Simultaneous Detection and Quantitation of Diethylene Glycol, Ethylene Glycol, and the Toxic Alcohols in Serum using Capillary Column Gas Chromatography

Robert H. Williams1,*, Steve M. Shah1, Jack A. Maggiore1, and Timothy B. Erickson2

1 Department of Pathology, Division of Clinical Pathology (M/C 750), University of Illinois at Chicago Medical Center, 840 South Wood Street, 201G CSB, Chicago, Illinois 60612 and 2 Department of Emergency Medicine, Division of Toxicology (M/C 724), University of Illinois at Chicago Medical Center, 808 South Wood Street, 471 CME, Chicago, Illinois 60612

Abstract

Determination of toxic glycols and alcohols in an emergency setting requires a rapid yet accurate and reliable method. To simultaneously determine diethylene glycol (DEG) along with ethylene glycol, methanol, isopropanol, acetone, and ethanol, we modified a previously developed gas chromatographic (GC) method. The system used a Hewlett-Packard 6890 GC with EPC, a Gooseneck splitless liner, and an Rtx-200 capillary column (30 m x 0.53-mm i.d., 3 mm). After serum samples were deproteinized using ultrafiltration (Millipore Ultrafree-MC), 1 mL of the protein-free filtrate was manually injected into the GC. Internal standards for alcohols (and acetone) and glycols were n-propanol and 1,3-butanediol, respectively. All compounds eluted within 3.5 min (linear temperature gradient from 40 to 260°C; total run time was 6.5 min. Limit of detection and linear range for all compounds were 1 or 2.5 mg/dL and 0–500 mg/dL, respectively. In addition, there was no interference from propionic acid, propylene glycol, and 2,3-butanediol. The modifications in the equipment and temperature program allowed increased resolution and thus, detection and reliable quantitation of DEG and other common toxic glycols and alcohols of clinical interest.

Introduction

Acute intoxication by the common toxic alcohols, methanol, isopropanol (and its metabolite acetone), and ethylene glycol, is well known in toxicology. Methanol and ethylene glycol through the generation of toxic metabolites produce a profound metabolic acidosis (1). The metabolites of methanol can cause severe damage to the optic nerve, whereas the metabolites of ethylene glycol cause renal and pulmonary damage (1). Isopropanol is also toxic—it is very caustic to the gastrointestinal tract—and its metabolite, acetone, is a major CNS depressant (1). However, because no acidic products are produced during the metabolism of isopropanol, metabolic acidosis is rarely observed unless the patient is very hypotensive (increased lactic acid production) (1). All three alcohols cause an increase in the osmolal gap.

Diethylene glycol (DEG) is also very toxic; however, it is not routinely determined as part of a toxic alcohol panel. DEG is a derivative of ethylene glycol with both compounds causing acute renal failure (2,3). DEG, like ethylene glycol, can increase the osmolal gap. However, unlike ethylene glycol, DEG does not cause a metabolic acidosis (2,3).

DEG has occurred as a contaminant or has been used as a substitute for propylene glycol and glycerin in the pharmaceutical industry in some countries (2). In 1937, 105 patients died in the United States when 72% DEG was used as a diluent in the antibiotic preparation called Elixir Sulfanilamide (2). Consequently, the 1938 the Federal Food, Drug, and Cosmetic Act was passed (2). Since that time, poisoning by diethylene glycol in North America has been rare. However, it has been associated with several international epidemics having numerous fatalities over the past 60 years (3) (Table I). Because diethylene glycol, unlike ethylene glycol (or methanol), does not produce a profound metabolic acidosis, it is often missed in the differential diagnosis of a patient that presents to the emergency department with a mild acidosis and some signs of nephrotoxicity. In addition, the development of

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1937</td>
<td>United States</td>
<td>105</td>
</tr>
<tr>
<td>1967</td>
<td>South Africa</td>
<td>7</td>
</tr>
<tr>
<td>1985</td>
<td>Spain</td>
<td>5</td>
</tr>
<tr>
<td>1986</td>
<td>India</td>
<td>14</td>
</tr>
<tr>
<td>1990</td>
<td>Nigeria</td>
<td>47</td>
</tr>
<tr>
<td>1990–1992</td>
<td>Bangladesh</td>
<td>51*</td>
</tr>
<tr>
<td>1992</td>
<td>Argentina</td>
<td>26</td>
</tr>
</tbody>
</table>

* An additional 157 cases were also likely due to DEG poisoning, but not confirmed.
severe metabolic acidosis from ingestion of ethylene glycol (or methanol) depends upon the time of ingestion and presentation to the emergency department because the formation of toxic “acidic” metabolites is time dependent and highly variable, especially if high quantities of ethanol have been consumed (1). Thus, it is not uncommon for patients who present soon after ingestion of these compounds to have a normal acid-base status or be mildly acidic.

The American Academy of Clinical Toxicology has developed a set of guidelines for laboratories that serve as regional toxicology treatment centers (4). To be clinically useful, results for toxic alcohol levels need to be available within 2 h (4). Thus, a rapid set of guidelines for laboratories that serve as regional toxicology treatment centers (4). To be clinically useful, results for toxic metabolites of these compounds to have a normal acid-base status or be mildly acidotic.

Most alcohols of clinical interest are volatile, and thus can be readily determined by direct injection or headspace gas chromatography (GC) using flame ionization detection (12,13). In the past, diols required derivatization to form boronic esters prior to GC analysis because underderivatized diols chromatographed poorly and yielded low sensitivity for flame ionization detectors (14,15). However, formation of boronic esters with diethylene glycol is not possible (16), so gas chromatography–mass spectrometry (GC–MS) has been used to determine DEG and ethylene glycol (16). Methods have been developed for diol analysis that do not require derivatization; however, they are not suitable for alcohol analysis (17,18). Enzymatic methods, although used for screening purposes, have had limited success and cannot be used to determine alcohols and diols simultaneously (12,19-21).

Recently, Livesey et al. (22) developed a GC method that has the capabilities to simultaneously determine many alcohols and diols of clinical interest without interference from compounds noted in the past, such as 2,3-butanediol, propylene glycol, propionic acid, and valproic acid (19). However, the method was not optimized to detect diethylene glycol. Thus, the objective of this work was to modify the method of Livesey et al. (22) to detect DEG in addition to the common alcohols and diols encountered in the emergency setting.

Methods and Materials

Instrumentation

Analysis was performed in the splitless mode with a Hewlett-Packard (Palo Alto, CA) model 6890 GC equipped with electronic pneumatic control, a flame ionization detector, and a Restek Rtx-200 capillary column (Crossbond® trifluoropropylmethyloxane, 30 m × 0.53-mm i.d., 3-µm film thickness, Restek Corp., Bellefonte, PA). The injection port liner was a Gooseneck splitless liner (Restek Corp.). The temperature of the injector port and detector was set at 250°C. The oven temperature program consisted of 1 min at 40°C followed by a temperature gradient from 40°C to 260°C at a rate of 70°C/min. The total run time was 6.5 min. Helium carrier gas flow rate was 80.1 mL/min; for the hydrogen-air flame, the hydrogen flow rate was 40 mL/min, and the airflow rate was 400 mL/min. Integration of peak area was obtained using an HP ChemStation/Integrator data system.

Reagents, supplies, and samples

Preparation of standards. All reagents were analytical/high-performance liquid chromatography (HPLC) grade. DEG, 1-n-propanol (n-propanol), 2-propanol (isopropanol), and acetone were purchased from Sigma (St. Louis, MO). Ethylene glycol, methanol, ethanol, 1,3-butandiol, 2,3-butanediol, and propylene glycol were purchased from Aldrich (Milwaukee, WI). Stock standards for DEG, ethylene glycol, methanol, isopropanol, acetone, and ethanol were either prepared from pure chemicals (listed) at a concentration of 1000 mg/dL or purchased commercially as a custom alcohol/glycol mixture (0.1% or 1000 mg/dL) from Restek Corp. A working standard containing a mixture of alcohols/diols at a concentration of 100 mg/dL was prepared by adding 1 mL of each compound to a 10-mL volumetric flask using distilled-deionized water as the diluent. Internal standards for alcohols and acetone (n-propanol) and for glycols (2,3-butanediol) were prepared by making a 1:10 dilution of the stock standard (800 mg/dL) to obtain a final concentration of 80 mg/dL.

Control materials for precision and accuracy. Quality-control materials used to assess precision were UTAK Volatiles Plus

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Retention time (min)</th>
<th>Retention time window (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1.84</td>
<td>0.80-0.88</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.07</td>
<td>0.98-1.15</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>1.28</td>
<td>1.24-1.32</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>1.34</td>
<td>1.30-1.58</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.76</td>
<td>1.71-1.81</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>2.59</td>
<td>2.54-2.64</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>2.69</td>
<td>2.61-2.77</td>
</tr>
<tr>
<td>2,3-Butene diol</td>
<td>2.73</td>
<td>2.65-2.81</td>
</tr>
<tr>
<td>1,3-Butene diol*</td>
<td>3.07</td>
<td>2.99-3.15</td>
</tr>
<tr>
<td>Diethylene glycol</td>
<td>3.44</td>
<td>3.35-3.53</td>
</tr>
</tbody>
</table>

* Target concentration = 100 mg/dL.
* Target concentration = 100 mg/dL.
* Internal standard for alcohols.
* Internal standard for diols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>UTAK Volatiles Plus 10 mg/dL - CV (%)</th>
<th>UTAK Volatiles Plus 25 mg/dL - CV (%)</th>
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</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>3.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.4</td>
<td>8.6</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>5.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Acetone</td>
<td>4.8</td>
<td>9.6</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>9.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Diethylene glycol</td>
<td>9.8</td>
<td>8.8</td>
</tr>
</tbody>
</table>
serum-based controls (UTAK Corp., Valencia, CA) containing DEG, ethylene glycol, methanol, isopropanol, acetone, ethanol, propylene glycol, and 2,3-butanediol at final concentrations of 10 and 25 mg/dL. For accuracy studies, UTAK custom-made, serum-based calibrators containing diethylene glycol, ethylene glycol, methanol, isopropanol, acetone, ethanol, propylene glycol, and 2,3 butanediol at final concentrations of 10, 50, 100, 200, and 300 mg/dL were purchased. Additional calibrators at concentrations of 400 and 500 mg/dL were purchased for ethanol. The UTAK calibrators were also used for calibration verification.

Ultrafiltration units. To filter the standards or obtain a protein-free filtrate from serum samples, Ultrafree-MC filter units having a 10,000-Da-cutoff were purchased from the Millipore Corp. (Bedford, MA). The ultrafiltration membranes were preserved with triethylene glycol (not glycerol) to avoid potential interference.

Patient samples. The Institutional Review Board at the University of Illinois at Chicago Medical Center approved the use of this method to analyze serum samples obtained from patients who presented to our emergency department suspected of a toxic alcohol or diol ingestion.

Procedure

Calibration. To prepare standards for calibration, 25 μL of internal standard (containing 80 mg/dL of n-propanol/1,3-butanediol) was added to an Ultrafree-MC filter unit followed by the addition of 100 μL of the working standard (100 mg/dL alcohols/diols). Samples were vortex mixed briefly then centrifuged for 5 min. One microliter of the centrifuged standard was manually injected directly onto the column using a Hamilton 1-μL syringe. Calibration is set to 100 mg/dL for alcohols/diols.

Sample analysis. In this method 25 μL of internal standard (containing 80 mg/dL of n-propanol/1,3-butanediol) was added to labeled Ultrafree-MC filter units followed by the addition of 100 μL of patient sample, working standard alcohol/diol mixture (duplicate), and UTAK controls to the appropriate filter units. Samples were vortex mixed for 15 s followed by centrifugation for 5 min. After centrifugation, 1 μL of the protein-free ultrafiltrate containing the internal standards was manually injected directly onto the column using a Hamilton syringe.

Results

Table II shows the average retention time and retention time window in minutes for some of the common alcohols and diols encountered in an emergency department setting. Several chromatograms using this direct injection method with a protein-free ultrafiltrate are depicted in Figure 1. A serum-based calibrator spiked with a target value of 100 mg/dL is shown Figure 1A, and a serum-based calibrator with a target value of 10 mg/dL is illustrated in Figure 1B. Regardless of concentration the chromatographic peaks are distinct and symmetrical and baseline separation was achieved. Figure 1 also illustrates three chromatograms of samples obtained from patients who presented to our emergency department with isopropanol ingestion (Figure 1C), ethylene glycol/diethylene glycol poisoning (Figure 1D), and methanol poisoning (Figure 1E). It should be noted that the ethanol peak due to ethanol administration as part of the therapeutic regimen is also depicted in both cases (Figure 1D and 1E).

Figure 1. Chromatogram of the high alcohol/diol standard, concentration of 100 mg/dL of each alcohol and diol (A). Chromatogram of the low alcohol/diol standard, concentration of 10 mg/dL of each alcohol/diol (B). Chromatogram of a patient positive for isopropanol ingestion (C). Chromatogram of patient positive for ethylene glycol and diethylene glycol poisoning (D). Chromatogram of patient positive for methanol poisoning (E).
the isopropanol case, acetone is separated from the other common alcohols/diols; in the case of ethylene glycol poisoning, DEG is also clearly separated and distinguishable on the chromatogram. Alcohols and diols of clinical interest evaluated by this method eluted within 3.5 min. No interfering peaks appeared to be present in the chromatograms with this method modification.

Precision, accuracy, and carryover
The day-to-day precision expressed as the percent coefficient of variation (%CV) for the two levels of the UTAK commercial control (10 mg/dL, 25 mg/dL) over a period of 10 days is given in Table III. All are within acceptable limits (less than 10%) and comparable to values reported in the literature with the use of GC. The CV for within-run precision was less than 5% for all compounds.

Accuracy was performed on the UTAK commercially spiked, serum-based samples with target values ranging from 10 to 500 mg/dL, depending on the compound. Table IV shows the relative accuracy for DEG, ethylene glycol, methanol, isopropanol, acetone, and ethanol with their corresponding CVs over a period of four days. At 10 mg/dL and 50 mg/dL, all CVs were less than 15%; at all other levels, the CVs were less than 10%.

Carryover for each alcohol or diol was assessed by analyzing a high-level serum-based calibrator (300 mg/dL) followed by three consecutive serum-blank samples. No detectable carryover was noted with any of the compounds.

Linearity and limits of detection (LOD) and quantitation (LOQ)
Linearity was assessed using blank serum and serum spiked with each alcohol or diol over the range of 10 to 500 mg/dL. All calibration curves were linear over the entire concentration range; the calibration curves along with the linear regression lines are illustrated in Figure 2. Limits of detection were determined by analyzing, in triplicate, specimens that contained 0 to 10 mg/dL of each compound. The LOD and LOQ for the alcohols and acetone were determined to be 1.0 mg/dL and 2.5 mg/dL, respectively. For DEG and ethylene glycol, the LOD was 2.5 mg/dL and the LOQ was 5 mg/dL.

Discussion
Concomitant analysis of alcohols and diols poses several problems since most methods are not amenable to both classes of compounds. Most alcohols of common clinical interest are volatile, and thus these compounds are readily analyzed without pretreatment by direct injection using headspace GC (12,13). Compounds such as ethylene glycol and DEG generally require manipulation of the sample by derivatization prior to
Figure 2. Linearity plots using regression analysis for ethanol (A), methanol (B), isopropanol (C), acetone (D), ethylene glycol (E), and diethylene glycol (F). Theoretical concentrations were 0, 10, 50, 100, 200, 300, 400, and 500 mg/dL.
analysis with GC (as with ethylene glycol) or GC–MS (as with DEG) (14–16).

Methods have been developed to measure ethylene glycol and/or DEG that use direct injection onto the column in a GC (17,18), however, they cannot be adapted to simultaneously measure the common alcohols. One of the problems encountered with GC of diols, especially with direct injection onto the column, is incomplete elution from the column, causing contamination at the GC inlet/liner interface, carryover, and peak tailing. Unlike other methods in use, the present method did show a problem with carryover or peak tailing after 100 consecutive manual injections. Use of protein-free ultrafiltrates rather than whole serum permitted direct column injection for the analysis of DEG and the other common toxic diols and alcohols. By using the Hewlett-Packard 6890 GC in the splitless mode along with modification of the liner and temperature program, an increase in resolution was noted compared to the original method of Livesey et al. (22). Simultaneous detection of DEG along with some of the other common diols and alcohols of clinical interest was also achieved.

The increase in inlet/detector temperature and higher temperature gradient provided complete elution and excellent baseline separation of DEG and ethylene glycol along with the common toxic alcohols. This method is rapid (calibration, controls, and patient samples analyzed within an hour) and thus meets the criteria established by the American Academy of Clinical Toxicology to measure toxic alcohols. This method is rapid (calibration, controls, and patient samples analyzed within an hour) and thus meets the criteria established by the American Academy of Clinical Toxicology for turnaround time for toxic alcohol and diols (4). This method has been in clinical use for over a year for patients seen in our emergency department and has had a positive impact on the treatment of our patients because of the reduction in turnaround time.

References


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