

CCQM-K27-Subsequent: Key Comparison (subsequent) for the Determination of Ethanol in Aqueous Matrix

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ABSTRACT

Ethanol is important both forensically (“drunk driving” or driving while under the influence, “DWI”, regulations) and commercially (alcoholic beverages). Blood-and breath-alcohol testing can be imposed on individuals operating private vehicles such as cars, boats, or snowmobiles, or operators of commercial vehicles like trucks, planes, and ships. The various levels of blood alcohol that determine whether these operators are considered legally impaired vary depending on the circumstances, and locality. Accurate calibration and validation of instrumentation is critical in areas of forensic testing where quantitative analysis directly affects the outcome of criminal prosecutions, as is the case with the determination of ethanol in blood and breath. Additionally, the accurate assessment of the alcoholic content of beverages is a commercially important commodity.

In 2002, the CCQM conducted a Key Comparison (CCQM-K27) for the determination of ethanol in aqueous matrix with nine participants. A report on this project has been approved by the CCQM and can be found at the BIPM website [1]. CCQM-K27 was comprised of three samples, one at low mass fraction of ethanol in water (nominal concentration of 0.8 mg/g), one at high level (nominal concentration of 120 mg/g), and one wine matrix (nominal concentration of 81 mg/g). Overall agreement among eight participants using gas chromatography with flame ionization detection (GC-FID), titrimetry, isotope dilution gas chromatography/mass spectrometry (GC-IDMS), and gas chromatography-combustion-isotope ratio mass spectrometry (ID-GC-C-IRMS) was good. The ninth participant used a headspace GC-FID method that had not been validated in an earlier pilot study (CCQM-P35).

A follow-on Key Comparison, CCQM-K27-Subsequent, was initiated in 2003 to accommodate laboratories that had not been ready to benchmark their methods in the original CCQM-K27 study or that wished to benchmark a different method. Four levels of ethanol in water were used in the subsequent study (nominal concentrations of 0.2 mg/g, 1 mg/g, 3 mg/g, and 60 mg/g). The three participants in the CCQM-K27-Subsequent Key Comparison demonstrated their ability to measure ethanol in aqueous matrix in the concentration range of 0.2 mg/g to 60 mg/g.

SUMMARY OF ORIGINAL CCQM-K27 STUDY

A Key Comparison on the determination of ethanol in water, CCQM-K27a for forensic matrices and CCQM-K27b for commercial matrices, was conducted in 2002 with Laboratory of the Government Chemist (LGC) as the coordinating laboratory. Nine laboratories participated in this Key Comparison:

<u>Country</u>	<u>Institution</u>
Australia	National Analytical Reference Laboratory, NARL ^a
China	National Research Centre for Certified Reference Materials, NRCCRM
France	Laboratoire National d'Essais, LNE
Germany	Bundesanstalt für Materialforschung und –prüfung, BAM
Japan	National Metrology Institute of Japan, NMIJ
Korea	Korea Research Institute of Standards and Science, KRISS ^c
Russia	D.I. Mendeleev Institute for Metrology, VNIIM ^b
United Kingdom	Laboratory of the Government Chemist, LGC
USA	National Institute of Standards and Technology, NIST

The details of the study can be found at the BIPM website [1]. The conclusion of the study was that the participating NMIs demonstrated the ability to make accurate and precise measurements of ethanol in aqueous matrix at the range of concentrations provided.

CONDUCT OF THIS STUDY (CCQM-K27-SUBSEQUENT)

Participants

The following three countries participated in this study:

<u>Country</u>	<u>Institution</u>
South Africa	CSIR-NML
Russia	D.I. Mendeleev Institute for Metrology, VNIIM
United Kingdom	Laboratory of the Government Chemist, LGC

CSIR-NML participated as a new laboratory while VNIIM and LGC used the subsequent study to assess a different method than they used in CCQM-K27. NIST served as the coordinating laboratory for this Key Comparison.

Methods Used for the CCQM-K27-Subsequent Comparison

It is the policy of the CCQM Organic Working Group that participants in the CCQM Key Comparisons use methods that are used to deliver that laboratory's measurement services. The laboratories in this study chose to use GC-FID, ID-GC-C-IRMS, and titrimetry.

Materials Used for the CCQM-K27-Subsequent Comparison

Six ampoules each of four levels of ethanol in water were sent to the participants by the coordinating laboratory, NIST. Three of the four solutions used (nominal concentrations 0.2 mg/g, 1 mg/g, and 3 mg/g) are part of candidate NIST SRM 1828b, Ethanol-Water Solutions (Blood-Alcohol Testing: six levels), while the fourth solution (nominal concentration 60 mg/g) is part of candidate NIST SRM 1847, Ethanol-Water Solutions (Breath-Alcohol Testing: three levels). These solutions were prepared at NIST by weighing and mixing known masses of ethanol and organic-free water. Each solution was mixed overnight (a minimum of 16 h). The total mass of each solution was measured, and the concentration of each solution was calculated from this gravimetric procedure. These gravimetric concentrations were adjusted for the purity estimation of the ethanol, which was determined using GC-FID with two stationary phases of different polarities, differential scanning calorimetry, and Karl Fischer analysis for water content. The bulk solution was chilled slightly, and 1.2 mL aliquots were dispensed into 2-mL amber glass ampoules, which were then flame sealed for the first three levels. For the fourth level, 10 mL aliquots were dispensed into 10-mL glass ampoules, which were then flame sealed. The homogeneity of each solution was checked at NIST by analyzing two aliquots each of nine ampoules selected using randomized stratified sampling. These analyses confirmed that there was no significant heterogeneity in the pool of samples and that basing the KCRV on the gravimetric value was appropriate.

Measurement Protocol and Calculation of Uncertainty

LGC requested that they be supplied only with the Level 1 (nominal concentration 0.2 mg/g) and Level 4 (nominal concentration 60 mg/g) samples. CSIR-NML and VNIIM requested all four levels. For each level received, participants were requested to analyze two aliquots taken from each of four ampoules (eight determinations in all). The results were to be reported on an absolute basis (corrected for chemical purity of the calibration material used by the participant) together with the expanded uncertainty. Space was provided at the end of the data reporting sheets for inclusion of a full uncertainty budget, including definition of terms and assessment of which components made significant contributions.

RESULTS

Results for the CCQM K27 Subsequent Comparison are summarized in Table 1 and in Figures 1 through 4. The uncertainty bars in the figures represent expanded uncertainties as reported by the participating laboratories. The gravimetric preparation value (corrected for purity as described above) is shown along with the upper and lower limits of an expanded uncertainty of the gravimetric value based on the results of the original CCQM-K27 Comparison (see discussion).

The sources of uncertainty in CCQM-K27-Subsequent as noted by the laboratories are summarized below:

Institute	Sources of Uncertainty Identified
CSIR-NML	Repeatability of titration measurements Concentration of potassium chromate Conversion factor Extent of oxidation Influence of impurities Blanks Titer volume
LGC	Measured isotope ratios Between blend variation Purity of absolute ethanol used Masses Dilution factors (where necessary) Isotopic amount ratio of primary standard and spike* Molecular mass of ethanol and spike*
VNIIM	Preparation of the calibration solutions Transfer of calibration solution and addition of internal standard to the calibration solutions prior to analysis Transfer of study sample and addition of internal standard to the study sample prior to analysis Standard deviation of the mean measurement results

*Negligible impact on uncertainty (0.1% of combined uncertainty).

DISCUSSION

Two of the three participating laboratories, LGC and VNIIM, participated in the original CCQM-K27 Key Comparison. [1]. Both laboratories participated in the subsequent study to benchmark new methods being used in their laboratories. In the subsequent study, VNIIM used liquid injection GC-FID instead of the static headspace GC-FID used in the original study. In the original study, VNIIM's results were low for each of the samples (between 0.8% and 3.0%). LGC's data were very precise and accurate for both the original and this study.

The concentrations used in CCQM-K27-Subsequent (nominal concentrations of 0.2 mg/g, 1 mg/g, 3 mg/g, and 60 mg/g) were different than those used in CCQM-K27 (nominal concentrations of 0.8 mg/g, 80 mg/g, and 120 mg/g). Indirect statistical methods are thus needed to compare the results of these two studies and to evaluate the assumption that the relationship between the ethanol level and the expanded uncertainty observed in CCQM-K27 is appropriate for use with the CCQM-K27-Subsequent results.

Figure 5 displays the relationships between the mean and standard deviation values for the samples in the CCQM-K27 and CCQM-K27-Subsequent studies. The standard deviations for the three samples of CCQM-K27 (solid circles), expressed as a percentage of the consensus value or %RSD, is on average 0.74% (relative). The %RSD for the four samples of CCQM-K27-Subsequent is 0.95% (relative). Combining the results for these

seven samples suggests that the expected %RSD of "higher order" measurements of ethanol in aqueous matrices is about 0.85% (relative).

Figure 6 displays the percentage differences of each laboratory's results from the relevant KCRVs for the three CCQM-K27 levels and the gravimetric values for the four CCQM-K27-Subsequent levels. The agreement between the two sets of data confirms that the relative expanded uncertainty from CCQM-K27 is appropriate for relating CCQM-K27-Subsequent results to those of CCQM-K27.

The percentage differences displayed in Figure 6 are combined in Figure 7 with the expected %RSD of Figure 5. This Concordance/Apparent Precision or "Target" plot [2] provides a compact comparison of the performance characteristics of all participants in the two studies. The upper segment of Figure 7 displays the composite results for the three samples for CCQM-K27; the lower segment displays results for the four samples of CCQM-K27-Subsequent. The horizontal or "Concordance" axis displays the average difference between the measured and reference values relative to the 0.85% expected %RSD. The vertical or "Apparent Precision" axis displays the standard deviation of the relative differences. Concordance is roughly equivalent to "trueness" or lack of bias. A concordance value of zero indicates that the measured values are the same as the reference values; a concordance of 0.85% indicates that the measured values on average are one %RSD larger than the reference values; a concordance of -0.85% indicates that the values are on average one %RSD smaller than the reference values. An apparent precision of zero indicates that the participant's concordance values are the same for all samples; an apparent precision of 0.85% indicates that the individual concordance values vary by one %RSD. The inner circle of each plot represents a total comparability, calculated as the square-root of the concordance and apparent precision values, of one %RSD (0.85%); the middle circle represents two %RSD (1.70%); and the outer circle represents three %RSD (2.55%).

The measurement characteristics of all participants in both studies fall within the three %RSD ring. All of the CCQM-K27-Subsequent participants and most of the CCQM-K27 participants fall within the one %RSD ring. The abilities demonstrated by the laboratories that provided measurements comparable to the KCRV for each of the samples in both comparisons should be indicative of their ability to provide reference measurements for ethanol content in aqueous samples for forensic and commodities applications (0.2 mg/g to 100 mg/g).

CONCLUSIONS and HOW FAR THE LIGHT SHINES

The three participants in the CCQM-K27-Subsequent Key Comparison demonstrated their ability to measure ethanol in aqueous matrix in the concentration range of 0.2 mg/g to 60 mg/g. The measurement performance figures of merit demonstrated for the four samples measured in CCQM-K27-Subsequent are very similar to those for the three samples used in the original CCQM-K27 Key Comparison. The abilities demonstrated by three participants should be indicative of their ability to provide reference measurements for ethanol content in aqueous samples for both forensic and commodities applications (0.2mg/g to 100 mg/ g).

REFERENCES

- [1] http://kcdb.bipm.org/AppendixB/appbresults/ccqm-k27.a/ccqm-k27.a_final_report.pdf.
- [2] Duewer, D.L., Kline, M.C., Sharpless, K.E., Brown Thomas, J., Gary, K.T., and Sowell, A.L., *Anal. Chem.*, 1999, 71, 1870-1878.

Table 1. Concentration of ethanol in water (% by mass) as reported by laboratory in the CCQM-K27-Subsequent study

Laboratory ID	Technique	Level 1 (gravimetric 0.194 mg/g)			Level 2 (gravimetric 1.01 mg/g)			Level 3 (gravimetric 2.97 mg/g)			Level 4 (gravimetric 60.4 mg/g)		
		Mean Result (mg/g)	Std. Unc. (mg/g)	Exp. Unc. (mg/g)	Mean Result (mg/g)	Std. Unc. (mg/g)	Exp. Unc. (mg/g)	Mean Result (mg/g)	Std. Unc. (mg/g)	Exp. Unc. (mg/g)	Mean Result (mg/g)	Std. Unc. (mg/g)	Exp. Unc. (mg/g)
CSIR-NML	titrimetry	0.195	0.002	0.004	1.002	0.006	0.011	2.981	0.017	0.033	60.5	0.3	0.7
LGC	ID-GC-C-IRMS	0.19339	0.00021	0.00042	Not Analyzed			Not Analyzed			60.06	0.13	0.27
VNIM	GC-FID	0.19400	0.0006	0.0012	1.013	0.003	0.006	2.967	0.0100	0.0200	60.17	0.24	0.48

Figure 1: Level 1 – Results for CCQM-K27-Subsequent study showing gravimetric value (0.194 mg/g) and upper and lower limits of the expanded uncertainty of the gravimetric value (± 0.002 mg/g) based on the CCQM-K27a study

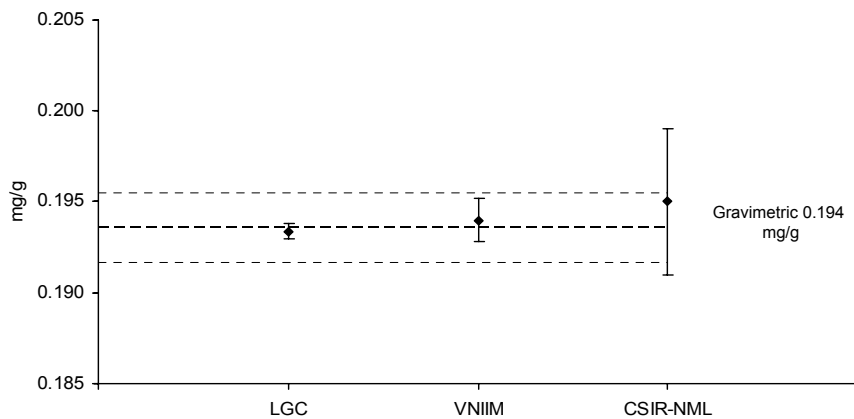


Figure 2: Level 2 – Results for CCQM-K27-Subsequent study showing gravimetric value (1.01 mg/g) and upper and lower limits of the expanded uncertainty of the gravimetric value (± 0.01 mg/g) based on the CCQM-K27a study

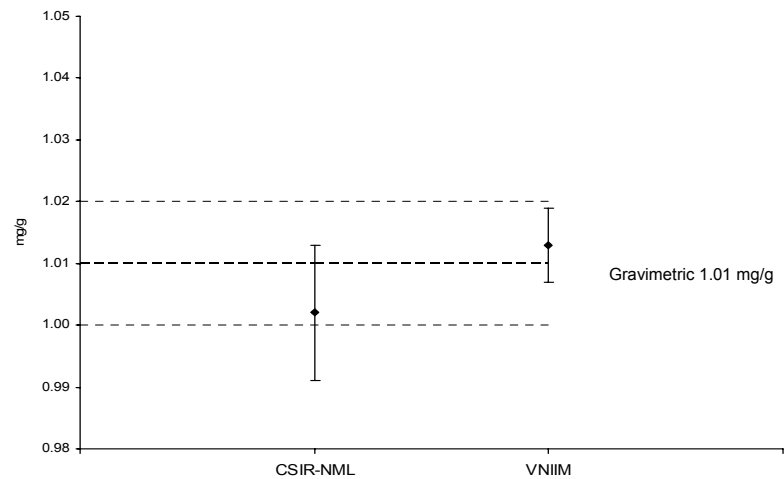


Figure 3: Level 3 – Results for CCQM-K27-Subsequent study showing gravimetric value (2.97 mg/g) and upper and lower limits of the expanded uncertainty of the gravimetric value (± 0.03 mg/g) based on the CCQM-K27a study

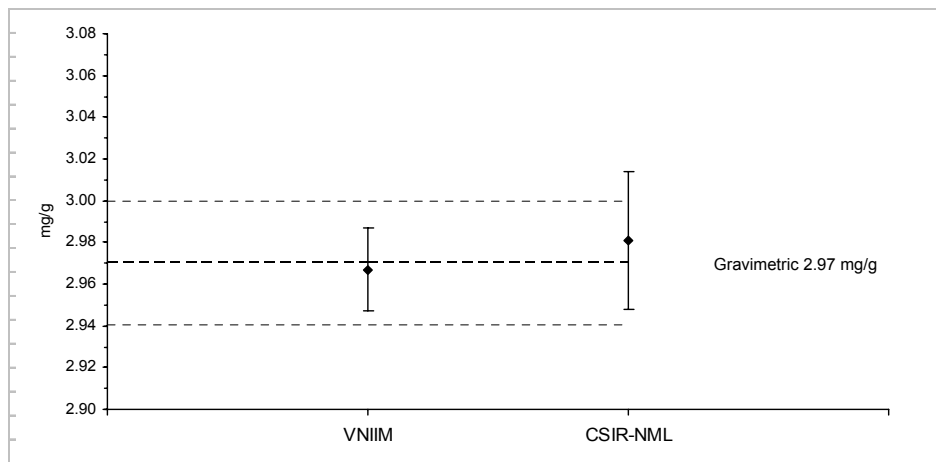


Figure 4: Level 4 – Results for CCQM-K27-Subsequent study showing gravimetric value (60.4 mg/g) and upper and lower limits of the expanded uncertainty of the gravimetric value (± 0.18 mg/g) based on the CCQM-K27a study

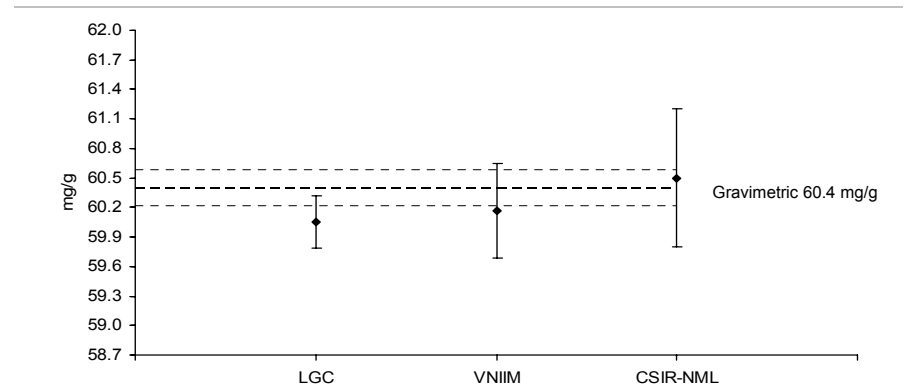
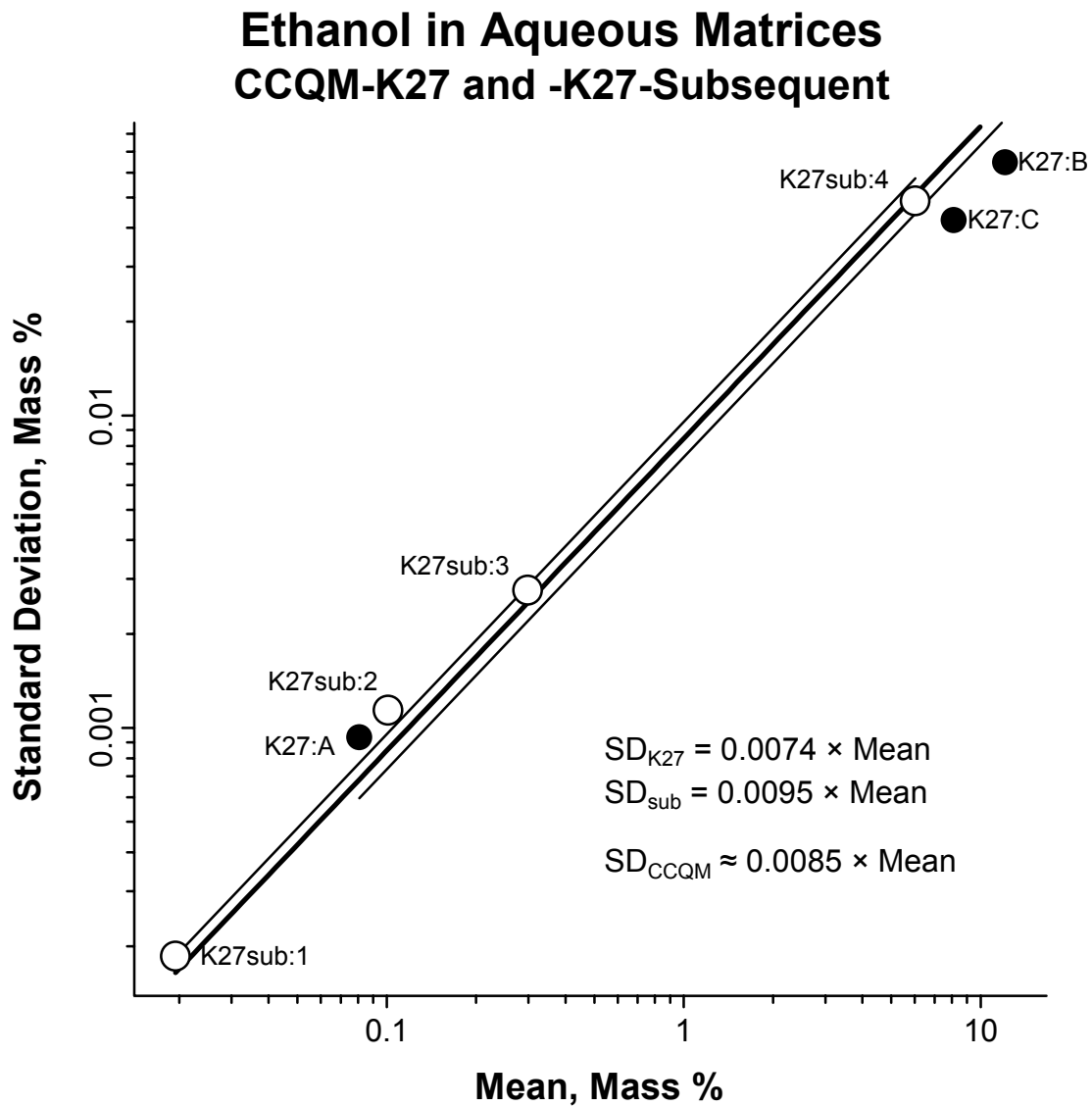


Figure 5. Relationship between the mean value and the standard deviation of the mean value in the CCQM-K27 study and in the CCQM-K27-Subsequent study.



CCQM-K27, Ethanol in Aqueous Matrix, Original and Subsequent Study - Relative Results

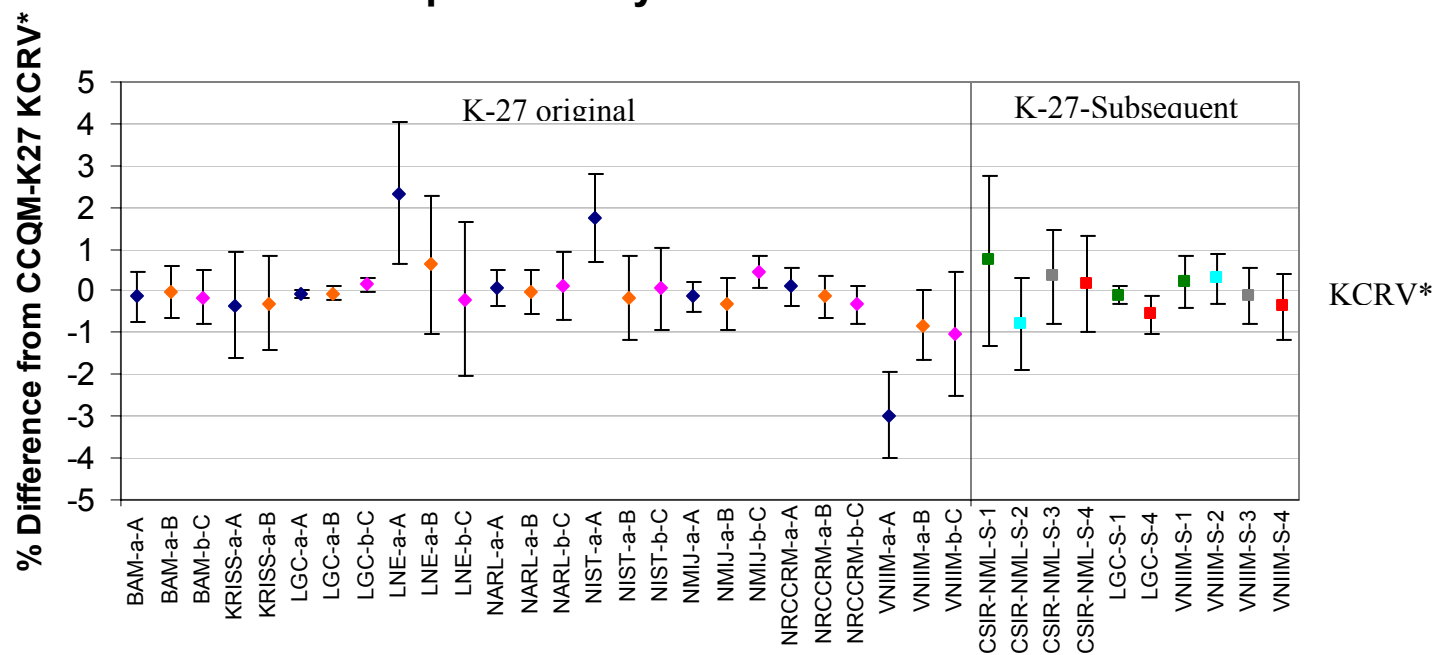
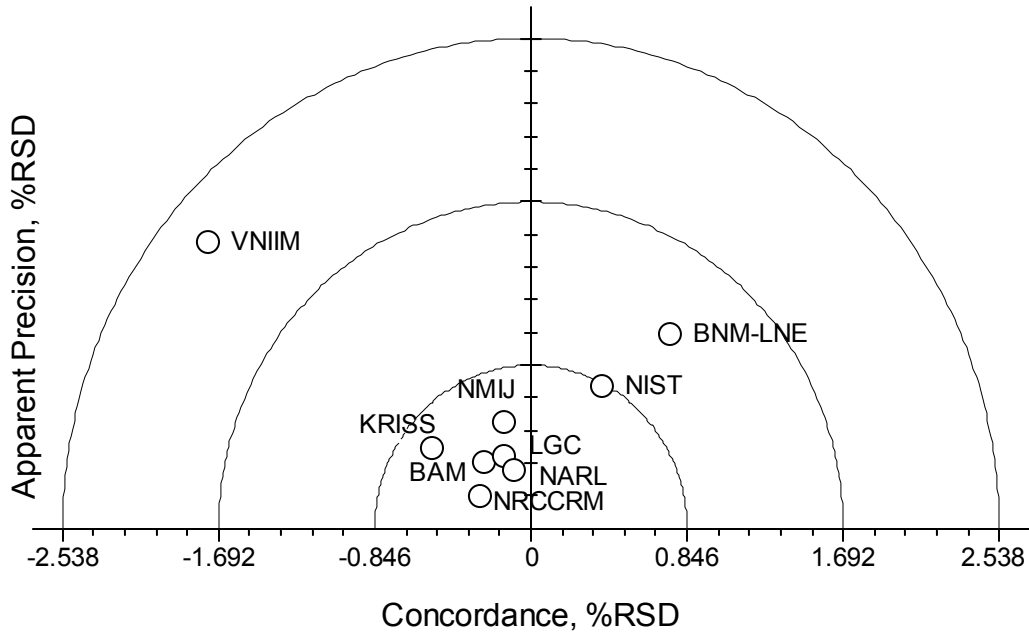


Figure 6. Percentage differences for the CCQM-K27 original (triangles) and CCQM-K27-Subsequent (squares) results plotted relative to the KCRV* values for the original study and to the gravimetric value for the subsequent study. Note that the expanded uncertainty for Levels A and B in the original study and Levels 1 through 3 in the Subsequent study is 0.995% while the expanded uncertainty for Level C in the original study and Level 4 in the Subsequent study is 0.295%. The different concentration levels are indicated by colors: blue triangles – Level A; orange triangles – Level B; pink triangles – Level C; green squares – Level 1; turquoise squares – Level 2; grey squares – Level 3; and red squares – Level 4.

Figure 7. Concordance plots for participants in CCQM-K27 and CCQM-K27 subsequent. The horizontal axis displays the average difference between the measured and reference values relative to the expected %RSD of 0.85%. The vertical axis displays the standard deviation of the relative differences. A concordance value of 0.00 indicates that the measured values are the same as the reference values.

CCQM-K27, Ethanol in Aqueous Matrices and in Commercial Red Wine



CCQM-K27 Subsequent, Ethanol in Aqueous Matrices

