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In Vitro Accuracy and Precision Studies Comparing Direct and Delayed Analysis of the Ethanol Content of Vapor

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ABSTRACT: In vitro accuracy and precision studies were conducted using silica gel, magnesium perchlorate, and indium encapsulation breath collection tubes in conjunction with three infrared breath ethanol analyzers (BAC Verifier, Intoxilyzer 5000, and Intoximeter 3000), the Breathalyzer® 900A, and the GC Mark IV. Statistical analyses revealed good accuracy and precision and correlation between direct and delayed vapor ethanol analyses for each combination of instruments and collection devices (range = 0.000 to 0.250 g/210 L, $N = 42$/instrument, $r > 0.99$). Delayed vapor ethanol analysis utilizing each instrument and collection device combination appears to predict satisfactorily original vapor ethanol concentrations.

KEYWORDS: toxicology, breath-alcohol testing devices, alcohol, ethanol

The implication of the laws regarding the measurement of breath ethanol concentrations of accused drivers continues to increase in significance. Hence, methods of breath testing producing accurate, precise, and unchallengeable results for court are needed to be effective in the enforcement of "driving while intoxicated" laws. There have been a number of efforts to improve testing procedures. Among these, saving a sample of the ethanol content of breath, either from the actual breath sample previously analyzed or from a sample taken coincidentally with another testing procedure, has become an important consideration. The validity of the previous research on this subject has not been fully established since the work has often been conducted or influenced by instrument manufacturers. In addition, a number of the previous studies were limited in scope and comprehension.

Most of the newer advanced quantitative infrared (IR) breath ethanol analyzers, in addition to measuring breath ethanol, have been designed to collect vapor ethanol for delivery to various sorbents for subsequent delayed ethanol analysis. IR ethanol analysis is nondestructive, and therefore, preservation of the same breath sample used in the direct ethanol analysis may be possible. Numerous technical approaches have been attempted including: (1) employment of a sample collection cylinder equipped with a low speed piston to deliver vapor ethanol to a breath collection tube, (2) recirculation of vapor ethanol through the breath collection tube, and (3) use of a high speed pump to flush vapor ethanol out of the sample

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cell into a breath collection tube. Although, the investigation of vapor ethanol preservation employing these new IR breath ethanol analyzers (BAC Verifier, Intoxilyzer 5000, and Intoximeter 3000) with silica gel and magnesium perchlorate has not been very extensive, several studies using silica gel in combination with the CMI Intoxilyzer Models 4011A, 4011AS, and 4011AW have been reported [*1,2*].

This study presents in vitro accuracy and precision studies of the collection and delayed analysis of the ethanol content present in vapor ethanol samples using the BAC Verifier, Intoxilyzer 5000, Intoximeter 3000, and Breathalyzer® 900A in combination with silica gel or magnesium perchlorate or both. In addition, the indium encapsulation technique in conjunction with the GC Mark IV was evaluated.

Materials and Methods

Instruments

Before use in this study, all instruments were evaluated in terms of accuracy and precision using a protocol adapted from portions of the U.S. Department of Transportation's (DOT) "Standard for Devices to Measure Breath Alcohol" [*3*]. Results of these studies have been previously reported [*4,5*]. The following instruments were used to collect vapor ethanol samples:

(1) BAC Verifier (Verax Systems, Inc., Fairport, NY),
(2) Intoxilyzer 5000 (CMI, Inc./Federal Signal Corp., Chicago, IL),
(3) Intoximeter 3000 (Intoximeters, Inc., St. Louis, MO),
(4) Breathalyzer 900A (Smith and Wesson Company, G.O.E.C., Pittsburgh, PA), and
(5) GC Mark IV (Intoximeters, Inc., St. Louis, MO).

Smith and Wesson Mark IIA breath ethanol simulators (Smith and Wesson Company, G.O.E.C., Pittsburgh, PA) operated at 34 ± 0.2°C were used to deliver various vapor concentrations of ethanol. Simulators were arranged tandemly to prevent ethanol depletion during the testing sequences [*6*].

A Perkin-Elmer F-45 headspace analyzer (Perkin-Elmer Corp., Norwalk, CT) was used for all gas chromatographic (GC) analyses.

Preparation of Ethanol Solutions

A stock ethanol solution (60.50 g/L) was prepared by diluting 308 mL of absolute ethanol (U.S.P. 200 proof; Warner Graham Co., Cockeysville, MD) with sufficient distilled water to constitute 4 L. The concentration of the stock solution was confirmed by dichromate oxidation and headspace gas chromatography [*5*].

Using the stock solution, ethanol simulator solutions were prepared in 4-L quantities; simulator solutions at 34°C containing 0.605, 1.210, 1.815, and 3.025 g ethanol/L yield vapor ethanol effluents of 0.050, 0.100, 0.150, and 0.250 g/210 L, respectively [*7*]. Therefore, for each 0.010-g/210-L ethanol concentration desired, 8.0 mL of stock solution was used. The concentrations of the working simulator solutions were confirmed by headspace gas chromatography which used calibration standards analyzed against potassium dichromate.

Breath Collection Tubes

Silica gel breath collection tubes (ToxTrap; Lots 24, 26, and 28) were supplied by Federal Signal Corp. (Chicago, IL). Magnesium perchlorate breath collection tubes and indium encapsulation tubes were purchased from Intoximeters, Inc. (St. Louis, MO).

Collection Protocol

Using the BAC Verifier, Breathalyzer 900A, Intoxilyzer 5000, and Intoximeter 3000, vapor ethanol samples were collected onto silica gel. Vapor ethanol samples were preserved with magnesium perchlorate using the Intoximeter 3000. Samples prepared for the GC Mark IV were collected with an indium crimper unit (Intoximeters, Inc., St. Louis, MO). The following general testing sequence was employed:

1. A 500-mL aliquot of the appropriate ethanol solution was placed in each Mark IIA Simulator and allowed to reach operational temperature.
2. Before the commencement of testing, the simulators were vented into the atmosphere. A sample was then delivered into the instrument.
3. Four "blank" analyses were performed on each instrument.
4. Twelve consecutive tests were performed at each concentration on each instrument. The following concentrations were used: 0.050, 0.100, 0.150, and 0.250 g/210 L.
5. A maximum of 25 samples were delivered from any set of simulators.

Delayed Ethanol Analysis by Headspace Gas Chromatography

The ethanol content of the silica gel and magnesium perchlorate were determined within 24 h of collection with the following procedure:

1. The contents of the breath collection tubes ($N = 52$) were transferred into 22-mL gas chromatograph headspace vials.
2. To the silica gel, 1.00 mL of an internal standard/desorption solution (0.01% v/v *n*-propanol and 20% sodium chloride) was added. The magnesium perchlorate was mixed with 2.0 g of sodium chloride and dissolved in 3.00 mL of internal standard/desorption solution (0.0015% v/v *n*-propanol).
3. The headspace vials were immediately sealed, swirled gently, and incubated at 60°C for a minimum of 60 min. Thirty minutes before analysis, the vials were gently swirled again.
4. The samples were analyzed by automated headspace gas chromatography using the Perkin-Elmer F-45 (Perkin-Elmer, Norwalk, CT). The gas chromatograph was equipped with a 10-ft by 1/8-in. (3-m by 0.3-cm) stainless steel column packed with 0.2% Carbowax 1500 on 80-100 Carbopak C. Typical retention times for ethanol and *n*-propanol were 1.21 and 2.47 min, respectively.

Since the precise volume of vapor ethanol delivered to the breath collection devices was unknown, 2 samples were randomly selected from each of the 5 concentrations ("blank," 0.050, 0.100, 0.150, and 0.250 g/210 L) to serve as simultaneous calibrators. Linear regression analyses were performed and the best fitting straight line was applied to the data. This regression line was then used to predict the ethanol concentration of the 42 remaining samples. As a measure of precision, standard deviation was determined [*8*].

The ethanol content of the indium encapsulation tubes was analyzed in accordance with the GC Mark IV operator's manual. Before use, the GC Mark IV was calibrated with vapor ethanol delivered from simulator solutions.

Results

The correlation between direct vapor ethanol determinations and delayed vapor ethanol determinations of the same vapor ethanol sample using the various instruments and adsorption material combinations is summarized in Table 1. A linear relationship was evident. The average standard deviations for each instrument and device combination at vapor ethanol concentrations 0.050, 0.100, and 0.150 g/210 L were compiled and are summarized in Table

TABLE 1—*Linear regression analysis: correlation between direct vapor ethanol determinations and delayed vapor ethanol determinations.*[a]

Instrument	Collection Technique	Slope	*y*-Intercept	Correlation Coefficient	Coefficient of Determination
BAC Verifier	silica gel	0.999	−0.0001	0.998	0.996
Intoxilyzer 5000	silica gel	1.038	−0.0045	0.996	0.992
Intoximeter 3000	silica gel	1.006	+0.0011	0.999	0.998
Intoximeter 3000	magnesium perchlorate	0.963	+0.0074	0.995	0.990

[a]Linear regression analysis with total $N = 42$ at vapor ethanol concentrations 0.000, 0.050, 0.100, 0.150, and 0.250 g/210 L:

x = direct vapor ethanol determination (g/210 L) and
y = delayed vapor ethanol determination (g/210 L).

2. The data used to generate the results presented in Tables 1 and 2 are shown in detail in Tables 3 through 8.

No erroneous digital responses were observed during any testing series, including the analysis of vapor from "blank" simulator effluents. All test sequences were free from interruption and all instruments operated without failure.

Discussion

Utilizing a range of vapor ethanol concentrations, statistical analyses revealed good accuracy, precision, and correlation at each concentration for each instrument and collection device combination. The results of direct vapor ethanol determinations did not differ significantly from those for delayed ethanol determinations.

Although, the Breathalyzer was not designed specifically to collect vapor ethanol samples for preservation onto silica gel, its performance was exceptional. The average standard deviation at vapor ethanol concentrations 0.050, 0.100, and 0.150 g/210 L by direct analysis was 0.0014 g/210 L and by delayed analysis was 0.0016 g/210 L. The BAC Verifier, Intoxilyzer 5000, and Intoximeter 3000 in combination with silica gel adsorption were slightly less pre-

TABLE 2—*Delayed vapor ethanol analyses: average standard deviation.*

Instrument	Collection Technique	Average Standard Deviation at Vapor Ethanol Concentrations 0.050, 0.100, and 0.150 g/210 L (g/210 L)[a]	
		Direct Analysis	Delayed Analysis
BAC Verifier	silica gel	0.0013	0.0025
Breathalyzer 900A[b]	silica gel	0.0014	0.0016
Intoxilyzer 5000	silica gel	0.0014	0.0062
Intoximeter 3000	silica gel	0.0009	0.0026
Intoximeter 3000	magnesium perchlorate	0.0011	0.0046
GC Mark IV[b]	indium encapsulation	0.0008	0.0019

[a]Total $N = 30$, 10 at each concentration.
[b]Direct analysis was coincidental to delayed analysis.

TABLE 3—*Delayed vapor ethanol analyses: accuracy and precision using the BAC Verifier with silica gel.*[a]

Target Concentration	0.050	0.100	0.150	0.250
Number	10	10	10	10
Mean direct (S.D.)	0.050 (0.0012)	0.101 (0.0011)	0.151 (0.0014)	0.250 (0.0017)
Mean delayed (S.D.)	0.053 (0.0021)	0.097 (0.0032)	0.151 (0.0022)	0.251 (0.0079)
Range (direct)	0.049-0.053	0.099-0.102	0.149-0.153	0.248-0.254
Range (delayed)	0.049-0.057	0.091-0.100	0.148-0.154	0.235-0.261
Difference (direct-delayed) range	+0.001 to −0.008	+0.010 to −0.001	+0.003 to −0.004	+0.015 to −0.007

[a]All concentrations expressed in terms of g/210 L.

TABLE 4—*Delayed vapor ethanol analyses: accuracy and precision using the Breathalyzer 900A and silica gel.*[a]

Target Concentration	0.050	0.100	0.150	0.250
Number	10	10	10	10
Mean direct[b] (S.D.)	0.050 (0.0011)	0.100 (0.0013)	0.150 (0.0018)	0.247 (0.0029)
Mean delayed (S.D.)	0.052 (0.0008)	0.100 (0.0012)	0.145 (0.0028)	0.251 (0.0032)
Range (direct)	0.048-0.052	0.099-0.103	0.148-0.153	0.243-0.251
Range (delayed)	0.051-0.053	0.098-0.101	0.140-0.149	0.247-0.255
Difference (direct-delayed) range	−0.001 to −0.003	+0.002 to −0.001	+0.010 to +0.001	+0.003 to −0.005

[a]All concentrations expressed in terms of g/210 L.
[b]Direct analysis was coincidental to delayed analysis.

TABLE 5—*Delayed vapor ethanol analyses: accuracy and precision using the Intoxilyzer 5000 with silica gel.*[a]

Target Concentration	0.050	0.100	0.150	0.250
Number	10	10	10	10
Mean direct (S.D.)	0.050 (0.0016)	0.102 (0.0014)	0.152 (0.0012)	0.250 (0.0013)
Mean delayed (S.D.)	0.050 (0.0033)	0.099 (0.0061)	0.151 (0.0091)	0.258 (0.0097)
Range (direct)	0.047-0.051	0.100-0.104	0.150-0.154	0.248-0.252
Range (delayed)	0.046-0.056	0.090-0.109	0.140-0.162	0.244-0.278
Difference (direct-delayed) range	+0.005 to −0.007	+0.010 to −0.006	+0.010 to −0.009	+0.007 to −0.028

[a]All concentrations expressed in terms of g/210 L.

cise than the Breathalyzer (average standard deviation of delayed vapor ethanol analyses at 0.050, 0.100, and 0.150 g/210 L $\geq$ 0.0025 g/210 L).

Notable differences were found to exist between the magnesium perchlorate and silica gel vapor ethanol preservation techniques. Under identical experimental conditions using the same instrument and vapor ethanol solutions, the magnesium perchlorate technique was less precise (average standard deviation at vapor ethanol concentrations 0.050, 0.100, and 0.150

TABLE 6—*Delayed vapor ethanol analyses: accuracy and precision using the Intoximeter 3000 with silica gel.*[a]

Target Concentration	0.050	0.100	0.150	0.250
Number	10	10	10	10
Mean direct (S.D.)	0.050 (0.0010)	0.102 (0.0007)	0.149 (0.0010)	0.250 (0.0011)
Mean delayed (S.D.)	0.055 (0.0034)	0.101 (0.0020)	0.149 (0.0025)	0.254 (0.0039)
Range (direct)	0.048-0.051	0.100-0.103	0.147-0.150	0.248-0.252
Range (delayed)	0.053-0.055	0.098-0.105	0.145-0.152	0.247-0.262
Difference (direct-delayed) range	−0.002 to −0.005	+0.004 to −0.003	+0.004 to −0.002	+0.003 to −0.013

[a]All concentrations expressed in terms of g/210 L.

TABLE 7—*Delayed vapor ethanol analyses: accuracy and precision using the Intoximeter 3000 and magnesium perchlorate.*[a]

Target Concentration	0.050	0.100	0.150	0.250
Number	10	10	10	10
Mean direct (S.D.)	0.050 (0.0010)	0.101 (0.0011)	0.150 (0.0013)	0.249 (0.0016)
Mean delayed (S.D.)	0.054 (0.0023)	0.107 (0.0023)	0.152 (0.0091)	0.247 (0.0103)
Range (direct)	0.048-0.052	0.099-0.102	0.148-0.152	0.247-0.251
Range (delayed)	0.049-0.057	0.103-0.112	0.134-0.163	0.225-0.261
Difference (direct-delayed) range	+0.002 to −0.007	−0.002 to −0.011	+0.016 to −0.014	+0.026 to −0.008

[a]All concentrations expressed in terms of g/210 L.

TABLE 8—*Delayed vapor ethanol analyses: accuracy and precision using the GC Mark IV with indium encapsulation tubes.*[a]

Target Concentration	0.050	0.100	0.150	0.250
Number	10	10	10	10
Mean direct[b] (S.D.)	0.050 (0.0007)	0.101 (0.0008)	0.150 (0.0008)	0.252 (0.0011)
Mean delayed[c] (S.D.)	0.050 (0.0011)	0.100 (0.0016)	0.150 (0.0030)	0.248 (0.0040)
Range (direct)	0.048-0.051	0.100-0.102	0.149-0.151	0.250-0.254
Range (delayed)	0.048-0.051	0.097-0.101	0.144-0.153	0.242-0.253
Difference (direct-delayed) range	+0.002 to −0.001	+0.003 to −0.001	+0.006 to −0.003	+0.008 to −0.003

[a]All concentrations expressed in terms of g/210 L.
[b]Direct analysis was coincidental to delayed analysis.
[c]Average compiled from three analyses per indium tube.

g/210 L for direct versus delayed analysis: silica gel = 0.0009 versus 0.0026 g/210 L and magnesium perchlorate = 0.0011 versus 0.0046 g/210 L).

Based on personal experience in our laboratory, silica gel adsorption appears to be the preferred technique. In addition to optimization of accuracy, precision, and correlation, supply cost is low, several simple methods of analysis are possible, and silica gel collection

tubes are easily adaptable to the Breathalyzer and several IR instruments. In contrast, magnesium perchlorate collection tubes are more expensive, only available for use with the Intoximeter 3000, and more difficult to prepare for analysis. Finally, indium encapsulation tubes are very expensive, thus prohibiting regular use by most law enforcement agencies.

Conclusion

In conclusion, we have conducted an in vitro accuracy and precision study with commercially available equipment and materials. We, therefore, believe that delayed vapor ethanol analysis using each instrument and collection device combination described in this paper appears to predict satisfactorily original vapor ethanol concentrations. It is essential to note, however, that the research was conducted under relatively strict and ideal conditions, thus eliminating many sources of experimental error. Consequently, a lesser degree of accuracy and precision should be expected if these techniques are used in the field with multiple operators and instruments.

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