SDPD Forensic Science Section
Forensic Alcohol Analysis using Headspace Gas Chromatography
Force Method Validation

Introduction

The Forensic Chemistry Unit quantitatively analyzes blood and urine samples for the presence of alcohol using a heated headspace gas chromatography method. The method is calibrated with secondary alcohol standards at concentrations of approximately 0.10, 0.20, and 0.30 grams%. An aqueous internal standard is incorporated in known amounts into each sample and standard. The data handling software derives the linear calibration from these standards and then compares the areas under the ethanol and internal standard peaks to quantitate the amount of ethanol in samples. The method also includes the analysis of a control (quality control reference material containing alcohol) at a concentration approximating 0.150 grams%, in accordance with California Department of Health regulations for forensic alcohol analysis as specified in Title 17, Article 6, Section 1220.3.

This validation sets forth to supplement the existing blood and urine alcohol analysis method in two ways. The linear fit for the calibration curve of ethanol will still be incorporated; however, the curve will be forced through the origin (force method), and thus the y-intercept will always be zero. The curve will be further evaluated with controls bracketing the preexisting 0.150 grams % control. The additional controls will be at target ethanol concentrations of 0.05 grams% and 0.40 grams%, allowing for the appraisal of the regression fit for samples at higher and lower ethanol concentrations. The purpose of this validation is to demonstrate the linearity and accuracy of the force method for quantitating ethanol concentrations between 0.00 and 0.40 grams%.

Materials and Method

Control samples approximating 0.05, 0.15, and 0.40 grams% of ethanol were prepared by the addition of 5, 15, and 40 ml, respectively, of 10 grams% ethanol stock solution to a final volume of 100 ml with nanopure water. The concentrations of the controls were established by repetitive injections using the validation methodology. Duplicates of the controls were analyzed over a set of ten days, producing 20 replicates of each control.

For analysis, 50.0 ul of control was diluted with 1.00 ml of 0.01 grams% n-Propanol internal standard. The analysis was performed using a Perkin Elmer Clarus 500 Gas Chromatograph with an attached headspace sampler, and a flame ionization detector. TotalChrom Workstation Software computed the calibration curve, produced a calibration report, and quantitated the ethanol concentration of each control.

The method relates amount ratios with response ratios to compute the calibration curve. The amount ratio is the amount of component (ethanol) in the calibration standard divided by the amount of internal standard (n-Propanol) in the same sample. The response ratio is the area of the component peak divided by that of the internal standard peak. The amount and response ratios at each calibration level contribute a data point to the calibration curve of ethanol. The

data handling software computes the calibration curve and produces a report of calibration with the amount ratio as the x-coordinate (the independent variable) and the response ratio as the y-coordinate (the dependent variable).

The curve that was established is a first order polynomial (linear) curve specified by the equation: $y = c_0 + c_1x$, wherein c_0 is the y intercept and c_1 is the slope of the line. The validation method forces the curve through the origin, and thus refines the first order polynomial equation to $y = c_1x$.

Results

Twenty replicates for each control were reported to four decimal places. Table 1 details the results for each injection. The averages of the replicates were calculated and will be rounded to three decimal places to establish the final values. The spread of the results (standard deviation) and the reliability of the established values (95% confidence interval) were determined. Plots 1, 2, and 3 show the scatter plots of the replicates.

Table 1

Target	0.05 grams%	0.15 grams%	0.40 grams%
Results (grams%)	0.0476	0.1490	0.4011
	0.0476	0.1489	0.4001
	0.0479	0.1488	0.4016
	0.0479	0.1491	0.3999
	0.0478	0.1489	0.3994
	0.0477	0.1491	0.4029
	0.0477	0.1485	0.3983
	0.0475	0.1479	0.3985
	0.0476	0.1471	0.3985
	0.0480	0.1480	0.4020
	0.0480	0.1483	0.3998
	0.0476	0.1483	0.3984
	0.0477	0.1477	0.3980
	0.0472	0.1489	0.4001
	0.0472	0.1474	0.3996
	0.0473	0.1495	0.4009
	0.0472	0.1474	0.3987
	0.0475	0.1483	0.4008
	0.0473	0.1484	0.3973
	0.0475	0.1492	0.3989
Average	0.04759	0.148435	0.39974
Std Dev	0.000257314	0.000676893	0.001469121
Confidence Interval df = n-1 = 19 95% Confidence level t = 2.093	0.04747-0.04771	0.14812-0.14875	0.39905-0.40043

The new controls were compared against a linear curve established from NIST-traceable calibration standards. The NIST-traceable standards were at ethanol concentrations of 0.10, 0.20, and 0.30 grams %, the same target values as the secondary alcohol standards usually implemented in this methodology. Table 2 lists the results for the duplicate injections of each control compared to the established target values for the controls (average rounded to three decimal places). A range of +/- 0.01 grams% of the established value is also listed.

Table 2

Target: grams % (+/- 0.01 grams% range)	0.048 (0.038 - 0.058)	0.148 (0.138 - 0.158)	0.400 (0.390 - 0.410)
Results	0.048 0.048	0.149 0.151	0.406 0.404
Average	0.048	0.150	0.405
Std Dev	0	0.001414214	0.001414214

To evaluate the effect of forcing the curve through the origin (forced curve), replicates of the secondary alcohol standards were analyzed after the linear curve was established. Table 3 lists the results of the analysis of the secondary alcohol standards, rounded to three decimal places. The target values of the secondary alcohol standards are determined through a method of direct oxidation and are listed in Table 3, along with a range of +/- 5% of their values.

Table 3

Target: grams % (+/- 5% range)	0.099 (0.094 - 0.104)	0.196 (0.186 - 0.206)	0.300 (0.285 - 0.315)
Results	0.096 0.098 0.097 0.097 0.098 0.098 0.097 0.096 0.096 0.097	0.199 0.198 0.198 0.198 0.197 0.200 0.197 0.197 0.196 0.197	0.299 0.299 0.299 0.298 0.298 0.300 0.298 0.299 0.299 0.301
Average	0.097	0.198	0.299
Std Dev	0.000816497	0.001159502	0.000942809

NIST-traceable standards were evaluated against a forced curve established with the usual secondary alcohol standards. The NIST-traceable standards were at the following concentrations: 0.015, 0.05, 0.08, 0.10, 0.20, 0.30, and 0.40 grams%. To evaluate the effects of the forced curve on a sample containing a low-level of ethanol, replicates of the NIST-traceable 0.015 grams% standard were injected an additional twenty times. The results of all of these injections rounded to three decimal places are listed in Table 4.

Table 4

Target	0.015	0.05	0.08	0.10	0.20	0.30	0.40
Results	0.014	0.047	0.076	0.095	0.194	0.295	0.401
	0.014	0.047	0.077	0.096	0.194	0.294	0.397
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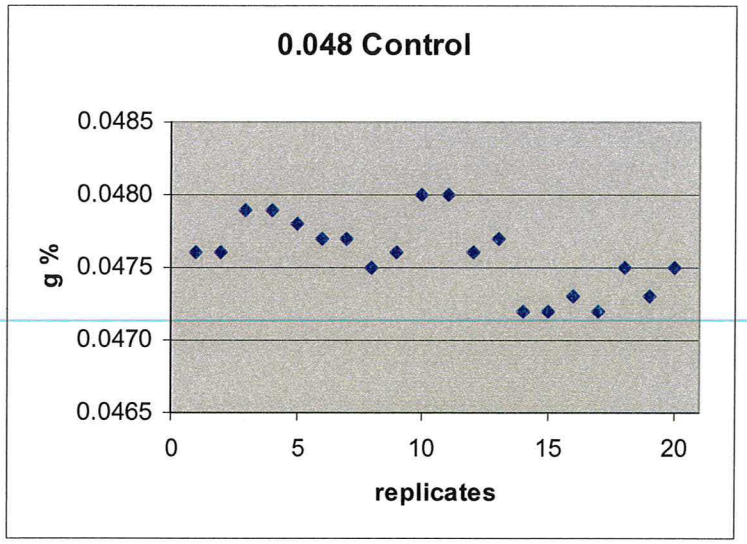
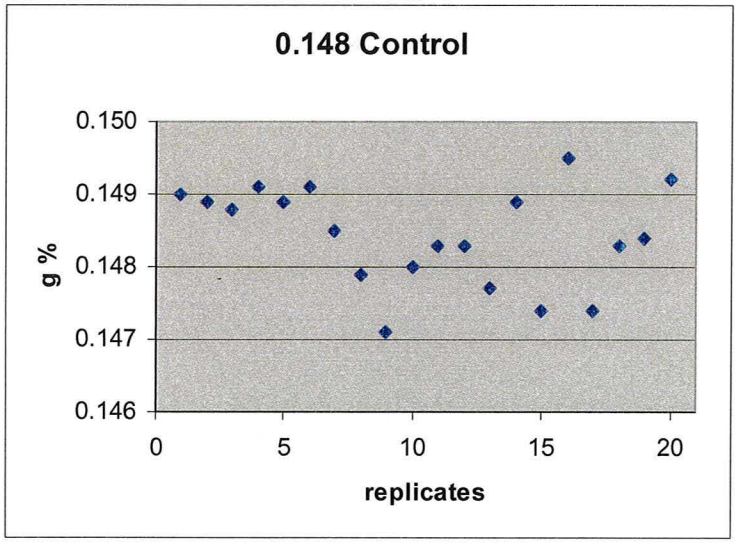
Average	0.0139	0.047	0.077	0.096	0.194	0.294	0.399
Std Dev	0.00047	0	0.00071	0.00071	0	0.00071	0.00283
% Difference Target - Average	8%	6%	4%	4%	3%	2%	<1%

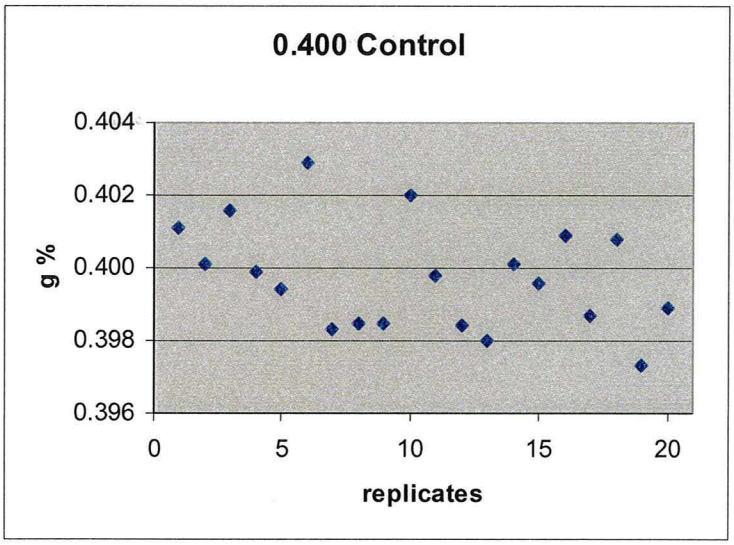
Plot 1



Plot 2



Plot 3



Conclusions

The results of replicate analyses were used to determine the values of the prepared 0.05, 0.150, and 0.400 grams% controls. San Diego Police Department Forensic Science Section standard methodology was used in the analyses of the controls, with the exception of a modification in the linear curve employing a forced origin (curve forced through the origin). The averages were rounded to three decimal places to establish that the control samples contain ethanol concentrations of 0.048, 0.148, and 0.400 grams%. The 95% confidence interval illustrates that the values of the controls are well within the established values.

The controls were analyzed against a linear curve forced through the origin employing NIST-traceable calibration standards. Article 6, section 1220.3 of Title 17 prescribes that an upper and lower limit of 0.01 grams% from the mean values be calculated from the established control values, and that acceptable variation in the method mandates the control values being within that range. Employing a NIST-traceable standard curve forced through the origin, the control samples produced results well within this prescribed acceptable range.

The modification of the 1st order polynomial curve to include the origin was assessed with re-injections of the secondary alcohol standards and with injections of NIST-traceable standards. The linear curve was derived from secondary alcohol standards, as per San Diego Police Department Forensic Science Section procedures. Article 6, section 1220.1 of Title 17 mandates that the method be capable of the analysis of a reference sample of known alcohol concentration within accuracy and precision limits of +/- 5% of the value, and that this limit is applied to alcohol concentrations which are 0.10 g% and higher. All analyses of the secondary alcohol standards fell well within this prescribed range. The NIST-traceable standards, except for the 0.015 and 0.05 grams%, fell within the prescribed range. The methodology produced accurate results and demonstrated that the accuracy increases as the ethanol concentration increases. The linear fit of the curve is more accurate for higher ethanol concentrations, with accuracy within 1% for the 0.400 grams% standard. The accuracy of a sample with low ethanol concentration approaching zero, as demonstrated by the analysis of the 0.015 grams% standard, was precise and accurate to within 10% of its value.

This validation demonstrates that the linear fit for the calibration curve which forces the regression through the origin (forced method) gives accurate and reproducible results for quantitating ethanol between 0.00 and 0.40 grams%. Since the accuracy of the force method was demonstrated up to the 0.40 grams% level, results surpassing this level will be reported as greater than 0.40 grams%. The use of controls at concentrations approximating 0.05, 0.150, and 0.40 grams % are effectively used to assess the response of samples to the linear fit of the curve. The three controls will be analyzed with each forensic run and their results will be evaluåted based on the requirements of Title 17. This requirement states that upper and lower limits of +/- 0.01 grams% are calculated around the values of the controls. Whenever the analysis of controls falls outside this acceptable range, the entire run will be regarded in error and the results will not be reported.

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Analyst _____ Date 8-14-07

Technical Review _____ Date 8-15-07

Administrative Review _____ Date 8-15-07

Quality Assurance Manager _____ Date 8-15-07