Measurement of Ethanol in Gaseous Breath Using a Miniature Gas Chromatograph

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Abstract

We designed and built a novel, miniature gas chromatograph (mGC) to use exhaled breath to estimate blood ethanol concentrations that may offer GC quality sensitivity and specificity, but with portability, reduced size, and decreased cost. We hypothesized that the mGC would accurately estimate the serum ethanol concentration using exhaled breath. Human subjects (n = 8) were dosed with ethanol employing the Widmark criteria, targeting a blood concentration of 0.08 g/dL. Serum and breath samples were collected concurrently over an hour. Ethanol concentrations in serum were measured using a CLIA-approved laboratory. Ethanol concentrations in conventional breath were assayed using a calibrated mGC or Intoxilyzer 400PA. Data were analyzed using Bland-Altman analysis using serum concentrations as a “gold standard”. For the mGC, the regression line (correlation coefficient), bias, and 95% limits of agreement were y = 1.013x – 0.031 (r = 0.91), –0.008 g/dL, and –0.031 to 0.016 g/dL, respectively, for 30 specimens. For the Intoxilyzer 400PA, the regression line (correlation coefficient), bias, and 95% limits of agreement were y = 0.599x + 0.008 (r = 0.86), 0.024 g/dL, and –0.049 to 0.002 g/dL, respectively, for 71 specimens with a large magnitude effect. We concluded that the mGC, using exhaled breath, performed well to estimate the serum ethanol concentrations.

Introduction

Alcohol1 abuse endures as a major public health problem with important repercussions for individuals, the health care system, and society in general. Current devices used to measure ethanol in human breath rely on technologies [e.g., infrared (1), fuel cell (2)] that were deployed several decades ago. A novel, breath-based toxicological tool enabled by modern technology with superior capabilities would be a welcome, additional tool to help address alcohol abuse by clinical, research, and law enforcement personnel. To meet this need, we designed and built a new generation of a breath-based ethanol detection device based on miniature gas chromatography (mGC) with a metal oxide sensor for real-time, point-of-need detection of ethanol concentrations. The fundamental improvements that enabled traditional gas chromatography (GC) to be reduced from a laboratory bench top to a handheld analytical instrument are not only miniaturization of parts, but also use of scrubbed, ambient air as a carrier gas. The employment of ambient air as a carrier gas affords key advantages such as avoidance of compressed gas cylinders, portable instrumentation, reduced size, and decreased cost. In addition, the mGC has substantially greater potential to not only discriminate between ethanol and competing interferants, but also to ascertain the identity and concentrations of these interferants if desired. Therefore, the primary purpose of this investigation was to study the utility of the mGC to measure ethanol concentrations in exhaled human breath.

Many years ago, GC was used for the determination of the alcohol content of breath (3). The Gas Chromatograph Intoximeter Mark II and IV (Intoximeters, St. Louis, MO) utilized a Porapak Q column and a flame-ionization detector interfaced to a compressed fuel/carrier gas consisting of hydrogen and nitrogen (4,5). The Alco-Analyzer Gas Chromatograph (Luckey Laboratories, San Bernardino, CA) utilized a Porapak S column with a thermal conductivity detector interfaced to a compressed carrier gas consisting of helium (6). Although both instruments were approved by the National Highway Traffic Safety Administration as evidential breath testing devices, neither gained wide acceptance. The present investigators believe that the mGC technology may offer some advantages compared to these previous GC devices, such as use of...
scrubbed room air as a carrier gas instead of coupling the GC to a helium cylinder. Therefore, to evaluate the performance characteristics of the mGC, we measured the ethanol concentrations in time-matched serum and breath (using the mGC and conventional a conventional Intoxilyzer 400PA unit) from healthy volunteer subjects who orally consumed ethanol in a controlled environment.

Experimental

mGC general description

The breath ethanol analyzer is based on a novel mGC sensor that allows highly selective detection of ethanol in spite of the presence of many other organic vapors that may be present in human breath. Carrier gas for the mGC is derived from the ambient air, thereby eliminating the need for compressed gas cylinders and imparting portability to the instrument. Chromatographic separations are performed on two micro-packed, isothermal GC columns to minimize power consumption. The entire mGC “engine” occupies a volume of approximately 96 cubic inches (8 × 4 × 3 in.) and is illustrated schematically in Figure 1. This volume includes the injection valve, column oven, carrier gas generator, sampling pump, microcomputer, and all associated electronics. The instrument incorporates a highly readable vacuum fluorescent display and pushbutton for user interactions, an audible alarm, replaceable air scrubber cartridge, external power jack, and an RS232 serial communication port. Power is provided to the instrument from an external 12 V DC laptop-PC-style power supply that operates from the 120 V AC line. Alternatively the instrument can be powered by a 13.6 V DC automotive battery. Warm-up is completed within 10 min. The warm-up includes the time required to bring the column and detector to their proper operating temperatures, stabilize the carrier gas flow, and purge the injection valve with clean, dry air from the scrubber.

A prototype mGC was used for this research that consisted of a concentrator trap containing 4 mg of Tenax TA (Sigma-Aldrich, St. Louis, MO), a small compressor pump to supply the scrubbed air carrier gas, and dual 5-m × 0.53-mm i.d. metal-clad GC columns attached to metal oxide semiconductor detectors. A breath sample from a human subject, blank air, or ethanol gas standard is blown through a plastic mouthpiece attached to the front of the mGC. The ethanol gas standards were produced by Airgas Specialty Gases (Radnor, PA) to be traceable consistent with the U.S. National Institute of Standards and Technology (NIST) Traceable Reference Material (NTRM) Program for Gas Standards (7) for ethanol and purchased from Intoximeters.

The inlet port of the mGC is located half-way along the length of the mouthpiece such that a side-stream sample can be taken. A 10-mL sample is aspirated during a single breath from the mouthpiece over 2 s through the inlet port to the concentrator trap with the trap at room temperature. Thereafter, the trap temperature is increased to 130°C and the ethanol transferred to two different GC columns operated at 45°C attached in parallel to the trap. The effluent from each GC column then flows to separate metal oxide detectors. Separation of ethanol from other volatile organic compounds present in the sample (e.g., acetone and isoprene) occurs in less than 2 min.

mGC operation

To start the instrument, the user presses the button on the front panel. Audible beeps are provided whenever a new instruction is presented. After each instruction, the device waits for the user to press the button, thereby acknowledging that each step is completed. Samples are introduced by the gas flowing across the inlet to the instrument. Once the individual subject is ready to provide a breath sample through a plastic “straw” and the button is pressed, the analyzer functions completely automatically. Breath is supplied by the subject by blowing into a straw with triggering of the vacuum pump by the operator. In this study, the operator triggered pump activation in the last half of the breath. A small vacuum pump draws a sample from the inlet source into a Tenax concentrator that traps a reproducible volume of sample. After a predetermined time, the sample pump stops, and several solenoid valves are activated on the manifold block that cause clean carrier gas to sweep through the heated Tenax concentrator, thereby pushing the sample vapors into the GC column for analysis. After a precisely timed injection period, the sample loop is then back flushed to remove residual vapors. Digital

![Figure 1. Schematic diagram of the mini gas chromatograph (mGC) used to measure ethanol concentrations in human breath.](https://academic.oup.com/jat/article-lookup/doi/10.1093/jat/35.3.134)
control of the valve timing and sequencing assures highly reproducible injections. During the chromatographic analysis, active electronic control of the chromatograph operation is maintained at all times. A pressure sensor and control electronics regulate the power to a small compressor pump that delivers carrier gas to the column. This method is a key feature of the system and provides highly reproducible carrier gas flow rates in spite of ambient atmospheric pressure variations. A temperature sensor and control electronics regulate the power supplied to the oven heater to provide very reproducible oven temperatures. During sample elution from the analytical column the signal from the solid-state detector is logged twice each second by the on-board computer.

The basic operating sequence consists of the following steps: 1. The sample pump is energized to draw a sample from the breath sample into the Tenax concentrator. 2. The valves are energized to allow trapped vapors to flow into the GC columns. 3. The sample vapor constituents are separated based on boiling point and polarity and then detected as they emerge from the separation column. The detector signal magnitude is proportional to the vapor concentration eluting from the column. The arrival time of each vapor peak is indicative of the vapor type.

The analyzer hardware is partitioned onto two main circuit boards that are 4 × 6 in. in size and stacked to reduce the overall footprint. The “CPU board” contains all of the digital microcomputer hardware and associated clocks, counters and interface logic for RS232 communication, digital display, push-button input, and audible tone generation and control signals for all pumps, valves, and heaters. The “Pneumatic Board” contains the drive circuitry for all of the pumps, valves, and heaters and an analog controller hardware for temperatures and pressures. An independent metal plate serves as the mounting point for the manifold, valves, pumps, GC oven, and detector hardware. This plate is stacked on top of the “Pneumatic Board”. Electrical connections from the individual pumps, valves, and heaters are made to appropriate push-on connectors on the “pneumatic board”. An Intel 80C32 8-bit microprocessor is used to control the instrument. Output is currently designed to numerically and graphically display on a laptop personal computer loaded with custom-written software.

mGC software

A peak selection algorithm locates the retention time and peak height of every compound that elutes during the predetermined chromatographic window. If a peak is found in the ethanol “window” then the computer logs a successful “hit” and calculates the ethanol concentration from the measured peak height. The processor supports a Basic language interpreter. All software is written in Basic and stored in executable form on the on-board, non-volatile EEPROM. The specific sequencing of pumps and valves and the temperature and pressure set point is determined by a programmable parameter list stored in EEPROM on the CPU Board. This list also contains peak detection criteria and retention times of the vapors to be analyzed. This allows the analyzer to be adaptable to a variety of different chemical markers by simple changes to the GC operating configuration. The analysis time for breath ethanol is less than 60 s.

mGC dual column considerations

To facilitate the separation of volatiles and improve the specificity of the device, the mGC was built using dual separation columns and detectors to allow definitive resolution of ethanol from other co-eluting chemical species. The actual column design was specified so that if ethanol did not resolve from a particular confounding chemical in column 1, then it would definitely separate in column 2 (and vice-versa). After evaluating many commercially available columns, we found a wax column (Restek, Bellefonte, PA) and an alcohol column (Restek) provided the best resolution from other chemicals potentially found in breath (Table 1). We selected and designed the mGC so that at least 21 chemical species could be separated. The particular species include acetaldehyde, acetone, acetonitrile, benzene, 2-butanol, cyclohexane, di-

Table 1. Tabulated Data of Retention Time of Ethanol, Possible Confounding Volatile Species Emanating from Breath, and Traditional Analytical Standards/Carriers for Two Independent Columns (BAC1 and Wax) and Detectors

<table>
<thead>
<tr>
<th>Species</th>
<th>BAC1 Column Retention Time (s)</th>
<th>Species</th>
<th>Wax Column Retention Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>42</td>
<td>n-Pentane</td>
<td>35</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>44</td>
<td>n-Hexane</td>
<td>35</td>
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<tr>
<td>Ethanol</td>
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<td>Diethylether</td>
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<tr>
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<td>n-Heptane</td>
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<tr>
<td>Isoprene</td>
<td>53</td>
<td>Isoprene</td>
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<tr>
<td>Diethylether</td>
<td>54</td>
<td>Cyclohexane</td>
<td>39</td>
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<td>Acetone</td>
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<td>49</td>
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<tr>
<td>1-Propanol</td>
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<td>1,1,1-Trichloroethane</td>
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<td>Ethyl acetate</td>
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<td>Methyl ethyl ketone</td>
<td>60</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>192</td>
<td>Methanol</td>
<td>61</td>
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<tr>
<td>Ethyl acetate</td>
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<td>Tetrachloroethane</td>
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ethylether, ethyl acetate, n-heptane, n-hexane, isoprene, isopropanol, methanol, methyl ethyl ketone (MEK), n-octane n-pentane, 1-propanol, tetrachloroethylene, 1,1,1-trichloroethane, trichloroethylene, toluene, and xylene. Representative chromatograms obtained from human exhaled breath before and after dosing a fasted subject with ethanol are shown in Figure 2.

**Human studies**

**Ethics.** This investigation was reviewed and approved by the University of Florida Institutional Review Board and the Executive Committee of the Clinical and Translational Science Institute (CTSI) at Shands Hospital at the University of Florida, Gainesville, FL, under IRB-01 protocol number 98-2009.

**Subject inclusion and exclusion criteria.** Male and female healthy, moderate drinking subjects ($n=8$) between the ages of 21 and 59 years (verified by driver’s license or other government-issued ID) were recruited. Subjects were volunteers recruited by word-of-mouth from the general community and through outreach efforts associated with the University of Florida CTSI. Subjects previously completed a minimum of 10 years of education and had no history of treatment for ethanol use disorders or other major psychiatric disorders (e.g., Major Axis I disorders including Bipolar Disorder or Psychotic Disorders as per DSM-IV criteria) (8). Using standard procedures, typical and maximum drinking levels for the prior six months were ascertained. Subjects reported a minimum level of 1 drink/week and may have reported as many as 5 (for men) or 4 (for women) drinks on a single occasion, if these episodes occurred not more than once a week or no more than 3 times/month. This range was selected to provide sufficient variability in drinking levels across non-treated populations. Subjects were excluded if they reported significant medical disorders, including disorders that had direct effects on brain function, were hypertensive (treated or untreated), were borderline or diagnosed diabetics, or had other metabolic disorders that might affect the pharmacokinetics of ethanol. No persons who had reduced ethanol intake due to medical consequences in the previous year were eligible.

**Subject screening phase.** Subjects provided written, informed consent prior to initiating screening. The screening materials included assessments of affective state, demographic information, and a detailed history of current and lifetime substance use. General health status was also assessed including current, recent, or chronic disorders; current medications; and psychiatric symptomatology using questionnaires and checklists. Affective state was assessed through standard research instrumentation; the Beck Depression Inventory-II (BDI-II) (9). Individuals with scores > 20 on the BDI-II (moderate depressive symptomatology) were not eligible. Subjects endorsing suicidal intent/ideation would be referred through research staff to the Psychiatry Department. The Michigan
Alcoholism Screening Test (MAST) (10) was administered to screen for undetected ethanol use disorders. Subjects meeting criteria for current potential problem drinking were discontinued from participation and provided information regarding “hazardous” drinking, as provided by the National Institutes on Alcohol Abuse and Alcoholism (NIAAA).

After successfully completing screening, subjects were scheduled for their trial in the CTSI for a subsequent day. Upon arrival (being transported by research staff), subjects were met by a staff member and provided informed written consent for this phase. The staff then conducted a brief interview reviewing inclusionary/exclusionary criteria. CTSI nurses administered urine collection (all subjects) and pregnancy tests (women only). The staff used the collected urine for an onsite test of illicit drugs. Baseline breath assessments for ethanol were obtained by investigators using a conventional breath alcohol analyzer (Intoxilyzer 400PA, CMI, Owensboro, KY) employing fuel cell technology and the mGC device. For subjects with negative drug, pregnancy, and ethanol tests, CTSI nurses inserted an indwelling catheter in the subjects’ non-dominant arms for the blood draws. In the event that alcohol or illicit substances were detected or the subjects reported use of prohibited over-the-counter (OTC) or prescription medication, the subjects were discontinued from the study and rescheduled, if appropriate.

Protocol. Subjects were fasted for at least 3 h before entering the protocol and remained fasted during the duration of the study. Subjects meeting selection criteria were administered a dose of ethanol (200-proof ethanol, Decon Labs, King of Prussia, PA) in non-caffeinated diet soda divided into two equal volumes, accounting for gender differences using a modification of the Widmark formula to achieve a blood ethanol concentration of approximately 0.08 g/dL (11).

For males:

\[
Dose\ (mL) = \left( \frac{[20.03 - 0.1183(AGE)] + [0.3626(MASS)]}{0.8} \right) \times 0.79
\]

For females:

\[
Dose\ (mL) = \left( \frac{[-2.097 + 0.1069(HEIGHT)] + [0.2466(MASS)]}{0.8} \right) \times 0.79
\]

where Dose is the volume of neat ethanol in milliliters, AGE is the age of the subject in years, MASS is the body mass of the subject, and HEIGHT is the length of the subject’s body in centimeters. A physician (TEM) determined the dosage of ethanol using this calculation. Shands Hospital at the University of Florida Investigational Drug Service measured and dispensed the ethanol in two separate containers per subject that were room temperature (22°C). CTSI nursing staff administered the ethanol drinks to the subjects. Exactly 5 min following conclusion of dosing, post-administration breath measurement began using the Intoxilyzer 400PA occurred every 5 min thereafter on the ascending limb. Prior to the first measurement, subjects were required to rinse their mouths 3 times with water, spitting the residue into a sink and thereby removing ethanol from the oral cavity. The mGC and breath measurements occurred at 10-min intervals on the ascending limb. Given individual variability in the time to achieve peak ethanol concentrations, 3–6 phlebotomy draws (2 mL/draw) were conducted with blood collected in serum separator tubes (Gold BD Hemogard Vacutainer® SST II Plus, BD, Franklin Lakes, NJ) and analyzed the same day of collection. Subjects were required to inhale, hold their breath, and then exhale into an attached tube. The resultant breath ethanol concentrations were displayed on the digital screen in grams-per-210-L units to 3 significant digits (i.e., thousandths place; e.g., 0.006). For the purposes of this study, the screen was opaquely covered (i.e., black tape) and read and recorded by the study staff outside the view of the subjects.

Ethanol analysis

Blood samples were placed in serum tubes, labeled, placed on ice, and sent by courier to the Clinical Laboratories of Shands Hospital at the University of Florida (Gainesville, FL) for analysis by a CLIA-approved laboratory and professional laboratory technicians. This method (Ethanol Gen. 2, COBAS Integra 400/700/800, Roche Diagnostics, Indianapolis, IN) uses alcohol dehydrogenase and is highly specific for ethanol. Ethanol is oxidized to acetaldehyde, and NAD is reduced to NADH. The NADH formed during the reaction, measured photometrically as a rate of change in absorbance at 340 nm, is directly proportional to ethanol concentration. The reported lower detection limit of this method for serum is 10 mg/dL (12). The within-run coefficients of variation for 93.0 and 194 mg/dL ethanol are 1.2 and 1.1%, respectively, whereas the between-run coefficients of variation for 100 and 197 mg/dL are 2.4 and 3.9%, respectively (12).

The Intoxilyzer 400PA directly burns ethanol in a fuel cell assembly within the device and provides a digital output of the breath ethanol concentration in grams per deciliter. Detection principles of the mGC device were previously described herein. Using 0.010% ethanol standard in 1.0-L Tedlar bags (SKC, Eighty Four, PA) with blank human breath as a diluent, the within-day signal relative standard deviation was 1.4%.

Figure 3. Time-concentration relationship for the mGC response at three different concentrations of ethanol for two columns and detectors prior to testing in a subject. Individual data points are shown along a correlation line.
During the day of each human experiment, the mGC was calibrated using ethanol concentrations of 0.038, 0.082, and 0.100 g/210 L. The correlation coefficients ($r$) for the linear function on each individual day varied from 0.9925 to 0.999. An example of a typical calibration is illustrated in Figure 3.

Statistical analysis
Summary data of subject characteristics, including age, weight, sex, and race, were prepared. The concentration-time relationship for blood ethanol was determined. We compared the breath ethanol concentrations from the mGC device to the serum ethanol concentration (“gold standard”) using linear regression to calculate $r$, standard deviation of $r$, 95% confidence interval for the regression line, and statistical significance of the correlation for each relationship. Thereafter, Bland-Altman analysis was performed to determine potential bias, limits of agreement, 95% confidence intervals for the upper and lower limits of agreement, and possible presence of a magnitude effect (13). The magnitude effect is a circumstance wherein the bias of the Bland-Altman is a function of the magnitude of the endpoint (e.g., blood ethanol concentration). Continuous data are presented as mean ± standard deviation.

**Results**

**Subject enrollment**
Nine subjects (7 males, 2 females; all Caucasian) were enrolled after providing written informed consent. Eight subjects completed the study as intravenous access to acquire blood could not be secured in one subject (male). For those completing the study ($n = 8$), the age and body mass were 25 ± 4 years and 76 ± 11 kg, respectively. Subjects consumed a mean 53.9 ± 8.5 mL of alcohol with a range of 41–64 mL. No adverse events were observed or reported as a consequence of this investigation. The serum ethanol concentration increased rapidly from baseline (0.00 g/dL) to approximately 0.08 g/dL. This concentration was maintained for over 1 h and allowed adequate time to conduct the sample collection for this study.

**mGC device to serum ethanol concentration comparison**
The serum ethanol concentration determined from the breath specimens by the mGC was compared to direct measurement of serum ethanol concentrations as shown in Figure 4. Overall, a moderate-to-high correlation coefficient was observed ($r = 0.91 ± 0.01$) between the mGC and direct serum ethanol concentration ($p < 0.001$). We also observed two outlying data points at times 5 and 15 min that were the first two data points acquired in the entire study (Figure 4, boxed points). Exclusion of these two outliers yielded a regression of $y = 1.069x - 0.007$ with an $r$ value of 0.96. In addition, the slope for line of regression approached unity, and no magnitude effect was observed. These data were re-plotted to compare the mGC device to whole blood ethanol concentrations (Figure 5). In this case, we calculated the whole blood ethanol concentrations by dividing the serum concentrations by 1.15. Regression of the points for whole blood yielded a linear equation of $y = 1.176x - 0.009$ with a similar correlation coefficient. Finally, the performance of the device appeared to be independent of the phase of absorption of ethanol.

**mGC to Intoxilyzer ethanol concentration comparison**
The performance of the mGC was compared to the Intoxilyzer analyzer, a device also designed to determine the blood concentration of ethanol.
concentration using breath analysis. Overall, a high degree of correlation ($r = 0.96$) was noted between the two devices as shown in Figure 6 ($p < 0.001$). Bland-Altman analysis revealed a bias of $-0.014$ g/210 L, but a large magnitude effect was observed with increasingly positive bias with larger concentrations of breath ethanol. Additionally, the two outlying points noted earlier (Figure 4A, boxed points) no longer were outlying values when comparing gas analyses exclusively.

**Intoxilyzer to serum ethanol concentration comparison**

To further understand the source of the magnitude effect in Figure 6 (mGC-Intoxilyzer) that was not noted in Figure 4 (mGC-serum), we next conducted Bland-Altman comparing the Intoxilyzer device and serum ethanol concentrations. The Intoxilyzer device had a high degree of correlation ($r = 0.86$) to serum ethanol concentrations, but tended to underestimate the serum values (Figure 7), especially at greater serum concentrations. This effect was evident as a magnitude effect in the Bland-Altman analysis (Figure 7B) wherein the bias of the measurement was a function of the magnitude of the ethanol concentration. In addition, the outlying points noted from mGC analysis (boxed points in Figure 4) were now also evident with breath analysis by the Intoxilyzer device compared to serum concentrations. Dividing the serum concent-

**Figure 6.** Bland-Altman analysis of the mGC compared to Intoxilyzer ethanol concentrations in human subjects ($n = 8$). A: Correlation of ethanol concentrations from the mGC device compared to time-matched Intoxilyzer specimens. The solid black line is a line of regression, and dashed lines are 90% confidence intervals. The dotted line shows a unity relationship (1:1). B: Bland-Altman analysis for ethanol concentrations from the Intoxilyzer and time matched blood specimens obtained from human breath. The dashed lines are the upper and lower limits of agreement (LOA), and the dotted lines are the 95% confidence intervals for the LOA lines.

**Figure 7.** Bland-Altman analysis of the calculated serum ethanol concentrations determined by the Intoxilyzer compared to measured serum ethanol concentrations human subjects ($n = 8$). A: Correlation of ethanol concentrations in blood from the Intoxilyzer device compared to time-matched serum specimens. The solid black line is a line of regression, and dashed lines are 90% confidence intervals. The dotted line shows a unity relationship (1:1). The two boxed points indicate outliers that were the first two points acquired from the first subject participating in this investigation. B: Bland-Altman analysis for ethanol concentrations from the Intoxilyzer and time matched blood specimens obtained from human breath. The dashed lines are the upper and lower limits of agreement (LOA), and the dotted lines are the 95% confidence intervals for the LOA lines.

**Figure 8.** Correlation of ethanol concentrations in serum with those from the Intoxilyzer device before and after correction for whole blood using a divisor of 1.15. The solid black line is a line of regression for serum, and the dashed line is that for serum/1.15. The dotted line shows a unity relationship (1:1).
tations by 1.15 to more accurately reflect blood concentrations, less bias was noted in the Intoxilyzer device (Figure 8), although a large magnitude effect still existed. A greater number of points were evident for Intoxilyzer observations as the local institutional review board mandated use of this device as a safety measure to assure that subjects returned to ≤ 0.010 g/210 L prior to discharge from the protocol.

Discussion

mGC performance
The mGC device was used in this clinical investigation to determine the serum and blood ethanol concentrations based on the exhaled breath ethanol concentrations. We found that the device was transportable and well accepted by both the human subjects and the investigators operating the device. The mGC performed well over a range of blood ethanol concentrations from 0.02 to 0.14 g/dL used in this investigation. In other laboratory investigations (data not shown), the device has detection limits conservatively measured to be approximately 0.001 g/210 L breath ethanol concentration. Reviewing Figure 4, the mGC underestimated the serum ethanol concentration by approximately 5–10% over the range studied. Of note, serum concentrations underestimate whole blood concentrations by about 10–15%. Therefore, the mGC actually may more closely approximate the line of unity once one accounts for this fact (Figure 5). In the future, measurement of alcohol in whole blood is preferable for this type of work to avoid this potentially confounding issue of estimating whole blood concentrations from serum values.

We noted two outlying points during the experiments and hypothesize that these values were spurious due to errors in blood handling or measurement. Two pieces of evidence support this position. First, these two points were the first data points obtained from the first subject at times 10 and 20 min following consumption of ethanol. One would expect this time period to entail an ascending concentration of ethanol as gastrointestinal absorption occurs. For this subject, these values at 10 and 20 min were 0.079 g/dL by serum analysis (for both time points) with subsequent values at times 30 and 40 min of 0.079 g/dL as well. Second, the mGC and Intoxilyzer data for these time points agree much more closely with one another than with serum concentrations. That is, the estimated blood concentrations at time 10 min by mGC and the Intoxilyzer device were 0.042 and 0.035 g/dL, respectively, whereas the values at 20 min were 0.035 and 0.030 g/dL, respectively. Because the serum data for these two points do not concur with ethanol pharmacokinetics and because the breath-based devices were in concordance, we believe that the serum concentrations are spurious for these points.

We did not detect any potential interferants in the human subjects studied in this investigation. To validate the clinical utility of the mGC to specifically discriminate between confounding species (as shown in Table 1), additional investigations would be needed in subjects with native production of these potential interferants. Additionally, a subsequent generation of this prototype device should incorporate a method to handle breath in a more precise manner so that alveolar samples are measured for ethanol concentration.

Intoxilyzer performance
In this investigation, the Intoxilyzer 400PA unit had a high degree of correlation to serum ethanol concentrations, but an overall underestimation of the serum values with a magnitude effect. Similarly, Cowan and colleagues (14) also noted that ethanol concentrations measured by an Intoxilyzer 8000 had a high correlation to blood ethanol concentrations \((r = 0.94)\), but tended to underestimate the blood concentrations in every subject. Although not specifically analyzed, review of their data also suggests a magnitude effect. Likewise, the Intoxilyzer 5000S correlated well with venous blood ethanol concentrations \((r = 0.99)\), but again erred towards reporting a lower concentration than measured in blood (15). Jones and colleagues (16) provided similar observations with an Intoxilyzer 5000. Overall, these present data are in concordance with these previous reports, indicating an overall high degree of correlation between breath (measured by Intoxilyzer devices) and blood ethanol concentrations, but that the Intoxilyzer units biased towards providing lower concentrations than actually existed in blood.

Not surprisingly, the data indicated that the mGC performed more poorly when compared to the Intoxilyzer than when compared to serum data. That is, the bias increased over four-fold and developed a large magnitude effect when the “gold standard” of ethanol measurement was switched from serum ethanol concentrations to Intoxilyzer-based ethanol concentrations (although both analysis methods maintained a high degree of overall correlation). These observations strongly concur with the founding essence of the Bland Altman analysis technique: 1. two methods designed to measure the same endpoint almost inevitably have a high degree of correlation and 2. variability in the “gold standard” may contaminate the interpretation of the accuracy and precision of the new method to measure the endpoint. For these reasons, future investigations on the performance characteristics of the mGC will use blood-based ethanol concentrations measurements as the standard.

Conclusions

We conclude that this first generation mGC using exhaled breath performed well in estimating serum ethanol concentrations in the present prototype device. Measurement of ethanol using this mGC is feasible and deserves additional consideration and development.

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References


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