Forensic laboratories face the need to analyze a large number of samples of human blood and body fluids for alcohol content. When faced with this challenge factors that need to be considered are sample throughput, resolution, and carryover.

A successful method for these analyses should be fast, precise, and accurate. Current methods used in these analyses use a gas chromatograph coupled to a static headspace sampler and flame ionization detector (FID). The x, y, z robotic autosampler used in this study has a capacity of up to 128 headspace samples, which is a distinct advantage compared to other samplers commercially available. Results obtained with the instrument and methodology described in this report meet the specifications set by the California Department of Justice Blood Alcohol Operating Procedures (Title 17) [2]. We also configured and tested a separate dual-column/dual-FID system that adds confirmation because of the different elution order of ethanol on the two columns.

**Experimental**

**Instrumentation**
Analyses were performed on a 6890 GC equipped with single or dual FID (Agilent Technologies), and a GERSTEL MPS 2 MultiPurpose sampler configured for static headspace injection.

**Reagents**
- Ethyl alcohol, absolute, 200 proof, 99.5%, A.C.S. reagent grade
- Methyl alcohol, 99.8%, A.C.S. reagent grade
- Acetone 99.5%, A.C.S. reagent grade
- n-Propanol (1-propanol) 99.5% A.C.S. reagent grade
- Isopropanol (2-propanol), 99.5%, A.C.S. reagent grade
- Blood alcohol mix resolution control standard (Restek, # 36256). 0.100 g/dL in water of 8 compounds: acetaldehyde, acetone, acetonitrile, ethanol, ethyl acetate, isopropanol, methanol and methyl ethyl ketone (MEK).

**Preparation of standards**
- Secondary standard (SS). 0.25 mL of absolute (200 proof) ethanol and 0.125 mL of n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water.
- Quality control standard (QC). 0.15 mL of absolute (200 proof) ethanol and 0.125 mL of n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water.
- Resolution standard (RS). 0.25 mL of absolute (200 proof) ethanol, 0.1 mL methanol, 0.1 mL isopropanol, 0.01 mL acetone and 0.125 mL of n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water.
- Blank standard. 0.125 mL of n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water. All standards above were diluted 1:6 in bottled water prior to use. 500 µL of standard was then pipetted into a 20 mL headspace vial. 1 mL of 1000 µg/mL internal standard (n-propanol) and 1 mL of the blood alcohol mix resolution control standard (Restek, # 36256). 0.100 g/dL in water of 8 compounds: acetaldehyde, acetone, acetonitrile, ethanol, ethyl acetate, isopropanol, methanol and methyl ethyl ketone (MEK).
and Secondary Standard (SS) for California Compliance.

Figure 2. FID overlay of Blank/Internal Standard (IS), Resolution Standard (RS) dual-FID system.

Table 3. Method parameters for dual-column configuration.

<table>
<thead>
<tr>
<th>GC 6890 (Agilent Technologies)</th>
<th>Inlet: Split/splitless, 100 °C</th>
<th>Column: 30 m DB-ALC2 (Agilent)</th>
<th>di = 0,53 mm, df = 2,0 µm</th>
<th>Oven: isotherm, 40 °C</th>
</tr>
</thead>
</table>

Table 1. Method parameters for California compliance.

Table 2. Example of calculation and check of K factor.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average peak area</th>
<th>Standard deviation</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>ALC1</td>
<td>ALC2</td>
<td></td>
</tr>
<tr>
<td>0.766</td>
<td>0.765</td>
<td>0.765</td>
<td>0.33%</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00%</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0.766</td>
<td>0.765</td>
<td>0.33%</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00%</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>0.766</td>
<td>0.765</td>
<td>0.33%</td>
</tr>
<tr>
<td>MEK</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

Table 3. Method parameters for dual-column dual-FID system.

Results and Discussion

California DOJ Blood Alcohol Operating Procedure (Title 17). The instrumentation used in this study was a GERSTEL MPS autosampler that can be programmed to be used with headspace syringes. For this study we used a 2.5 mL syringe that can also be programmed to be used with headspace syringes from 0.25 mL up to 2.5 mL. The headspace syringe adaptor is heated and the gas chromatographic conditions include the use of a capillary GC column instead of the packed column currently used for these analyses in California. The gas chromatographic conditions include the use of a capillary GC column instead of the packed column currently used for these analyses in California.

Conclusions

- The GERSTEL MPS 2 robotic autosampler is capable of delivering performance for blood alcohol analysis that meets or exceeds the California Title 17 Forensic Alcohol Analysis and Breath Alcohol Analysis performance criteria.
- Testing during a 3-month period showed good robustness and reproducibility.
- This instrumentation provides increased sample throughput by accommodating up to 128 samples and by using the “prep ahead” function to equilibrate multiple samples simultaneously.
- The GERSTEL MPS 2 autosampler performed well when used with a dual column blood alcohol confirmation method.
Detection, identification, and quantitation of ethanol and other low molecular weight volatile compounds in liquid matrices by headspace gas chromatography–flame ionization detection (HS–GC–FID) and headspace gas chromatography–mass spectrometry (HS–GC–MS) are becoming commonly used practices in forensic laboratories. Although it is one of the most frequently utilized procedures, sample preparation is usually done manually. Implementing the use of a dual-rail, programmable autosampler can minimize many of the manual steps in sample preparation.

The autosampler is configured so that one rail is used for sample preparation and the other rail is used as a headspace autosampler for sample introduction into the gas chromatograph inlet. The sample preparation rail draws up and sequentially adds a saturated sodium chloride solution and internal standard (0.08%, w/v acetonitrile) to a headspace vial containing a biological sample, a calibrator, or a control. Then, the analytical rail moves the sample to the agitator for incubation, followed by sampling of the headspace for analysis. Using DB-624 capillary columns, the method was validated on a GC–FID and confirmed with a GC–MS. The analytes (ethanol, acetonitrile) and possible interferences (acetaldehyde, methanol, pentane, diethyl ether, acetone, isopropanol, methylene chloride, n-propanol, and isovaleraldehyde) were baseline resolved for both the GC–FID and GC–MS methods. This method demonstrated acceptable linearity from 0 to 1500 mg/dL. The lower limit of quantitation (LOQ) was determined to be 17 mg/dL and the limit of detection was 5 mg/dL.