

The preparation, assay and certification of aqueous ethanol reference solutions

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Abstract Internationally, certified ethanol reference materials are required to calibrate breathalysers and blood-alcohol measurement instruments. The CSIR National Metrology Laboratory of South Africa provides certified aqueous ethanol solutions with traceability to the SI. Ethanol solutions in the concentration range 10 mg/100 g to 20 g/100 g are prepared gravimetrically by mixing ethanol and reagent quality water. To verify the concentration of the ethanol it is oxidized to acetic acid with potassium dichromate in the presence of sulphuric acid. The unreacted potassium dichromate is back-titrated with sodium thiosulphate. The method utilizes gravimetry and titrimetry, which are both primary methods. This paper addresses aspects of ethanol-certified reference materials that have not been previously published: traceability, stability of unpreserved ethanol solutions, homogeneity, quality control measures and the effect of reproducibility on the measurement uncertainty.

Keywords Ethanol · Certified reference material · Traceability · Titrimetry · Gravimetry · Primary methods

Introduction

South Africa's requirements

In South Africa, blood and urine samples have been analysed for ethanol content by headspace gas chromatography for over 40 years [1]. Traditionally, the ethanol solutions used

for the calibration of the gas chromatographs were prepared by the analyst. The concentrations were verified in-house, but in the field of forensic ethanol analyses, problems were encountered when results obtained using in-house standards were to be defended in court. In 1999, South African laboratories approached the CSIR National Metrology Laboratory (CSIR NML) with a request to produce certified ethanol reference materials (CRMs) in practical concentrations and volumes.

Ethanol reference solutions are prepared, bottled and labelled according to the ISO guides [2–4] and the homogeneity and stability are established according to these guides. The standard ISO [4, 5] and EURACHEM/CITAC [6] guides to the estimation of uncertainty are applied as well as the guide to the use of validation data by Barwick and Ellison [7].

This paper describes the procedures for preparation of the ethanol solutions, verification of their concentrations, homogeneity and stability by primary titrimetry, calculation of uncertainty, the establishment of traceability, and the quality control measures applied to assure the quality of the CRMs.

Established ethanol assays

Barwick and Ellison [8] oxidised ethanol to acetic acid with an excess of potassium dichromate in the presence of sulphuric acid and titrated the excess potassium dichromate with ferrous ammonium sulphate. Vogel questioned the use of ferrous ammonium sulphate for accurate work [9] because the ferrous ion is easily oxidised, leading to errors if stringent precautions are not taken. Van Gent [1] made an extensive study of the use of sodium thiosulphate as a titrant. Vogel [10] provided detailed instructions on titrations of potassium dichromate with sodium thiosulphate. We employ dichromate oxidation and thiosulphate titration.

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Procedures

Preparation of the ethanol solutions

High-purity ethanol was obtained from Merck Chemicals (Uvasol) or Riedel-de Haën (Absolut puriss.). The purity was verified by assay (see below). Initially, the water content was verified by Karl Fischer titration, but following consistent results over 3 years, the Karl Fischer titration is now only done if the assay does not match the stated concentration.

Aqueous ethanol solutions are prepared by mixing measured weights of assayed ethanol and organic-free water ($18 \text{ M}\Omega \cdot \text{cm}$) from a Waters Milli-Q system). Solutions are made in batches of 1, 2 or 5 l. Weighing both components ensures that solutions are produced with accurately known concentrations, but limits the maximum volume of a single batch to what can be conveniently weighed in the laboratory.

Batches of solution are dispensed in 50-ml, 500-ml or 1-l portions in labelled bottles, according to ISO [2–4]. The concentrations and homogeneity (see below) are then verified by the oxidation/ back-titration method.

Assay and certification

The method of Van Gent [1] was adapted for automated titrations using a Mettler-Toledo DL55 autotitrator with a combined platinum ring electrode. Reagent and titrant concentrations were as follows: potassium dichromate 0.017 mol/l (4.9071 g/kg), sodium thiosulphate 0.05 mol/l (12.4 g/l), potassium iodate 0.017 mol/l (3.64 g/kg). Sample size is adjusted according to ethanol concentration in order to consume a volume of potassium dichromate solution that is within the range of the autotitrator burette.

For each set of assays, a set of 12 potassium dichromate blanks, covering the expected dichromate consumption for the samples, is run and used to calculate h (Eq. (2) below). Figure 1 represents a flowchart of the procedure.

Homogeneity

After the batches are bottled and sealed, at least four different bottles are sampled to provide eight aliquots. To be considered homogeneous, the relative standard deviation of the eight titrimetric results must be less than the repeatability uncertainty for the measurements. The repeatability uncertainty limits for homogeneity (Table 2) were established as the mean within-run repeatability for ten batches.

Verified concentration

At least four bottles from different places in the batch are sampled to provide eight aliquots, and assayed according to the oxidation/ back-titration method.

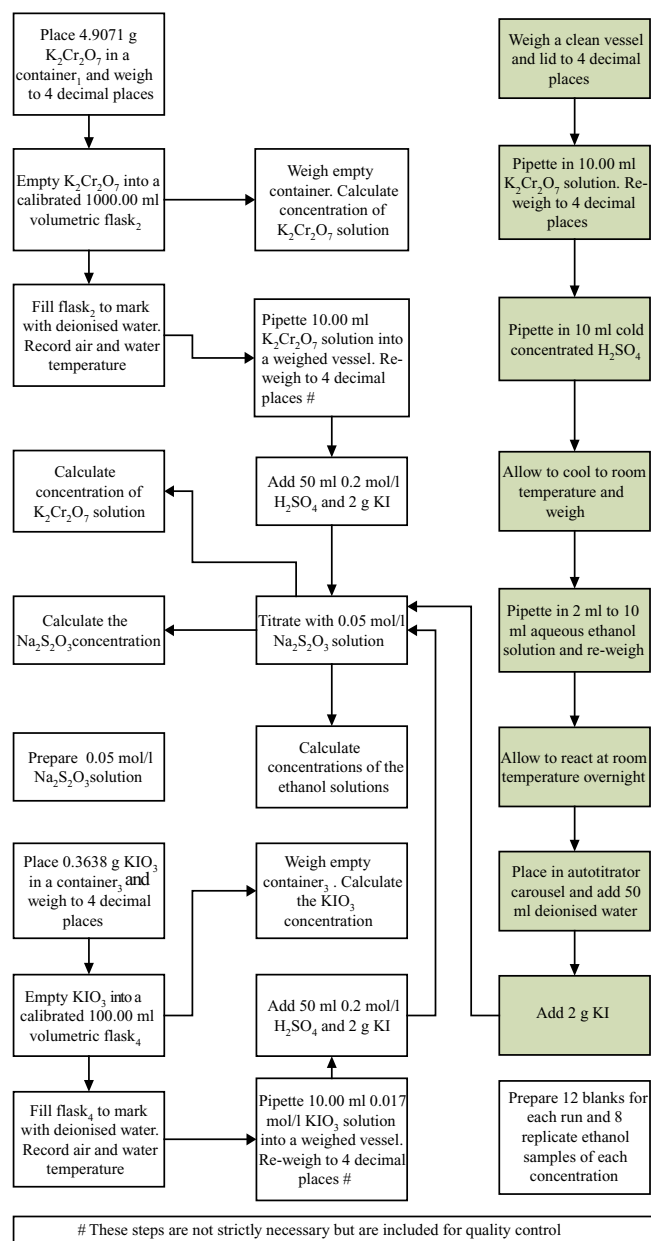


Fig. 1 Flowchart of the assay procedure for aqueous ethanol reference materials

Certification

The mean of the verification assay results may not differ from the gravimetric concentration by more than the relative expanded uncertainty for that concentration as listed in Table 2. For example, if a solution was gravimetrically prepared to be 175.78 mg/100 g, the mean of the titrimetric results must lie between 174.02 and 177.54 mg/100 g. The certified concentration (in g/100 g) is the mean of the prepared concentration and the eight titrimetric results. If the mean of the eight results is 176.6 mg/100 g (0.47%

Table 1 Replicate analyses in procedure

Procedure	Replicates
Blank K ₂ Cr ₂ O ₇ solutions	12
Sample solutions	8
Verification [K ₂ Cr ₂ O ₇]	4
Standardisation Na ₂ S ₂ O ₃	4

Table 2 Homogeneity limits and relative reported uncertainties of certified aqueous ethanol solutions ($k = 2$)

Concentration of ethanol solution	Homogeneity limit (% RSD)	Reported relative expanded uncertainty (%)
10 mg/100 g	4.0	4.0
18 to 49 mg/100 g	1.5	2.5
50 to 59 mg/100 g	0.8	1.3
60 to 500 mg/100 g	0.7	1.0
1 to 20 g/100 g	0.7	1.2

higher than prepared), then the certified concentration will be 176.2 mg/100 g \pm 1.8 mg/100 g. If both the verification and the homogeneity assays conform to the set requirements the batch can be certified.

Stability

Eight aliquots of each batch are tested for stability at intervals of 5 months. The whole range of solutions has remained stable over a period of at least 18 months when stored between 4 and 10°C. Solutions stored at 20°C were found to be stable over a period of at least 12 months. Figure 2 is a summary of stability tests on a solution stored between 4 and 10°C over a period of 23 months. The initial certification period was 8 months from date of preparation, but enough data is now available over the whole concentration range that the certification period could be increased to 12 months.

To establish the ruggedness of the CRMs to environmental abuse during transport, solutions over the whole concentration range were placed in a non-air-conditioned vehicle that was used for commuting during the South African summer. A calibrated temperature- and humidity-logging device was kept with the solutions. The temperature cycled from 13 to 43°C and the relative humidity ranged from 15 to 80%. After 2 weeks, the solutions were assayed. There was no detectable change in concentration.

Distribution

The reference material is distributed in volumes from 50 to 1,000 ml, depending on the application.

Quality control

Quality-control procedures are crucial to the validity of the procedure. This aspect has not been addressed in previous publications.

Potassium dichromate is often used as a primary standard in titrimetry. It is obtainable at high purities and when dried, solutions of accurately known concentration can be prepared. Nevertheless, the concentration of the potassium dichromate is verified by titration against a standard sodium thiosulphate solution every time it is used. If the gravimetric concentration differs from the titrated concentration by more than 0.3% relative, then the titration is repeated. If the concentration of the potassium dichromate cannot be verified, a fresh solution is prepared.

The concentration of the 0.05 mol/l sodium thiosulphate solution need not be known to calculate the concentration of the aqueous ethanol solutions (Eq. (1) below). However, in order to verify the potassium dichromate concentration, the sodium thiosulphate solution is standardised against 0.017 mol/l potassium iodate.

The back-titration is validated using blanks containing weights of potassium dichromate corresponding to the expected quantity of potassium dichromate left in the samples after oxidation.

All quality-control checks and sample assays are replicated as shown in Table 1.

Uncertainty

The uncertainty was treated according to ISO GUM [5] and CITAC/EURACHEM [6].

The ethanol concentration is calculated from:

$$[\text{EtOH}] = \left(\frac{100 \cdot A}{4.2565 \cdot B} \right) \cdot \left(C - \left(\frac{D}{h} \right) \right) \quad [\text{g}/100 \text{ g}] \quad (1)$$

$$h = \frac{\sum_{b=1}^n \frac{E_b}{F_{E_b}}}{n} \quad [\text{ml}/\text{g}] \quad (2)$$

where A is the concentration of the potassium dichromate solution (g/kg), B is the weight of the ethanol sample aliquot (g), C is the weight of the potassium dichromate solution added to the sample (g), D is the sodium thiosulphate titre for the sample (ml), h is the blank value for the analytical batch (that is, the average of the ratio of the sodium thiosulphate titre to the weight of the potassium dichromate titrated), E_b is the sodium thiosulphate titre for the blank (ml), F_{E_b} is the weight of the potassium dichromate used for the blank (g) and n is the number of blank determinations made.

Fig. 2 Results of titrimetric analyses of a certified ethanol solution for stability over a period of 23 months

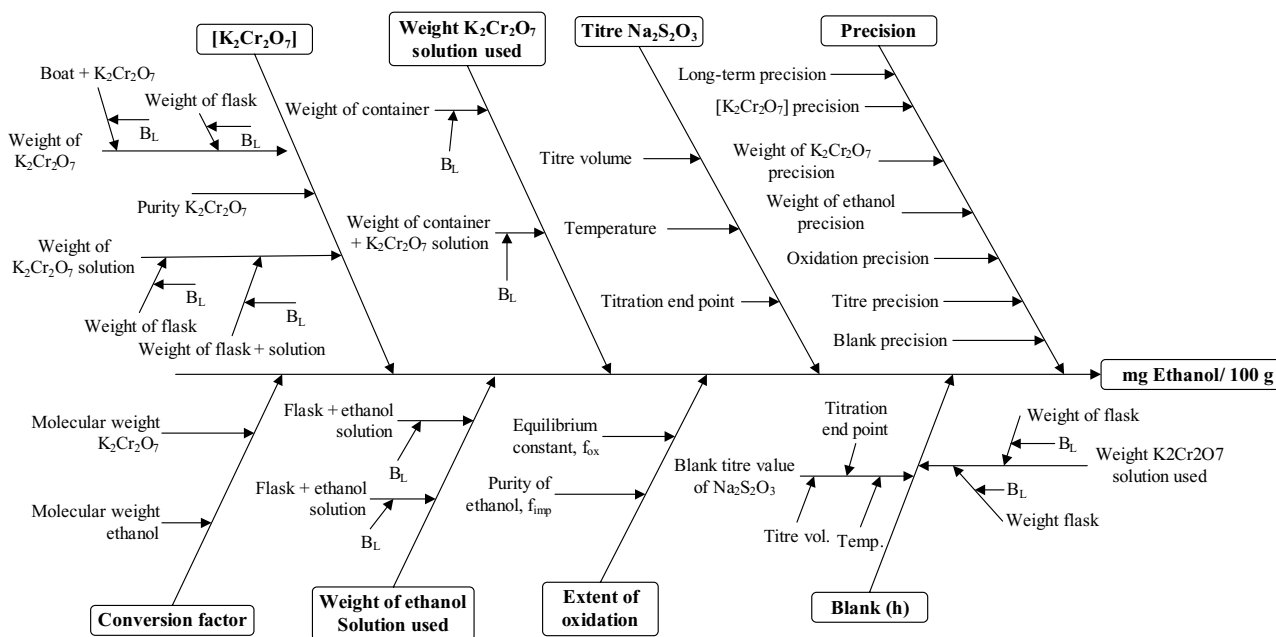
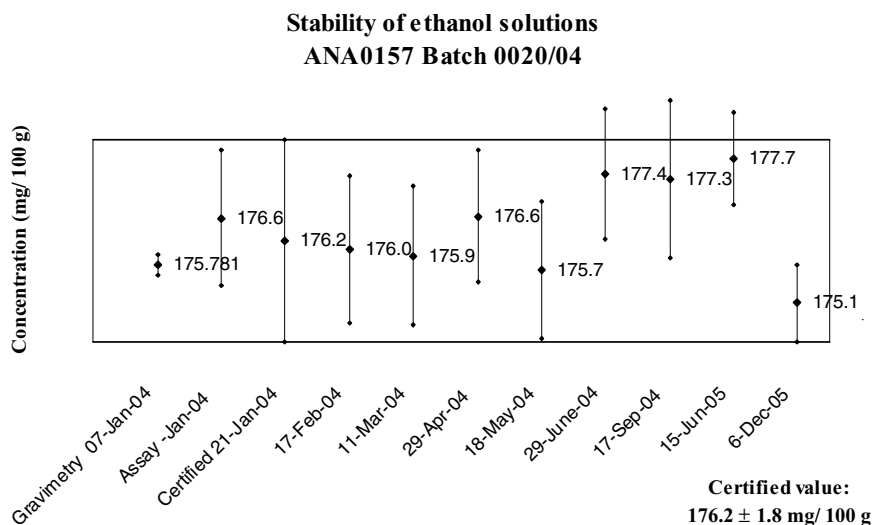


Fig. 3 Cause-and-effect diagram for the certification of aqueous ethanol solutions

The cause-and-effect diagram is given in Fig. 3.

In order to estimate reproducibility uncertainty, selected batches of aqueous ethanol covering the whole concentration range were analysed at least six times by different analysts and using different batches of reagents over at least 7 months. The expanded uncertainty quoted on the CSIR NML certificates includes reproducibility contributions.

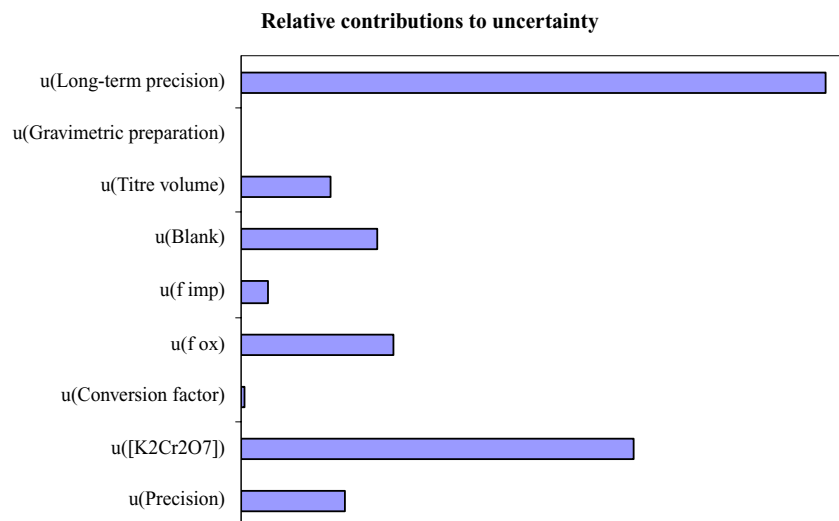
Although the gravimetric (prepared) concentrations of the solutions are used to calculate the certified concentration, the uncertainty of the gravimetric preparation is not included in the cause-and-effect diagram because the contribution of the gravimetric preparation can be shown to be negligible (Fig. 4).

Table 2 lists the relative expanded uncertainties for the whole concentration range. The assay of solutions containing more than 500 mg/100 g ethanol includes a dilution step, which results in a slightly increased relative uncertainty.

Traceability

The question of traceability of aqueous ethanol solutions has not been addressed in previous publications. The CSIR NML aqueous ethanol certified reference material is traceable to the kilogram since all reagents are weighed on calibrated balances. The CRMs are also traceable to the mole since the assay is a primary method with known reaction mechanisms. The CSIR NML CRMs are traceable to CRMs from

Fig. 4 Comparison of relative contributions to the uncertainty budget



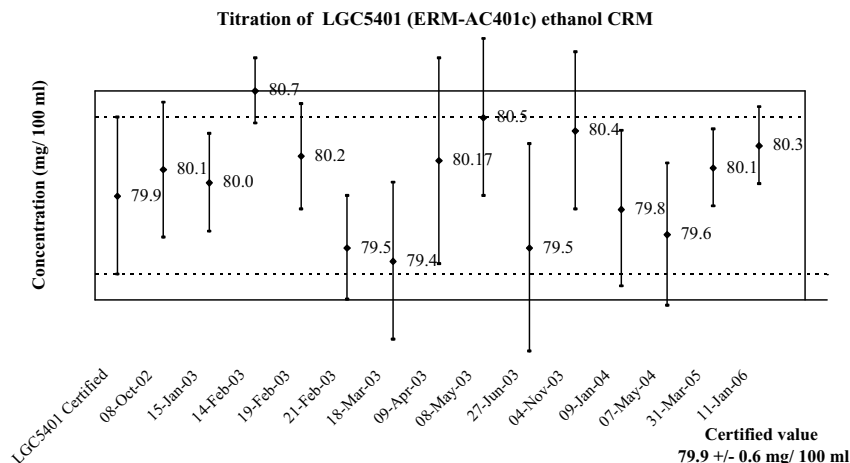
where:

The long-term precision is represented by eleven analyses by different analysts using different batches of reagents, over thirteen months (between-run precision or reproducibility).

u(Precision) is the precision for the analysis of eight aliquots by a single analyst on one day (within-run precision or repeatability).

u(f imp) and u(f ox) represent the uncertainties of the impurities in the ethanol and the extent of the ethanol oxidation, respectively.

Fig. 5 Results of titrimetric analyses of ethanol CRM LGC5401 from October 2002 to January 2006 by different analysts. The dotted horizontal lines indicate the uncertainty limits set by LGC, while the solid horizontal lines indicate the CSIR NML tolerance of $\pm 1\%$ for this concentration



a National Metrology Institute: Laboratory of the Government Chemist (LGC), UK., which are used to verify method performance (Fig. 5). A traceability chart is given in Fig. 6.

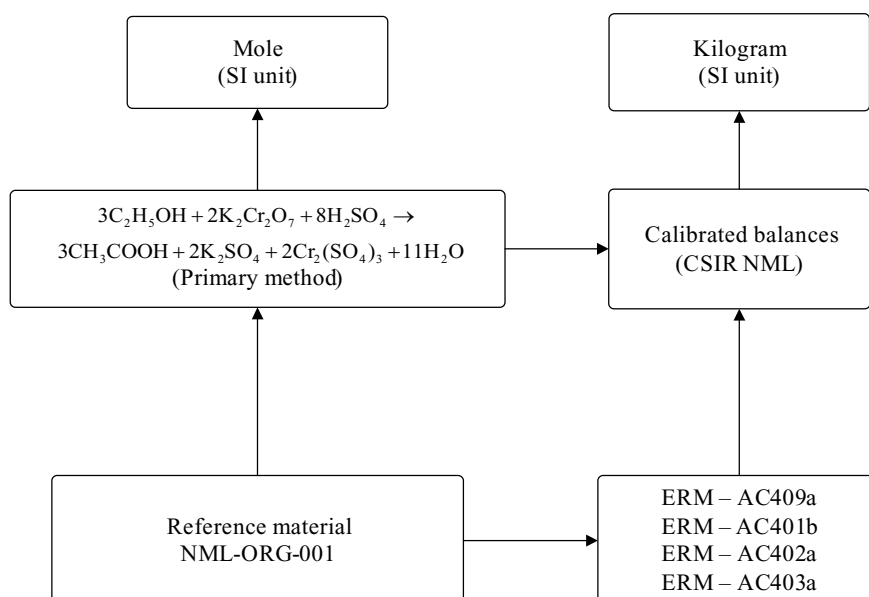
Discussion and conclusions

Barwick and Ellison [8] discussed the analysis of a single batch of aqueous ethanol. They used four potassium dichromate blanks that consumed 88 ml of titrant, while their samples consumed 31 ml. In our hands, assaying samples with different ethanol concentrations at one time, it

was found that four blanks gave reproducibilities that varied beyond our control limits. Analysing 12 blanks with end-points over the whole expected sample endpoint range, gives day-to-day reproducibility within the control limits listed in Table 2.

A detailed study of a repeatability uncertainty budget was made by Barwick and Ellison [8], who reported an expanded uncertainty for ethanol concentration of 0.28% relative (coverage factor, $k = 2$) at an ethanol concentration of 80 mg/100 ml. Reproducibility (between-run) effects were not included in their study. At the CSIR NML, the relative standard deviations of the repeatability range from

Fig. 6 Traceability chart for aqueous ethanol solutions prepared at the CSIR National metrology Laboratory, South Africa



0.27 to 0.63%, but when the reproducibility uncertainty is included the relative expanded uncertainty increases to 1.1% ($k = 2$). From Figs. 2 and 5 it is evident that the within-run repeatability is not robust, and will underestimate the overall uncertainty of the certified property value.

Primary methods, such as gravimetry and titrimetry used in this procedure, are especially important in the production of certified reference materials. Because small (1–5 L) batches are prepared the ethanol solutions can be prepared gravimetrically, with lower uncertainties than volumetry. The titrimetric assays are then a verification of the concentration. Because the assay is a primary method, impurities in the reagents or samples will cause erroneous results. The quality-control procedures detect such errors in the analytical process.

Many certified ethanol reference materials contain mercury (II) chloride as a preservative, but no data have been published on the stability of unpreserved ethanol solutions. To avoid possible environmental issues and toxic hazards, no preservative is added to CSIR NML ethanol reference materials. Despite the lack of preservative, the CRMs are certified as stable for 12 months when stored between 4 and 10°C.

The aqueous ethanol certified reference solutions prepared at the CSIR NML fill several needs for South African laboratories, especially those laboratories concerned with the analysis of ethanol in blood and urine. At present, eight different concentrations are routinely prepared for the calibration of gas chromatographs. These provide the widest range available from NMIs to date. The solutions have been used for the calibration of breath-alcohol instruments by the

so-called wet method. The South African wine industry has used CSIR NML ethanol CRMs to verify methods for estimating ethanol content by measuring the density of wine distillates, and interest is being shown in their application to ethanol measurements in wine by infra-red spectrometry. The ethanol CRMs have also been used as proficiency testing samples.

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