Preparation, Uncertainty, & Certification of Ethanol Standards

Authors Ning Chang, PhD, Isil Dilek, PhD, Bryan Dockery, Kevin Gates, Huahua Jian, PhD, Mitzi Rettinger, Krystal Silva,

Introduction

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

Ethanal standards are widely used in forenzic and toxicology applications for determination of blood lacohal content. Results of blood alcohol stanig have significant legal inglications and are frequently used as evidence in courts of law. The blood alcohol analysis process must therefore be reliable and detersultie.
A critical component of blood alcohol analysis is the calibrator used for quantitation of numli. Enhand relevance standards run widely available for this purpose and rere sold in many format – bottled and ampulade. The occurracy and uncertainty associated with these standards are important contributors to the occuracy and associated uncertainty of the information of the standard be within the margins of the uncertainty of the information standards be within the margins of the blood disciolal leading uncertainty and the the centified conjunction on blood disciolal leading uncertainty and the the centified conjunction to blood disciolal leading uncertainty and the the centified conjunction to blood disciolal leading uncertainty and the the centified conjunction to the standard disciolation of the standard comparement on the standard blood blo

Critical Elements · Proper certification of the neat material Dispensing, packaging and stability Analytical verification Traceability to SI units of measure & traceability to
 NIST SRM Certification & Uncertainty

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.

 $PurityFactor = (100 - (wt%H_2O)\left(\frac{ChromPurit}{100}\right) \pm U$

 $H_{(ChemPosty)} = \frac{0.25 \text{ N}}{\sqrt{3}} = 0.144 \text{ N}$

U represents the combined uncertainty of the purity factor at ~95%

Uncertainty of residual water content is based on repeatability experiments on the Karl Fischer Coulometric method (USP-Q21-5). $u_{ij} = 0.03990\% \text{ w/w}$

All instruments are fully availified and calibrated

Requalification is performed annually and system suitability is performed daily

Balances are qualified and calibrated.

All weighings are traceable to SI units

confidence and includes uncertainty of bo and the residual water analysis

Uncertainty of the Purity Factor

Uncertainty of the neat ethanol purity factor was achieved by evaluating th uncertainty of the analytical tests uses 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%.

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 SIW ethanol standards. The preparation, certification and uncertainty of these standards is presented in this poster.

Purity Factor Calculation

Certification of the Neat Ethanol

Complete & accurate characterization of the neat ethanol is

Certification Considerations

channol is widely divaliate in high purity and is sta many years when stored appropriately – What is the grade of ethanol used? – Uhat are the specifications of the ethanol pro-use in the standard? – How is the ethanol centified?

Cerilliant 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 Certification ensures traceability through certification by an
- The neat ethanol is stored in 5 mL ampoules, flame sealed under argon to protect from moisture absorption during

Characterization of neat ethanol

nation of purit Chromatographic purity by GC/FID using 2 different • Vecifi n of identity

- By GC/MS nation of residual water co etermination or resulut water content - Karl Fischer Caulometry -USP921> - Ethanol is hygroscopic. Residual water content must be determined and included in purify factor calculations for use of ethanol in quantitative applications

Assignment of a mass balance purity factor value for use in preparation of the solution standard

Results were combined in a Kratgen Spreadsheet⁽¹⁾ to determine uncertainty of the neat ethanol purity factor

Kragten Spreadsheet for Uncertainty of the Purity Factor

Variable name, symbol	Input Value	Units	Uncertainty source description	Reported uncer.	Typ	e Distrib	ution	Factor to normalize	Standard uncer., v _i			
Water Content, wt%H2O	0.0745	‰v/w	QC Specification	0.0399	В	comb. std	., k = 1	1	3.99E-02			
ChromPurity	99.997	%	Test Specification	0.250	В	Unit	orm	0.57735027	1.44E-01			
Sequential Pertu	hara			u(wt%H2	20)	u(ChromPurity)	1					
sequennial Pertu	roanon			0.03	3990	0.14434	1					
			ďf	99	9999	99999						
Measurement	Equation in	puts	Value									
w%H2O			0.0745	0.11	440	0.07450	Purity	Factor = (100 - (wt))	$actor = (100 - (wr\%H_2O))\left(\frac{ChromPur}{100}\right)$			
Chron	nPurity		99.9970	99.99	700	100.14134			, 100			
Re	Result 99.9		99.922502	99.8	826	100.0667	1					
differ	ence			-0.03	3990	0.14423	1	RESI	JLTS			
	U _C		0.14965	1.59191	E-03	2.08023E-02	1	PF (wi%)	99.9225			
ChromPurity Result difference b dif k		df		0.07109		0.92891		k	2			
		k	2			0.99926			0.29929			
		U	0.29929	0.3	00%	U _{rolativo} , %		U (w1%)	0.27729			
				0.1	50%	Urplative, %						



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Solution Standard Preparation and Uncertainty

Mass Measurement

Aass Measurement Accuracy / Traceability iliant requires minimum sample masses (specified for each ance) to limit relative uncertainty to <0.1% as prescribed by USP ance selection and minimum weighings are outlined in standard rating procedures and were determined through the combination nanufacturer tolerances and repeatability experiments performed operating procedure of manufacturer toler Improper weighing technique can increase uncertainty. Proper weighing techniques are outlined in standard operating procedures Weighing technologies and Gualification and Traceability Each balance has been fully qualified in its installed state, is Each balance has been fully qualified in its installed state, is Each balance has been fully qualified in its installed state, is calibrated semi-annually to manufacturer tolerances and adjusted weekly with NST traceable weights. Calibration verified prior to each use using NIST traceable weights.



Water Density vs. Temperature

·····

16 18 20 22 24 26 28 30

Temperature (°C)

Thermal expansion will affect volumetric accuracy of calibrated flasks

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

 $u_{z} = \frac{0.001}{\sqrt{3}} = 0.000577 \text{ g/mL}$

messared and are specific to the totalics unable of the balances sensitivity befores
 - Includes the uncertainty of the balances balan interence weight used for internal calibrations
 - Balance monutocurre calibrations touches that the balances balance to the balances.
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0.995

(Tur) 10,998

C Density

Diluent Addition - Gravimetric vs. Volumetric Methods





Actual solution mass calculated back into volume to report concentration as mg/dL Advantages of Gravimetric Approach

nsures lotto-lat consistency – Measurement of volume by mass liminates temperature dependence of flask accuracy and allows all olutions to be consistently prepared at the same chosen reference temperature. Eliminates the subjectivity of visual fill line in valumetric additi Mass measurements provide traceability to SI units of measur

Mass measurements provide traceability to SI units of measure Weigh topes provide an audit trail Allows accurate formulation of batch volumes well beyond the constribution of Clarach Backs Incertainty of Diluent Addition

Uncertainty related to diluent addition arises from uncertainty in the density value used for the solution. Barad an instrument talarances for dearity measurement (Type B)

Dispensing Process

Identification and Control of Critical Parameters

The Dimension Process was Analy

- ne Dispersing Process was Analyzes: I 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 eveporation induced concentration in late
- Dilution is eliminated and consistency of volume ensured by purging the lines with product prior to filling.
- Evaparative lasses are controlled through protection of the bulk container during dispensing and through speed of the dispensing Process speed: Typical Cozzoli speed is fast, 50 containers per minute [11 in 17 minutes], minimizing degradation and potential for evaporative losses.

in 12 minutes), minimizing degradation and potential for evoporative losses. Ecoparative losses were further evoluted in evoporative adules where the evoporation of solvent from cpen containers was measured gravimetrically. Ecoparative loss of solvend utrian gravoling on the Cozcali dispensery/secter was meddeed and determined to be <0.000% over 4 hrs. Not a significant contributor to solution standard uncentainty.



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 Punity Factor; Mass Measurement uncertainty and Diluent Addition uncertainty.









The Kragten Spreadsheet shows the calculation of uncertainty and contributions of each uncertainty component

Kragten Spreadsheet - Uncertainty of the Certified Concentration

standard uncertainty of mass measurements:	0.00035*Net Mass Ial		Input descriptionw	Symbol	Value	Units	Reported uncer.	Туре	Distribution	Factor to normalize	Standard uncer., u	ReLu,
	0.0015		mass of vial	п,	5	9	0.038529876	A	comb. Std.	1	0.038530	0.771%
tandard uncertainty of adjustment factor	0.0015		mass of visi+analyte	m.,,	115.08536	9	0.038529876	A	comb. Std.	1	0.038530	0.033%
standard uncertainty of density	0.000577 a/ml	11	mass of flask	m,	100	9	9.625245	A	comb. Std.	1	9.625245	9.625%
	' [mass of fask+solvent	n.,,	27660.7	9	9.625245	A	comb. Std.	1	9.625245	0.035%	
$C = \frac{(m_{x+x} - m_{x})d}{(m_{x+x} - m_{x})d} + U$			adjustment factor	р	1.00078	9/0	0.0015	в	comb. Std.	1	0.001500	0.150%
$C = \frac{1}{(m_1, \dots, m_r)}$	- [solution density	d	1	ginL	0.001	в	unform	1.732050808	0.000577	0.058%	



Accuracy

Validation Summary

NIST SRM Calibrator

Area Area

esuit (mg/dL)

4.99985 0.528 -2.9431

300.00299 0.918 0.58511

Accuracy demonstrates consistency and reproducibility

 Conc
 a (eppended uncert)
 atd uncert
 Unita
 k
 Conv Fact

 0.01501
 0.00018
 0.00000
 wt %
 2
 99800

 19.47008
 0.17924
 0.08982
 mg/dt.

 $C_{ur} = \frac{Area}{Area} + C_{ABT} \pm U$

1 2.358094 1.1455

Expanded Uncert

25.3485 a 0.849132 mg/dL

The validated GC/HS method can adequately detect and quantitate enhanol concentrations ranging from 5 to 600 mg/dL.
 The method is robust to slight modifications in temperature ramp, injection time, and vial incolection time, but is sensitive to changes in

When all analyses were evaluated from precision, intermediate preci and linearity, the overall %25D was 1.145%, representative of the uncertainty of the instrument response including day to day, operator, instrument and sample preparation variability.

 Neput Description
 Symbol
 Yakue
 Ubits
 Reported uncer.
 Type
 Distribution
 Practor to normalize
 Standard uncer.
 Rest u(%)

 Area of aid
 Area_{wa}
 268.11466
 response
 3.050915347
 A
 cmh. bid.
 1
 3.060915
 1.4555

Area_{NET} 295.94708 response 2.358094098 A comb. Std.

u(AreaNIST) u(CNI 3.06992 2.35809 0.089

271.18478 258.11486 258.1 205.94708 208.30518 255.9 19.47058 19.47038 19.59

0.0542 46.733 0.094

why Q.5% confider

25.63879 25.06159 25.465 0.29024 -0.28596 0.116

40 0.082343 0.01367 % 45.681% 7.5867

3.350% U_{nister} %

menhility to SI units of measure

nation of uncertainty are necessary to ensure

e for the stated cor

C_{NET} 19.47035 mg/dL 0.0895

Value 268.11485 205.94708 19.47098 25.34855

0.04913

a, 0.42456619

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 liquing stated uncertainties.

Analytical Verification & Method Validation

- A Validated Analytical Method is used to Verify Solution

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

Mefhad Validation – Linearity Low Range (5 - 100 mg/dl) High Range (100 - 600 mg/dl)

ted to the analytical method and instrument response uncertainty reported on the value assigned to the NIST

Measurement equation for uncertainty of analytical concentration verification

 $C_{urr} = \frac{Area_{ud} * C_{NEST}}{d} \pm U$

Area NDT

Factors Impacting Uncertainty of the Analytical Verification

Linearity is previously of the Analytical Verification Uncertainty specific to the analytical Verification CC Hoodspace FID, http://www.inter.interview.com/ CC Hoodspace methods can within technique to CC Hoodspace methods can will be previous dependent variables includes sample preparation used. Variables includes sample preparation regular tabing instrument response, instrument parameters included on time, pair into, edit.

included. If a curve is run, analytical uncertainty applies to <u>each</u> curve point analyzed and must be factored into determination of the overall uncertainty. The biggest contributor to uncertainty in our study is the GC Headbace analytical method, representing approximately 90% of the uncertainty.

Traceability is Provided from Beginning to End

Fill volume is anavimetrically verified during the dispensing process

The uncertainty value is reported with a coverage factor. k=2, represe

· The neat material traceability and test data are provided on the COA.

 Weekly and pre-use calibration verifications performed using NIST traceable weights - pre-use v Gravimetric preparation for analyte and diluent – weigh tapes included in solution standard batch record – tra

Balance tolerances experimentally verified for the manufacturing process and included in uncertainty calculation.

The concentration is reported with uncertainty in accordance with ISO/IEC 17025 and ISO Guide 34.

An understanding of vendor preparation and certification practices as well as factors included in the dete

Analytical verification of concentration and homogeneity by ISO/IEC 17025 accredited testing lab utilizing validated methods.

The accuracy and traceability of calibrators used in the determination of blood alcohol content is critical to the outcome and defensibility of the analysis.

Compliance wini regionary requestments are a supporting interpret Certificant Certification Reference Standards are sublicle for use in forencic investigations. Certificat standards are manufactured and certified to the highest industry standards to ensure occuracy and precision including ISO Guide 34 and ISO/IEC 17025 requirements and are traceable to SI units and to NIST SRM.

Process Controls Ensure Consistency of Fill Volume & Lot Homogeneity

Certificite ethnole solution structured are dispersed into consultantal andre organopolise using a Cozard dispersing/soluting system.
 Angoles are purged with argan prior to flome soluting.
 Soluting effectively is writed darky and weekly using day tests.
 New koling is used for each product to eliminate tisk of contamination.
 Is due and systems are down data for a solution of the solution of the soluting effective and systems are down data for a solution of the values considering of the values.
 Is an are purged with product prior to ampound to eliminate deal values considering of the values.
 If it is a verified gravimetically using a statisfied roadem sampling plan in balance calcineation gravit and the values.

an balances calibrated semi-annually and vertified before use to INDS traceable weights. Concentration and homogeneity are verified analytically using a stratified random sampling plan developed from an analysis of critical points in the filing/sealing process.

The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty collustrate and in therefore, excluded

Certilliant Ethanol Solution Standard Stability

microbial arowth. Expiration is established through real-time stability studies. Solution purity and concentration are re-evaluated at multiple intervals Stability is established as long as purity and concentration continue to most activity and concentration continue to

 Five Years of shelf life has been established. Stability is not a significant contributor to uncertainty and is, therefore

The gravimetrically prepared concentration is the "True Value" and is reported on the centricate of randysis with statietd uncertainty as "rmg".(d). Verification of the solution standard concentration is performed post ampouling demonstrating accuracy and argoould is ompoule consistency (batch homogenelly). Validated Headspace GC/FD method with known uncertainty

· Preparation and certification by ISO Guide 34 and ISO/IEC 17025 accredited company Neat material certification by ISO/IEC 17025 accredited testing lab.

Conclusions

compliance with regulatory regu

industry standards to ensure ethanol standards.

- · The purity of the neat material is included in the uncertainty of the standard pre-Balances installed, qualified and calibrated semiannually by ISO/IEC 17025 accredited testing lab utilizing NIST traceable weights.
- Concentration verified against NIST SRM and Cerilliant Control Control is prepared from a different lot of ethanol and qualified
 against NIST SRM
- against Nici Joky Solution purity is verified to demonstrate no contamination or degradation has occurred during preparation = samples are pulled from across the batch to demonstrate homogeneity. The %XSD of results is reported on the COA

Accuracy was assessed using a minimum of nine determinations over at least three concentration levels 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 different lot of neat ethanol which has been certified. Control is covering the specified range. Each sample was 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 amposles plus one per every 400 ampoules dispensed. prepared in triplicate and %RSD values represent the reproducibility of the method

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

Linearity

 \bullet 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 method is linear from 5 to 600 mg/dL Ethanol in Water.

or Equation y=9.9448x - 4.4537 Linear Equation y=10.0568x - 17.65

i^a 1.000 i^a Linearity ensures the analytical method is reliable for quantitation across a range of concentrations

Uncertainty of the Analytical Verification

Where: Area, a crea response of the standard Area, area response of the NST SBM