

CCQM-K140: Carbon Stable Isotope Ratio Delta Values in Honey

Final Report

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1 - INTRODUCTION

Isotope ratio measurements are applied in a number of different fields including archaeology, environmental science, geochemistry, forensic science and ecology. This is because there can be small but measurable differences in isotope ratio between different sources of the same element/compound/material. For example the chemical composition of sucrose obtained from sugar beet and sugar cane is identical, however the $^{13}\text{C}/^{12}\text{C}$ isotope ratio of the two different sucroses is not. Isotope ratios for the light elements (H, C, N, O and S) are typically reported as delta values which are isotope ratios expressed relative to an internationally agreed standard (this standard is the zero-point on the scale). Absolute isotope ratios which are traceable to the SI have also been reported. An example delta value expression for carbon isotope ratios is given below where ^{13}R is the $^{13}\text{C}/^{12}\text{C}$ ratio:

$$\text{Equation 1} \quad \text{sample } \delta^{13}\text{C}_{std} = \left(\frac{{}^{13}R_{\text{sample}}}{{}^{13}R_{\text{std}}} \right) - 1$$

Delta values are typically very small and so the result is often multiplied by a factor of 1000 and thereby expressed in permil (in an analogous way to multiplying a fraction by a factor of 100 and thereby expressing the result in percent). Delta scales are for the most part defined by one or more artefacts with exact delta values which have been assigned via consensus. For example in the case of carbon, Urey *et al* (1951) reported the use of a Cretaceous Belemnite material from the Pee Dee formation in South Carolina as a laboratory standard, which was later proposed as the international reference material (known as PDB, with $\delta^{13}\text{C}_{\text{PDB}} = 0 \text{ ‰}$ by definition) by Craig (1957). This material has since been exhausted and therefore a new international scale known as Vienna Pee Dee Belemnite (VPDB) as defined based upon assigning an exact $\delta^{13}\text{C}_{\text{VPDB}}$ value to NBS-19 limestone of +1.95 (a value derived from an inter-laboratory consensus of measurements of NBS-19 vs. the original PDB). The new scale therefore had exactly the same numerical delta values as the original PDB (Coplen, 1995; Hut, 1985; Friedman *et al*, 1982). More recently VPDB has been re-defined by the isotope ratio of LSVEC, a lithium carbonate reference material ($\delta^{13}\text{C}_{\text{VPDB}} = -46.6$ exactly) in addition to that of NBS-19 to account for scale expansion (Coplen *et al*, 2006a; 2006b).

Anchoring of instrumentally measured isotope ratios to the appropriate international reporting scale is achieved via the analysis of reference materials with calibrated delta values on the appropriate scale. These secondary reference materials link measurements to the, scale-defining artefacts and thereby provide traceability to the delta scales. For delta scales defined by two artefacts two or more secondary reference materials should be used for scale calibration (Coplen, 2011). A list of secondary reference materials can be found in the appropriate IUPAC Technical Report (Brand *et al* 2014). Laboratories can also use secondary reference materials to calibrate their own in-house standards that can in turn be used for scale calibration, albeit with an extended traceability chain.

2 - RATIONALE

The Inorganic Analysis Working Group (IAWG) of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) has previously organised several pilot studies on isotope ratio determination (CCQM-P75: Stable isotope delta values in methionine, 2006; CCQM-P105: Sr isotope ratios in wine, 2008; CCQM-K98: Pb isotope ratios in bronze with additional delta values in CCQM-P134, 2011). It has, however, been a number of years since delta values of light elements have been considered and there has been no key comparison (KC).

The IAWG has been granted a traceability exception for the use of arbitrary delta scales until SI traceability can be established at the required level of uncertainty but this goal is some years away. Therefore, the IAWG has included the need for a KC based on an arbitrary delta scale in its future program, to support ongoing requirements to demonstrate core capabilities as well as specific claims of measurement capability (CMCs) in this area. This KC, CCQM-K40, has been coordinated by LGC Ltd, UK and TUBITAK UME, Turkey. A parallel pilot study (CCQM-P175) was also co-ordinated and the results can be found in a separate report.

3 - LIST OF PARTICIPANTS

Table 1 provides information regarding the participants in CCQM-K140. With 5 NMIs participating this was an improvement over the previous pilot study on stable isotope delta values (CCQM-P75 Stable isotope delta values in methionine had only four NMI participants).

Table 1 – Participants in CCQM-K140.

Abbreviation	Contact Person	NMI/DI	Country
JSI	Nives Ogrinc	Jožef Stefan Institute	SI
LGC	Heidi Goenaga-Infante	LGC Ltd	GB
NIM	Lu Hai	National Institute of Metrology, P.R. China	CN
NMIA	Paul Armishaw	National Measurement Institute, Australia	AU
TUBITAK	Ahmet Ceyhan Gören	TÜBİTAK National Metrology Institute	TR

4 - SCHEDULE

Call for participation	June 2015
Registration Deadline	31st August 2015
Sample Distribution	by end of October 2015
Deadline for Reporting Results	29th February 2016
First presentation of results	IAWG Meeting April 2016
Issue of Draft Preliminary Report	End of July 2016

5 - SAMPLE

5.1 - MATERIAL

Each participant received at least two amber borosilicate vials, each containing 2 g of honey and was requested to confirm the delivery of the samples by regular mail or e-mail using the sample receipt form as soon as the samples had arrived.

5.2 - HOMOGENEITY STUDY

Homogeneity study between the units was performed with number of samples representing the whole batch. Twenty units (ten spare) were selected by using random stratified sampling software (TRaNS) and reserved for the study of homogeneity between units. Homogeneity tests were carried out for all candidate CRMs by measuring three sub-samples under the same repeatability conditions. The method used for these measurements was validated and the samples to be analysed were introduced to the instrument by random order to find out any trend arising from analytical and/or filling sequences. Certified reference materials and samples were analysed in the same run. All measurements were carried out using EA-IRMS.

5.3 - SHORT/LONG-TERM STABILITY STUDIES

Short term stability studies were performed with isochronous design which is cited in the ISO Guide 35. For the Short Term Stability (STS) test, two different temperatures (4°C and 60 °C) and four time points (1, 2, 3 and 4 weeks) were tested. Ten samples for each CRM were selected by randomly. 8 samples were subjected to the test temperatures for the specified time intervals.

Samples were moved to +18°C (reference temperature) after completion of the test time. All samples were analysed at the same time. Three replicate samples were prepared from each unit and were analyzed by EA-IRMS under the repeatability conditions for ¹³C/¹²C isotope ratio delta values.

25 °C was chosen as the test temperature for long term stability tests and in total 52 units (26 spare) for each CRM were reserved for this study. Samples were selected by randomly and kept at 25 °C for 12 months. Two units for each time point (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months) were stored at 25 °C and transferred to 18 °C (reference temperature) after completion of the test time. Three replicate samples were prepared from each unit and were analyzed by EA-IRMS under the repeatability conditions for ¹³C/¹²C isotope ratio delta values.

Homogeneity and short/long term stability tests were carried out by TUBITAK UME and the results indicated that the honey material isotopic composition was not significantly different between vials (ten random vials analysed in triplicate gave a SD of 0.07 ‰) and that the isotope ratio would be stable over the time period of this key comparison ($F_{\text{Statistic}}$ for storage at 4 or 60 °C for up to 4 weeks were 1.11 and 0.61, respectively, both of which were less than the F_{Critical} of 2.76; long term stability of up to 12 months at 25 °C gave $F_{\text{Statistic}}$ of 1.09 which again was less than the critical value of 1.90). Statistical results (ANOVA) for homogeneity, short-term stability and long-term stability are given in Table 2, 3 and 4, respectively.

Table 2. ANOVA results for homogeneity study of honey CRM 1312

ANOVA Test for Homogeneity		
Analyte	F-statistic	F-critic
Honey CRM 1312	1.66	2.39

Table 3. ANOVA results for short-term stability study of honey CRM 1312

ANOVA Test for Homogeneity		
Analyte	F-statistic	F-critic
CRM 1312 at 4 °C	1.11	2.76
CRM 1312 at 60 °C	0.61	2.76

Table 4. ANOVA results for long-term stability study of honey CRM 1312

ANOVA Test for Homogeneity		
Analyte	F-statistic	F-critic
CRM 1312 at 25 °C	1.09	1.90

6 - INSTRUCTIONS FOR PARTICIPANTS

There were no specific storage requirements for the vials of honey, although it was recommended that they were kept at room temperature. Vials could be opened multiple times during use. The amount of material to analyse was to follow the usual protocols of participants' laboratory.

The mandatory measurand for the sample was the $\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ value of the bulk honey multiplied by a factor of 10^3 and thereby expressed in permil (‰). A delta value with uncertainty was to be reported as well as the results from at least 5 independent replicates. Participants were requested to provide details over their methods including amount of sample analysed, corrections applied to instrumental data (including but not limited to ^{17}O , drift, linearity, carryover and blank corrections) and method applied for scale calibration (including details over reference materials used to ensure traceability to the VPDB-LSVEC scale).

7 - METHODS OF MEASUREMENT

The participants were free to choose any suitable method for example isotope ratio mass spectrometry (IRMS), cavity ring down spectroscopy (CRDS) or some other form of isotope ratio infrared spectroscopy (IRIS) provided that they included a full description of the method of analysis. This description was to include details over all corrections to instrumental data where they were applied including e.g. those for isobaric interferences (^{17}O correction for IRMS measurements on CO_2), blank, etc. and the source of traceability to the VPDB-LSVEC delta value scale. It was recommended that at least two organic reference materials should be used for scale calibration and that the delta values assigned to these reference materials should be those recommended in the IUPAC technical report (Brand et al, 2014).

Each laboratory was to report a full uncertainty budget as part of their results report. Contributions to the overall uncertainty would arise from the repeatability of the sample preparation, the repeatability of instrumental determination, scale calibration using suitable reference materials and any other parameter specific to the method of analysis chosen by the participant.

8 - CCQM-K140 PARTICIPANTS' RESULTS AND DISCUSSION

8.1 - MEASUREMENT TECHNIQUES

All participants used IRMS to determine the $\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ value of the honey, with an elemental analyser (EA) to convert the honey into CO_2 (isotope ratio mass spectrometers are gas-source instruments). JSI used an Elementar Vario PYRO Cube EA coupled to an Isoprime 100 mass spectrometer. All other participants used Thermo Scientific systems: LGC used a Flash EA/HT with a MAS 200R autosampler (with NoBlank Device) coupled *via* a ConFlo IV to a Delta V

Advantage mass spectrometer; NIM used a Flash 2000 EA coupled to a MAT 253 mass spectrometer; NMIA used two different EA systems, a Flash EA 1112 and a Flash 2000 which were coupled *via* a Conflo IV to a Delta V Plus mass spectrometer; and finally TUBITAK used a Flash 1112 EA coupled *via* a Conflo III to a MAT 253 mass spectrometer.

8.2 - AMOUNTS OF HONEY ANALYSED

Typical amounts of honey analysed ranged from just over 100 to 2500 µg per analysis (Table 5). TUBITAK had the smallest range of masses analysed, with JSI the largest.

Table 5 – Typical masses of honey analysed at each institute given as a range or mean value with standard deviation.

Institute	Typical mass of honey µg
JSI	2000 to 2500
LGC	103 (stdev 66)
NIM	600 to 1000
NMIA	900 ± 100
TUBITAK	200 ± 10

8.3 - CORRECTIONS APPLIED TO RAW DATA

Table 6 details the corrections each participant applied to their raw data (excluding scale calibration which is covered later in this report). These are discussed in more detail in the following sections.

Table 6 – Corrections applied to raw data.

Institute	¹⁷ O	Blank	Linearity	Drift	Memory
JSI	Craig	n	n	y	n
LGC	IUPAC	Mass balance	n*	n*	n*
NIM	IUPAC	Mass balance	n*	y	n*
NMIA	SSH	n*	n*	y	n**
TUBITAK	SSH	Mass balance	n	n	n

*none observed and therefore a correction was deemed unnecessary.

**Memory effect was observed in materials following graphite analyses but affected data were excluded from calculations so a correction was unnecessary.

The raw data for the majority of the participants (JSI, NIM and TIBITAK) were delta values for the sample gas measured relative to a working gas (WG) of known/calibrated isotopic composition calculated by the instrumental software. LGC performed the raw delta value calculation offline and therefore used ion current ratios as the raw input data in their calculations. NMIA used absolute isotope ratios throughout their calculations (¹³R values) until the final conversion into a delta value. Their raw data were therefore ¹³R values for the sample gases as determined by the instrumental software (this again relies on the known and measured isotopic composition of the WG).

8.3.1 - ¹⁷O CORRECTION

For IRMS measurements of carbon isotope ratios on CO₂ gas, the isotopic composition of the oxygen must be taken into account as the m/z 45 ion current will reflect not only the ¹³C¹⁶O¹⁶O isotopologue, but also the ¹²C¹⁶O¹⁷O and ¹²C¹⁷O¹⁶O isotopologues. The latter isotopologues contribute some 6-7 % to the m/z 45 signal and this is typically accounted for by using the m/z

46 signal as a proxy for the ^{18}O isotopic composition of the CO_2 ($^{12}\text{C}^{16}\text{O}^{18}\text{O}$ and $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ account for >99 % of the m/z 46 signal) and then relating this to the ^{17}O isotopic composition.

The ^{17}O corrections are typically carried out by instrumental software packages but can also be applied offline and use one of three different sets of algorithms. The so-called Craig correction (Craig, 1957) is the simplest to apply but makes assumptions regarding oxygen isotopic fractionation which are not supported by experimental evidence. JSI used the Craig correction approach for ^{17}O . The improved algorithm suggested by Santrock Studley and Hayes (Santrock *et al*, 1985, commonly known as SSH) is typically employed in Thermo Scientific IRMS systems. This is an exact approach but requires an iteration procedure to determine the $^{18}\text{O}/^{16}\text{O}$ ratio in the sample CO_2 . Two participants, NMIA and TUBITAK, used the SSH algorithm. The final approach is the ^{17}O algorithm endorsed by IUPAC which is a linear approximation rather than an exact solution and also uses more up-to-date values for the absolute isotope ratios of VPDB than the SSH approach (Brand *et al*, 2010). Two participants, LGC and NIM, used the IUPAC approach.

If the same raw ion current ratios are processed using each of the three algorithms then the differences obtained between the raw delta values are expected to be less than 0.06 permil (Brand *et al*, 2010).

8.3.2 - OTHER ISOBARIC INTERFERENCES AND CORRECTIONS

The presence of water in the ion source of a mass spectrometer can lead to in-situ protonation of CO_2 ions forming interfering species at m/z 45 and 46 (e.g. $^1\text{H}^{12}\text{C}^{16}\text{O}^{16}\text{O}$ is isobaric with $^{13}\text{C}^{16}\text{O}^{16}\text{O}$). The presence of these protonated species is minimised by ensuring that the amount of water within the ion source is as low as possible (Leckrone and Hayes, 1998). Instruments with metal to metal seals for the ion source (e.g. Thermo MAT 253) have significantly lower water backgrounds than those relying on rubber seals (e.g. Thermo Delta V). Provided that the water background is constant within a measurement, then all gases analysed within the ion source should be protonated to the same extent. Only LGC mentioned checking that the level of water within the ion source was below acceptable limits.

8.3.3 - BLANK CORRECTION

The presence of carbon within the tin capsules used to hold the samples during EA-IRMS analysis contributes to the magnitude and isotopic composition of the sample gas. This contribution must be assessed and, if deemed necessary, corrected for.

Three laboratories, LGC, NIM and TUBITAK, found a blank level that required correction and therefore carried out a blank correction of their raw delta values using a mass balance approach as described in the FIRMS Good Practice Guide for IRMS (Carter & Barwick, 2011). The magnitude and isotopic composition of the blank were determined in each case via the analysis of empty tin capsules. TUBITAK applied the blank correction within the instrument proprietary software, for LGC and NIM the procedure was carried out offline. NMIA found negligible background contamination and therefore deemed a blank correction unnecessary. JSI did not apply a blank correction.

The significance of the blank contribution depends largely on whether additional dilution of the sample CO_2 gas is carried out or not. If the sample gas is diluted then larger amounts of honey can be analysed and the blank contribution will be relatively very small. Where there is no dilution of the sample gas, the blank contribution will be more significant.

8.3.4 - LINEARITY CORRECTION

It is a well-known phenomenon that isotope ratio mass spectrometers measure different isotope ratios for the same material depending on the mass of sample analysed (even when the material is isotopically homogeneous). This so-called linearity effect is usually overcome by analysing materials in equal amounts in terms of carbon, rather than application of a specific correction to raw data, although linearity corrections can be performed (the magnitude of the required correction can be determined by analysis of a matrix-matched quality control material at different amount levels). All participating laboratories controlled the mass of honey analysed to some degree, with some having very low tolerances while others having much wider ranges of acceptable mass (Table 5). LGC, NIM and NMIA and TUBITAK deemed a linearity correction was unnecessary within the ranges of mass of honey analysed, JSI also did not carry out a linearity correction.

8.3.5 - DRIFT CORRECTION

Change in raw delta value with time during a sequence of analyses is also a known phenomenon in IRMS. Drift can be corrected for using sample-standard bracketing whereby the reference materials (RMs) used for scale calibration are analysed regularly throughout the analytical sequence and the pairs of RMs used to scale-calibrate the samples between them. Alternatively a RM or quality control (QC) material can be analysed regularly throughout the sequence and the obtained delta values checked for drift with time. If drift is discovered, then these QC data can be used to determine a drift correction for samples in the same sequence. A final option is to run the scale calibration RMs at the beginning and end of each sequence and then to compare the calibration plots obtained for the two sets. If there is no significant difference in slope (measured vs certified delta value for the RMs), then there is no significant drift.

LGC did not find evidence of drift within their analytical sequences and therefore did not apply a drift correction. LGC's sequence design included scale calibration reference materials at the beginning and end which were all used for scale calibration and may therefore account for this lack of observable drift – this could be seen as sample-standard bracketing. Three participating laboratories carried out some form of drift correction: NMIA employed a sample-standard bracketing technique within each sequence; JSI used the results from regularly analysed control materials throughout each analytical sequence to correct for drift; and NIM used either linear or quadratic polynomial drift corrections based upon the analysis of reference materials throughout their analytical sequences. .

8.3.6 - MEMORY CORRECTION

Memory effects (also known as carryover effects) can occur in EA-IRMS analyses and result in carbon from one sample still being in the instrument when the next sample is analysed. It is most clearly visible when two materials of very different isotopic composition are analysed sequentially. Neither LGC nor NIM nor TUBITAK observed any evidence of a significant memory effect and therefore did not deem a memory correction necessary. JSI also did not apply a memory correction. NMIA observed carryover from graphite into subsequent analyses; however the affected analyses were discarded rather than a memory correction being applied.

8.4 - SCALE CALIBRATION APPROACH AND SOURCES OF TRACEABILITY TO VPDB-LSVEC

No two participating laboratories used the same suite of reference materials for scale calibration. Three participants used a two point linear regression approach (JSI, NMIA and TUBITAK), while the remaining two (LGC and NIM) used a multiple point linear regression each

with four RMs. The RMs used by each participant as well as calibration approach can be found in Table 7. The values assigned to these RMs were those from the IUPAC Technical Report (Brand *et al*, 2014). The advantage of multiple point linear regression where n is greater than 2 is that random error associated with the analysis of any one reference material can be detected via the correlation coefficient (Paul *et al*, 2007).

Table 7– Scale calibration approach and RMs used.

Institute	Scale calibration approach	Reference materials used		
		Name	NIST SRM	Material
JSI	Two point	IAEA-CH-6	8542	Sucrose
		IAEA-CH-7	8540	Polyethylene
LGC	Multiple point	NBS-22	8539	Mineral Oil
		IAEA-CH-6	8542	Sucrose
		USGS40	8573	l-Glutamic Acid
		USGS41	8574	l-Glutamic Acid
NIM	Multiple point	IAEA-CH-6	8542	Sucrose
		IAEA-C7	8540	Polyethylene
		IAEA-600*		Caffeine
		USGS40	8573	l-Glutamic Acid
NMIA	Two point	NBS-22	8539	Mineral Oil
		USGS24	8541	Graphite
TUBITAK	Two point	NBS-22	8539	Mineral Oil
		IAEA-CH-6	8542	Sucrose

*IAEA 600 Caffeine is not distributed by NIST.

8.5 - QUALITY CONTROL (QC) MATERIALS

To check the quality of the obtained delta values within a single sequence it is common to analyse one or more quality control material(s) within each sequence for which the delta value is well known. This can be a RM or an in-house calibrated standard, or in an ideal situation, a material matrix-matched to the sample. If the results for the QC material(s) following all corrections (including scale calibration) are within the expected range then the results for unknown samples can be assumed to be reliable. The quality control material(s) used by each laboratory can be found in Table 8.

Table 8 – Quality control material(s) used by participants.

Institute	QC Material(s) used
JSI	
LGC	Olive oil from FIRMS PT Scheme
	Chitin from FIRMS PT Scheme
	USGS42 Hair
	In-house calibrated glycine*
NIM	Two in-house calibrated honey samples
NMIA	USGS40 l-Glutamic Acid
TUBITAK	

* used for long-term instrumental monitoring.

Only NIM used truly matrix matched QC materials within their analytical sequences. LGC and NMIA used certified reference materials and/or materials with known delta values from a proficiency testing scheme amongst their QC materials. JSI used quality control materials to

determine and correct for instrumental drift but gave no indication as to their nature. TUBITAK did not report the use of any quality control materials.

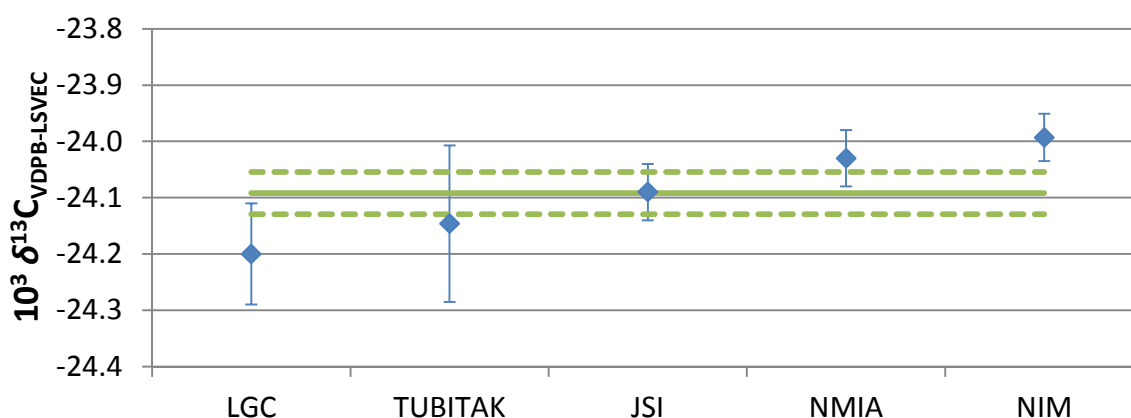
8.6 - REPORTED CARBON ISOTOPE RATIO DELTA VALUES

The delta values on the VPDB-LSVEC scale reported by the participants can be found in Table 9 and Figure 1. The mean value between the participants was -24.09 ‰. Two participants (NIM and TUBITAK) reported values to three decimal places, the others (LGC, JSI and NMIA) only to two.

Table 9 – Reported $\delta^{13}C_{VPDB-LSVEC}$ values for the honey.

Institute	$10^3 \delta^{13}C_{VPDB-LSVEC}$	u	U	k
JSI	-24.09	0.05	0.11	2
LGC	-24.20	0.09	0.18	2
NIM	-23.993	0.042	0.084	2
NMIA	-24.03	0.05	0.11	2
TUBITAK	-24.146	0.139	0.278	2

Figure 1 – Reported $\delta^{13}C_{VPDB-LSVEC}$ values for the honey. The error bars represent the reported standard uncertainty ($k=1$) while the solid green line is the arithmetic mean value and the dashed green lines are this arithmetic mean value plus or minus its standard uncertainty ($k=1$).



8.7 - MEASUREMENT UNCERTAINTY

Each participant was required to provide an estimate of the measurement uncertainty for their reported results. As with the calibration/data reduction, no two participants used the same approach to estimation of their measurement uncertainty, or included exactly the same contributing factors in their calculations. All participants did include the uncertainty associated with the calibration of raw delta values to the VPDB-LSVEC scale using reference materials (including not only the uncertainty in the certified delta values for the reference materials but also the uncertainty in their measured delta values or from the calibration plot). NIM only included the certified uncertainty of one of their four scale calibration reference materials, all other participants (JSI, LGC, NMIA and TUBITAK) included the contributions from all scale calibration reference materials. Of the three participants employing a blank correction, only LGC included the blank determination within their uncertainty budget while NIM and TUBITAK did not. Two participants (LGC and NMIA) also included the influence of the ^{17}O correction in their uncertainty budgets, although the contribution determined by LGC was $<0.1\%$. The raw delta

values for all participants were determined relative to a WG and the precision estimates for the honey delta values includes the uncertainty in the measured isotopic composition of this WG. The assigned/calibrated delta value of the WG has no influence over scale calibrated delta values (as raw delta values sample and reference materials are all measured relative to the same WG), nevertheless TUBITAK included this contribution to uncertainty in their calculations (resulting in a contribution of 0.7 %). The uncertainty budgets for each participant can be found in Table 10 below. To allow better comparison the contributions to uncertainty provided by each participant have been grouped into similar categories.

Table 10 – *Uncertainty budgets for each participant.*

Institute	Factor	Contribution %
JSI	Measured values of honey	19.3
	Certified values of RMs	38.2
	Slope and intercept of calibration curve	42.6
LGC	WG $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$	<0.1
	Constants for ^{17}O correction	<0.1
	Certified values of RMs	10.9
	Measured values of blank*	4.9
	Measured values of CRMs*	43.1
NIM	Measured values of honey	5.5
	Linearity of calibration curve	62.7
	Certified value of RM**	31.8
NMIA	Certified values of RMs***	29.7
	Measured values of honey****	3.2
	Bias from ^{17}O correction and calibration	67.1
TUBITAK	Certified values of RMs	60.6
	Measured values of CRMs	13.0
	Measured values of honey	25.8
	Working gas carbon isotope ratio	0.7

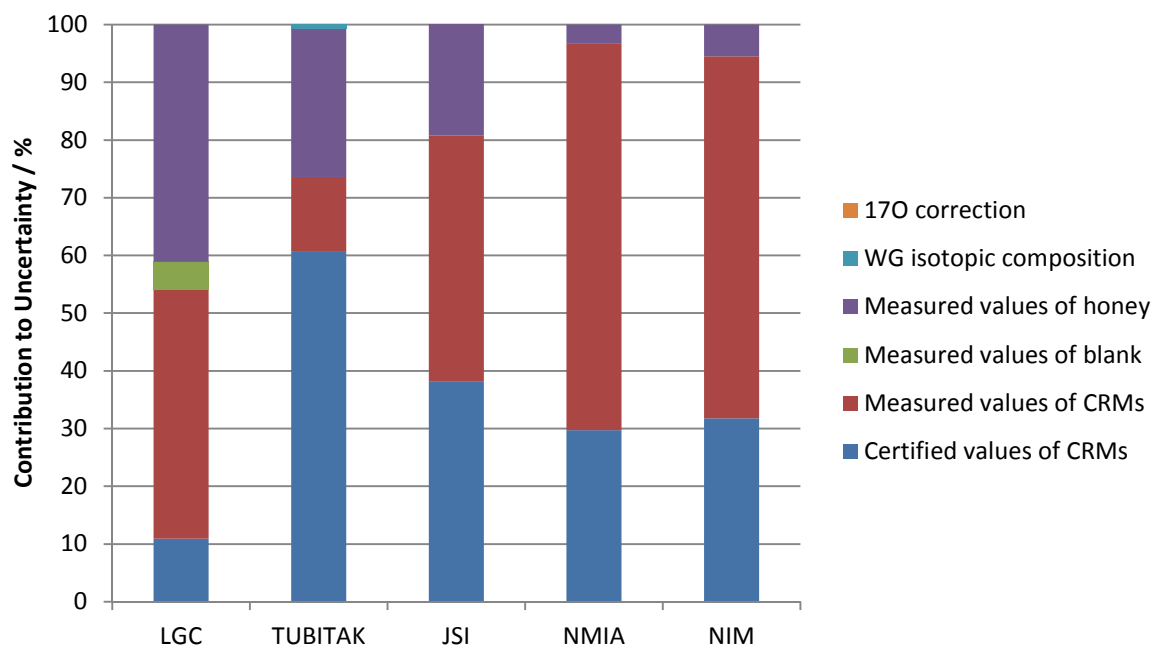
*For LGC the measured values were raw ion current ratios and peak areas, while for other participants the raw data were delta values calculated by instrumental software.

**Only one RM stated in uncertainty budget despite four being used for scale calibration because that RM (USGS 40) was reported to have a significantly larger uncertainty than the other RMs used.

***Converted to ^{13}R values using a literature value for $^{13}\text{R}_{\text{VPDB}}$ and the standard delta equation (Equation 1)

****As ^{13}R values that include both repeatability and reproducibility using a different EA-IRMS system

Figure 2 – Uncertainty budgets for the participants. Note that NMIA included the contribution from the ^{17}O correction within their budget for measured values of the CRMs.



9 - KCRV AND ASSOCIATED UNCERTAINTY

Inspection showed no strong outliers among the data and only a little evidence of overdispersion (the between laboratory standard deviation was a little larger than the root mean squared uncertainty representing the average within-laboratory variation). Under these circumstances, robust estimators are unnecessary. Of the commonly used approaches for estimating the KCRV from a Key Comparison (median, arithmetic mean and weighted mean), the median/ MAD_E is not appropriate due to the low number of participants. While the weighted mean does take into account the uncertainty estimates reported by the participants and these uncertainties are large in comparison to the overall standard deviation of the reported values; the IAWG deemed there to be insufficient consistency in the measurement uncertainty calculation approach between participants and therefore the weighting of the uncertainties was considered inappropriate. The IAWG therefore selected the arithmetic mean as the KCRV on the basis of the low number of participants. The DerSimonian-Laird estimator and the Mandel-Paule algorithm (commonly used for cases where overdispersion is present) were also potential estimates of the KCRV, but are robust estimators which are unnecessary as discussed earlier.

Table 11 –KCRV and associated uncertainty. Note that the uncertainty shown does not incorporate explicit allowances for any inhomogeneity in the test materials. The uncertainty is expanded using coverage factors based on Student's t for the appropriate degrees of freedom.

Method	$10^3 \delta^{13}C_{VPDB-LSVEC}$	u	U	k
Arithmetic Mean	-24.095	0.039	0.107	2.776

10 - DEGREES OF EQUIVALENCE

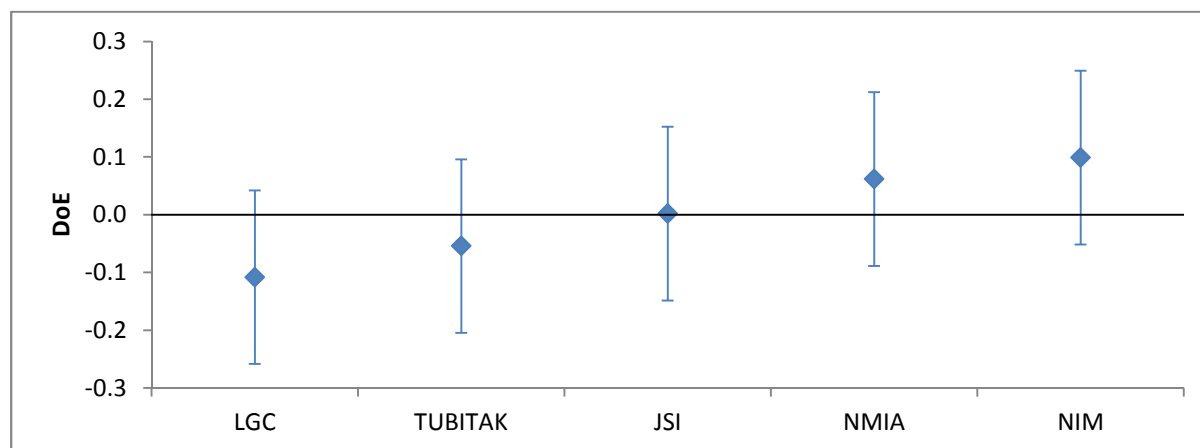
Given the values x_i (submitted by the participating NMIs) with standard uncertainties u_i and a KCRV x_K calculated as the arithmetic mean with standard uncertainty u_K , the degree of

equivalence d_i is $(x_i - x_K)$. The standard uncertainty in the degree of equivalence must include contributions from the uncertainties in the individual participant results and from the uncertainty in the KCRV together with any covariance between these contributions. The covariance between each participant result and the KCRV is important to consider in this KC because there are few participants. The standard uncertainty in the degrees of equivalence are calculated as $u(di) = s(x) \cdot (1 - 1/n)^{0.5}$ where $s(x)$ is the standard deviation of participant results and n is the number of participating laboratories. The calculated degrees of equivalence and their associated uncertainties are shown in Table 12 while Figure 4 shows degrees of equivalence with error bars showing the uncertainty component of the degree of equivalence expressed as an expanded uncertainty with a coverage factor $k=2$. Results enclosing zero within their uncertainty interval are considered to be consistent with the KCRV

Table 12 – CCQM-K140 – degrees of equivalence.

Institute	x	u	U	Units	d	$u(d)$	$U(d)$	$d/U(d)$
JSI	-24.09	0.05	0.11	‰	0.002	0.075	0.150	0.012
LGC	-24.20	0.09	0.18	‰	-0.108	0.075	0.150	-0.720
NIM	-23.993	0.042	0.084	‰	0.099	0.075	0.150	0.657
NMIA	-24.03	0.05	0.11	‰	0.062	0.075	0.150	0.411
TUBITAK	-24.146	0.139	0.278	‰	-0.054	0.075	0.150	-0.361

Figure 4 - Degrees of equivalence with error bars showing the uncertainty component of the degree of equivalence expressed as an expanded uncertainty with a coverage factor $k=2$. Results enclosing zero within their uncertainty interval are considered to be consistent with the KCRV



11 - CMC SUPPORT

Participation in CCQM-K140 supports CMCs relating to bulk carbon isotope ratio determination in solid and liquid materials within the isotopic range of reference materials available for scale calibration (i.e. $\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ between -47.32 ‰ and +535.3 ‰, Brand *et al* 2014). In terms of matrix, honey is a mixture of sugars with a small protein component and is therefore easily combusted within an elemental analyser. CMCs for EA-IRMS involving the carbon isotopic analysis of more complex matrices such as plant material (e.g. freeze-dried leaves) or more proteinaceous materials (e.g. meat) are supported provided that the complete conversion of the sample carbon into CO_2 can be demonstrated. Where continuous flow IRMS has been applied

this KC can also support nitrogen isotope ratio determinations, again provided that quantitative conversion of the nitrogen within the sample to nitrogen gas can be demonstrated, as the same principles apply (here the $\delta^{15}\text{N}_{\text{AirN}_2\text{-USGS32}}$ range covered by reference materials is between -30.41 ‰ and +375.3 ‰). The use of cavity ring down spectroscopy or other infrared absorption techniques in this KC can only support carbon isotope ratio determination. Bulk hydrogen and oxygen isotope ratio measurements are not supported as they have a number of different considerations which are not tested by this KC, in particular the needs to ensure samples are completely dry and to account for the presence of extrinsic and exchangeable hydrogen.

12 - CONCLUSIONS

The performance of all of the CCQM-K140 participants was very good, illustrating their ability to obtain accurate results for carbon isotope ratios, within the calibration range afforded by internationally agreed reference materials ($\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ between -47.32 ‰ and +535.3 ‰, Brand *et al* 2014) with measurement uncertainties of between 0.08 and 0.28 ‰. This was despite that no two participants used exactly the same approach in terms of instrumentation or data treatment.

13 - ACKNOWLEDGEMENTS

Thanks to Ahmet Ceyhan Gören, Mine Bilsel and Adnan Şimşek (TUBITAK UME) for providing their candidate reference material for use in these studies; for shipping the honey samples to the participants (not as straightforward a process as we'd imagined!) and providing their data from the homogeneity and stability testing of the honey. Thanks to Dmitriy Malinovskiy (LGC) for careful checking of this report as well as LGC's data and also for valuable discussions over results. Thanks to Sarah Hill and Heidi Goenaga-Infante (LGC) for their help and advice in coordinating these studies, planning the practical work, discussions over the results obtained and reviewing this report. Thanks to Simon Cowen (LGC) for the statistical analyses contained herein.

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ANNEX 1: INVITATION TO PARTICIPATE IN THE KEY COMPARISON CCQM-K140

Invitation to participate in the key comparison CCQM-K140 on the measurement of carbon stable isotope ratio delta values in honey

Dear Colleagues,

We kindly invite you to participate in the CCQM key comparison CCQM-K140 “Carbon stable isotope ratio delta values in honey.” Organisations which are a national metrological institute (NMI), or an appropriate designated laboratory in accordance with the CIPM MRA, are invited to participate in this key comparison. Please find in the attached documents the registration form, the technical protocol and the results submission form.

If you decide to participate in CCQM-K140, please complete the registration form and return it to us by regular mail or e-mail before 31st August 2015. Although a number of institutes have already expressed an interest, we ask all participants to register by regular mail or e-mail.

Following the registration deadline the samples will be shipped to all participants. Further details can be found in the attached technical protocol. The schedule for the study can also be found in the technical protocol. After the submission deadline has passed, a report will be drafted and circulated to all participants for comments and corrections. The results of the key comparison will be presented in the form of a report to the CCQM, available to participants and to members of the IAWG. The report will identify the results with the names of the participating institutes. Preliminary (A) and final (B) drafts of the report will be circulated to participants for comment and correction. The approved report will be submitted to the BIPM’s Key Comparison Database (KCDB) and the results will be publicly available. A similar report will be prepared for the pilot study, for participants and members of the IAWG. A scientific paper describing the study may be published separately in an appropriate journal provided participants agree to this.

If you have any further comments or questions, please do not hesitate to contact us.

Yours sincerely

Philip Dunn
Researcher in IRMS
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Tel: +44(0)20 8943 7454

ANNEX 2: REGISTRATION FORM

CCQM-K140 – Carbon stable isotope ratio delta values in honey

Registration Form

Name (contact person): _____

Institute: _____

Shipping address: _____

Telephone: _____

E-Mail: _____

Participation CCQM-K140: Yes No

Signature: _____

Date: _____

Please return the completed form by regular mail or e-mail no later than 31st August 2015 to:

Philip Dunn
LGC
Queen's Road
Teddington
Middlesex
TW11 0LY
UK
philip.dunn@lgcgroup.com
Telephone +44 (0)20 8943 7454

CCQM-K140 – Carbon stable isotope ratio delta values in honey

Technical Protocol

Rationale

Isotope ratio measurements are applied in a number of different fields including archaeology, environmental science, geochemistry, forensic science and ecology. Isotope ratios of carbon are used to differentiate plants with different photosynthetic mechanisms, which is particularly useful to identify food adulteration (e.g. adulteration of honey with corn syrup). They are typically reported as delta values against internationally agreed primary reference materials on the VPDB-LSVEC scale, although absolute carbon isotope ratios traceable to the SI have also been reported.

There have been several previous IAWG CCQM pilot studies on isotope ratio determination (CCQM-P75: Stable isotope delta values in methionine, 2006; CCQM-P105: Sr isotope ratios in wine, 2008; CCQM-K98: Pb isotope ratios in bronze with additional delta values in CCQM-P134, 2011). It has, however, been a number of years since delta values of light elements have been considered and there is no key comparison. Therefore, the IAWG has included the need for such a KC in its future program of key comparisons, supporting ongoing requirements to demonstrate core capabilities as well as specific CMCs in this area. This KC, CCQM-K40, will be coordinated by LGC Ltd, UK and TUBITAK UME, Turkey.

Participation in CCQM-K140 will support CMCs relating to bulk carbon isotope ratio determination in solid and liquid materials within the isotopic range of reference materials available for scale calibration (i.e. $\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ between -47.32 ‰ and +535.3 ‰). Where continuous flow IRMS has been applied this KC can also support nitrogen isotope ratio determinations (the use of cavity ring down spectroscopy or other infrared absorption techniques can only support carbon isotope ratio determination); however, bulk hydrogen and oxygen isotope ratio measurements will not be supported as they have a number of different considerations which are not tested by this KC.

Sample

Each participant will receive the number of amber borosilicate vials indicated in their response to the questionnaire. Each vial contains 2 g of honey. Every participant is asked to confirm the delivery of the samples by regular mail or e-mail using the attached form as soon as the samples have arrived.

Homogeneity and short/long term stability tests have indicated that the honey material isotopic composition is not significantly different between vials (ten random vials analysed in triplicate gave a SD of 0.07 ‰) and that the isotope ratio is stable over the time period of this KC ($F_{\text{Statistics}}$ for storage at 4 or 60 °C for up to 4 weeks were 1.11 and 0.61, respectively, both of which are less than the F_{Critical} of 2.76; long term stability of up to 12 months at 25 °C gave $F_{\text{Statistic}}$ of 1.09 which again was less than the critical value of 1.90). There are no specific storage requirements, although we recommend that the vials are kept at room temperature. Vials may

be opened multiple times during use. The amount of material to analyse should follow the usual protocols of participants' laboratory.

Measurand and Reporting

The mandatory measurand for the sample is the $\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ value of the bulk honey multiplied by a factor of 10^3 and thereby expressed in permil. A delta value with uncertainty should be reported as well as the results from at least 5 independent replicates. Details can be found in the attached reporting form.

Methods of Measurement

The participants are free to choose any suitable method (e.g. IRMS, CRDS ...) but please include a full description of your method of analysis. This description should include details over all corrections applied to instrumental data where they have been applied including e.g. those for isobaric interferences (^{17}O), blank, scale calibration etc. and the source of traceability to the VPDB-LSVEC delta value scale. We recommend that at least two organic reference materials should be used for scale calibration and that the delta values assigned to these reference materials should be those recommended in the IUPAC technical report (Brand et al, Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report), Pure Appl. Chem., 2014, 86, 425-467).

Each laboratory should report a full uncertainty budget as part of the results. Contributions to the overall uncertainty will arise from the repeatability of the sample preparation, the repeatability of instrumental determination, scale calibration using suitable reference materials and any other parameter specific to the method of analysis chosen by the participant.

Planned Time Schedule

Call for participation	June 2015
Registration Deadline	31st August 2015
Sample Distribution	by end of October 2015
Deadline for Reporting Results	29th February 2016
Issue of Draft Preliminary Report	by end of July 2016

Contact Details

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ANNEX 3: RESULTS SUBMISSION FORM

CCQM-K140 – Carbon stable isotope ratio delta values in honey

Results Submission Form

NAME :

INSTITUTE :

ADDRESS :

TEL :

E-MAIL :

Report your result and uncertainties in the carbon isotope ratio delta value on the VPDB-LSVEC scale using the units in the table below. Details concerning the analysis of replicates, details of the method, calculation of results, and associated uncertainties should be given in the following pages of your report.

$10^3 \delta^{13}\text{C}_{\text{VPDB-LSVEC}}$	Unit	Standard Uncertainty	Expanded Uncertainty	k
	‰			

Date	
Signature	

Additional Information

Results for the analysis of replicate samples:

Determination	$10^3 \delta^{13}\text{C}_{\text{VPDB-LSVEC}}$	Unit	Standard Uncertainty	Expanded Uncertainty	k
1		‰			
2		‰			
3		‰			
4		‰			
5		‰			

Please report at least 5 independent replicates. If more than 5 determinations have been carried out, please insert more lines.

Method(s) used:

Further information and details can be added in pages below, or in a separate report if preferred. If you use a separate report, please provide a complete description of the method(s) used for the determination, including the following information as appropriate:

1. Details of sample handling, including weight taken and reference materials used.
2. Measurement technique and instrumentation.
3. Corrections applied to data including scale calibration procedure.
4. Any other relevant information.

An uncertainty calculation should be prepared as described in the study protocol.

Contact Details

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Sample preparation

Typical weight taken for each analysis:

Measurement Procedure

Principle (e.g. IRMS):

Instrumentation:

Corrections applied to raw instrumental data – please indicate which of the following have been applied and give brief description of the method employed.

- ^{17}O (e.g. IUPAC, Santrock et al, Allinson et al)?
- Blank?
- Linearity (change in delta value with sample mass)?
- Drift (change in delta value with time)?
- Memory (carry over between samples)?
- Other (please give details)?

Traceability to VPDB-LSVEC scale including reference material(s) used for scale calibration:

Uncertainty Budget

Please provide an uncertainty budget for your delta values as described in the technical protocol:

ANNEX 4: CORE COMPETENCY SUMMARY TABLE FOR EA-IRMS

Inorganic Core Capabilities Summary Table

CCQM Study: CCQM-K140

Institute(s): LGC, TUBITAK UME, JSI, NIM China, NMIA

Method: EA-IRMS

Analyte(s): $\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$ of Bulk Honey

Instructions:

List in the appropriate column (as NIST, PTB, LGC, etc.) the institutes which did or did not demonstrate each capability. Where the table includes multiple analytes add the element symbols or 'All' in parenthesis after each institute - e.g. LGC (As, Ca). Provide a brief summary of the challenges encountered in the final column, highlighting any aspects where this measurement presented an unusually high degree of difficulty. This should be a consensus agreed with all participants except where there is a valid reason for it to be different at a specific institute. This also requires explanation. Please add rows for any other capabilities which were used but which have not been included in this table.

Capabilities/Challenges	Not tested	Tested	Specific challenges encountered
<p>Sample preparation All techniques and procedures used to prepare samples for IRMS analysis (includes online conversions to analyte gases); procedures to minimize contamination with the elements of interest (highest difficulty for elements that are ubiquitous in the sample preparation environment)</p>		LGC, TUBITAK, JSI, NIM China, NMIA	Transfer of viscous liquid into tin capsules for analysis.
<p>Determination of peak areas (complex chromatograms/small peaks) Procedures used to determine peak areas. (e.g., high difficulty for small peak areas on complex backgrounds or determination of areas for multiple unresolved peaks.)</p>	LGC, TUBITAK, JSI, NIM China, NMIA		Bulk analysis so n/a.
<p>Correction for isobaric/polyatomic interferences Any techniques used to remove, reduce or mathematically correct for interferences caused by mass overlap of analyte isotopes with isobaric or polyatomic species. Includes offline purification and online chromatographic separation. The relative concentrations and sensitivities of the analyte isotopes and the interfering species will affect the degree of difficulty.</p>		LGC (^{17}O , $^1\text{HCO}_2$), TUBITAK (^{17}O), JSI (^{17}O), NIM China (^{17}O), NMIA (^{17}O)	Correction for ^{17}O required for mass spectrometric isotope ratio determination of C on CO_2 . Presence of water in the ion source can lead to protonation.
<p>Scale calibration Procedures used to calibrate raw instrumental values to international reporting delta scales (e.g. VSMOW-SLAP, VPDB-LSVEC, AirN2-USGS32).</p>		LGC, TUBITAK, JSI, NIM China, NMIA	
<p>Linearity effect Procedures or techniques used to avoid, reduce or mathematically correct for linearity in</p>	TUBITAK, JSI	LGC, NIM China,	Control of sample mass to avoid manifestation of linearity effect. Success of this avoidance process

Capabilities/Challenges	Not tested	Tested	Specific challenges encountered
<i>measured delta values (i.e. where the analysis of different amounts of the same material yields different delta values).</i>		NMIA	must be checked.
Drift effect <i>Procedures or techniques used to avoid, reduce or mathematically correct for drift in delta values with time</i>	TUBITAK	LGC, JSI, NIM China, NMIA	
Memory (carry-over) effect <i>Procedures or techniques used to avoid, reduce or mathematically correct for the carry-over of analyte between two consecutively measured standards and/or samples.</i>	LGC, TUBITAK, JSI, NIM China,	NMIA	Only NMIA observed a memory effect and this was addressed by excluding the affected data rather than via correction.
Exchangeable fractions <i>Procedures or techniques used to avoid, reduce or mathematically correct for the presence of exchangeable fractions of the element analysed, (e.g. H in -OH, -COOH and/or NH₂ functional groups).</i>	LGC, TUBITAK, JSI, NIM China, NMIA		n/a