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Short communication

# Comparison of SPME and static headspace analysis of blood alcohol concentration utilizing two novel chromatographic stationary phases

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ABSTRACT

Headspace gas chromatography, coupled to flame ionization detection (GC–FID) analysis of blood alcohol concentration is a routine analysis carried out in forensic laboratories. A common concern with this analysis is the possible co-elution of a variety of other commonly encountered compounds with the target compound ethanol. By use of application specific columns, improved separation of ethanol, as well as the other potential components can be achieved. The presented method includes the evaluation of blood alcohol concentration by both direct gas headspace and SPME utilizing a new combination of GC columns. An investigation of method detection limits (MDLs) was also conducted in order to determine a reporting limit as well as the degree of uncertainty at the common threshold value of 0.08 g/dL. The study showed that under the conditions of this work, static headspace analysis with the use of t-butanol as an internal standard provided the most accurate and precise data with an MDL of 0.002 g/dL for the Rtx® – BAC PLUS 1 column and 0.005 g/dL for the Rtx® – BAC PLUS 2 column. The study also showed that the SPME analysis using a Carboxen/PDMS (65 μm) fiber with the use of t-butanol provided the lowest overall MDL of 0.0006 g/dL for both Rtx® – BAC PLUS 1 and 2 columns without loss of accuracy or precision.



## 1. Introduction

In the United States, alcohol abuse is associated with automobile fatalities, industrial accidents, and numerous other incidents such as alcohol poisoning and drug facilitated sexual assault. Due to its enormous impact on society, a rapid and precise methodology for the determination of blood alcohol concentration (BAC) is desired. BAC is the amount of ethyl alcohol in a person's bloodstream [1]. However, there may be a number of other compounds present such as methanol, isopropanol, acetaldehyde, and acetone that potentially interfere with the identification and quantitation of ethanol [2]. To accurately determine a subject's blood alcohol concentration, numerous factors must be taken into account, including the qualitative and quantitative analysis of the alcohols and their metabolites. Scheme 1 illustrates the metabolism of ethanol to acetic acid which occurs in the liver. The hydrogen which is released when alcohol dehydrogenase (ADH) turns alcohol into acetaldehyde, and acetaldehyde into acetic acid, is bound to a compound called NAD+ (Nicotinamide Adenine Dinucleotide) to form NADH (Nicotinamide Adenine Dinucleotide plus Hydrogen) [3]. Identification and quantification of these compounds using GC–FID are usually performed using an internal standard, typically n-propanol or t-butanol, but care must be taken to avoid incorrect identity assignment which may result from using a non-selective detector [2]. Additionally, quantification bias will occur if there is any co-elution with the various target compounds or internal standards. Given the range of common laboratory contaminants and metabolic pathways, this requires a relatively sophisticated separation.

Common current analytical methods include either direct gas headspace (under static or dynamic conditions) [4–7], or solid-phase microextraction (SPME) [8–11] as preparation techniques. Utilizing headspace sampling prevents buildup of non-volatile contaminants in the injector and on the column that may occur using other possibilities. It also helps to maintain consistent performance and extends the lifetime of the column [12]. Static and SPME headspace analyses are sensitive, stable, and reproducible techniques, that are extremely useful for the analysis of biological samples due to their ability to isolate volatile target compounds from high molecular components such as proteins in the matrix [13,14].

In BAC analysis, baseline resolution for all analytes of interest is desired, as is the case for any separation method. In addition, fast analysis times are essential due to the high throughput nature of this analysis in most forensic laboratories [13]. The goal of this study was to determine if the use of two new gas chromatographic

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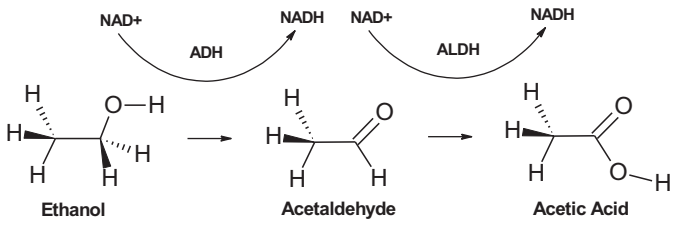


**Scheme 1.** The major route of ethyl alcohol metabolism in the body (liver) is the oxidation of ethyl alcohol by alcohol dehydrogenase (ADH) [3].

column stationary phases positively impacts the separation, and also to evaluate spiked samples in an effort to gauge the limit of quantification and uncertainty of the analysis when using two different sample introduction techniques.

## 2. Materials and methods

### 2.1. Materials

All experiments were performed using an Agilent Technologies 6890 Series GC coupled with dual flame ionization detectors (GC–FID). The instrument was equipped with the Rtx® – BAC PLUS 1 (30 m × 0.32 mm ID × 1.80 μm) and Rtx® – BAC PLUS 2 (30 m × 0.32 mm ID × 0.6 μm) columns (Restek, cat# 18004 and 18006 respectively; Bellefonte, PA). The dual-column system provides for primary and confirmatory analyses in a single injection by coupling both columns through a universal "Y" Press-Tight® connector (Restek, CAT# 20406-261; Bellefonte, PA) to a single injection port on the GC using a short section (ca. 20-cm) of 0.32-mm i.d. deactivated fused silica (Restek cat# 10044; Bellefonte, PA). In order to obtain reliable results for both primary and confirmatory analyses, the GC columns must employ the use of two different selectivities such that the target compounds elute with different capacity factors on both columns, and ideally with a significant change in elution order. This aids in the confirmation of ethanol and also allows for a potential reduction in possible interferences or co-elutions with ethanol (due to different elution times) [15].

The GC dual-FID system was equipped with a Gerstel® MultiPurpose Sampler (MPS 2L), which was employed as the automated headspace sampler (Gerstel USA; Baltimore, MD). A 0.75 mm ID Straight/SPME Inlet Liner (Restek, CAT# 21111; Bellefonte, PA) was utilized in the injection port. The SPME fibers examined included: Polydimethylsiloxane/Divinylbenzene (PDMS/DVB, 65 μm), Carbowax-Polyethylene Glycol (CW-PEG, 60 μm), Carboxen/PDMS (85 μm), and Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS; 50, 30 μm) (all SPME fibers were obtained from Supelco®; Bellefonte, PA).

### 2.2. Analytical procedure

The first objective was to optimize a method for the separation of all eight target compounds in the resolution control standard (Restek, CAT# 36256; Bellefonte, PA) on each column. After the separation was optimized a calibration curve was prepared with ethanol standards ranging from 0.02 g/dL to 0.30 g/dL (Restek, CAT# 36249, 36251, 36260, 36252, 36253, 36254, and 36255; Bellefonte, PA). Both n-propanol and t-butanol (J.T. Baker, CAT# 9086-01 and 9056-01; respectively; Center Valley, PA) were utilized as internal standards for quantification. All standards were prepared in class A volumetric glassware and all pipettes were calibrated before use.

The second objective was to measure precision and accuracy of the BAC analysis. Ten replicate samples containing ethanol at a concentration of 0.08 g/dL (Restek, CAT# 36263; Bellefonte, PA) were prepared for the evaluation of the uncertainty at the common threshold value. Additionally, ten replicate samples containing ethanol at a concentration of 0.02 g/dL (Restek, CAT# 36249; Bellefonte, PA) were prepared for the evaluation of the MDL. Each set of the ten replicate samples were prepared by the addition of 100 μL of the ethanol standard (0.08 g/dL, or 0.02 g/dL) to 4.9 mL of organic-free water (>18.2 MΩ) for a total sample volume of 5 mL in a 20 mL headspace sample vial. Both n-propanol and t-butanol were each added at a concentration of 0.12 g/dL.

### 2.3. Conditions of GC–FID analysis

Tables 1 and 2 are the conditions used for the static and SPME headspace analyses respectively. Fig. 1 illustrates the optimized separation of the resolution control standard on the Rtx® – BAC PLUS 1 and 2 columns using static headspace sampling. It should be noted that the results presented utilized a 5-min GC program, but this could be easily shortened to 3.0 min. Initial experimentation utilized the longer isothermal hold, but this was ultimately not necessary once optimized conditions were determined.

**Table 1**
Static headspace analysis GC parameters.

| | |
|---|---|
| Instrument control parameters | |
| Injector temp. | 250 °C |
| Mode | Split (50:1) |
| Carrier gas | Helium |
| Detector temp. | 250 °C |
| Maestro configuration | |
| Incubation temp. | 65 °C |
| Incubation time | 5.00 min |
| Agitator on/off time | 10 s/1 s |
| Injection volume | 500 μL |
| Injection speed | 1000 μL/s |
| Isothermal temperature program | |
| 40 °C | 5.0 min |

**Table 2**
SPME headspace analysis GC parameters.

| | |
|---|---|
| Instrument control parameters | |
| Injector temp. | PDMS/DVB: 270 °C<br>CW(PEG): 240 °C<br>Carboxen/PDMS: 320 °C<br>DVB/CAR/PDMS: 270 °C |
| Mode | Split (5:1) |
| Carrier gas | Helium |
| Detector temp. | 250 °C |
| Headspace extraction configuration | |
| Incubation temp. | 80 °C |
| Incubation time | 5.00 min |
| Agitator on/off time | 10 s/1 s |
| Extraction time | DVB/CAR/PDMS: 2.00 min<br>Other Fibers: 5.00 min |
| Desorption time | 120 s |
| Isothermal temperature program | |
| 40 °C | 5.00 min |

## 3. Results and discussion

The basis of assessment of sobriety is BAC, but knowledge of the magnitude of analytical error occurring during analysis is essential for correct interpretation of the obtained results [16]. The average results for each column were determined along with standard deviation and percent relative standard deviation (%RSD). The MDL data was calculated with the use of the EPAs online MDL calculator [17]. The MDL provided the limit of quantification (LOQ), the lower confidence limit (LCL) and the upper confidence limit (UCL) for the data sets. The MDL online calculator was limited to the use of only nine replications. A second MDL was then calculated including all ten replicates with the use of the calculations provided by the EPA site. All of the calculations were conducted with the appropriate values for a 99% confidence limit.

Two different quantification methods were considered, external and internal, in order to compare the accuracy and the stability of the headspace sampling methods. The external quantification method was compared to quantification using the two different internal standards, t-butanol and n-propanol.

The results for the static headspace method showed that internal standard data provided accurate results with a reduction in the standard deviation when compared to quantification using external standard alone. The headspace data also showed a slight bias with the use of n-propanol and better column-to-column data with the use of t-butanol (Table 3). The static headspace MDL data sets for both the external and internal standards showed no significant improvement of the average or the standard deviation (Table 4).

The comparison of the data for the SPME analysis showed a decrease in accuracy as well as an increase in the standard