CCQM-K27.2 Second Subsequent Study: Determination of Ethanol in Aqueous Media Final Report

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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. A report on this project has been approved by the CCQM and can be found at the BIPM website [1].

A second follow-on Key Comparison, CCQM-K27.2 Second Subsequent, was initiated in 2006 to accommodate laboratories that had not been ready to benchmark their methods in the previous two CCQM-K27 studies. Two levels of ethanol in water were used in the second subsequent study ranging in concentration between 0.5 mg/g and 4 mg/g. Four of the five participants in the CCQM-K27.2 Second Subsequent Key Comparison demonstrated their ability to measure ethanol in aqueous matrix in that concentration range.

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:

Country	Institution
Australia	National Analytical Reference Laboratory, NARL
China	National Research Centre for Certified Reference Materials, NRCCRM
France	Laboratoire National d'Essais, LNE
Germany	Bundesanstalt fűr Materialforschung und –prűfung, BAM
Japan	National Metrology Institute of Japan, NMIJ
Korea	Korea Research Institute of Standards and Science, KRISS
Russia	D.I. Mendeleyev Institute for Metrology, VNIIM
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.

SUMMARY OF FIRST SUBSEQUENT STUDY, CCQM-K27 SUBSEQUENT

A Subsequent Key Comparison on the determination of ethanol in water, CCQM-K27S was conducted in 2003 with NIST as the coordinating laboratory. Three laboratories participated in this Subsequent Key Comparison:

Country	Institution
Russia	D.I. Mendeleyev Institute for Metrology, VNIIM
South Africa	CSIR NML
United Kingdom	Laboratory of the Government Chemist, LGC

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-K27.2 SECOND SUBSEQUENT)

Participants

The following five countries participated in this study:

Country	Institution
Argentina	INTI
Brazil	INMETRO
Chile	Centro de Matrologia Quimica de Fundacion Chile
México	CENAM
South Africa	CSIR NML

NIST served as the coordinating laboratory for this Key Comparison.

Methods and Materials Used for the CCQM-K27.2 Second 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 and titrimetry.

Four ampoules each of two levels of ethanol in water were sent to the participants by the coordinating laboratory, NIST. 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. 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

For both levels, participants were requested to analyze two aliquots taken from each of three ampoules (six 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 K27.2 Second Subsequent Comparison are summarized in Table 1 and in Figures 1 and 2. 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 as discussed in the CCQM-K27S report available on the BIPM website [1].

For preparation of the calibration solutions, Argentina, Brazil, and Mexico used a commercial sources of ethanol at purities of >99.8%, Chile used SRM 2897 Ethanol-Water Solution (nominal 2% by mass), and South Africa used a primary method. Argentina prepared a six point calibration curve, Brazil and Mexico prepared eight point calibration curves, Chile used a bracketing method, and South Africa used oxidation and back-titration for calibration.

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

Institute	Sources of Uncertainty Identified
CENAM	Ethanol purity
	Internal standard purity
	Solution preparation (gravimetry)
	Calibration curve
	Variations in the same solution
	Variations between ampoules
	Difference between maximum and minimum
CSIR NML	Repeatability of titration measurements
	Concentration of potassium dichromate
	Conversion factor
	Extent of oxidation
	Influence of impurities
	Blanks
	Titer volume
Fundacion Chile	Masses of ethanol and internal standard in the samples and standards

	Repeatability of area ratios, areas of ethanol and internal standard in the samples and standards
	Uncertainty of reference material
	Internal standard purity
INMETRO	Repeatability of area ratio
	Mass of sample solution
	Mass of internal standard
	Repeatability of analysis
	Calibration curve
	Purity of ethanol standard
INTI	Uncertainties from linear least squares calibration

DISCUSSION

As discussed in the first subsequent study report (CCQM-K27S), the expected %RSD of "higher order" measurements of ethanol in aqueous matrices is about 0.85% (relative). As for the previous key comparison studies for ethanol in aqueous media, the gravimetric concentration corrected for the purity of the ethanol used to prepare the samples is the Key Comparison Reference Value (KCRV). As shown in Figures 1 and 2, the concentrations reported by four of the five laboratories participating in CCQM-K27.2 agreed with the KCRV to within ± 1.5% with the fifth laboratory reporting concentrations for the two levels that were >12% lower than the KCRV.

One of the five participating laboratories, CSIR NML, also participated in the first subsequent CCQM-K27S Key Comparison [1] using the same titrimetric method. CSIR NML's data were very precise and accurate for both subsequent studies.

Figure 3 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 and two CCQM-K27.2 Second Subsequent levels. The agreement among the three sets of data confirms that the relative expanded uncertainty from CCQM-K27 is appropriate for relating data from both CCQM-K27-Subsequent Studies to those of CCQM-K27.

The abilities demonstrated by the laboratories that provided measurements comparable to the KCRV for each of the samples should be indicative of their ability to provide reference measurements for ethanol content in aqueous samples for forensic and commodities applications (0.5 mg/g to 4 mg/g).

CONCLUSIONS and HOW FAR THE LIGHT SHINES

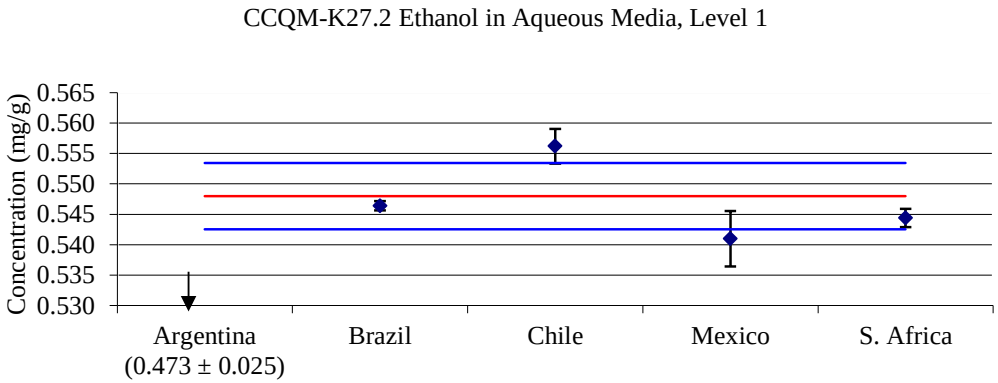
Four of the five participants in the CCQM-K27.2 Second Subsequent Key Comparison demonstrated their ability to measure ethanol in aqueous matrix in the concentration range of 0.5 mg/g to 4 mg/g. The abilities demonstrated by the four participants should be indicative of their ability to provide reference measurements for ethanol content in aqueous samples for both forensic and commodities applications.

REFERENCES

[1] http://kcdb.bipm.org/AppendixB/appbresults/ccqm-k27.a/ccqm-k27.a_final_report.pdf.

Table 1. Summary of data from CCQM-K27.2 Second Subsequent Key Comparison

Country	Method	Level 1 Concentration (mg/g)		% difference	Level 2 Concentration (mg/g)		% difference
		reported	exp unc	from KCRV	reported	exp unc	from KCRV
Argentina	GC-FID	0.473	0.005	-13.69%	2.73	0.03	-14.18%
Brazil	GC-FID	0.546	0.002	-0.29%	3.18	0.01	0.06%
Chile	GC-FID	0.556	0.006	1.50%	3.22	0.03	1.07%
Mexico	GC-FID	0.541	0.009	-1.28%	3.14	0.07	-1.29%
S. Africa	titrimetry	0.544	0.003	-0.66%	3.19	0.01	0.28%
KCRV	gravimetry	0.548	0.005		3.18	0.03	



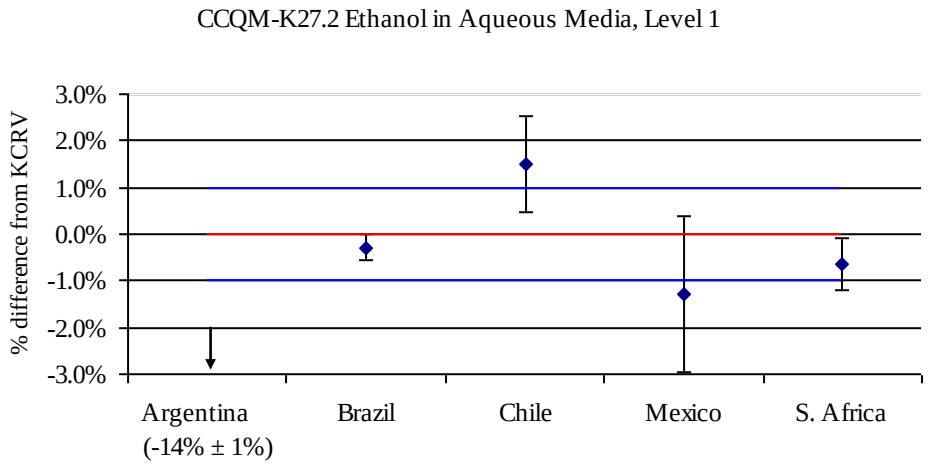


Figure 1. Results of CCQM-K27.2 Second Subsequent Study Level 1 sample showing in terms of absolute concentrations (each laboratory's error bars represent their reported standard uncertainty) and % deviations (each laboratory's error bars represent their reported expanded uncertainty) from the KCRV (red line). The blue lines represent the expanded uncertainty of the KCRV based on the CCQM-K27a study.

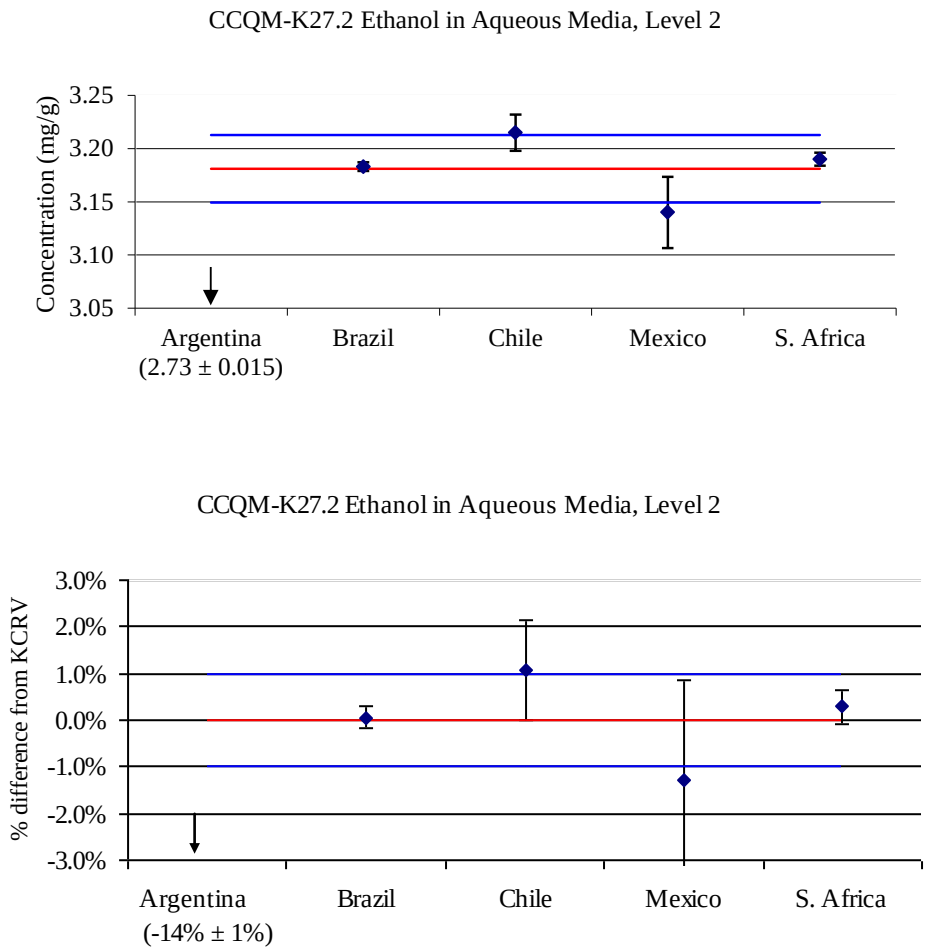


Figure 2. Results of CCQM-K27.2 Second Subsequent Study Level 2 sample showing in terms of absolute concentrations (each laboratory's error bars represent their reported standard uncertainty) and % deviations (each laboratory's error bars represent their reported expanded uncertainty) from the KCRV (red line). The blue lines represent the expanded uncertainty of the KCRV based on the CCQM-K27a study.

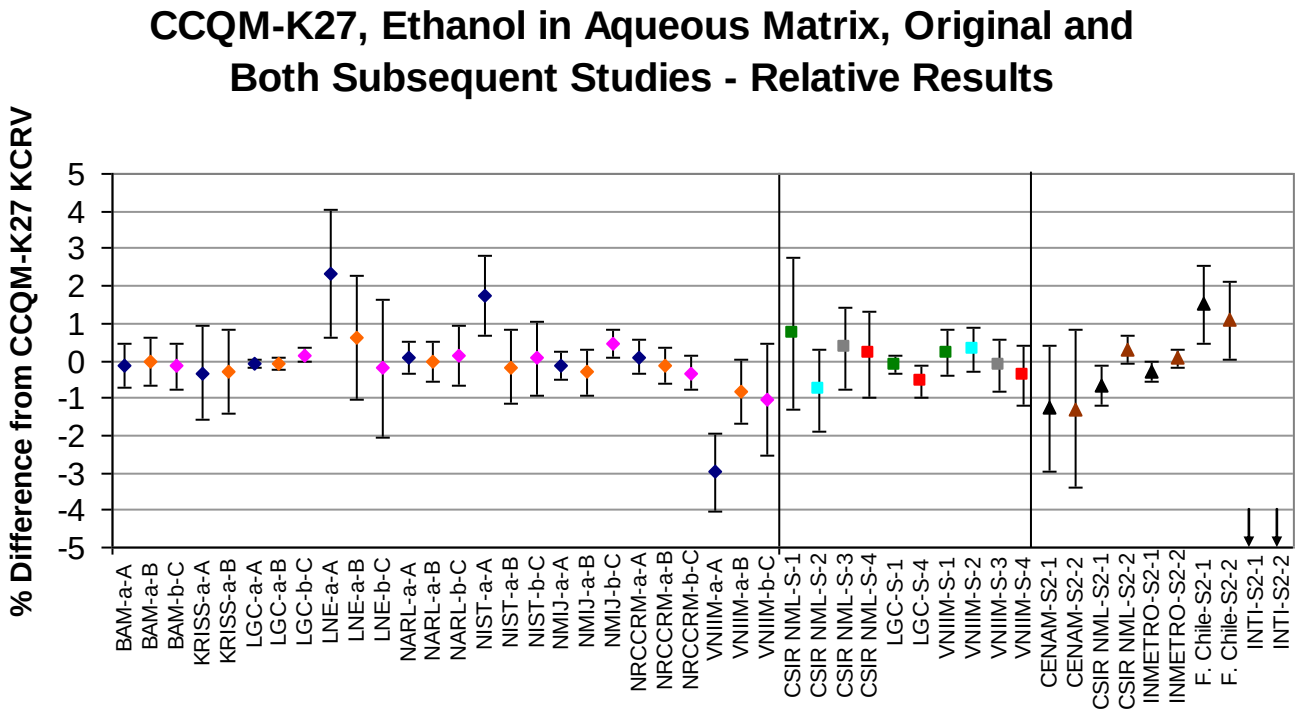


Figure 3. Percentage differences for the CCQM-K27 original (diamonds), CCQM-K27-Subsequent (squares), and CCQM-K27.2 Second Subsequent (triangles) results plotted relative to the KCRV values for the original study and to the gravimetric value for the two subsequent studies. Note that the expanded uncertainty for Levels A and B in the original study, Levels 1 through 3 in the Subsequent study, and Levels 1 and 2 in the Second Subsequent Study is 0.995% while the expanded uncertainty for Level C in the original study, Level 4 in the Subsequent Study is 0.295%. The different concentration levels are indicated by colors: blue diamonds – Level A (0.804 mg/g); orange diamonds – Level B (121 mg/g); pink diamonds – Level C (81.2 mg/g); green squares – Subsequent Level 1 (0.194 mg/g); turquoise squares – Subsequent Level 2 (1.01 mg/g); grey squares – Subsequent Level 3 (2.97 mg/g); red squares – Subsequent Level 4 (60.4 mg/g); black triangles- Second Subsequent Level 1 (0.548 mg/g); and brown triangles – Second Subsequent Level 2 (3.18 mg/g).