)benzoic acid as ISTD in MEOD.

$$m_{qNMR(HCl)} = \frac{m_{qNMR(MA)} + m_{qNMR(AceK)} + m_{qNMR(BA)} + m_{qNMR(BFBA)}}{4} \qquad (8)$$

where,
$m_{qNMR(MA)}$ is the mass fraction of oxytetracycline HCl determined using maleic acid as ISTD in 0.01 N DCl D2O by qNMR,
$m_{qNMR(AceK)}$ is the mass fraction of oxytetracycline HCl determined using AceK as ISTD in 0.01 N DCl D2O by qNMR,
$m_{qNMR(BA)}$ is the mass fraction of oxytetracycline HCl determined using benzoic acid as ISTD in CD3OD by qNMR,
$m_{qNMR(BFBA)}$ is the mass fraction of oxytetracycline HCl determined using 3,5 bis(trifluoromethyl)benzoic acid as ISTD in CD3OD by qNMR.

Measurement equation for final reported result:

$$x_{report} = \frac{m_{MB(HCl)} + m_{qNMR(HCl)}}{2} \qquad (9)$$

where,
x_{report} is the reported mass fraction of oxytetracycline HCl,
$m_{MB(HCl)}$ is the mass fraction of oxytetracycline HCl determined by mass balance approach,
$m_{qNMR(HCl)}$ is the mass fraction of oxytetracycline HCl determined by qNMR approach.

Measurement uncertainty equation for mass balance approach:
The combined standard uncertainty of the mass fraction of the oxytetracycline HCl using mass balance approach 1, $u(m_{MB\ (HCl)\_1})$, is calculated from mathematical equations related to the standard uncertainty of each component (I_{RSI}, F_{VO}, F_W, F_{IR}) and the corresponding sensitivity coefficient:

Appendix F: Summary of measurement equations and uncertainty budgets

$$u(m_{MB(HCl)\_1}) = \sqrt{c_{I_{RSI}}^2 u_{I_{RSI}}^2 + c_{F_{VO}}^2 u_{F_{VO}}^2 + c_{F_W}^2 u_{F_W}^2 + c_{F_{IR}}^2 u_{F_{IR}}^2} \quad (10)$$

The sensitivity coefficients of each component can be expressed as follows:

$$c_{I_{RSI}} = \frac{\delta m}{\delta I_{RSI}} = -(1000 - F_{VO} - F_W - F_{IR} - F_{HCl})/1000 \quad (11)$$

$$c_{F_{VO}} = \frac{\delta m}{\delta F_{VO}} = -(1000 - I_{RSI})/1000 \quad (12)$$

$$c_{F_W} = \frac{\delta m}{\delta F_W} = -(1000 - I_{RSI})/1000 \quad (13)$$

$$c_{F_{IR}} = \frac{\delta m}{\delta F_{IR}} = -(1000 - I_{RSI})/1000 \quad (14)$$

The combined standard uncertainty for the mass fraction of the oxytetracycline HCl using mass balance approach 2, $u(m_{MB\,(HCl)\_2})$, is calculated as follow:

$$u(m_{MB(HCl)\_2}) = m_{MB(HCl)\_2} \times \sqrt{\left(\frac{u(m_{MB(base)})}{m_{MB(base)}}\right)^2 + \left(\frac{u(MW_{oxytetracycline\ HCl})}{MW_{oxytetracycline\ HCl}}\right)^2 + \left(\frac{u(MW_{oxytetracycyline\ base})}{MW_{oxytetracycline\ base}}\right)^2} \quad (15)$$

The combined standard uncertainty for the final mass fraction of oxytetracycline HCl using mass balance is calculated as follows:

$$u(m_{MB(HCl)}) = \sqrt{\left(\frac{u(m_{MB(HCl)\_1})}{2}\right)^2 + \left(\frac{u(m_{MB(HCl)\_2}))}{2}\right)^2 + u_B^2} \quad (16)$$

where,
$u(m_{MB(HCl)\_1})$ is the uncertainty of mass fraction of oxytetracycline HCl determined by mass balance approach 1,
$u(m_{MB(HCl)\_2})$ is the uncertainty of mass fraction of oxytetracycline HCl determined by mass balance approach 2,
u_B is the uncertainty from method bias estimated based on rectangular distribution of the difference between the two results.

<u>Measurement uncertainty equation for qNMR approach:</u>

Appendix F: Summary of measurement equations and uncertainty budgets

In general, the combined standard uncertainty from qNMR approach, $u(m_{qNMR})$ was calculated as follows:

$$u(m_{qNMR}) = m_{qNMR} \times \sqrt{\left(\frac{u(MP)}{m_{qNMR}}\right)^2 + \left(\frac{u(P_{ISTD})}{P_{ISTD}}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(M_x)}{M_x}\right)^2 + \left(\frac{u(m_{ISTD})}{m_{ISTD}}\right)^2 + \left(\frac{u(M_{ISTD})}{M_{ISTD}}\right)^2 + \left(\frac{u(F_{Diff})}{F_{Diff}}\right)^2}$$

(17)

where,
$u(m_{qNMR})$: the uncertainty in mass fraction of oxytetracycline HCl using qNMR approach
u(MP): the uncertainty in method precision
$u(P_{ISTD})$: the uncertainty in the mass fraction of the internal standard
$u(m_X)$: the uncertainty in the mass of sample weighed (including uncertainty of F and RHx in the calculation of m_{RH50})
$u(m_{ISTD})$: the mass of the internal standard weighed
$u(M_X)$: the uncertainty in the molecular weight of the analyte (oxytetracycline HCl)
$u(M_{ISTD})$: the uncertainty in the molecular weight of the internal standard
$u(F_{Diff})$: the uncertainty of the factor representing bias in the results due to different parameters (e.g. neutral vs acidic solvent)

$$u(m_{qNMR(HCl)}) = \sqrt{\left(\frac{u(m_{qNMR(MA)})}{4}\right)^2 + \left(\frac{u(m_{qNMR(AceK)})}{4}\right)^2 + \left(\frac{u(m_{qNMR(BA)})}{4}\right)^2 + \left(\frac{u(m_{qNMR(BFBA)})}{4}\right)^2 + u_B^2}$$

(15)

where,
$u(m_{qNMR(MA)})$ is the uncertainty of mass fraction of oxytetracycline HCl determined using maleic acid as ISTD in 0.01 N DCl D2O by qNMR,
$u(m_{qNMR(AceK)})$ is the uncertainty of mass fraction of oxytetracycline HCl determined using acesulfame potassium as ISTD in 0.01 N DCl D2O by qNMR,
$u(m_{qNMR(BA)})$ is the uncertainty of mass fraction of oxytetracycline HCl determined using benzoic acid as ISTD in CD3OD by qNMR,
$u(m_{qNMR(BFBA)})$ is the uncertainty of mass fraction of oxytetracycline HCl determined using 3,5 bis(trifluoromethyl)benzoic acid as ISTD in CD3OD by qNMR,
u_B is the uncertainty from method biases expressed as the standard deviation of the results from the four methods.

<u>Measurement uncertainty equation for final reported result:</u>

$$u(x_{report}) = \sqrt{\left(\frac{u(m_{MB(HCl)})}{2}\right)^2 + \left(\frac{u(m_{qNMR(HCl)})}{2}\right)^2 + u_B^2} \quad (16)$$

where,
$u(m_{MB(HCl)})$ is the uncertainty of mass fraction of oxytetracycline HCl determined by mass balance approach,
$u(m_{qNMR(HCl)})$ is the uncertainty of mass fraction of oxytetracycline HCl detenasermined by qNMR approach,
u_B is the uncertainty from method bias estimated based on rectangular distribution of the difference between the two results.

Appendix F: Summary of measurement equations and uncertainty budgets

Table 1. Uncertainty budget for oxytetracycline HCl using mass balance approach 1

Parameter	Source of data	Source(s) of uncertainty	Results			Type of uncertainty	Contribution to overall U, %
			I_w, mg/g	u_x, mg/g	c_x		
I_{RSI}	HPLC-DAD	The combined standard uncertainty was calculated from the standard deviation of results in HPLC-DAD measurement using ODS-AQ column, differences in results obtained using two columns (ODS-AQ vs C8) divided by two, differences in results obtained using 275 nm and 254 nm divided by two.	49.5	4.3	0.8936	Combined Type A uncertainty	19.87
		Standard uncertainty of organic impurity not detected in HPLC-DAD, $u(I_{WO})$, assuming there are 5 of them.		0.022			
		Standard uncertainty of non-resolved organic impurities, $u(I_{WR})$, assuming there are 5 of them.		0.065			
F_{Others}							
F_W	Karl Fischer titration	The standard deviation of the results in the measurement of moisture in the study material after correction for atmospheric moisture and drift, correction of results to RH50 according to study protocol, differences in results between direct addition and oven transfer method divided by two, the bias of the results estimated using NIST SRM 2890.	106.4	4.0	-0.9505	Type A and B uncertainty	19.26
F_{WO}	TGA	The standard uncertainty in measurement of organic solvent was estimated with the assumption of rectangular distribution. The LOD of the instrument is 2.3 mg/g. The value of the standard uncertainty is $LOD/(2\sqrt{3})$.	0.024	0.66	-0.9505	Type B uncertainty	0.54
F_{IR}	TGA	The standard uncertainty in measurement of non-volatiles/inorganics was estimated with the assumption of rectangular distribution. The LOD of the instrument is 5.0 mg/g. The value of the standard uncertainty is $LOD/(2\sqrt{3})$.	0.00	1.44	-0.9505	Type B uncertainty	2.56
$F_{HCL\_Type\ B}$	Stoichiometric ratio	The standard uncertainty of the assumption of 1:1 stoichiometric ratio of oxytetracycline base:HCl was estimated based on a triangular distribution of the difference of the calculated stoichiometric ratio of oxytetracycline base: HCl (1: > 1). The mass fraction of oxytetracycline base using mass balance approach and mass fraction of HCl determined by IC were used in the calculation.	0.00	6.86	-0.9505	Type B uncertainty	57.77

Appendix F: Summary of measurement equations and uncertainty budgets

Combined uncertainty, $u(m_{MB(HCl)\_1})$, mg/g	8.6
Effective degrees of freedom (υ_{eff})	246.05
k (at 95% CI)	2.00
Expanded uncertainty (U), mg/g	17.1

Table 2. Uncertainty budget for oxytetracycline HCl using mass balance approach 2

Parameter	Value	Standard Uncertainty
$m_{MB(base)}$, mg/g	786.3	5.8
$MW_{oxytetracycline}$, g/mol	460.433	0.01299
$MW_{oxytetracycline\ HCl}$, g/mol	496.892	0.01339

Combined uncertainty, $u(m_{MB(HCl)\_2})$, mg/g	6.3
Effective degrees of freedom (υ_{eff})	62.48
k (at 95% CI)	2.00
Expanded uncertainty (U), mg/g	12.6

Table 3. Uncertainty budget for final mass fraction of oxytetracycline HCl using mass balance approach

Parameter	$m_{MB(HCl)\_1}$	$m_{MB(HCl)\_2}$
Value, mg/g	849.3	848.6
Standard uncertainty, mg/g	8.6	6.3
Arithmetic mean, mg/g	848.9	
Combined uncertainty, $u(m_{MB(HCl)})$, mg/g	5.3	
Effective degrees of freedom (υ_{eff})	308.52	
k (at 95% CI)	2.0	
Expanded uncertainty (U), mg/g	10.6	

Table 4. Uncertainty budget for oxytetracycline HCl using qNMR approach (This table shows the MU budget using Acesulfame potassium as ISTD in 0.01 N DCl D2O)

Parameter	Value	Standard Uncertainty	Remarks
MP (mg/g)	834.0	13.2	
P_{ISTD} (mg/g)	999.2	2.5	
m_{ISTD} (g)	0.0090195	0.0000148	
m_x (g)	0.0107670	0.0000152	
M_{ISTD} (g/mol)	201.245	0.00551	
Mx (g/mol)	496.892	0.01338	
$F_{diff\_integration}$	1	0.00365	Bias in the results due to integration by different analyst

Appendix F: Summary of measurement equations and uncertainty budgets

F_{diff_peak}	1	0.00973	Bias in the results due to integration on peak 4.3 ppm vs 1.8 ppm
$F_{diff\_solvent}$	1	0.01336	Bias in the results due to different solvent (0.01 N DCl D2O vs D2O)

Combined uncertainty, $u(m_{qNMR(AceK)})$, mg/g	19.5
Effective degrees of freedom (υ_{eff})	9.05
k (at 95% CI)	2.26
Expanded uncertainty (U), mg/g	44.2

Table 3. Uncertainty budget for final mass fraction of oxytetracycline HCl using qNMR approach

Parameter	$m_{qNMR(MA)}$	$m_{qNMR(AceK)}$	$m_{qNMR(BFBA)}$	$m_{qNMR(BA)}$
Value, mg/g	824.3	834.0	827.7	829.0
Standard uncertainty, mg/g	10.3	19.5	1.6	6.8
Arithmetic mean, mg/g	828.8			
Combined uncertainty, $u(m_{qNMR(HCl)})$, mg/g	7.0			
Effective degrees of freedom (υ_{eff})	50.25			
k (at 95% CI)	2.0			
Expanded uncertainty (U), mg/g	14.1			

Table 4. Uncertainty budget for final report result of oxytetracycline HCl using using both mass balance and qNMR approaches

Parameter	$m_{MB(HCl)}$	$m_{qNMR(HCl)}$
Value, mg/g	848.9	828.7
Standard uncertainty, mg/g	5.3	7.0
Arithmetic mean, mg/g	838.8	
Combined uncertainty, mg/g	7.3	
Effective degrees of freedom (υ_{eff})	358.77	
k (at 95% CI)	2.0	
Expanded uncertainty (U), mg/g	14.6	

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: NMISA

1. $P_{OTC.HCl\_QNMR} = \frac{A_A}{A_{is}} \frac{N_{is}}{N_A} \frac{m_{is}}{m_A} \frac{M_A}{M_{is}} P_{is}$

POTC.HCl_QNMR Purity of analyte A (OTC.HCl)

A_A Absolute peak integral of the analyte A

A_{is} Absolute peak signal integral of the internal standard (is)

N_A number of protons represented by analyte A peak being integrated **N_{is}** number of protons represented by internal standard (is) peak being integrated

m_A mass of analyte A sample

m_{is} mass of internal standard (is)

M_{is} Molecular mass of the internal standard (is)

M_A Molecular mass of the analyte A

Pis Purity of internal standard (is)

2. $W_{OTC.HCl:QNMR} = P_{OTC.HCl:\,QNMR} - W_{imp\,LC}$

The final OTC.HCl NMR signal is determined by subtracting the structurally related impurities determined by LC (mass balance approach) from the full OTC signal determined by QNMR. Structurally-related impurities* were estimated underneath the signals of interest, except for a the minor IsoCTC peak visible on the aromatic proton signal. Refer to spreadsheet tab Results (QNMR) for combining uncertainty. *4-Epitetracycline (4 ETC) 4-epioxytetracycline (4EOTC) Tetracycline (TC) Chlortetracycline (CTC) 4-epianhydrotetracycline (4EATC) totaling 19.8 mg/g.

Appendix F: Summary of measurement equations and uncertainty budgets

UNCERTAINTY BUDGET	value	uncertainty (u_x)	rel uncertainty (u/x)	vi	rel u^4/vi
PURITY P_{std}	998.7	2.0	0.002029198	100	1.6955E-13
MOLECULAR MASS					
OTC.HCl (M_s)	460.43	0.007368784	1.6004E-05	100	6.5602E-22
TCNB (M_i)	260.88	0.011290379	4.32775E-05	100	3.5079E-20
MASS					
mass OTC.HCl (m_s)	16.07	8.01E-03	4.981E-04	100	6.158E-16
mass TCNB (m_i)	5.05	8.01E-03	1.586E-03	100	6.3192E-14
NMR-spectra integral intensities (I_s and I_{std})					
method repeatability $s_r = \sqrt{MS_{within}}$	878.6	2.6	0.00299	15	5.31E-12
between bottle homogeneity					
standard combined u_c			0.0040	veff	45.08
Expanded uncertainty U95% (k=2)	**878.6**	**±**	**7.0**	**mg/g k=**	**2.0**

	Mass Fraction (mg/g)	Combined Standard Uncertainty (mg/g)	Expanded Uncertainty (mg/g)
4-Epitetracycline (4 ETC)	1.21	0.07	0.18
4-epioxytetracycline (4EOTC)	4.03	0.17	0.37
Tetracycline (TC)	6.57	0.32	1.02
Chlortetracycline (CTC)	6.79	0.13	0.26
4-epianhydrotetracycline (4EATC)	1.2	0.1	0.2
LC impurities	19.80	1.15	
QNMR result	878.6	7.03	**U95%(k=2)**
W OTC.HCl purity result	859	7.1	**14**

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: BIPM

The mass balance value was calculated according to equation 1.

$$w = 1000 - (\sum_i w_i + w_w + w_{VOC} + w_{NV}) \qquad \text{(Eq. 1)}$$

Where:

w : mass fraction (mg/g) of the main component in the material.

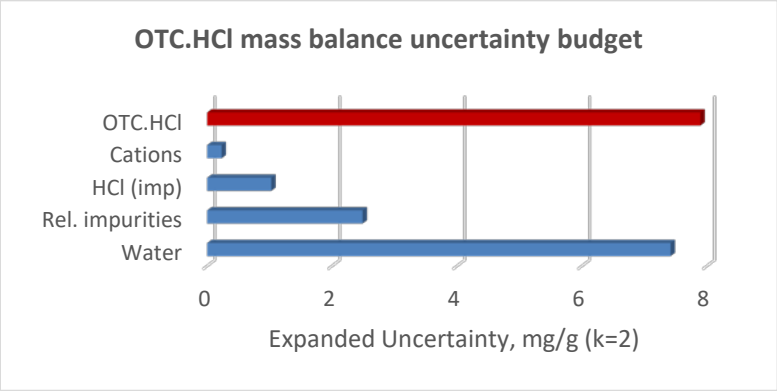
w_i: mass fraction (mg/g) of individual structurally related impurity *i* in the material.

w_w: mass fraction (mg/g) of water in the material.

w_{VOC}: mass fraction (mg/g) of residual solvent in the material.

w_{NV}: mass fraction (mg/g) of non-volatile residue in the material.

The uncertainty of the mass balance value was calculated by square root of the quadratic summation of the individual impurities mass fraction uncertainties.



Appendix F: Summary of measurement equations and uncertainty budgets

Participant: NMIJ

1-1. Measurement equation for Mass balance approach

$$w_{\mathrm{p}}(\mathrm{MBA}) = 1000 - w_{\mathrm{related}} - w_{\mathrm{water}} - w_{\mathrm{volatile}} - w_{\mathrm{non-volatile}}$$

1-2. Measurement equation for qNMR

$$w_{\mathrm{p}}(\mathrm{qNMR}) = \frac{S_{\mathrm{x}}}{S_{\mathrm{s}}} \cdot \frac{M_{\mathrm{OTC.HCl}}}{M_{\mathrm{s}}} \cdot \frac{N_s}{N_{\mathrm{x}}} \cdot \frac{m_s}{m_{\mathrm{x}}} \cdot P_{\mathrm{s}}$$

2. Measurement equation for combination of values

$$w_{\mathrm{p}} = \frac{w_p(\mathrm{MBA}) + w_p(\mathrm{qNMR})}{2}$$

Model equation for uncertainty evaluation of w_{p}

$$w_{\mathrm{p}} = \frac{w_{\mathrm{p}}(\mathrm{MBA}) + w_{\mathrm{p}}(\mathrm{qNMR})}{2} + f_{\mathrm{method}}$$

f_{method} = 0 mg g^{-1}

$$u(f_{\mathrm{method}}) = \frac{\left|w_{\mathrm{p}}(\mathrm{MBA}) - w_{\mathrm{p}}(\mathrm{qNMR})\right|}{\sqrt{12}}$$

Uncertainty components of w_{p} are measurement methods (mass balance approach and quantitative nuclear magnetic resonance) and difference between the methods. The standard uncertainties of the components were combined assuming they have no correlation.

Uncertainty budgets of w_{p} and w_{p}(MBA) are shown below.

Uncertainty budger of w_{p}

Symbol	Source of uncertainty	Value x_i / mg g^{-1}	Standard uncertainty $u(x_i)$ / mg g^{-1}	c_i	$c_i\,u(x_i)$ / mg g^{-1}	Contribution
w_{p}(MBA)	Mass balance approach	858.24	3.92	0.5	1.96	0.303
w_{p}(NMR)	Quantitative nuclear magnetic resonance	848.7	2.20	0.5	1.10	0.095
f_{method}	Diferrence between measurements	0	2.76	1	2.76	0.601
$u_{\mathrm{c}}(w_{\mathrm{p}})$	Combined standard uncertainty				3.56 mg g^{-1}	
$U(w_{\mathrm{p}})$	Expanded uncertainty				7.12 mg g^{-1}	
					(k = 2)	

Appendix F: Summary of measurement equations and uncertainty budgets

Uncertainty budget of w_p(MBA)

Symbol	Source of uncertainty	Value x_i / mg g^{-1}	Standard uncertainty $u(x_i)$ / mg g^{-1}	c_i	$c_i\,u(x_i)$ / mg g^{-1}	Contribution
$w_{related}$	Total related structure impurities	34.56	2.80	1	2.80	0.51
w_{water}	Water content	107.04	2.71	1	2.71	0.48
$w_{volatile}$	Total non-volatiles and inorganics	0.16	0.10	1	0.10	0.00
$w_{non\text{-}volatile}$	Volatile organics content	0	0.35	1	0.35	0.01
$u_c(w_p$(MBA))	Combined standard uncertainty				3.92 mg g^{-1}	
$U(w_p$(MBA))	Expanded uncertainty				7.84 mg g^{-1}	
					(k = 2)	

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: NMIA

Purity (%) = (100 - I“Organic”)*(100 - I"Other")

I"Organic" = Mass fraction of organic impurities of similar structure.

I"Other" = Mass fraction of volatile and non-volatile impurities.

Equation for qNMR

$$P_A = \frac{I_s}{I_{Std}} \frac{n_{Std}}{n_s} \frac{M_s}{M_{std}} \frac{m_{Std}}{m} P_{std}$$

All uncertainties are combined using the square root of the sum of the squares approach, using standard uncertainties or relative standard uncertainties as appropriate.
The major components of the uncertainty budget are
Uc from Karl Fischer analysis,
Uc from HPLC organic purity analysis,
Uc from non-volatile residues,

$$u_{Purity} = P \sqrt{\left(\frac{U_{Organic}}{I_{Organic}}\right)^2 + \left(\frac{U_{Other}}{I_{Other}}\right)^2}$$

The qNMR uncertainty was calculated using the relative standard uncertainties of all componenets in the measurement equation, as shown below.

$$u_{P_{Analyte}} = P_{Analyte} x \sqrt{\left(\frac{u_{P_{Analyte}}}{P_{Analtye}}\right)^2 + \left(\frac{u_{\rho_{IS}}}{\rho_{IS}}\right)^2 + \left(\frac{u_{\rho_{Analyte}}}{\rho_{Analyte}}\right)^2 + \left(\frac{u_{P_{IS}}}{P_{IS}}\right)^2 + \left(\frac{u_{Mwt_{Analyte}}}{Mwt_{Analyte}}\right)^2 + \left(\frac{u_{Mwt_{IS}}}{Mwt_{IS}}\right)^2 + \left(\frac{u_{wt_{IS}}}{wt_{IS}}\right)^2 + \left(\frac{u_{wt_{Analyte}}}{wt_{Analtye}}\right)^2}$$

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: NIM

The measurement equation (Eqn. 1) of the Mass Balance to assign the purity of Oxytetracycine in CCQM-K148.b is:

$$P_{MB} = 1000 - X_{RS} - X_W - X_{Cl} - X_{NV} - X_V \qquad (1)$$

Where

P_{MB} : mass fraction of Oxytertracyine

X_{RS} : mass fraction of total structurally related imputies

X_W : mass fraction of water content

X_{Cl} : mass fraction of Chloride ion

X_{NV} : mass fraction of total non-volatiles and inorganics

X_V : mass fraction of volatile organic content

Measurement equation for qNMR method:

$$P_{QNMR} = \frac{I_s}{I_{std}} \frac{n_{std}}{n_s} \frac{M_s}{M_{std}} \frac{m_{std}}{m_s} P_{std} \qquad (2)$$

Where

P_{QNMR} : mass fraction of sample(Oxytetracycine HCl)

P_{std} : mass fraction of internal standard.

m_{std} : weight of internal standard.

M_{std} : molecular weight of internal standard.

n_{std} : number of hydrogen of the quantification peak of internal standard.

I_{std} : Peak area of quantification peak of internal standard.

m_s : weight of Oxytetracycine sample.

n_s : number of hydrogen of the quantification peak at the common structure part of homologues of Oxytetracycine sample.

I_s : Peak area of quantification peak of Oxytetracycine HCl sample.

Appendix F: Summary of measurement equations and uncertainty budgets

The value of Oxytetracycline is :

$$P = \frac{P_{MB}+P_{QNMR}}{2} \qquad (3)$$

1. Uncertainty evaluation from Mass balance

Evaluation of measurement uncertainty of mass fractions From Eq. 1, the uncertainty of mass fration of component is:

$$u(P_{MB}) = \sqrt{[u(X_{RS})]^2 + [u(X_{Cl})]^2 + [u(X_W)]^2 + [u(X_V)]^2 + [u(X_{NV})]^2}$$

（1）$u(X_{RS})$

The relative uncertainty $u_{rel}(X_{RS1})$ of known impurities is:

$$u_{rel}(X_{RS1}) = \sqrt{u_{rel}^2(p) + u_{rel}^2(R)}$$

$u_{rel}(p)$: The relative uncertainty of impurity purity;

$u_{rel}(R)$: The relative uncertainty from the repeatability of impurity measurement;

The relative uncertainty $u_{rel}(X_{RS2})$ of unknown impurities is:

$$u_{rel}(X_{RS2}) = \sqrt{u_{rel}^2(f) + u_{rel}^2(R)}$$

$u_{rel}(f)$: The uncertainty of the average influence factor of unknown impurities;

$u_{rel}(R)$: The relative uncertainty from the repeatability of impurity measurement;

The combined uncertainty $u(X_{RS})$ is:

$$u(X_{RS}) = u_{rel}(X_{RS}) * X = 2.1 \text{ mg·g}^{-1}$$

X is the concentration of impurity, mg·g^{-1}.

Taking a 95% confidence probability with a coverage factor of *k*=2, the expanded uncertainty $U(X_{RS})$ is:

$$U(X_{RS}) = u(X_{RS}) * k = 4.2 \text{ mg·g}^{-1}$$

（2）$u(X_{Cl})$

The relative uncertainty $u_{rel}(X_{Cl})$ of chloride ion determination results is:

Appendix F: Summary of measurement equations and uncertainty budgets

$$u_{rel}(X_{Cl}) = \sqrt{u_{rel}^2(S) + u_{rel}^2(M) + u_{rel}^2(D) + u_{rel}^2(R)}$$

$u_{rel}(S)$:The relative uncertainty of CRM for the analysis of chloride ions in water;

$u_{rel}(M)$:The relative uncertainty from mass of smaple;

$u_{rel}(D)$:The relative uncertainty from the dilution process of standard solutions;

$u_{rel}(R)$:The relative uncertainty from measurement repeatability;

The combined uncertainty *u* (X) is:

$$u(X_{Cl}) = u_{rel}(X_{Cl}) * X = 0.0106 * 2.62 = 0.028 \text{ mg·g}^{-1}$$

X is the concentration of chloride ions, mg·g^{-1}.

Taking a 95% confidence probability with a coverage factor of *k*=2, the expanded uncertainty $U(X_{Cl})$is:

$$U(X_{Cl}) = u(X_{Cl}) * k = 0.056 \text{ mg·g}^{-1}$$

（3）$u(X_W)$

The uncertainty of water is list in the table:

Source of uncertainty	Value	Absolute uncertainty	Relative uncertainty	Comment
RHx (%)	46.2	0.1	0.22%	From hygrometer
RHx－50	-3.8	0.1	2.63%	Combined with absolute uncertainties
F	0.00037	0.00003	8.11%	From the protocol
F·(RHx－50)	-0.00141	0.00012	8.52%	Combined with relative uncertainties
1+F·(RHx－50)	0.99859	0.00012	0.01%	Combined with absolute uncertainties
m_{RHX} (mg)	10.69	0.01	0.05%	From balance
$m_{RH50}=m_{RHX}/(1+F·(RHx－50))$ (mg)	10.7051	0.0059	0.06%	Combined with relative uncertainties
Wtoal (mg)	1.1086	0.01	0.90%	From titrator
Wblank (mg)	0.2297	0.0311	3.38%	From blank detections (*n*=16)
Wsam=Wtotal-Wblank (mg)	0.8789	0.0327	3.72%	Combined with absolute uncertainties
m_{RH50}-m_{RHX} (mg)	0.0151	0.0083	54.95%	Combined with absolute uncertainties of m_{RH50} and m_{RHX}
Wsam50=Wsam+(m_{RH50}-m_{RHX}) (mg)	0.8940	0.0337	3.77%	Combined with absolute uncertainties of Wsamand (m_{RH50}-m_{RHX})
X_W=W_{sam50}/m_{RH50} (mg/mg)	0.0835	0.0031	3.77%	Combined with relative uncertainties of W_{sam50} and m_{RH50}
Repeatability			2.55%	RSD of 6 determinations
Xw of 6 detmerinations (mg/g)	89.92	4.09	4.5%	Combined with relative uncertainties of Xw and repeatibility

*For addition or subtraction, absolute uncertainties are combined by square root of sum of squares

*for multiplication or division, relative uncertainties are combined by square root of sum of squares.

（4）$u(X_V)$

The relative uncertainty of volatile organic determination results is：

$$u_{rel}(X_v) = \sqrt{u_{rel}^2(m_s) + u_{rel}^2(m_{std}) + u_{rel}^2(P_{std}) + u_{rel}^2(R)}$$

$u_{rel}(m_s)$: uncertainty from mass of sample；

$u_{rel}(m_{std})$: uncertainty from mass of standard preparation；

$u_{rel}(P_{std})$: uncertainty from purity of standard；

$u_{rel}(R)$: uncertainty from measurement repeatability.

The combined uncertainty of methanol measurement $u(X_V)$ is:

$$u(X_V) = u_{rel}(X_v) * \mathrm{X} = 0.0194 \text{ mg·g}^{-1}$$

X is the concentration, mg·g^{-1}.

Taking a 95% confidence probability with a coverage factor of k=2, the expanded uncertainty $U(X_V)$ is:

$U(X_V) = u(X_V) * k = 0.04$ mg·g^{-1}

（5）$u(X_{NV})$

The uncertainty of of total non-volatiles and inorganics is：

$$u(X_{NV}) = \sqrt{[u(P)]^2 + [u(R)]^2 + [u(L)]^2}$$

Where

$u(P)$: uncertainty from the CRM of inorganics solution;

$u(R)$: uncertainty from measurement repeatability;

$u(L)$: uncertainty from Linear of standard curve.

Taking a 95% confidence probability with a coverage factor of k=2, the expanded uncertainty $U(X_{NV})$ is:

$U(X_{NV}) = u(X_{NV}) * k = 0.018$ mg·g^{-1}

Appendix F: Summary of measurement equations and uncertainty budgets

2. Uncertainty evaluation from QNMR

The uncertainty evaluation for the results was carried out from weighing of sample, internal standard, molecular weight of sample and measurement of the equipment. In general, the measurement uncertainty is mainly due to measurement of the equipment .

Evaluation of measurement uncertainty of mass fractions From Eq 2, the uncertainty of mass fraction of component is:

$$\frac{u(P_{QNMR})}{P_{QNMR}} = \sqrt{\left(\frac{u(I_s/I_{std})}{I_s/I_{std}}\right)^2 + \left(\frac{u(M_s)}{M_s}\right)^2 + \left(\frac{u(M_{std})}{M_{std}}\right)^2 + \left(\frac{u(m_{std})}{m_{std}}\right)^2 + \left(\frac{u(m_s)}{m_s}\right)^2 + \left(\frac{u(P_{std})}{P_{std}}\right)^2}$$

Where

$\frac{u(I_s/I_{std})}{I_s/I_{std}}$: uncertainty from NMR measurement, including baseline correction, integration of peak area and measurement repeatability.

$\frac{u(M_s)}{M_s}$: uncertainty from molecular weight of sample (Oxytetracycine).

$\frac{u(M_{std})}{M_{std}}$: uncertainty from molecular weight of internal standard.

$\frac{u(m_{std})}{m_{std}}$: uncertainty from mass of internal standard.

$\frac{u(m_s)}{m_s}$: uncertainty from mass of sample.

$\frac{u(P_{std})}{P_{std}}$: uncertainty from purity (expressed as mass fraction) of internal standard.

The combined uncertainty (u_c) can be calculated by:

$$u(P_{QNMR}) = P_{QNMR} * \frac{u(P_{QNMR})}{P_{QNMR}} = 3.04 \text{ mg·g}^{-1}$$

The expanded uncertainty U can be calculated with coverage factor k=2 corresponds to a confidence interval of 95%.

$$U = ku_C$$

Appendix F: Summary of measurement equations and uncertainty budgets

Component (units)	xi	$U=ku_C$ u(xi)	u(xi)/xi (%)	
M_{std} (g mol^{-1})	166.1739	0.00421	0.00253%	
M_s (g mol^{-1})	496.8949	0.01033	0.00208%	
m_s (mg)	9.0	0.00065	0.00719%	
m_{std} (mg)	3.5	0.00029	0.00829%	
P_{std}(mg g^{-1})	999.7	0.25	0.02501%	
I_s/I_{std} (mg g^{-1})	788.6	3.0328	0.38458%	
P_{QNMR} (mg g^{-1})		788.6	3.04	0.38551%

3. The combined Uncertainty

$$u_s = \sqrt{\left(\frac{P_{MB}-P_{NNMR}}{2}\right)^2 + \left(\frac{u(P_{MB})}{2}\right)^2 + \left(\frac{u(P_{QNMR})}{2}\right)^2} = 5.2 mg/\text{g}$$

$$U = u_s \times k = 10.4\, mg/g$$

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: GLHK

1a. Mass balance method:

$$m_{OTC} = (1000 - m_{RS,rel}) \times \left(\frac{1000 - (m_W + m_{OS} + m_{NV} + m_{Cl} + m_H)}{1000}\right) mg/g$$

1b. qNMR method:

$$P_{sample} = \frac{I_{Analyte}}{I_{IS}} \times \frac{N_{IS}}{N_{Analyte}} \times \frac{M_{Analyte}}{M_{IS}} \times \frac{m_{IS}}{m_{Sample}} \times P_{IS}$$

2. Measurement equation for combined results:

$$purity_{combined} = \sum_{i=1}^{N} w_i x_i$$

$$w_i = \frac{1}{u_i^2}$$

where w_i is the weighing factor

x_i is purity of OTC by mass balance or qNMR

1a. Mass balance method:

$U(X_{OTC}) = U(\Sigma X_{IC})$ where the major components of $U(X_{IC})$ include purities of reference standard, precision, recovery and estimation for unknown impurities

1b. qNMR method:

$U(X_{OTC}) = U(\Sigma X_{IC})$ where the major components of $U(X_{IC})$ include the following: purity of IS, integration, molecular weight of IS, molecular weight of analyte, mass of analyte, mass of IS, precision and repeatability

3. Calculation of Measurement Uncertainty of combined results:

$$u_{combined} = \frac{1}{\sqrt{w_{MB} + w_{qNMR}}}$$

$$w_i = \frac{1}{u_i^2}$$

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: INMETRO

qNMR measurement equation

$$P_a = \frac{I_a}{I_{IS}} * \frac{M_a}{M_{IS}} * \frac{m_{IS}}{m_a} * \frac{N_{IS}}{N_a} * P_{IS}$$

Considering that we used the whole aromatic range, which is overlapped with structurally related impurities, we used the LC-PDA area normalization (with the calculated response factors) value multiplied by the raw qNMR result as a correction to obtain the final qNMR result:

$$qNMR_{final} = qNMR_{RAW} * N_A$$

Mass balance measurement equation

$$w_A(mg/g) = (1000 - \sum w_{imp}) \times N_A$$

The combination of the results was performed by a simple average.

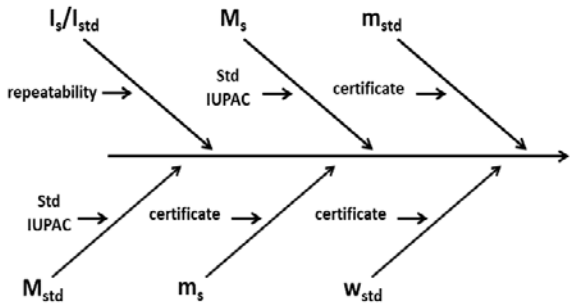
Mass balance –

Mass fraction:	Value	u	Relative contribution
Water	101.8108	1.2282	
Volatile organic compounds	0.2286	0.0094	
Total volatile impurities*	102.0394	1.2282	
Inorganic impurities*	1.7980	1.0852	
Partial purity (disregarding area normalization)	896.1626	1.6390	29.4054
Area normalization	0.9628	0.0027	70.5946
Main compound	862.7916	2.9099	

The first step in the mass balance approach is to calculate (1000 - total volatile impurities - inorganic impurities). These results in a partial purity value and the uncertainties of the water content, VOCs and inorganic impurities are all combined as relative uncertainties which resulted in the 1.6390 mg/g uncertainty for the partial purity. This value is then multiplied by the area normalization to yield the final mass balance result and the uncertainties are combined as relatives once more. For this sample, the main uncertainty source for the mass balance was the area normalization since the impurity content is relevant and each of the impurities have large uncertainties associated to their response factors. The area normalization accounts for about 70 % of the final uncertainty.

Appendix F: Summary of measurement equations and uncertainty budgets

qNMR - Provided for one of the systems as an example



Uncertainty sources	Value	u	u Component
IA	60545	-	-
IIS	40631	-	-
IA/IIS (repeatability)	1.4901	0.000705739	4.21E-01
Analyte Molar Mass	496.8924	0.013384718	2.39E-02
IS Molar Mass	260.8832	0.013175058	4.48E-02
IS weighed Mass	4.92	0.007	1.26E+00
Analyte weighed Mass	5.23	0.007	1.19E+00
IS purity	997.8	1.10000000	9.79E-01
Overall			2.04

The two qNMR values as well as the qNMR + Mass balance combinations are done by simple averaging while the associated measurement uncertainties are performed taking into account also the differences between the results by using the equation:

$$u_c = \sqrt{\frac{\left(\sum_{j=1}^{m}(Y_j - \bar{Y})^2/m - 1\right) + \left(\sum_{j=1}^{m} u_{(Y_j)}^2/m\right)}{m}}$$

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: EXHM

Measurement equations

$$w_{OCT_{\square}} = A_{OCT,n}\left(1 - \frac{w_{H_2O}+w_{vol}+w_{in}}{1000}\right)$$

Mass balance method:

OCT fraction (mg/g) is given by the following equation:

$$w_{OCT_{\square}} = A_{OCT,n}\left(1 - \frac{w_{H_2O}+w_{vol}+w_{in}}{1000}\right)$$

where

w	:	mass fraction (mg/g)
$A_{OCT,n}$	:	normalized $OCT_{\square}$ peak area in the HPLC- DAD chromatogram on a mass basis
H_2O	:	water (mg/g)
vol	:	residual volatiles (mg/g)
in	:	inorganics and non-volatile material (mg/g)

The normalized OCT area on a mass basis is given by the following equation

$$A_{OCT_n} = \frac{A_{OCT}\ \frac{Rf_{OCT}}{mw_{OCT}}}{A_{OCT}\ \frac{RRf_{OCT}}{mw_{OCT}} + \sum A_{SRI,i}\ \frac{RRf_{SRI,i}}{mw_{SRI,i}}}$$

where

A_{OCT}	:	$OCT_{\square}$ peak area in the HPLC - DAD chromatogram
$A_{SRI,i}$	:	SRI_i peak area in the HPLC- DAD chromatogram
SRI_i	:	i^{th} Structure Related Impurity
RRf_{OCT}	:	relative OCT response factor (= 1)

$RRf_{SRI,i}$: relative ith SRI response factor
mw : molar mass

SRI determination:

The mass fraction of each structurally-related impurity was determined as the area fraction of the respective peak in the HPLC-DAD chromatogram.

$$w_{sri} = SRI_{i,n}\ (1 - \frac{w_{H_2O}+w_{vol}+w_{in}}{1000})$$

where

$SRI_{i,n}$: normalized SRI_i peak area in the HPLC- DAD chromatogram (calculated in the same way as $A_{OCT,n}$)

Water determination:

The equation describing water determination by coulometric Karl Fisher titration is given by the following equation:

$$w_{H2O} = \frac{Q}{z\ F}\ \frac{mw}{m_{sample}} - w_{blank}$$

where, w_{H2O} = water mass fraction

Q = amount of charge

z = number of electrons exchanged

F = electrochemical equivalent

MW = molar mass

m_{sample} = sample mass

w_{blank} = water in blank

Appendix F: Summary of measurement equations and uncertainty budgets

Volatile / Inorganic impurities determination:

To determine these impurities, an amount of the sample is used to form a particular solution, either by simply dissolving it in a suitable solvent system, or by using treatment such as digestion/dissolution, and determining the impurities.

The equation describing the determination of volatile and inorganic impurities by means of chromatographic and spectrometric techniques is given by the following generic equation:

$$w_{vol/in} = \frac{R_{soln}}{R_{std}} C_{std} \frac{m_{soln}}{m_{sample}}$$

where, $w_{vol/in}$ = volatile/inorganic mass fraction

$R_{soln, std}$ = solution/standard response

C_{std} = standard concentration

m_{soln} = solution mass

m_{sample} = sample mass

In the particular case, no volatiles nor any inorganics were determined above the LOQ (0.02 %) and therefore the value is set as zero with an uncertainty of

5b. Uncertainty budget

The uncertainty of **oxytetracycline free base and oxytetracycline hydrochloride** was calculated using the following equation:

$$u(w_{OCT,SRI}) = \sqrt{\frac{(SD_R)^2}{n} + (C_i u_{H2O})^2 + (C_i u_{vol})^2 + (C_i u_{im})^2}$$

where SD_R is the standard deviation under reproducibility conditions, n the number of determinations and C_i appropriate sensitivity coefficients.

The uncertainty of the total structure-related impurities was calculated as the sum of the uncertainties of the individual components.

The uncertainty for the **determination of residual water** is provided by the following generic equation:

$$u(w_{H2O}) = \sqrt{\frac{(SD_{R,H2O})^2}{n} + \left(C_i u_{sample\ mass}\right)^2}$$

The uncertainty for the **determination of volatile mater and inorganic/non volatile impurities** is provided by the following generic equation:

$$u\left(w_{vol,in}\right) = \sqrt{\frac{(SD_R)^2}{n} + (C_i u_{Cstd})^2 + \left(C_i m_{sample}\right)^2 + (C_i m_{soln})^2}$$

qNMR

Purity was determined by qNMR and checked by the mass balance approach. The respective uncertainties were calculated via the following equations:

$$P_s = \frac{I_s}{I_{is}} \frac{N_{is}}{N_s} \frac{mw_s}{mw_{is}} \frac{m_{is}}{m_s} P_{is}$$

where

P	:	purity (mg/g)
I	:	signal intensity
N	:	number of protons
mw	:	molecular weight
m	:	mass
s	:	sample (OCT)
is	:	internal standard (maleic acid)

Appendix F: Summary of measurement equations and uncertainty budgets

UNCERTAINTY BUDGETS

Mass balance

OTC Normalization uncertainty budget

impurity acc. to E.P.	compound	factor	value	unc	Ci	Ci x ui	(Ci x ui)^2	NORM AREA	uncertainty	free base (mg/g)	uncertainty (mg/g)
	OTC	A	951,46	0,60	0,026	0,016	0,0002	960,25	3,12	797,50	4,67
		Rf	1,00	0	25,02	0,000	0,0000				
		MW	460,44	0,02	0,054	0,001	0,0000				
A	4epiOTC	A	3,15	0,06	0,998	0,064	0,0040	3,18	0,06	2,64	0,06
		Rf	1,00	0,01	3,143	0,031	0,0010				
		MW	460,44	0,02	0,007	0,000	0,0000				
B	TC	A	5,96	0,09	0,963	0,087	0,0076	6,23	0,09	5,18	0,08
		Rf	1,00	0,01	5,712	0,057	0,0033				
		MW	444,44	0,02	0,013	0,000	0,0000				
C	2-ADOTC	A	14,85	0,06	0,996	0,059	0,0035	15,02	0,08	12,47	0,09
		Rf	1,00	0,01	14,785	0,148	0,0219				
		MW	459,45	0,02	0,032	0,001	0,0000				
D	a-APOTC	A	6,08	0,38	0,996	0,380	0,1443	3,19	0,19	2,65	0,16
		Rf	0,50	0,01	14,785	0,148	0,0219				
		MW	442,42	0,02	0,032	0,001	0,0000				
F	AOTC	A	3,43	0,43	0,996	0,424	0,1797	3,60	0,43	2,99	0,35
		Rf	1,00	0,01	14,785	0,214	0,0457				
		MW	442,42	0,02	0,032	0,001	0,0000				
E	b-APOTC	A	5,00	0,36	0,996	0,362	0,1313	2,63	0,18	2,18	0,15
		Rf	0,50	0,01	14,785	0,148	0,0219				
		MW	442,42	0,02	0,032	0,001	0,0000				
E	b-APOTC	A	6,26	0,70	0,996	0,695	0,4825	3,29	0,35	2,73	0,29
		Rf	0,50	0,01	14,785	0,148	0,0219				
		MW	442,42	0,02	0,032	0,001	0,0000				
	ATC	A	0,55	0,02	0,996	0,019	0,0004	0,60	0,02	0,50	0,02
		Rf	1,00	0,01	14,785	0,148	0,0219				
		MW	426,40	0,02	0,032	0,001	0,0000				
	RRT 13,7	A	0,26	0,02	0,996	0,018	0,0003	0,29	0,02	0,24	0,02
		Rf	1,00	0,01	14,785	0,148	0,0219				
		MW	426,40	0,01	0,032	0,000	0,0000				
	RRT 14,4	A	0,26	0,02	0,996	0,018	0,0003	0,29	0,02	0,24	0,02
		Rf	1,00	0,02	14,785	0,296	0,0874				
		MW	426,40	0,01	0,032	0,000	0,0000				
	RRT 14,8	A	0,36	0,02	0,996	0,018	0,0003	0,40	0,02	0,33	0,02
		Rf	1,00	0,02	14,785	0,296	0,0874				
		MW	426,40	0,01	0,032	0,000	0,0000				
	RRT 15,7	A	0,31	0,01	0,996	0,012	0,0002	0,34	0,01	0,28	0,01
		Rf	1,00	0,02	14,785	0,296	0,0874				
		MW	426,40	0,01	0,032	0,000	0,0000				
	RRT 16,5	A	0,39	0,01	0,996	0,012	0,0002	0,42	0,01	0,35	0,01
		Rf	1,00	0,01	14,785	0,185	0,0342				
		MW	426,40	0,02	0,032	0,001	0,0000				
	RRT 17,7	A	0,23	0,02	0,996	0,016	0,0003	0,08	0,01	0,06	0,00
		Rf	0,31	0,01	14,785	0,185	0,0342				
		MW	426,40	0,02	0,032	0,001	0,0000				
	RRT 17,8	A	0,19	0,02	0,996	0,019	0,0004	0,20	0,02	0,17	0,02
		Rf	1,00	0,01	14,785	0,185	0,0342				
		MW	426,40	0,02	0,032	0,001	0,0000				
	unknown SRIs	A	1,41	1,04	0,959	0,994	0,9881	0,00	2,96	0,00	2,46
		Rf	1,00	0,01	1,349	0,013	0,0002				
		MW	442,42	20,00	0,003	0,061	0,0037				
								1000,00	4,51	830,51	

Appendix F: Summary of measurement equations and uncertainty budgets

OTC HCl PURITY UNCERTAINTY BUDGET

component	unit	value	uncertainty
HYDRO CHLORIDE	mg/g	63,13	1,38
FREE BASE	mg/g	797,50	4,67
OTC HCl fraction	mg/g	860,64	
combined uncertainty	mg/g	5,04	
k=2			
expanded uncertainty	mg/g	10,09	

KF uncertainty budget					
uncertainty component	value	units	uncertainty	uncertainty	squared RU
determination repeatability	105327,0	µg g^{-1}	643,00	0,006	0,000
blank determination	71,0	µg g^{-1}	0,10	0,001	0,000
sample mass at 43 %RH	50,000	mg	0,02	0,000	0,000
sample mass at 50 %RH	50,128	mg	0,03	0,001	0,000
water content result	105327,0	µg g^{-1}			0,00
combined standard uncertainty	660,7	µg g^{-1}			
coverage factor (k, n=6)	2,0				
expanded uncertainty (k=2)	1321,4	µg g^{-1}			

chloride uncertainty budget (standard additions)					
uncertainty component	value	units	ncertainty	ncertainty	squared RU
determination repeatabili	62,4	mg/g	1,30	0,021	1,690
sample mass	10,0	g	0,02	0,002	0,000
added Cl solution mass	100,0	mg	0,02	0,000	0,000
Cl standard solution mass	998,0	mg	0,40	0,000	0,160
standard addition model	62,6	mg	0,48	0,008	0,000
chloride mass fraction	62,4	µg g^{-1}			62,40
combined standard uncerta	1,4	µg g^{-1}			
coverage factor (k, n=8)	2,0				
expanded uncertainty (k=2	2,7	µg g^{-1}			

Appendix F: Summary of measurement equations and uncertainty budgets

qNMR

qNMR uncertainty budget							
uncertainty component	value	units	u_i	u_i/x_i	C_i	C_iu_i	$(C_iu_i)^2$
OXT/MA signal ratio (ppm 3.8)	0,1258		0,00102	8,108E-03	6836,65	6,9734	4,863E+01
OXT molecular mass	496,890	g mol^{-1}	0,00600	1,208E-05	1,73	0,0104	1,079E-04
MA molecular mass	116,070	g mol^{-1}	0,00600	5,169E-05	-7,41	-0,0445	1,977E-03
no of protons in signal integrated for OXT	1	nucl/mol	0,00040	1,800E-05	-860,05	-0,3440	1,183E-01
no of protons in signal integrated for MA	2	nucl/mol	0,00040	1,800E-05	430,03	0,1720	2,959E-02
OXT mass	6,6076	mg	0,00100	1,513E-04	-130,16	-0,1302	1,694E-02
MA mass	5,2772	mg	0,00100	1,895E-04	162,97	0,1630	2,656E-02
boyancy correction	1,0000		0,00000	4,065E-06	860,05	0,0035	1,222E-05
MA	999,80	mg g^{-1}	0,50000	5,001E-04	0,86	0,4301	1,850E-01
OXT purity							**860,06**
combined standard uncertainty		**mg g^{-1}**					**7,00**
expanded uncertainty (k=2)		mg g^{-1}					14,00

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: UME

Mass Balance

$wA = mA / mA + \sum mx \quad = \quad nA*M(A) / mA + \sum mx$

wA mass fraction of main component A in the material

mA mass of A in an aliquot of the material

Σmx summed mass of minor components (impurities) in the same aliquot

nA moles of A in an aliquot of the material

M(A) Molar mass of A

$w_A = 1000- (W_{RS} + W_W + W_{VOC} + W_{NV})$

w_{RS} = mass fraction of structurally related impurities in the material

w_W = mass fraction of water in the material

w_{VOC} = mass fraction of residual solvent (volatile organics) in the material

w_{NV} = mass fraction of non-volatile compounds in the material

qNMR equation

$$Px = \frac{Ix}{Istd}\frac{Nstd}{Nx}\frac{Mx}{Mstd}\frac{mstd}{mx}Pstd$$

The standard uncertainty of the material of mass balance approach $u(w_{MB})$ is given by the equation below:

$$u(w_{MB}) = \sqrt{u(w_{RS})^2 + u(w_W)^2 + u(w_{VOC})^2 + u(w_{NV})^2}$$

The uncertainty of the material, qNMR approach:

$$u(P_x) = P_x\sqrt{\left(\frac{u(I_x/I_{std})}{I_x/I_{std}}\right)^2 + \left(\frac{u(M_x)}{M_x}\right)^2 + \left(\frac{u(M_{std})}{M_{std}}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(m_{std})}{m_{std}}\right)^2 + \left(\frac{u(P_{std})}{P_{std}}\right)^2}$$

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: KRISS

1-1. LC-UV (structurally related impurities)

$$P_{related\ structure\ impurity,i} = \frac{A_{impurity,i}}{A_{main} + \sum A_{impurity,i}}$$

$P_{structurally\ related\ impurity,I}$: mass fraction of the structurally related impurity $A_{impurity,I}$: peak area of the impurity A_{main} : peak area of the main component

1-2. KF titration (water content)

$$P_{water} = (ICEQ/10.712 - Time \times Drift - Blank)/m \times C$$

P_{water} : mass fraction of water in the sample ICEQ: total consumed electric charge Time: total KF measurement time Drift: systematic water content measured by KF titration before the analysis in time Blank: systematic water content in empty vial m: weight of the sample C: constant, 1×10^6

1-3. TGA (non-volatile impurities)

$$P_{non-volatile\ impurities} = \frac{W_{non-volatile\ impurities}}{W_{sample}}$$

$P_{non\text{-}volatile\ imputies}$: mass fraction of non-volatile impurities $W_{non\text{-}volatile\ imputies}$: weight of non-volatile impurities W_{sample} : weight of the sample

1-4. Headspace-GC/MS (volatile organics)

$$P_{volatile\ organic} = \frac{\sum W_{volatile\ organic,i}}{W_{sample}}$$

$$W_{volatile\ organic} = \frac{A_{volatile\ solvent} - y_{intercept}}{Slope}$$

$W_{volatile\ organic,i}$: weight of volatile organics W_{sample} : weight of the sample $y_{intercept}$: intercept of the calibration curve Slope: slope of the calibration curve

1-5. IC-CD (chloride ion content)

Appendix F: Summary of measurement equations and uncertainty budgets

$$P_{chloride\ \mathrm{ion}} = \frac{C_{std} \times A_{sample}}{C_{tc} \times A_{std}}$$

Pchloride ion : mass fraction of chloride ion in sample Cstd : Concentration of chloride ions in standard solution Ctc : Concentration of tetracycline.HCl in sample solution Astd : Chloride peak area in standard solution Asample : Chloride peak area in sample solution

2. Combination of value:

$$P_{\mathrm{OTCHCl}} = (1 - \sum P_{\mathrm{impurity}}) \times P_{\mathrm{chromatography}} \times P_{chloride\ ion} \times \frac{M_{OTC.HCl}}{M_{Cl}}$$

P_{OTC} : mass fraction of oxytetracycline·HCl

$P_{impurity}$: mass fraction of imputities (including structurally related impurities, water, non-volatile impurities, and volatile organics)

$P_{chromatography}$: mass fraction of oxytetracyclin measured by LC-UV

$P_{chloride\ ion}$: mass fraction of chloride ion measured by IC-CD

M_{Cl} : molecular weight of chloride

M_{OTCHCl} : molecular weight of oxytetracyclinHCl

3. qNMR

$$P_a = \frac{I_a N_s M_a W_s}{I_s N_a M_s W_a} P_s$$

p_a: purity of analyte

I_a: integral area of quantification peak of analyte

I_s: integral area of quantification peak of internal standard

N_s : number of protons of the quantification peak of internal standard

N_a: number of protons integrated for quantification of analyte

M_a: molecular weight of analyte

M_s: molecular weight of internal standard

W_s: weight of internal standard

Appendix F: Summary of measurement equations and uncertainty budgets

W_a: weight of analyte p_s: purity of internal standard

1. LC-UV (structurally related impurities)

$$u_{chromatography} = \frac{SD_{main}}{\sqrt{n}}$$

SD_{main}: standard deviation of main component content measured by LC-UV n: number of sample

2. KF titration (water content)

$$u_{KF} = \frac{SD_{water}}{\sqrt{n}}$$

SD_{water}: standard deviation of water content measured by KF titration n: number of sample

3. TGA (non-volatile impurities)

$$u_{TGA} = \frac{SD_{non-volatile\ impurities}}{\sqrt{n}}$$

$SD_{non\text{-}volatile\ impurities}$: standard deviation of non-volatile impurities content measured by TGA n: number of sample

4. Headspace-GC/MS (volatile organics)

$$u_{HS-GC/MS} = \sqrt{\sum_{j=1}^{n} \left(u_{volatile\ organics,j}\right)^2}$$

case1: peak S/N < 3

$$u_{volatile\ organics} = \frac{LOD}{\sqrt{3} \times W_{sample}}$$

LOD: limit of detection W_{sample} : weight of the sample

case2: peak S/N > 3

Appendix F: Summary of measurement equations and uncertainty budgets

$$u_{volatile\ organics} = \frac{SD}{Slope}\sqrt{\frac{1}{p}+\frac{1}{n}+\frac{C_0 - C_m}{S_{xx}}} \div W_{sample}$$

$$S_{xx} = \sum_{j=1}^{n}\left(C_j - C_m\right)^2$$

SD : standard deviation Slope: slope of the calibration curve p : number of measurements to determine C_0 n: number of measurements for the calibration C_0 : determined volatile organic content C_m : mean value of the different calibration standards C_j : volatile organic content obtain the calibration curve W_{sample} : weight of the sample

5. IC-CD (chloride ion content)

$$u_{IC-CD} = \sqrt{(u_{std})^2 + \left(\frac{SD_{chloride}}{\sqrt{n}}\right)^2}$$

μ std : uncertainty of chloride standard solutions $SD_{chloride}$: standard deviation of chloride contents in samples measured by IC-CD n: number of sample

6. Combination of value

$$u_{OTCHCl} = \sqrt{\left(u_{imputies}\right)^2 + \left(u_{chromatography}\right)^2 + (u_{IC-CD})^2}$$

$$u_{impurities} = \sqrt{(u_{KF})^2 + (u_{TGA})^2 + \left(u_{HS-GC/MS}\right)^2}$$

The uncertainty was pooled with the standard uncertainty of mass balance result.
Major uncertainty contribution was from measurements of structure related impurities, water content and chloride ions.

7. qNMR

$$u_{NMR} = \sqrt{\left(\frac{u(I_a/I_s)}{I_a/I_s}\right)^2 + \left(\frac{u(M_a)}{M_a}\right)^2 + \left(\frac{u(M_s)}{M_s}\right)^2 + \left(\frac{u(W_a)}{W_a}\right)^2 + \left(\frac{u(W_s)}{W_s}\right)^2 + \left(\frac{u(P_s)}{P_s}\right)^2}$$

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: KIMIA

Organic purity = 100 % – I_{SRO}

$$F_{Residual} = \frac{m_{OV}}{m_{sample}} \qquad F_{Water} = \frac{m_{w,corrected}}{m_{sample}} \qquad F_{TNV} = \frac{m_{residue}}{m_{sample}}$$

$$P_{MB} = (1000 - I_{SRO}) \times (1000 - F_{Residual} - F_{Water} - F_{Cl} - F_{TNV})/1000 \text{ mg/g}$$

$$u(P_{MB}) = \sqrt{c_{I_{SRO}}^2 u_{I_{SRO}}^2 + c_{F_{Residual}}^2 u_{F_{Residual}}^2 + c_{F_{Water}}^2 u_{F_{Water}}^2 + c_{F_{Cl}}^2 u_{F_{Cl}}^2 + c_{F_{TNV}}^2 u_{F_{TNV}}^2}$$

Components in purity analysis						
	Value	u(x)	Rel. u(x)	Sensitivity Coefficient (c)	c^2*u^2	Contribution to U
1. Structurally related organic substances (SRO)						
100% - I_{SRO} %	96.6039%	0.20280%	0.20993%			
F_{NR}	1%	0.16667%	16.66667%	0.92853	6.15637E-06	25.087%
F_{ND}	1%	0.05000%	5.00000%			
100% - I_{SRO} %	96.6039%	0.26722%	0.27661%			
2. Water	6.9418%	0.40235%	5.796%	-0.96604	1.510766E-05	61.563%
3. Residual solvent	0.1800%	0.12000%	66.7%	-0.96604	1.343853E-06	5.476%
4.Total non-volatiles (TNV)	0.0251%	0.14390%	573.2%	-0.96604	1.932461E-06	7.875%
Combined	89.700%	0.495%	0.552%		2.45403E-05	

Property value of a reference material and the accosiated uncertainty can be expressed as:				
Purity	89.700%			
u(x)	0.495%			
k (at 95% level)	2			
U(x) with *k* =2	0.991%			
%U(x)	1.105%			
95% Confidence Interval			(Note that the upper limit of this purity range may exceed	Confidence Level = 95%
88.71%	89.70%	90.69%		*k* = 2

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: VNIIM

$$w_{OTC.HCl} = 1000 - w_{RS} - w_{TNV} - w_{H2O} - w_{VOC} - w_I$$

w_{RS}- mass fraction of structurally related Imp

w_{NV} – mass fraction of total non-volatiles and inorganics

w_{H2O} – mass fraction of water

w_{VOC} – mass fraction of volatile organics content

w_I – mass fraction of other ions (Br^-, F^- , ets.)

Other ions (Br^-, F^- , ets.) are not detected (<0,03 mg/g)

$$w_{RS} = \sum_{i=1}^{13} w_{imp}$$

$$w_{VOC} = w_{CH_3CN} + w_{CH_3OH} + w_{C_2H_5OH}$$

$$U^0 = 2\sqrt{u_{RS}^2 + u_{TNV}^2 + u_{H2O}^2 + u_{VOC}^2}$$

$$u_{RS} = \sqrt{u_{imp1}^2 + u_{imp2}^2 + u_{imp3}^2 + u_{imp4}^2 + u_{imp5}^2 + u_{imp6}^2 + u_{imp7}^2 + u_{imp8}^2 + u_{imp9}^2 + u_{imp10}^2 + u_{imp11}^2 + u_{imp12}^2 + u_{imp13}^2}$$

$$u_{(RS)i} = \sqrt{u_A^2 + u_{cal}^2 + u_{samp}^2}$$

i - identified *Imp. A, Imp. B, Imp. D, Imp. E*

$$u_{(RS)j} = \sqrt{u_A^2 + u_{cal}^2 + u_{samp}^2 + u_{un}^2}$$

j - unidentified Imp. 2, Imp. (5-12)

u_A — SD of RS measurement results, mg/g

u_{cal} — uncertainty due to calibration, mg

u_{samp} - — uncertainty due to sample preparation, mg

u_{un} - — uncertainty due to unknown RRF for unidentified Imp, mg

u_{NV} — combined standard uncertainty of non-volatiles mesurement,mg/g

$u_{TNV} = \frac{LLOQ}{\sqrt{3}} = \frac{0{,}0004}{\sqrt{3}}$; ***LLOQ*** - low limit of quantitation of TGA method

u_{H2O} — combined standard uncertainty of water mesurement, mg/g

$$u_{H2O} = \sqrt{u_a^2 + u_b^2} = \sqrt{\left(\frac{SD}{\sqrt{n}}\right)^2 + \left(\frac{u_{KFtitrator}}{\sqrt{3}}\right)^2}$$

Appendix F: Summary of measurement equations and uncertainty budgets

$u_{KF\ titrator}$ - uncertainty due to titrator characteristics, mg

$$u_{VOC} = \sqrt{u_{CH3CN}^2 + u_{CH3OH}^2 + u_{C2H5OH}^2}$$

$$u_{(VOC)i} = \sqrt{u_A^2 + u_{cal}^2 + u_{samp}^2}$$

i = CH3CN, CH3OH, C2H5OH

u_A — SD of VOC measurement results, mg/g

u_{cal} — uncertainty due to calibration, mg

u_{samp} - — uncertainty due to sample preparation, mg

Source of uncertainty	mg/g
u_{RS}	0,93
u_{NV}	0,002
u_{H2O}	1,21
u_{VOC}	0,007
Combined Standard Uncertainy	1,5
Expanded Uncertainty (k=2)	3,0

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: NIMT

Mass balance

$w_A = [1000 - (w_W + w_{NV} + w_{OS})] * w_{Org}$

w_W : Mass Fraction of Water in sample

w_{NV}: Mass Fraction of Nonvolatile Materials in sample

w_{OS} :Mass Fraction of Residual Organic Solvent in sample

w_{Org} :Mass fraction of structurally related impurities in sample

Mass balance

$$u(w_A) = \sqrt{u(w_{Org})^2 + u(w_W)^2 + u(w_{OS})^2 + u(w_{NV})^2}$$

where;

u_{wOrg} standard uncertainty of sample–structurally related impurities in sample

u_{ww} standard uncertainty of water in sample

u_{OS} standard uncertainty of organic solvent in sample

u_{NV} standard uncertainty of non-volatile in sample

Uncertainty budget

Parameter	Source of uncertainty	xi	u(xi)	u(xi)^2
M(H2O)	Mass fraction of H2O (mg/g)	59.06	16.64	276.98945
M(OTC)	Mass fraction of OTC (mg/g)	968.76	0.70	0.49000
M(V)	Mass fraction of volatiles (mg/g)	0.00	1.44	2.08225
M(NV)	Mass fraction of non-volatiles (mg/g)	5.42	0.41	0.16810
Impurities (H_2O, NV and OS) (mg/g)		64.48		
Oxytetracycline .HCL content (mg/g)		906.29	16.725	
Expanded uncertainty (k=2) (mg/g)		33.45		

qNMR

$$P_{Analyte} = \frac{I_{analyte}}{I_{std}} x \frac{N_{std}}{N_{analyte}} x \frac{M_{analyte}}{M_{std}} x \frac{m_{std}}{m_{analyte}} x P_{std}$$

Where: $I_{analyte}$ = integrated signal area of analyte

I_{Std} = integrated signal area of standard

N_{Std} = number of H in the integrated signal area of standard

$N_{analyte}$ = number of H in the integrated signal area of analyte

Appendix F: Summary of measurement equations and uncertainty budgets

$M_{analyte}$ = molar masses of the analyte

M_{Std} = molar masses of the standard

m_{Std} = the mass of the standard

$m_{analyte}$ = the mass of the analyte

P_{Std} = the purity of the standard

Uncertainty budget

$$u_c(P_{Analyte}) = \sqrt{\left(\frac{u\left(I_{analyte}/I_{std}\right)}{\left(I_{analyte}/I_{std}\right)}\right)^2 + \left(\frac{u\left(M_{analyte}\right)}{M_{analyte}}\right)^2 + \left(\frac{u\left(M_{std}\right)}{M_{std}}\right)^2 + \left(\frac{u\left(m_{std}\right)}{m_{std}}\right)^2 + \left(\frac{u\left(m_{analyte}\right)}{m_{analyte}}\right)^2 + \left(\frac{u\left(P_{std}\right)}{P_{std}}\right)^2}$$

Where: $u(I_{analyte}/I_{std})$ = the std. uncertainty of integrated signal area of analyte

$u(M_{analyte})$ = the std. uncertainty of molar masses of the analyte

$u(M_{Std})$ = the std. uncertainty of molar masses of the standard

$u(m_{Std})$ = the std. uncertainty of the mass of the standard

$u(m_{analyte})$ = the std. uncertainty of the mass of the analyte

MU budget for Oxytetracyclin.HCl purity value, determined by ^{1}H NMR					
Source of uncertainty	Value	u(x)	rel u (%)	Veff	Rel.U4/Vi
Method Repeatability		0.0057			
BB in-homogeneity Uncertainty		0.0165			
PANALYTE,mean/%	91.0%	1.742%	1.915%	9	1.4927E-08
$\rho_{internal\ std.}$	1	0.000	0.000%	61	0
$\rho_{ANALYTE}$	3	0.000	0.000%	61	0
Pinternal std./%	99.8%	0.190%	0.190%	30	4.3807E-13
m internal std.	0.055	0.001%	0.025%	4	1.0527E-15
m analyte	0.10	0.001%	0.014%	4	9.3216E-17
$MW_{ANALYTE}$	496.8924	0.003	0.001%	4	2.9006E-22
$MW_{internal\ std.}$	260.8832	0.006	0.002%	10	2.7978E-20
	uc =	**1.75%**	K =	2.26	
	U =	**4.0%**			

Appendix F: Summary of measurement equations and uncertainty budgets

mass balance combined qNMR

$$P_{final} = \frac{P_{MB} + P_{qNMR}}{2}$$

Uncertainty budget

$$u_{\text{final}} = \sqrt{\left(P_{MB} - P_{qNMR}\right)^2 + u_{MB}^2 + u_{qNMR}^2}$$

Parameter	Source of uncertainty	xi	u(xi)	u(xi)^2
P_{MB}	Mass fraction of Oxytetracycline .HCL from mass balance (mg/g)	906.29	16.7	278.89
P_{qNMR}	Mass fraction of Oxytetracycline .HCL from qNMR (mg/g)	910.00	17.5	306.25
Different value between mass balance and qNMR (mg/g)		3.71		
P_{final}	Mass fraction of Oxytetracycline .HCL from mass balance combined qNMR (mg/g)	908.2	24.47	
Expanded uncertainty (k=2) (mg/g)		48.7		

Appendix F: Summary of measurement equations and uncertainty budgets

Participant: NML Phil

Karl Fischer:

Corrected H2O = Raw H2O- Blank H2O

%H2O = Corrected H2O/ Mass

HPLC-UV:

$$Raw\ Purity\ (\%) = \frac{Area_{Analyte}}{\sum_{n=1}^{x} Area_{Peak}}\ x\ 100$$

$Area_{Analyte}$ = Integrated area under the analyte

$Area_{Peak}$ = Integrated area under a peak

x = Number of peaks

TGA-DSC:

$$\%\,\text{Im purity} = \frac{(M_i - M_d) + M_f}{M_i} \times 100\,\%$$

$$= I_{OT}$$

Where:

M_i = Initial sample weight

M_d = Dried sample weight at moderate temperature(e.g., in the range of 120 to 180°C)

M_f = Residue weight at high temperature (e.g. 850°C)

I_{OT} = Impurity level detected by TGA

Combination of values:

Purity (%) = (100 - $I_{"Organic"}$)*(100 - I_{Other})

Equation 1

Where

$I_{"Organic"}$ = Mass fraction of organic impurities of similar structure.

I_{Other} = Mass fraction of volatile and non-volatile impurities.

The overall uncertainty of the purity value (U_{Purity}) is calculated from the individual uncertainty components associated with $I_{"Organic"}$ and I_{Other} using equation 2.

$$u_{Purity} = P\sqrt{\left(\frac{U_{Organic}}{I_{Organic}}\right)^2 + \left(\frac{U_{Other}}{I_{Other}}\right)^2}$$

Equation 2

Appendix F: Summary of measurement equations and uncertainty budgets

	Value	u(x)	Rel. u(x)	% uncertainty contribution
1. Organic purity which is concerned with the mass fraction of impurities with similar	95.318%	0.48%	0.50%	**55.02%**
2. Method repeatability	1	0.08%	0.08%	**8.80%**
3. Variance between samples	1	0.04%	0.04%	**4.40%**
4. Impurity not resolved from the main analyte in HPLC	1	0.058%	0.058%	**6.35%**
5. Impurities below the LOD in the HPLC	1	0.02%	0.02%	**2.20%**
6. Combined uncertainty associated with the volatile impurity and non-volatile residue	0.934	0.20%	0.21%	**23.22%**
			0.91%	**100.00%**

APPENDIX G: Core competency claims by participants

CCQM-K179	*HSA*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*(1) Structural elucidation by NMR spectroscopy; and* *(2) Comparison of retention time and UV absorption profile of the comparison material with those of the reference standards of oxytetracycline HCl from different source (Dr Ehrenstorfer).*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*Approach 1: Deduction of four classes of impurities from 1,000 mg/g using the mass balance approach and conversion of mass fraction of oxytetracycline free base (determined from mass balance approach as reported in CCQM-K148.b) to oxytetracycline HCl based on molecular weight; and* *Approach 2: Direct determination of the main component (oxytetracycline HCl) using quantitative nuclear magnetic resonance spectroscopy via internal standard method.*
Oxytetracycline.HCl content (mg/g)	✓	*838.8 ± 14.6*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*(1) HPLC-DAD for identification and quantification of structurally related impurities using relative peak area approach; and* *(2) LC-MS/MS for identification of structurally related impurities.*
Total structurally related impurity (mg/g)	✓	*44.3 ± 17.1*
Assignment of water content	✓	*Karl Fischer Coulometer*
Category of water content assignment[1]	✓	*Polar organic solid, water content > 20 mg/g*
Water content (mg/g)	✓	*106.4 ± 7.9*
Assignment of chloride content	✓	*Ion chromatography* *(TQ-ICP-IDMS method for verification of chloride content).*
Chloride content (mg/g)	✓	*64.4 ± 4.6*
Assignment of residual solvent content	✓	*(1) GC-MS for identification and estimation of residual solvent;* *(2) NMR for identification and quantification of residual solvent; and*

Appendix G: Core competency claims by participants

		(3) TGA for quantification of residual solvent.
Total residual solvent (mg/g)	✓	*0.024 ± 2.11*
Assignment of inorganic content	✓	*(1) TGA for quantification of total non-volatiles/inorganics; and* *(2) ICP-MS for identification and quantification of inorganics.*
Non-volatiles (mg/g)	✓	*0 ± 3.27*

Appendix G: Core competency claims by participants

CCQM-K179	*NMISA*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*Summary of methods used to establish the qualitative identity (e.g., comparison with independent sample, mass spec., NMR, other)* *Proton and Carbon NMR, compared to literature references, and comparison against authentic reference standards for Oxytetracycline hydrochloride against retention time, UV absorbance and proton/carbon NMR spectroscopy.*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*Indicate method(s) used to quantify mass fraction of OTC in the material* *Q-NMR (internal standard technique) with impurity subtraction using NMR processing and chromatographic impurities, quantified by LC-UV external calibration with verification against mass balance and chloride estimation (ion chromatography)*
Oxytetracycline.HCl content (mg/g)		*Reported comparison result (± $U_{95\%}$)* *859 ± 14 mg/g*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*Indicate method(s) used to quantify mass fraction of structurally related impurities in the material* *LC-UV external calibration against authentic reference standards and relative response factors for unidentified impurities*
Total structurally related impurity (mg/g)		*Reported comparison result (± $U_{95\%}$)*
Assignment of water content	✓	*Indicate method(s) used to quantify mass fraction water content in the material* *KF coulometric titration with oven transfer (cross-check against direct insertion KF coulometry)*
Category of water content assignment[1]	✓	*Select from list below[1] the applicable category of general water content assignment competency* polar organic solid, water content > 20 mg/g
Water content (mg/g)		*Reported comparison result (± $U_{95\%}$)* 97.5 ± *5.8 mg/g* corrected to %RH50
Assignment of chloride content	✓	*Indicate method(s) used to quantify mass fraction chloride ion content in the material* *Ion chromatography with external calibration*
Chloride content (mg/g)		*Reported comparison result (± $U_{95\%}$)* 58.5 ± *3 mg/g* corrected to %RH50

Appendix G: Core competency claims by participants

Assignment of residual solvent content	✓	*Indicate method(s) used to quantify mass fraction residual solvent content in the material* *Headspace Gas chromatography with time-of-flight mass spectrometric identification and quantification by external calibration by HS-GC-FID and GC-TOFMS.*
Total residual solvent (mg/g)		*Reported comparison result (± $U_{95\%}$)* 0.47 ± *0.17 mg/g*
Assignment of inorganic content[2]	✓	*Indicate method(s) used to quantify mass fraction of non-volatile content in the material* *Below LOD*
Non-volatiles (mg/g)[2]	✓	*Reported comparison result (± $U_{95\%}$)* *Below LOD*

Appendix G: Core competency claims by participants

CCQM-K179	NMIJ	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	Comparison of mass spectrum and NMR spectrum with a commercial sample
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	qNMR and Mass balance approach (LC-UV, LC-CAD, GC-FID, KF, TG)
Oxytetracycline.HCl content (mg/g)	✓	853.5 ± 7.2 (k = 2)
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	LC (UV, CAD)
Total structurally related impurity (mg/g)	✓	34.56 ± 5.60 (k = 2)
Assignment of water content	✓	Coulometric Karl Fischer titration with oven transfer
Category of water content assignment[1]	✓	polar organic solid, water content > 20 mg/g
Water content (mg/g)	✓	107.04 ± 5.42 (k = 2)
Assignment of chloride content	✓	IC (CD)
Chloride content (mg/g)	✓	63.07 ± 0.14 (k = 2)
Assignment of residual solvent content	✓	GC (FID)
Total residual solvent (mg/g)	✓	0.00 ± 0.58 (k = 1.65)
Assignment of inorganic content[2]	✓	TG
Non-volatiles (mg/g)[2]	✓	0.16 ± 0.17 (k = 1.65)

Appendix G: Core competency claims by participants

CCQM-K179	*GLHK*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{ow} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	NMR, LC-UV, LC-MS, comparison with independent sample
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	Combination of mass balance method (indirect) and qNMR method (direct)
Oxytetracycline.HCl content (mg/g)		860.3 ± 8.9 (± $U_{95\%}$)
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	LC-UV
Total structurally related impurity (mg/g)		32.0 ± 6.5 (± $U_{95\%}$)
Assignment of water content	✓	Coulometric Karl Fischer titration with oven transfer, TGA as supporting
Category of water content assignment[1]	✓	polar organic solid, water content > 20 mg/g
Water content (mg/g)		103 ± 12 (± $U_{95\%}$)
Assignment of chloride content	✓	Ion chromatography (IC) with conductivity detector, IC-ICP-MS as supporting
Chloride content (mg/g)		64.7 ± 5.7 (± $U_{95\%}$)
Assignment of residual solvent content	✓	qNMR, HS GC-MS as supporting
Total residual solvent (mg/g)		0.021 ± 2 (± $U_{95\%}$)
Assignment of inorganic content	✓	TGA, ICP-MS
Non-volatiles (mg/g)		0.017 ± 2 (± $U_{95\%}$)

Appendix G: Core competency claims by participants

CCQM-K179	*Inmetro*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{ow} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*spectra according to OTC.HCl structure: -MS and UV (from LC-PDA-MS/MS) -NMR - X-ray fluorescence to determine the counter-ion*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*qNMR combined with mass balance (Mass balance considered structurally related substances, water, residual solvent and inorganics including chlorine)*
Oxytetracycline.HCl content (mg/g)	✓	*859.9 mg/g ± 7.2 mg/g (k=2)*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*LC-PDA, LC-MSMS, qNMR*
Total structurally related impurity (mg/g)	✓	*33.4 mg/g ± 4.8 mg/g (k=2)*
Assignment of water content	✓	*Karl Fischer direct sampling coulometric titration*
Category of water content assignment[1]	✓	*polar organic solid, water content > 20 mg/g*
Water content (mg/g)	✓	*101.8 mg/g ± 2.4 mg/g (k=2)*
Assignment of chloride content	✓	*Cloride = X-ray Fluorescence*
Chloride content (mg/g)	✓	*1.8 ± 2.2 mg/g (k=2) – corresponds to the excess chloride impurity after subtraction of the stoichiometric chlorine.*
Assignment of residual solvent content	✓	*HS-GC-MS (qualitative analysis) and qHNMR (qualitative and quantitative analysis)*
Total residual solvent (mg/g)	✓	*0.229 mg/g ± 0.019 mg/g (k=2)*
Assignment of inorganic content	✓	*Cloride= X-ray Fluorescence Elementary Analysis= ICP-MS and ICP-OES*
Non-volatiles (mg/g)	✓	*1.8 mg/g ± 2.2 mg/g (k=2)*

CCQM-K179	*EXHM*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*comparison with independent EP sample, mass spectroscopy, NMR*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*Mass balance verified by qNMR*
Oxytetracycline.HCl content (mg/g)	✓	*860.64 ± 10.08*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*HPLC-DAD-CAD, LCqTOF-MS verified by qNMR*
Total structurally related impurity (mg/g)		*34.44 ± 5.24*
Assignment of water content	✓	*Coulometric titration*
Category of water content assignment[1]	✓	*polar organic solid, water content > 20 mg/g*
Water content (mg/g)		*105.33 ± 1.33*
Assignment of chloride content	✓	*ION CHROMATOGRAPHY, ICP-MS*
Chloride content (mg/g)		*62.40 ± 2.72*
Assignment of residual solvent content	✓	*ION CHROMATOGRAPHY, ICP-MS*
Total residual solvent (mg/g)		*0.00 ± 0.02*
Assignment of inorganic content	✓	*ION CHROMATOGRAPHY, ICP-MS*
Non-volatiles (mg/g)		*64.16 ± 2.80*

Appendix G: Core competency claims by participants

CCQM-K179	*TÜBİTAK UME*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	HPLC-UV, NMR
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	Mass Balance (HPLC-UV, Karl-Fischer coulometry, HS GC-MS, Ion chromatography), qNMR
Oxytetracycline.HCl content (mg/g)	✓	879.5 ± 16.7
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	Mass Balance (HPLC-UV, Karl-Fischer coulometry, HS GC-MS, Ion chromatography), qNMR
Total structurally related impurity (mg/g)	✓	47.6 ± 0.8
Assignment of water content	✓	Coulometric Karl Fischer titration with oven transfer
Category of water content assignment[1]	✓	polar organic solid, water content > 20 mg/g
Water content (mg/g)	✓	73.4 ± 1.0
Assignment of chloride content	✓	Ion Chromatography
Chloride content (mg/g)	✓	61.3 ± 1.6
Assignment of residual solvent content	✓	HS GC-FID and qNMR
Total residual solvent (mg/g)	✓	0.17 ± 0.002
Assignment of inorganic content	NA	NA
Non-volatiles (mg/g)	NA	NA

Appendix G: Core competency claims by participants

CCQM-K179	KRISS	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{ow} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	Comparison with independent sample, LC-UV, LC-MS, and NMR
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	Mass balance method
Oxytetracycline.HCl content (mg/g)	✓	(867.4 ± 13.1) mg/g (with 95% of confidence level, *k*=2.0)
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	LC-UV
Total structurally related impurity (mg/g)	✓	(38.1 ± 1.6) mg/g (with 95% of confidence level, *k*=2.1)
Assignment of water content	✓	Coulometric KF titration with oven method
Category of water content assignment[1]	✓	polar organic solid, water content > 20 mg/g
Water content (mg/g)	✓	(78.1 ± 3.7) mg/g (with 95% of confidence level, *k*=2.1)
Assignment of chloride content	✓	IC-CD
Chloride content (mg/g)	✓	(64.6± 1.1) mg/g (with 95% of confidence level, *k*=2.6)
Assignment of residual solvent content	✓	Headspace GC-MS
Total residual solvent (mg/g)	✓	(0.1 ± 3.3) mg/g (with 95% of confidence level, *k*=2.0)
Assignment of inorganic content	✓	TGA
Non-volatiles (mg/g)	✓	(0.1 ± 1.3) mg/g (with 95% of confidence level, *k*=2.0)

Appendix G: Core competency claims by participants

CCQM-K179	*KIMIA*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*1) Comparison with reference standard* *2) FT-IR*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*1) HPLC-UV-PDA: Structurally related organic compound* *2) Coulometric Karl Fischer Titration: Water* *3) Headspace GC-FID: Residual solvent* *4) TGA: Total non-volatiles* *5) Ion chromatography: Chloride ion*
Oxytetracycline.HCl content (mg/g)	✓	*897 mg/g ± 9.9 mg/g*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*HPLC-UV-PDA*
Total structurally related impurity (mg/g)	✓	*33.97 mg/g ± 5.34 mg/g*
Assignment of water content	✓	*Coulometric Karl Fischer Titration* *TGA (as a consistency check)*
Category of water content assignment[1]	✓	*Polar organic solid, water content > 20 mg/g*
Water content (mg/g)	✓	*69.42 mg/g ± 8.04 mg/g*
Assignment of chloride content	✓	*Ion chromatography*
Chloride content (mg/g)	✓	*72.33 mg/g ± 4.84 mg/g*
Assignment of residual solvent content	✓	*Headspace GC-FID* *GC-MS (direct injection) &* *TGA (as consistency check)*
Total residual solvent (mg/g)	✓	*1.8 mg/g ± 2.4 mg/g*
Assignment of inorganic content	✓	*TGA (under high-temperature oxidative conditions)*
Non-volatiles (mg/g)	✓	*0.25 mg/g ± 2.88 mg/g*

Appendix G: Core competency claims by participants

CCQM-K179	*VNIIM*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*LC/MS mass-spectra*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*Mass balance approach:* *Structurally related imp.- LC/DAD;* *Residual solvent – GC/MS, GC/FID;* *Water - KF titration with oven;* *Non-volatiles – TGA* *Chloride ion - CE*
Oxytetracycline.HCl content (mg/g)	✓	*919,3 ± 3,0*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*LC/DAD*
Total structurally related impurity (mg/g)	✓	*17,75 ± 1,86*
Assignment of water content	✓	*KF titration with oven*
Category of water content assignment[1]	**N/A**	
Water content (mg/g)	✓	*62,34 ± 2,42*
Assignment of chloride content	✓	*CE*
Chloride content (mg/g)	✓	*72,86 ± 4,44*
Assignment of residual solvent content	✓	*GC/FID*
Total residual solvent (mg/g)	✓	*0,560 ± 0,014*
Assignment of inorganic content		*TGA*
Non-volatiles (mg/g)		*< 0,004*

Appendix G: Core competency claims by participants

CCQM-K179	*NMLPhil*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{ow} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	Comparison with independent sample source from Sigma Aldrich
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	Mass Balance approach: HPLC-UV to determine the organic impurities, Karl Fischer with oven transfer for water content, TGA for volatile and non-volatile matter
Oxytetracycline.HCl content (mg/g)		890.7 ± 10.4 mg/g ($U_{95\%}$)
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	HPLC-UV to determine the organic impurities
Total structurally related impurity (mg/g)		46.8 ± 9.6 mg/g ($U_{95\%}$)
Assignment of water content	✓	Karl Fischer with oven transfer
Category of water content assignment[1]	✓	polar organic solid, water content < 20 mg/g
Water content (mg/g)		58.0 ± 3.82 mg/g ($U_{95\%}$) – corrected at RH 50% 58.2 ± 3.82 mg/g ($U_{95\%}$) – observed at local RH
Assignment of chloride content		Ion Chromatography
Chloride content (mg/g)		68.6 ± 6.0 mg/g ($U_{95\%}$)
Assignment of residual solvent content	✓	Thermogravimetric Analyzer (TGA)
Total residual solvent (mg/g)		4.22 ± 3.88 mg/g ($U_{95\%}$)
Assignment of inorganic content	✓	Thermogravimetric Analyzer (TGA)
Non-volatiles (mg/g)		5.43 ± 0.78 mg/g ($U_{95\%}$)

Appendix G: Core competency claims by participants

CCQM-K179	*NIM*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{OW} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	*the qualitative identity was established by comparison with independent sample and the LC-MS/MS*
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	*Indicate methods used to quantify mass fraction of OTC in the material are mass balance and QNMR*
Oxytetracycline.HCl content (mg/g)	✓	*(854.5±10.4) mg/g (± $U_{95\%}$)*
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	*External calibration method was used to quantify mass fraction of structurally related impurities in the material*
Total structurally related impurity (mg/g)	✓	*(48.38±4.2) mg/g (± $U_{95\%}$)*
Assignment of water content	✓	*Karl Fischer titration method was used to quantify mass fraction water content in the material*
Category of water content assignment[1]	✓	*polar organic solid, water content > 20 mg/g*
Water content (mg/g)	✓	*(89.90±8.18) mg/g (± $U_{95\%}$)*
Assignment of chloride content	✓	*Ion Chromatography method was used to quantify mass fraction chloride ion content in the material*
Chloride content (mg/g)	✓	*(2.62±0.06) mg/g (± $U_{95\%}$) (chloride ion residue)* *(65.16±1.4) mg/g(± $U_{95\%}$) (total chloride content)*
Assignment of residual solvent content	✓	*headspace GC method was used to quantify mass fraction residual solvent content in the material*
Total residual solvent (mg/g)	✓	*(0.89±0.04) mg/g (± $U_{95\%}$)*
Assignment of inorganic content	✓	*ICP-MS with internal standards was used to quantify mass fraction total non-volatile content in the material*
Non-volatiles (mg/g)	✓	*(0.18±0.02) mg/g (± $U_{95\%}$)*

Appendix G: Core competency claims by participants

CCQM-K179	*NIMT*	Mass fraction of analyte in the salt form in a solid organic material
Scope of comparison: The measurement results are representative of the laboratory's capability for the purity assignment of organic compounds present as salts in the molar mass range 75 - 500 g/mol with pK_{ow} > -2.		
Competency	**✓,× or N/A**	**Specific Information**
• Value assignment of Primary Reference: Main component mass fraction and uncertainty		
Identity verification	✓	comparison with commercial OTC.HCl salt standard (Supelco) using HPLC-PDA and ^{1}H- NMR
Assignment of OTC.HCl salt mass fraction content of CCQM-K179	✓	Mass balance and qNMR
Oxytetracycline.HCl content (mg/g)		908.2 ± 48.7 (mg/g)
• Value assignment of Primary Reference: Impurity class mass fraction and uncertainty (required if using a mass balance method, otherwise optional)		
Assignment of structurally related impurity	✓	HPLC-PDA
Total structurally related impurity (mg/g)	✓	31.23 ± 1.42 (mg/g)
Assignment of water content	✓	Karl Fischer Titration (KFT)
Category of water content assignment[1]	✓	polar organic solid, water content > 20 mg/g
Water content (mg/g)	✓	59.07 ± 33.28 (mg/g)
Assignment of chloride content	✓	Ion chromatography
Chloride content (mg/g)	✓	61.66 ± 9.78 (mg/g)
Assignment of residual solvent content	✓	Thermogravimetric Analysis (TGA)
Total residual solvent (mg/g)	✓	0 ± 2.88 (mg/g)
Assignment of inorganic content	✓	Ion chromatography
Non-volatiles (mg/g)	✓	5.42 ±0.82 (mg/g)