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Observations on ToxTrap Silica Gel Breath Capture Tubes for Alcohol Analysis

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ABSTRACT: Experimental studies were carried out to investigate the accuracy, precision, and reliability of ToxTrap silica gel tubes relative to the capture, from Intoxilyzers®, and subsequent analysis of alcohol derived from Simulator vapors or breath samples. Factors influencing analytical results, such as the presence of moisture in the tubes, were investigated. Comparisons were made between immediate, direct Intoxilyzer results and ToxTrap tube results obtained by a gas chromatographic technique.

KEYWORDS: criminalistics, breath-alcohol testing devices, alcohol, ToxTrap, Intoxilyzer®, silica gel, breath, capture

The idea of capturing breath or components from breath for storage and subsequent independent analysis has frequently been presented as an attractive idea for use in checking the accuracy of breath-alcohol analyses carried out by police using evidentiary breath test instruments such as the Breathalyzer® or Intoxilyzer®. Indeed, such a process has been adopted in some states, for example, Colorado and Arizona. Thus, the captured, stored samples tend to become regarded as referee samples—with their analysis then serving as referee analyses.

Fundamentally, a referee procedure should be more accurate, precise, and reliable than the procedure it is checking. If it is not equally or more reliable, precise, and accurate, then, if there is a discrepancy between the original analysis and the referee analysis, the discrepancy will more likely be due to a problem with the referee analysis than the original analysis when the original analysis was in fact conducted correctly.

One publication concerning the use of silica gel tubes indicated that good accuracy and precision were obtained when certain quality control steps were taken [*1*]. On the other hand, in view of previous difficulties encountered by workers investigating silica gel or other adsorbent systems, it was decided to carry out an experimental series using ToxTrap silica gel tubes to ascertain, further, the accuracy, precision, and reliability of such tubes.

Materials and Apparatus

Materials

The materials used were:

ToxTrap Tubes, Lots 9, 16, 21 (made by ToxTrap, Inc., Smyrna, DE);
ethanol, absolute, reagent grade, U.S. Industrial Chemicals Co.;

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1-propanol, reagent grade, Baker;
detergent cleaner, Isoterge®, with 1 : 1000 sodium azide preservative;
serum vials, 1 mL, Wheaton;
serum vial septa, Teflon®-lined, 11 mm, aluminum seal, Wheaton; and
volumetric glassware, Type A.

Apparatus

The apparatus used were:

Intoxilyzer, CMI Model 4011A and Omicron Model 4011AW;
Simulator, Mark II, Smith & Wesson;
gas chromatograph, Hewlett Packard Model 5710A;
auto sampler, Hewlett Packard Model 7672A;
sampler/event control module, Hewlett Packard Model 1900A;
Integretor, Hewlett Packard Model 3390A;
pipet, Finnpipette®, to 1 mL;
crimper, for 1-mL serum vials, Wheaton; and
balance, Mettler, Type H15.

Experimental Procedure

The Intoxilyzer Model 4011A instruments were modified temporarily to receive ToxTrap tubes by disconnecting the exhaust tubing at the end of the chamber and replacing it with short 6.35-mm (¼-in.) inside diameter Tygon tubing to protrude through the right side of the Intoxilyzer by approximately 51 mm (2 in.). The one-way value was included in-line inside the chassis.

Simulator solutions were prepared by making a stock solution of 60.8 mg of ethanol per millilitre of water by the addition of 77.0 mL of absolute alcohol as 60.80 g of alcohol by weight to water in a volumetric flask and diluting to 1 L. Appropriate Simulator solutions were then prepared by the addition of 10 mL of stock solution per 500 mL of total aqueous solution for each 0.10% simulated blood alcohol concentration desired [*2*]. A maximum of eight samples were delivered from the Simulator into the Intoxilyzer, after which a fresh Simulator solution was used.

ToxTrap samples were collected by running Simulator samples in the Intoxilyzer Calibrator mode, recording the Intoxilyzer reading, removing the Simulator and immediately connecting the pump hose to the breath hose, attaching the ToxTrap tube to the end of the exhaust tube, turning the selector switch to the Air Blank cycle to purge the Intoxilyzer chamber air through the ToxTrap tubes, and finally the ToxTrap tubes were removed and recapped. Breath samples from volunteer subjects were taken in the Breath mode.

The internal standard solution was prepared by the addition of 0.80 mL of 1-propanol and 5.0 mL of Isoterge to water and diluting to 1 L with deionized water. The resultant internal standard solution contained 0.64 mg of 1-propanol per millilitre of solution.

A calibration solution was prepared to contain 0.650 g of ethanol, 0.644 g of 1-propanol, and 5.00 mL of Isoterge per litre of aqueous solution. The Intoxilyzer Model 4011A chamber, breath hose, and exhaust tube capacity—to the one-way valve—was measured to be 721 cm^3 for the Intoxilyzer used in the bulk of the studies (Intoxilyzer 1). This volume—at 55°C Intoxilyzer temperature—corresponded to 675 cm^3 at 33.8°C, the mean temperature held by the Mark II Simulator employed in these studies. One millilitre of calibration solution therefore contained 0.650 mg of ethanol, which if contained in 675 mL of Simulator vapor at 33.8°C, represented a 0.206% (w/v) blood alcohol concentration. For this purpose the equation

$$y = 0.04145e^{0.06583x}$$

was employed [3], where y = partion coefficient of ethanol between air and water $\times 10^3$, e = natural logarithm base, and x = temperature in centigrade. Hypothetically, 675 cm^3 of Simulator vapor at 33.8°C will, at a 0.206% equivalent blood alcohol concentration, contain 0.650 mg of ethanol. A 100% capture of ethanol in a ToxTrap tube should, therefore, be represented by trapping this quantity of ethanol in the tube at a 0.206% blood alcohol concentration. For research purposes, this approach enabled a comparison to be made between results that should be obtained and results actually obtained—to provide some insight relative to physicochemical processes and equilibria which may be relevant. The calibration solution and internal standard solution each contained the same concentration of 1-propanol.

In preparation for gas chromatographic analysis, the silica gel contents of the tubes were transferred carefully to the serum vials, 1 mL of internal standard solution was added by means of the Finnpipette, and the vials were capped. The tubes were swirled between the fingers—without shaking—and, in most studies, set aside for equilibration to occur overnight at room temperature. The calibration standard vials were prepared by addition of 1 mL of calibration solution to the silica gel contents of unused ToxTrap tubes. They were prepared at the same time as the other vials.

Direct liquid injection gas chromatographic (GC) analyses were carried out using the auto sampler system, with calibration based on Vial 1—the calibration standard—corresponding to 0.206% blood alcohol.

The gas chromatograph conditions were as follows:

Column: 1.8-m (6 ft, 1/8-in.) stainless steel packed with Carbopak C, 60-80 mesh, containing 0.2% Carbowax 1500 (Supelco);
column temperature: 100°C;
injection port temperature: 200°C;
flame ionization detector (FID) temperature: 200°C;
N_2 and H_2 flow: 30 cm^3/min;
air flow: 240 cm^3/min;
HP 5710A GC attenuation: range 10 and attenuation 1024;
Model 3390A Integrator attenuation: 5; and
Model 7672A auto sampler: short stroke.

Results and Discussion

Using a "0.20%" Simulator solution, the mean ToxTrap value for 30 tubes of Lot 9 was found to be 0.161%, with a range from 0.141 to 0.188%. An average underestimation of 0.04% was shown. Attachment of a second Intoxilyzer to the exit end of several tubes, operated in the Breath mode and in series with Intoxilyzer 1, disclosed that some alcohol passed completely through the tubes and was not captured.

The silica gel contents of four tubes were found to average 202 mg, ranging from 190 to 212 mg. The overall results suggested that significant quantities of atmospheric water has been adsorbed—to decrease the capacity of the tubes to trap alcohol. Several tubes were therefore heat-desiccated in an oven at 135°C for 2 to 3 h following removal of the polyethelene caps and ring inserts so that they would not melt. The average silica gel weight of six tubes following heat desiccation was 171 mg, ranging from 162 to 179 mg.

Before heat desiccation, other untreated tubes had been tested using a "0.10%" Simulator solution, giving a mean ToxTrap result of 0.078% and ranging from 0.069 to 0.084%. In this case, however, loss of alcohol by passage through the tubes was not apparent by attachment of a second Intoxilyzer in series during the Air Blank purge cycle. It might be argued that one could simply introduce an arbitrary "correction" factor to bring the mean values found to correspond to the Simulator value, however, use of such an arbitrary factor does not have a place in good analytical methodology. Following the above results, the remaining Lot 9 ToxTrap tubes were heat-desiccated and subsequently stored in a desiccator.

Using a "0.20%" Simulator solution, a series of 20 desiccated ToxTrap tubes were tested, with a resultant mean value of 0.196% and ranging from 0.184 to 0.208%. The standard deviation was ±0.0048%. In all cases the results were based on the calibration solution described previously, indicating that virtually 100% alcohol recovery into the ToxTrap tubes was obtained in the latter series, as opposed to an apparent recovery of the order of 80% in the former series.

A "0.10%" Simulator solution was used to test another series of 20 desiccated tubes, with a resultant mean value of 0.101% and ranging from 0.095 to 0.106%, with a standard deviation of ±0.0026%. A "0.30%" Simulator solution produced, using 22 desiccated ToxTrap tubes, a mean value of 0.296% and a range from 0.280 to 0.307%, with a standard deviation of ±0.0070%.

The Intoxilyzer® mean values for the three Simulator solution concentrations were 0.103, 0.198, and 0.291%. The 0.10% Simulator solution had also been checked by an oxidation-reduction titration procedure using potassium dichromate as a primary standard and found to be 0.103% (w/v). Table 1 summarizes the ToxTrap results. Table 2 compares the standard deviations found for the Intoxilyzer and the desiccated ToxTrap tubes.

A comparision was made of ToxTrap Lot 9 (desiccated), Lot 16, and Lot 21, with results as noted in Tables 3 and 4. Lot 9 had been packaged in bulk in Ziplock® plastic bags while Lots 16 and 21 had been packaged individually in plastic heat-sealed pouches by the manufacturer.

The results shown in Table 3 demonstrated that no significant difference existed among the

TABLE 1—*Mean concentrations found, ranges, and standard deviation values for ToxTrap tubes.*

Simulater Value, %	Method	Number of Tubes	Mean Concentration, %	Range Found, %	Standard Deviation, %
0.20	ToxTrap, untreated	30	0.161	0.141-0.188	±0.0100
0.10	ToxTrap, untreated	15	0.078	0.069-0.084	±0.0051
0.10	ToxTrap, desiccated	20	0.101	0.095-0.106	±0.0026
0.20	ToxTrap, desiccated	20	0.196	0.184-0.208	±0.0048
0.30	ToxTrap, desiccated	22	0.296	0.280-0.307	±0.0070

TABLE 2—*Standard deviation comparison of the Intoxilyzer and desiccated ToxTrap tubes using Simulator solutions.*

Simulator Solution, %	Standard Deviation	
	Intoxilyzer, %	ToxTrap Tubes (Desiccated), %
0.10	±0.0012	±0.0026
0.20	±0.0019	±0.0048
0.30	±0.0031	±0.0070

TABLE 3—*ToxTrap lot comparison relative to use of calibration solution, with calibration based on Lot 9 (desiccated).*

Calibration Solution, %	Lot No.	GC Result, %
0.206	9 (desiccated)	0.207
0.206	16	0.205
0.206	21	0.206

TABLE 4—*ToxTrap lot comparison studies using "0.20%" Simulator.*

Lot No.	Intoxilyzer Result, %	ToxTrap GC Result, %
9 (desiccated)	.200	.199
9 (desiccated)	.200	.193
9 (desiccated)	.198	.201
9 (desiccated)	.197	.196
9 (desiccated)	.196	.196
	mean .198	mean .197
	std. dev. ±.0018	std dev. ±.0031
16	.198	.174
16	.196	.190
16	.196	.151
16	.195	.180
16	.196	.170
	mean .196	mean .173
	std. dev. ±.0011	std dev. ±.014
21	.200	.200
21	.200	.201
21	.198	.195
21	.200	.196
21	.197	.191
	mean .199	mean .197
	std. dev. ±.0014	std dev. ±.0041

lot numbers relative to the calibration solution and the steady state equilibria attained—in this case after overnight standing at room temperature. Table 4 results show that while Lot 21 tubes behaved similarly to the desiccated Lot 9 tubes, the Lot 16 tubes behaved similarly to the Lot 9 untreated tubes, giving low results and a large standard deviation.

Table 5 lists ToxTrap desiccated tube results relative to Intoxilyzer results for four volunteer drinking subjects. The Intoxilyzer used for these tests was a different CMI Model 4011A in which two consecutive Air Blank purge cycles were essential to purge the chamber assembly free of sample. Therefore, double purges were carried out routinely in tests conducted with this instrument (Intoxilyzer 3). It was found, further, that Intoxilyzer 2, an Omicron Model 4011AW, also required two purge cycles to clear the chamber completely. Incomplete Air Blank purges will affect, not only the silica gel tube result, but also the next Intoxilyzer test in that in preparation for the next test the zero setting will be made with some alcohol in the chamber. Consequently, the next Intoxilyzer result will be low—measuring only the difference between the alcohol concentration in the chamber at the end of the test and the beginning of the test.

Five Intoxilyzers were used in these studies. The ToxTrap results did display an instrumental variation. Tubes from Intoxilyzer 2 (an Omicron model) averaged 4% higher results than tubes from Instrument 1 (Model 4011A). Instrument 3 was similar to 1 in ToxTrap results while

TABLE 5—*Intoxilyzer and ToxTrap results from volunteer drinking subjects.*

Subject No.	Intoxilyzer Result, %	ToxTrap (Desiccated) GC Result, %
1	0.096	0.095
2	0.090	0.085
3	0.092	0.096
4	0.063	0.067

Instruments 4 and 5 (both Model 4011A) gave ToxTrap results 5 and 6%, respectively, lower than Instrument 1.

The GC auto sampler system injects syringe samples which are reasonably constant in size. To examine ethanol and internal standard (1-propanol) equilibria in the water/silica gel phases in the sealed serum vials used for GC analysis, mean integrator counts were used. First, the ethanol and 1-propanol peak counts were taken for the calibration solution alone and not in the presence of silica gel—representing 0.206% ethanol concentration. One half of that ethanol count was taken to represent a 0.103% ethanol concentration, all in the water phase of this one-phase system. The 0.103% Simulator solution was used for capture of ethanol in desiccated ToxTrap tubes. The silica gel content in the tubes was estimated to weigh approximately 170 mg. In other tubes the silica gel content was doubled before alcohol capture. In this manner, the absolute peak counts as well as the ethanol to 1-propanol count ratios could be studied. The purpose of doubling the amount of silica gel in some tubes was to ensure that the influence of the presence of increased quantities of silica gel would be unambiguous and seen readily.

Based on total counts and count ratios, it was calculated that, after a 2-h extraction at 45°C in the presence of 170 mg of silica gel, the ethanol distribution was 86:14 in the water:silica gel phases, respectively. The 1-propanol distribution was 79:21. At the same time, in the presence of 340 mg of silica gel, the ethanol distribution was 84:16 while the 1-propanol distribution was 59:41. The effect of decreasing the amount of 1-propanol significantly in the water phase—as a result of an increased amount of silica gel being present to adsorb more propanol—was to increase the apparent amount of ethanol present. This was due to the increased ethanol to 1-propanol ratio in the water phase. Indeed, 0.138% ethanol was reported—versus a 0.10% correct value. Later, after overnight standing, the vials were reanalyzed. In the 170-mg silica gel vial the ethanol distribution was 84:16 (water:silica gel) while the 1-propanol distribution was 80:20. The small shift found indicated that a steady state equilibrium had almost been reached following a 2-h incubation period at 45°C. In the presence of 340 mg of silica gel, however, a considerable shift occurred during the overnight standing. The ethanol distribution had shifted to 77:23 (from 84:16 after 2-½ h) while the 1-propanol distribution had shifted to 65:35 (from 59:41). This shift changed the apparent ethanol concentration from 0.138% the previous afternoon to 0.116% the following morning.

A probable explanation of these observations lies in the fact that most of the captured ethanol will be adsorbed at the front end of the silica gel tube—thereby being concentrated on a small proportion of the silica gel. Then, on addition of the aqueous internal standard-containing solution, free adsorption sites will take up a considerable amount of 1-propanol (and water). Also, considerable desorption of ethanol into the water is expected to occur. Next, on continued standing, a steady state equilibrium will be reached after exchange of some ethanol and propanol (and water) to and from silica gel sites until their concentrations are equalized across the gel surfaces. The results have shown that the quantity of silica gel present affects the ethanol/1-propanol equilibrium in solution and the greater the quantity of silica gel present, the longer it takes for a steady state equilibrium to become established during the desorption stage.

Much manipulation is involved in the analysis of the silica gel samples. Each manipulative step contains potential sources of error. For reasonably accurate and precise results to be expected it is of importance that manipulative errors be avoided, for example, the addition of the wrong quantity of internal standard solution, and in addition, that errors caused by inadequate quality of the silica gel, for example, presence of moisture, be avoided. The following list itemizes sources of error to be avoided.

1. The silica gel must be free from atmospheric moisture. Silica gel is very hygroscopic. If moisture is present the trapping efficiency may decrease markedly.
2. No leaks must exist during the purging step from the Intoxilyzer through the silica gel tube.
3. The Intoxilyzer Air Blank purge cycle must be capable of exhausting the chamber completely.

4. The silica gel tubes must be properly packed to avoid channeling effects.
5. The tubes should be stored so that there is no alcohol loss before analysis. High temperatures should be avoided.
6. No loss of silica gel must occur during the transfer of silica gel from the tubes to the vials used for GC analysis. The transfer must be complete.
7. The quantity of internal standard-containing solution must be measured accurately for addition to the vials.
8. The calibration standard must be prepared and dispensed accurately.
9. The silica gel weight in the tubes and the mesh size should have good quality control.
10. Sufficient time must be given to permit extraction/equilibration of alcohol and internal standard in the vials during the desorption stage.
11. The vial caps (septa) should be inert and impermeable to alcohol, for example, Teflon-lined, to avoid alcohol loss.
12. The calibration standard, if used, should be mixed with the same type and amount of silica gel as the other samples. It is possible that alcohol and internal standard equilibration between the water and silica phases may vary among different silica gels.
13. The analytical procedure and calculations must be free of mathematical error.
14. The Intoxilyzer chambers and Intoxilyzer breath hose volumes may vary. Therefore silica gel tube results may vary from one instrument to another. Appropriate adjustment factors for some instruments at variance may be calculated.
15. The Intoxilyzer chamber temperature may vary somewhat from one instrument to another, thereby affecting the breath volume analyzed. There should be recognition of this factor.
16. If a headspace GC method is used close attention is necessary with respect to: (a) bath temperature and (b) syringe temperature.

Errors relative to Points 1, 2, 4, 5, 6, 7, 8, and 16 may not be detectable during or after analyses have been completed. After an error has been made there is often no means of checking it. In general, errors may be divided into two classes: (a) indeterminate errors and (b) determinate errors. Indeterminate errors are random and originate in the limited ability of the analyst to control all the variables affecting the measurements [*4*]. The other class of error, determinate error, encompasses systematic errors or constant errors rather than random ones. Determinate errors are numerous and include [*4*]:

(1) instrumental errors and those caused by apparatus and reagents,
(2) operative errors—generally associated with the manipulations of an analysis,
(3) personal errors—inability to make certain observations accurately or prejudice, and
(4) errors of method—have their origin in the chemical or physiochemical properties of the analytical system.

When these various sources of error are considered in the context of Intoxilyzer tests of breath for alcohol content and the capture, storage, and analysis of alcohol in silica gel tubes, it becomes evident that the possibility of one or more errors are inherently greater with the silica gel tubes than the Intoxilyzer. Moreover, if the Intoxilyzer or similar type of instrument is operated in accordance with instructions—especially under regulations such as those provided under Title 17 of the California Administrative Code—any errors of operation should be detectable. That is not to say that accurate results cannot be obtained with silica gel tubes because reasonably good accuracy and precision were obtained in the course of these experiments but only when all aspects of the quality of the tubes, the capture process, and the analyses were controlled fully—and all in one place. Also, in an experimental series using samples of known concentration, errors are normally detected because results are different from the results expected. If a laboratory is in receipt of one or two silica gel tubes and lacks other inormation, the possibility of producing erroneous results is very real. The weight that can be accorded such results must be considered in the light of the limitations of the method.

Conclusions

1. Under optimal conditions, the precision of the Intoxilyzer results was somewhat better than twice as good as the precision of the ToxTrap tube results. For example, at a "0.20%" Simulator alcohol concentration the Intoxilyzer standard deviation was ±0.0019% while the ToxTrap standard deviation was ±0.0048%.
2. The presence of moisture in silica gel tubes decreases the accuracy and precision of results markedly.
3. The reliability of silica gel tube results is less than that of the Intoxilyzer or similar type of instrument—the former being subject to a large variety of error sources, of which a number are not detectable.

Acknowledgment

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