

Preparation, Uncertainty, & Certification of Ethanol Standards

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Introduction

Ethanol Reference Standards are Critical to the Accurate Quantitation of Blood Alcohol in Forensic Analysis

Ethanol standards are widely used in forensic and toxicology applications for determination of blood alcohol content. Results of blood alcohol testing have significant legal implications and are frequently used as evidence in court of law. The blood alcohol analysis process must therefore be reliable and defensible.

A critical component of blood alcohol analysis is the calibration used for quantification of results. Ethanol reference standards are widely available for this purpose and are sold in many formats – bottled and ampoules. The blood alcohol test result is imperative that the standards are important contributors to the accuracy and associated uncertainty of the blood alcohol test result. It is imperative that the uncertainty of the reference standards be within the margins of the blood alcohol testing uncertainty and that the certified concentration is accurate and completely traceable to international units of measure.

Cerilliant Certified Ethanol Reference Standards are manufactured and certified to ISO Guide 34 and ISO/IEC 17025 standards, and are traceable to SI units and to NIST SRM ethanol standards. The preparation, certification and uncertainty of these standards is presented in this poster.

Critical Elements

- Proper certification of the neat material
- Accuracy of mass measurement
- Accuracy of diluent addition
- Dispensing, packaging and stability
- Analytical verification
- Traceability to SI units of measure & traceability to NIST SRM
- Certification & Uncertainty

Certification of the Neat Ethanol

Complete & accurate characterization of the neat ethanol is essential to accuracy of the solution.

Certification Considerations

- Ethanol is widely available in high purity and is stable for many years when stored appropriately
 - What is the grade of ethanol used?
 - Is the ethanol vendor accredited?
 - What are the specifications of the ethanol procured for use in the standard?
 - Is it the ethanol certified?

Certification Practice

- Ethanol procured for standards meets ACS/USP specifications
- Vendor COA provides complete testing information, vendor is certified to ISO9001:2000
- The ethanol is tested for identity, purity and water content and then certified by Cerilliant's ISO/IEC 17025 accredited testing lab.
- Certification ensures traceability through certification by an accredited testing lab.
- The neat ethanol is stored in 5 ml ampoules, flame sealed under argon to protect from moisture absorption during storage.

Characterization of neat ethanol

- Determination of purity
 - Chromatographic purity by GC/FID using 2 different columns
- Verification of identity
 - by GC/MS
- Determination of residual water content
 - Karl Fischer Coulometry <USP2>
 - Ethanol is hygroscopic. Residual water content must be determined and included in purity factor calculations for use of ethanol in quantitative applications
- Assignment of a mass balance purity factor for use in preparation of the solution standard

Purity Factor Calculation

- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.

$$Purity\ Factor = (100 - (w\%H_2O) \left(\frac{ChromPurity}{100} \right)) \pm U$$

- U represents the combined uncertainty of the purity factor at ~95% confidence and includes uncertainty of both the purity determination and the residual water analysis.

Uncertainty of the Purity Factor

- Uncertainty of the neat ethanol purity factor was achieved by evaluating the uncertainty of the analytical tests used in the Purity Factor equation.
- Uncertainty of chromatographic purity is based on specifications for chromatographic purity by two different methods to be within 0.5%.
- Uncertainty of residual water content is based on repeatability experiments on the Karl Fischer Coulometric method (USP921-1).

$$U_{Purity\ Factor} = \pm 0.144\%$$

$$w\%H_2O = 0.25\%$$

$$w\%H_2O = 0.03990\%$$

All instruments are fully qualified and calibrated. Requalification is performed annually and system suitability is performed daily. Balances are qualified and calibrated. All weighings are traceable to SI units.

Results were combined in a Kratgen Spreadsheet® to determine uncertainty of the neat ethanol purity factor

Kratgen Spreadsheet for Uncertainty of the Purity Factor

Variable name, symbol	Input Value	Units	Uncertainty source description	Reported uncer.	Type	Distribution	Factor to normalize	Standard uncer., u_i
Water Content, $w\%H_2O$	0.0745	%w/w	GC Specification	0.0399	B	comb. std., $k = 1$	1	3.99E-02
ChromPurity	99.997	%	Test Specification	0.250	B	Uniform	0.57735027	1.44E-01

Sequential Perturbation	df	u_i	u_i
$w\%H_2O$	0.0745	0.11440	0.07450
ChromPurity	99.9970	99.99700	100.14134
Result	99.922502	99.9826	100.06467
difference		0.03990	0.14423

Measurement Equation Inputs	Value
$w\%H_2O$	0.0745
ChromPurity	99.9970
Result	99.922502
difference	0.03990

$$Purity\ Factor = (100 - (w\%H_2O) \left(\frac{ChromPurity}{100} \right)) \pm U$$

RESULTS

PF [w%] **99.9225**

k **2**

U [w%] **0.29929**

Solution Standard Preparation and Uncertainty

Mass Measurement

Mass Measurement Accuracy / Traceability

- Certified requires mass balance (specified for each balance) to limit relative uncertainty to $\pm 0.1\%$ as prescribed by USP <921>
- Balance selection and minimum weighings are outlined in standard operating procedures and were determined through the combination of manufacturer tolerances and repeatability experiments performed.
- Improve weighing techniques can increase uncertainty. Proper weighing techniques are outlined in standard operating procedures.

Qualification and Traceability

- Each balance has been fully qualified in its installed state, is calibrated semi-annually to manufacturer tolerances and adjusted weekly with NIST traceable weights. Calibration verified prior to each use using NIST traceable weights.

Mass measurement uncertainty was determined from a combination of balance manufacturer specified tolerances for sensitivity and linearity and repeatability experiments following specified weighing procedures. Balance manufacturer tolerances alone are insufficient. Values are proportional to the net mass being measured and are specific to the balance utilized.

Uncertainty due to balance sensitivity tolerances

Includes the uncertainty of the balances built-in reference weight used for internal calibrations.

Balance manufacturer calibrations incorporate traceability to NIST SI units and their associated uncertainty in the sensitivity component

Uncertainty due to nonlinearity of the characteristic curve

from the balance manufacturer

Repeatability

Includes effects from readability, drift, static, ambient drafts, thermal drafts, vibration, gross/net weight, eccentric loading, temperature stability, electromagnetic interferences/facade frequency interferences, weighing procedure, installation, tare container geometry, adsorption/absorption, balance settings, and operator technique

Determined by tests of 20 replicate weighings conducted by multiple operators at various test loads and net weights on all balances used to prepare solution standards

Diluent Addition - Gravimetric vs. Volumetric Methods

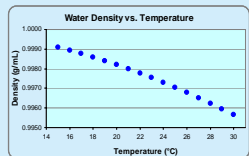
Cerilliant Process is Gravimetric

- Target solution mass calculated from target volume by adjusting for density.
- Actual solution mass collected back into volume to report concentration as mg/dL

Advantages of Gravimetric Approach

- Ensures lot-to-lot consistency - Measurement of volume by mass eliminates temperature dependence of flask accuracy and allows all solutions to be consistently prepared at the chosen stock reference temperature.
- Eliminate the subjectivity of visual fill line in volumetric addition
- Mass measurement provides traceability to SI measure
- Weight tapes provide an audit trail
- Allows accurate formulation of both volumes well beyond the capacity of Class A flasks

Thermal expansion will affect volumetric accuracy of calibrated flasks



0.21% difference in concentration of aqueous solutions when prepared volumetrically at 15°C vs. 25°C

$$u_x = \pm 0.001 \sqrt{\frac{1}{\rho^2}} = 0.000577 \text{ g/mL}$$

Uncertainty of Diluent Addition

Uncertainty related to diluent addition arises from uncertainty in the density value used for the solution.

Based on instrument tolerances for density measurement (Type B)

Dispensing Process

Identification and Control of Critical Parameters

The Dispensing Process was Analyzed:

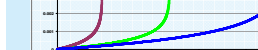
- In a test case, every ampoule, from the beginning to the end of a run, was tested analytically for concentration homogeneity.
- The study identified potential for dilution/lower fill volumes in the early ampoules and potential for evaporation-induced concentration in late ampoules.

- Dilution is eliminated and consistency of volume ensured by purging the lines with product prior to filling.
- Evaporative losses are controlled through protection of the bulk container during dispensing and through speed of the dispensing process.

- Process speed - Typical Czapoli speed is fast. 50 containers per minute (11 in 17 minutes), minimizing degradation and potential for evaporative losses.
- Evaporative losses were further evaluated in evaporative studies where the evaporation of solvent from open containers was measured gravimetrically.
- Evaporative loss of solvent during impinging on the Czapoli dispenser/sealer was modeled and determined to be <0.0006% over 4 hrs. Not a significant contributor to solution standard uncertainty.

Cerilliant Ethanol Solution Standard Stability

- The implored ethanol solution standards are autoclaved to control microbial growth.
- Expiration is established through real-time stability studies.
- Solution purity and concentration are reevaluated at multiple intervals. Stability is established as long as purity and concentration continue to meet original release criteria.
- Five Years of shelf life has been established.
- Stability is not a significant contributor to uncertainty and is, therefore, excluded.



Assessment and Reporting of Uncertainty of the Certified Concentration

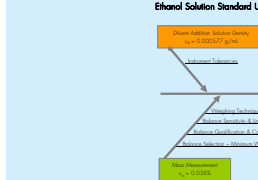
Solution Standard Certification & Uncertainty

Cerilliant evaluated every step involved in the preparation of its Certified Ethanol Solution Standards and determined that the primary contributing factors impacting uncertainty were: uncertainty of the Purity Factor, Mass Measurement uncertainty and Diluent Addition Uncertainty.

Measurement Equation for Concentration Uncertainty

$$C = \left(\frac{m_{std} - m_{dil}}{m_{std} - m_{dil}} \right) \pm U$$

Where: C = Concentration of solution (mass/volume)
 m_{std} = mass of standard
 m_{dil} = mass of diluent
 $m_{std} - m_{dil}$ = mass of final solution
 $m_{std} - m_{dil}$ = mass of empty flask
 $m_{std} - m_{dil}$ = density of solution
 $m_{std} - m_{dil}$ = density of diluent
 U = the assigned combined expanded measurement uncertainty



Cerilliant Model

- Concentration of solution (mass/volume) against NIST SRM
- Concentration verified against NIST SRM and Cerilliant Control
- Control is prepared from a diluent of ethanol and qualified against NIST SRM
- Solution purity is verified to demonstrate no denaturation or degradation has occurred during preparation
- Samples are pulled from across the batch to demonstrate homogeneity. The MSD of results is reported on the COA

Kratgen Spreadsheet - Uncertainty of the Certified Concentration

Input description	Symbol	Value	Units	Reported uncer.	Type	Distribution	Factor to normalize	Standard uncer., u_i	Rel. u_i (%)
Standard uncertainty of mass measurement	u_{mass}	0.000377	g/mass	0.000377	B	Uniform	1	0.000377	0.377%
Standard uncertainty of solution factor	u_{PF}	0.0011		0.0011	B	Uniform	1	0.0011	0.110%
Standard uncertainty of density	u_{ρ}	0.000577	g/mL	0.000577	B	Uniform	1	0.000577	0.0577%

Sequential Perturbation	df	u_i	u_i
u_{mass}	0.000377	0.000377	0.000377
u_{PF}	0.0011	0.0011	0.0011
u_{ρ}	0.000577	0.000577	0.000577
Result (mg/dL)	0.000377	0.000377	0.000377

Results
Cert. = Expanded Uncertainty (k=2)
398.883 ± 1.46068 mg/dL

Analytical Verification & Method Validation

A Validated Analytical Method is used to Verify Solution Concentration and Ampoule to Ampoule Consistency

Solution standard concentration is verified analytically by comparison to an appropriate NIST SRM.

- A calibration control is used in the analysis. Control is made from a diluent of neat ethanol which has been certified. Control is qualified to NIST SRM.

Homogeneity across the lot is verified by testing samples pulled from across the lot. A stratified random sampling plan is utilized and includes samples of the first and last ten ampoules plus one per every 400 ampoules dispensed.

Concentration and homogeneity are verified using a validated Headspace GC/FID method.

Validation ensures the analytical method is accurate, robust, repeatable and reliable

Linearity

- Linearity of the method was determined by plotting measured signals (peak area) as a function of analyte concentration (mg/mL) across the range.
- The linear relationship was evaluated by calculating a regression line by the method of least squares.
- The linear fit is linear from 5 to 600 mg/dL Ethanol in Water.

Low Range (3 - 100 mg/dL)	High Range (100 - 600 mg/dL)
Linear Equation: $y = 0.0048x - 0.0137$	Linear Equation: $y = 10.0358x - 17.2716$
$R^2 = 1.0000$	$R^2 = 1.0000$

Linearity ensures the analytical method is suitable for quantitation across a range of concentrations

Uncertainty of the Analytical Verification

Uncertainty assessment for verification includes uncertainty related to the analytical method and instrument response and uncertainty reported on the value assigned by the NIST SRM.

$$C_{cert} = \frac{Area_{std} - Area_{dil}}{Area_{std} - Area_{dil}} \pm U$$

Where: $Area_{std}$ = area response of the standard
 $Area_{dil}$ = area response of the diluent
 $Area_{std} - Area_{dil}$ = area of final solution
 $Area_{std} - Area_{dil}$ = area of empty flask
 $Area_{std} - Area_{dil}$ = density of solution
 $Area_{std} - Area_{dil}$ = density of diluent
 U = the assigned combined expanded measurement uncertainty

Factors Impacting Uncertainty of the Analytical Verification

- Uncertainty is specific to the analytical technique (GC/FID, GC/Headspace/FID, titration etc) and within technique to the specific instrument/method
- GC/Headspace methods can vary in precision depending on the specific instrument/method and parameters used
- Variables include sample preparation, analytical training, instrument response, instrument parameters (injection time, split ratio, etc.)
- Uncertainty of the calibrator concentration must also be included

If a curve is run, analytical uncertainty applies to each curve point analyzed and must be factored into determining the overall uncertainty.

The biggest contributor to uncertainty in our study is the GC/Headspace analytical method, representing approximately 90% of the uncertainty.

Traceability is Provided from Beginning to End

Traceability is the property of a measurement result whereby it can be related to stated references usually through national or international standards through an unbroken chain of comparisons all having stated uncertainties.

- Preparation and certification by ISO Guide 34 and ISO/IEC 17025 accredited company.
- Neat material certification by ISO/IEC 17025 accredited testing lab.
- The purity of the neat material is included in the uncertainty of the standard preparation.
- Balances installed, qualified and calibrated semiannually by ISO/IEC 17025 accredited testing lab utilizing NIST traceable weights.
- Weekly and pre-use calibration verifications performed using NIST traceable weights - pre-use calibration NIST traceable in solution standard batch record.
- Gravimetric preparation for analyte and diluent - weigh tapes included in solution standard batch record - traceability to SI units of measure.
- Balance tolerances experimentally verified for the manufacturing process and included in uncertainty calculation.
- Fill volume is gravimetrically verified during the dispensing process.
- Analytical verification of concentration and homogeneity by ISO/IEC 17025 accredited testing lab utilizing validated methods.
- The concentration is reported with uncertainty in accordance with ISO/IEC 17025 and ISO Guide 34.
- The uncertainty value is reported with a coverage factor, k=2, representing an approximately 95% confidence for the stated concentration.
- The neat material traceability and test data are provided on the COA.

Conclusions

- The accuracy and traceability of calibrators used in the determination of blood alcohol content is critical to the outcome and defensibility of the analysis.
- An understanding of vendor preparation and certification practices as well as factors included in the determination of uncertainty are necessary to ensure compliance with regulatory requirements and to supporting analytical results in courts of law.
- Cerilliant Certified Ethanol Reference Standards are suitable for use in forensic investigations. Cerilliant standards are manufactured and certified to the highest industry standards to ensure accuracy and precision including ISO Guide 34 and ISO/IEC 17025 requirements and are traceable to SI units and to NIST SRM ethanol standards.



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 3. EURACHEM/CITAC Guide 2nd ed., "Quantifying Uncertainty in Analytical Measurement," EURACHEM/CITAC, 2000, Section 8.2.5 and Appendix E