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The Trapping, Storing, and Subsequent Analysis of Ethanol in In-Vitro Samples Previously Analyzed by a Nondestructive Technique

REFERENCE: Wilkinson, D. R., Sockrider, D. W., Bartsch, C. L., Kataoka, Y. G., and Zettle, J. R., **"The Trapping, Storing, and Subsequent Analysis of Ethanol in In-Vitro Samples Previously Analyzed by a Nondestructive Technique,"** *Journal of Forensic Sciences,* JFSCA, Vol. 26, No. 4, Oct. 1981, pp. 671-677.

ABSTRACT: There is a need for a simple technique to collect breath samples of persons suspected of driving under the influence of alcohol. Solutions containing ethanol were analyzed using dichromate oxidation procedures. The standard solutions were placed in a breath alcohol simulator at 34°C and the vapors analyzed with a CMI Intoxilyzer, Model 4011AS, with one-way valves placed at either end to prevent air entering the outlet or leaving through the inlet. The analyzed 715-mL vapor sample was then pumped through an activated silica gel column. The trapped alcohol was removed from the column with water, and the resulting solution was analyzed by dichromate oxidation, liquid injection, and headspace gas chromatographic procedures. A very good linear relationship between concentration and peak height ratio was obtained by gas chromatography. The slope of the graph was used to calculate the percentage of blood alcohol for breath samples previously analyzed by the Intoxilyzer. The average deviation from the correct alcohol value was ±5%. Samples were collected, stored, and analyzed after 15, 90, and 120 days with no apparent loss of alcohol. The three methods of analyzing the trapped alcohol were compared. Over 100 trapped samples were collected in the field and analyzed, and the laboratory analyses were compared with the breath analyzer printouts.

KEYWORDS: pathology and biology, alcohol, breath-alcohol testing devices, trapped breath samples, nondestructive tests, gas chromatography

Several states have passed legislation requiring the collection of breath samples for future analysis. Legislation of this type has made the operation of breath alcohol testing programs very difficult. The collection of breath samples has been rather difficult to achieve from a practical, inexpensive viewpoint.

The Delaware State Police Laboratory, Delaware State College, and the Colorado State Health Laboratory agreed to test the concept of trapping the alcohol in vapor samples that had already been analyzed by a nondestructive technique. The approach used was to take the basic concepts of the SM-7 Mobat System [*1*], namely, trapping 2100 mL of deep lung air and exhausting the trapped breath through a silica gel column, and to apply this system

Received for publication 29 Sept. 1980; revised manuscript received 24 Feb. 1981; accepted for publication 9 April 1981.

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to a CMI Intoxilyzer. The Intoxilyzer is a nondestructive, infrared analyzer that traps approximately 715 mL of vapor in its cell. This represents approximately one third of the volume trapped in the SM-7 Mobat bags.

This paper represents a system to collect and store the actual vapor sample that has previously been analyzed by a nondestructive technique. The paper also evaluates various techniques used in analyzing the trapped alcohol samples.

Procedure

The Intoxilyzer is a nondestructive infrared analyzer [*2*]. Because of its numerous beam reflections, the instrument offers great sensitivity. One-way valves were placed in the sample inlet hose as well as in the sample outlet hose. The total volume of air trapped between the valves was 715 mL. After the sample was analyzed, an activated SM-7 silica gel column (Luckey Laboratories, San Bernadino, Calif.) was placed on the outlet hose and the pump was used to exhaust the system. The silica gel was carefully removed from the column and transferred into a 4-mL vial containing 1 mL of water when analyzed by dichromate oxidation procedures. The resulting mixture was mixed, allowed to sit for 1 h, mixed again, and analyzed by one of several analytical procedures including gas chromatography, dichromate oxidation, and spectrophotometry.

Experimental Work

A study was made of the release of ethanol from silica gel at room temperature as a function of time. The analysis was performed by gas chromatography. Figure 1 shows that ethanol was released not instantaneously but, rather, over a 30- to 40-min period. It was decided to allow the silica gel to remain in the water solution for at least 1 h. Figure 2 indicates that good linearity was obtained in the 0.05 to 0.20% ethanol range studied.

Four alcohol solutions were standardized with the dichromate oxidation procedures. The four solutions were placed in Mark IIA Simulators and heated to 34°C. By the use of the Intoxilyzer's pumping system, the headspace contents of each of ten replicate determinations of each of four standards were pumped into the Intoxilyzer, analyzed, and collected on silica gel columns, and the solutions were analyzed by gas chromatography. The results of these analyses are shown in Table 1. There was good agreement in the results obtained by the three procedures. The standard deviation ranged from 0.002 to 0.006. The reproducibility was quite good, being less than 10% average deviation from the mean. The procedure was repeated and the final solutions were analyzed by headspace procedures [*3*]. The results of these analyses are shown in Table 2. In this case, the solutions were first standardized to two significant figures with an Intoximeter. The results of the Intoximeter, the Intoxilyzer, and the gas chromatographic analysis were again in good agreement, differing by no more than 0.01%. The standard deviations were low and constant for the gas chromatographic procedure. The average deviation from the mean was less than 5% in all cases.

As shown in Tables 3 and 4, similar results were obtained when the final analyses of solutions without an internal standard were performed by oxidation-titration and spectrophotometric procedures. Again, the Intoxilyzer, titration, and spectrophotometric results were in good agreement, being within $\pm 0.01\%$ of the known standard. However, a much larger standard deviation was observed in the spectrophotometric analyses as well as a rather large average percentage of deviation from the mean in the lower concentration range. Blanks were run with all analytical procedures. In each case the blanks showed 0.00% alcohol, and a blank correction was not necessary.

Solutions of various ethanol concentrations were prepared and placed in simulators, and their headspace contents were pumped into an Intoxilyzer, analyzed, and collected on silica gel. Ten samples were collected by this procedure for each method of analysis used. A total

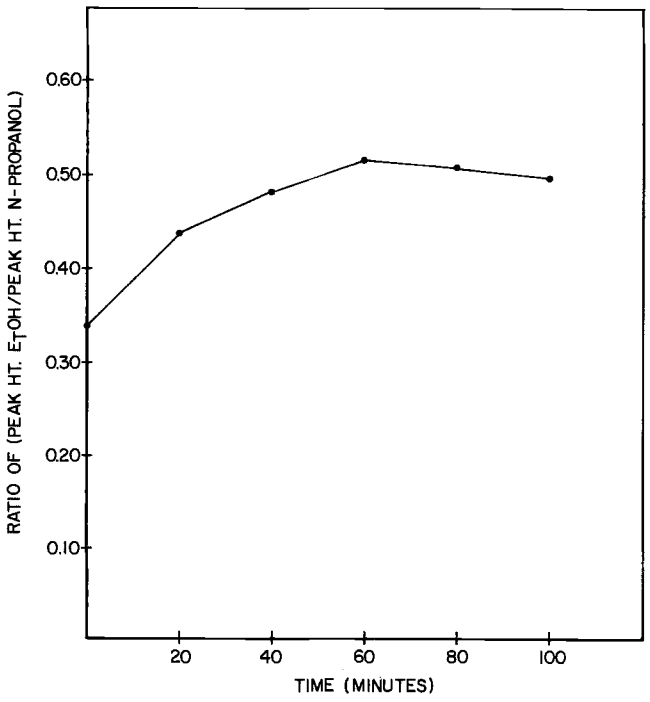

FIG. 1—*Release of ethanol from silica gel column.*

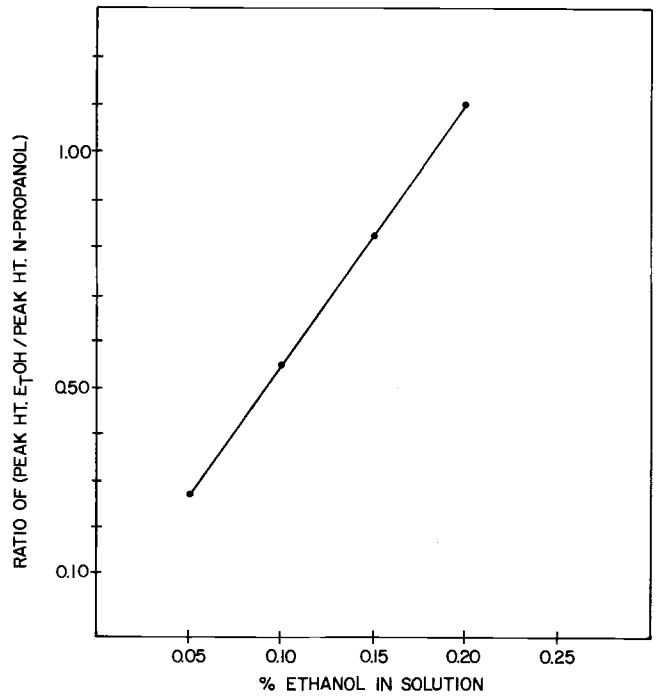

FIG. 2—*Linearity of response—peak height versus concentration.*

TABLE 1—*Analysis of standard solutions; gas chromatographic (GC) direct injection (ten samples each concentration).*

Standard Concentration, % w/v (Dichromate)	Concentration, Mean % w/v (Intoxilyzer)	Concentration, Mean % w/v (GC)	Standard Deviation	Average % Deviation from Mean	Range (% w/v) of Values from GC
0.050	0.048	0.048	0.006	8.91	0.032–0.052
0.105	0.096	0.104	0.002	0.90	0.095–0.108
0.155	0.154	0.159	0.002	3.31	0.150–0.159
0.200	0.196	0.195	0.004	4.48	0.188–0.202

TABLE 2—*Analysis of standard solutions: gas chromatographic (GC) headspace injection (ten samples of each concentration).*

Standard Concentration, % w/v (Standard Solution)	Concentration, Mean % w/v (Intoxilyzer)	Concentration, Mean % w/v (GC)	Standard Deviation	Average % Deviation from Mean	Range (% w/v) of Values from GC
0.08	0.078	0.078	0.002	4.09	0.073–0.084
0.10	0.099	0.098	0.002	3.59	0.095–0.101
0.16	0.162	0.160	0.002	2.75	0.157–0.166
0.20	0.198	0.194	0.002	2.58	0.190–0.199

TABLE 3—*Analysis of standard solutions; dichromate oxidation followed by titration (ten samples per concentration).*

Standard Concentration, % w/v (Dichromate)	Concentration, Mean % w/v (Intoxilyzer)	Concentration, Mean % w/v (Titration)	Standard Deviation	Average % Deviation from Mean	Range (% w/v) of Values from Gas Chromatography
0.060	0.059	0.055	0.007	8.33	0.048–0.062
0.098	0.095	0.097	0.001	1.02	0.093–0.097
0.148	0.152	0.145	0.007	3.38	0.149–0.154
0.258	0.258	0.254	0.008	2.32	0.255–0.260

TABLE 4—*Analysis of standard solutions; spectrophotometric determinations (ten samples per concentration).*

Standard Concentration, % w/v (Dichromate)	Concentration, Mean % w/v (Intoxilyzer)	Concentration, Mean % w/v (Spectrophotometer)	Standard Deviation	Average % Deviation from Mean	Range (% w/v) of Values from Gas Chromatography
0.060	0.059	0.056	0.009	11.67	0.043–0.062
0.098	0.095	0.088	0.002	10.20	0.082–0.098
0.148	0.152	0.156	0.001	5.40	0.146–0.159
0.258	0.258	0.264	0.007	5.81	0.243–0.259

of 40 samples were collected. These were grouped in four sets, each labeled "Unknown Sample 1" through "Unknown Sample 10." The samples were sealed and stored in a freezer at −18°C until analyzed. These "unknown" samples were analyzed by laboratory personnel who did not know the alcohol content of the columns. The samples were put into solution and analyzed by liquid and headspace injections into a gas chromatograph, as well as by oxidation-titration and spectrophotometric procedures. The data from these determinations are shown in Tables 5, 6, 7, and 8. The results were truncated. The liquid injection samples did not deviate by more than ±0.01%, with a few notable exceptions designated by asterisks in Table 6. It is believed that these samples were incorrectly collected in the field. At this time there is no explanation for these high results.

The stability of the trapped alcohol was studied at 0, 15, 90, and 120 days with 0.10 and 0.20% alcohol solutions. The samples were stored in a freezer at −18°C and then analyzed by gas chromatographic procedures using both headspace and direct injections. Table 9 shows the percentage recovery after these time periods. The average recovery of all samples stored longer than 30 days was 98%. More than 142 samples collected from detained subjects were stored at room temperature (20 to 25°C) for up to 381 days and analyzed by gas chromatography. The average recovery for these samples was approximately 100%. The amount of alcohol on silica gel columns was calculated and compared to the experimental

TABLE 5—*Determination of unknown samples by gas chromatographic, liquid injections (five injections per sample).*

Sample	Silica Gel Average	Intoxilyzer Average	Deviation
1	0.11	0.12	−0.01
2	0.13	0.12	+0.01
3	0.12	0.12	0.00
4	0.10	0.10	0.00
5	0.10	0.09	+0.01
6	0.07	0.06	+0.01
7	0.06	0.06	0.00
8	0.06	0.06	0.00
9	0.06	0.06	0.00
10	0.06	0.06	0.00

TABLE 6—*Determination of unknown samples by gas chromatographic headspace injections (five injections per sample).*

Sample	Silica Gel Average	Intoxilyzer Average	Deviation
1	0.20	0.21	−0.01
2	0.14	0.15	−0.01
3	0.16	0.16	0.00
4	0.14	0.15	−0.01
5	0.19	0.16	+0.03*
6	0.15	0.16	−0.01
7	0.27	0.27	0.00
8	0.24	0.16	+0.08*
9	0.15	0.15	0.00
10	0.15	0.17	−0.02

*See text for explanation.

TABLE 7—*Determination of unknown samples by dichromate oxidation (five injections per sample).*

Sample	Silica Gel Average	Intoxilyzer Average	Deviation
1	0.14	0.15	−0.01
2	0.15	0.15	0.00
3	0.09	0.09	0.00
4	0.26	0.25	+0.01
5	0.20	0.20	0.00
6	0.06	0.06	0.00

TABLE 8—*Determination of unknown samples by spectrophotometric analysis (five injections per sample).*

Sample	Silica Gel Average	Intoxilyzer Average	Deviation
1	0.15	0.15	0.00
2	0.15	0.15	0.00
3	0.08	0.09	−0.01
4	0.26	0.25	+0.01
5	0.19	0.20	−0.01
6	0.06	0.06	0.00

TABLE 9—*Recovery of alcohol (%) after storage of standard solutions (five injections per value).*[a]

Average Intoxilyzer Value (% Ethanol) at Time of Collection	Average Gas Chromatography Value (% Ethanol at Time T_1	Time Sample Was Stored on Silica Gel Column (T_1), days	% Recovery
0.100	0.100	0	100
0.100	0.100	15	100
0.100	0.100	90	99
0.100	0.099	120	99
0.200	0.200	0	100
0.200	0.195	15	98
0.200	0.205	90	100
0.200	0.194	120	97

[a]98% average recovery on all samples stored over 30 days.

value. The results indicated that 99.4% of the alcohol on the columns was removed by the recommended procedures.

Conclusions and Discussion

Samples analyzed by a nondestructive, infrared analyzer can be collected, stored more than 100 days, and analyzed in the laboratory with a 98% recovery. All of the alcohol on the

silica gel column can be removed, provided sufficient time is allowed for the silica gel to remain in contact with the water. Not only has the exact, previously analyzed sample been collected and stored on an inexpensive (75¢) silica gel column, but the resulting solution can be analyzed by one of several readily available analytical procedures including gas chromatography (liquid and headspace injections), dichromate oxidation-titration analysis, and spectrophotometric determinations.

We have, then, an inexpensive, versatile, and dependable method for collecting and storing breath alcohol samples for possible later analysis.

It is recommended that 0.66 mL of 0.20% *l*-propanol internal standard solution be added to the silica gel sample containing the trapped alcohol, be allowed to sit at room temperature for approximately 1 h, and then be analyzed by one of the previously mentioned techniques.

Many of the reported problems of poor reproducibility with silica gel columns have been caused by the 40-min delay in the complete release of ethanol. It is strongly recommended that analysts using this technique allow the silica gel to remain in the *l*-propanol solution at room temperature at least 1 h.

Addendum

After the completion of this work, a new batch of silica gel columns was ordered. These columns contained a greater weight of silica gel (30% more) and a larger particle size than the previous columns. The results were completely unacceptable when aqueous standards were used. Only when standards were run through a simulator and the trapped headspace contents were analyzed were the results reasonably consistent with the Intoxilyzer readings. Other researchers, including the Arizona Department of Health, reported a similar problem, namely, an inconsistent release of ethanol. The distributor of the tubes is aware of the problems and is attempting to correct them. Better quality control and batch or lot numbers are required. The Colorado and Delaware Laboratories are presently testing new batches of tubes that match the tubes tested in the past. If a good quality control program cannot be developed by the manufacturer of the tubes, an alternate column absorbent must be found. Several researchers, including Kurt Dubowski, have been working in this area.

For further information, please see page 864 of this issue.

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