Simultaneous Determination of Alcohols and Ethylene Glycol in Serum by Packed-or Capillary-Column Gas Chromatography

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We developed a packed-column chromatographic procedure capable of simultaneous quantitation of methanol, ethanol, isopropanol, acetone, and ethylene glycol. This method was then updated to a rapid, sensitive, wide-bore capillary method. The packed-column system uses direct injection of 1 μL of Na2WO4/H2SO4-deproteinized serum onto a 1.8 m × 2 mm (i.d.) column packed with 80/100 HayeSep R. A linear temperature gradient from 90 to 205°C allows complete elution of all components within 20 min; minimum detection limits are 2 mmol/L. The wide-bore capillary method uses 0.1 μL of sample deproteinized by ultrafiltration, injected onto a 30 m × 0.53 mm (i.d.) 3-μm Rtx-200 (Restek) column. Baseline resolution to a minimum detection limit of 0.1 mmol/L of all compounds is achieved in 5 min with a linear temperature gradient from 40 to 250°C and dual internal standards of n-propanol and 1,2-butandiol.

Indexing Terms: methanol/toxicology

Diagnosis and treatment of intentional and accidental poisonings after ingestion of methanol, isopropanol, or ethylene glycol require accurate, sensitive, rapid, and reliable methods of analysis. Previous chromatographic methods (1–7) have required separate columns and (or) chromatographic systems to analyze alcohols and diols, time-consuming and potentially incomplete derivatizations of ethylene glycol, and long analysis times. Enzymatic methods (8, 9) for these compounds have had only limited success and do not allow simultaneous quantitation of all alcohols and diols.

Here we describe two different single-run gas-chromatographic (GC) methods for the simultaneous quantitation of alcohols and diols, with minimal preanalytical sample preparation.4 The first uses packed columns, with a time between injections of 20 min. The second uses a wide-bore capillary column to attain >10-fold improvement in detection limits, with injections possible every 10 min.

Materials and Methods

Materials

Chemicals. OmniSolv®-grade methanol, acetone, 2-propanol, and sodium tungstate were obtained from BDH (Toronto, ON). Ethylene glycol (1,2-ethanediol), isobutyl alcohol, 1-butanol, isopentyl alcohol, 1-pentanol, formaldehyde, chloroform, dichloromethane, acetonitrile, ethyl acetate, isopropylacetone, 2,4-pentanediol, cyclohexanone, glycerol, and dimethyl sulfoxide were from Fisher Scientific (Nepean, ON). Propionic acid, n-propanol, 1,2-butanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,3-butanediol, 2-methyl-2,4-pentanediol, 1,4-butanediol, 2-butanone, 3-pentanone, 2-pentanone, and hydroxyzacetone were from Aldrich (St. Louis, MO). Oxalic acid was from Sigma (St. Louis, MO). Ethanol was obtained from Commercial Alcohols (Brampton, ON), sulfuric acid from J. T. Baker (Phillipsburg, NJ).

Standards and quality-control materials. We prepared an aqueous stock solution (generally 10 mL/L) of each alcohol and diol in distilled, deionized water. This stock was used to prepare fortified serum standards from a serum pool confirmed (by GC) to be alcohol- and diol-free. Aliquots (1 mL) were frozen (−20°C) in Nalgene cryovials until needed. After thawing, these serum standards were treated the same as ordinary serum samples, with internal standards (n-propanol for alcohols, 1,2-butanediol for diols) added at the time of analysis.

Packed-Column GC Analyses

Instrumentation. A Varian Model 3700 gas chromatograph (Varian Canada, Ottawa, ON) with dual columns and flame ionization detectors (FID) was used. The columns were 1.8 m × 2 mm (i.d.) packed with 80/100 HayeSep R (Supelco, Bellefonte, PA). The carrier gas was nitrogen at 45 mL/min. Injector and FID temperatures were 220°C; the oven temperature was programmed from 90 to 205°C at 10°C/min, and then held at 205°C for 2 min. For diols other than ethylene glycol, the final temperature was increased to 235°C. The 1-μL injections were made manually, every 30 min, with a 5-μL gas-tight syringe (Hamilton, Reno, NV). Detector output was monitored with a Recordall Series 5000 (Fisher Scientific, Nepean, ON) chart recorder.

Sample preparation. To deproteinize the serum/plasma samples and the fortified serum standards, we vortex-mixed 200 μL of each serum, 100 μL of internal standard (n-propanol, final concentration ~25 mmol/L), 100 g/L Na2WO4, and 1.3 mmol/L H2SO4 and then

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4 Nonstandard abbreviations: GC, gas chromatography-(ic); FID, flame ionization detector; and PEG, polyethylene glycol.

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centrifuged at 13 000g for 2 min. The supernates were
decanted and retained for injection.

Capillary-Column GC Analyses

Instrumentation. We used an HP 5890A GC
(Hewlett-Packard, Mississauga, ON) with an
unpurged, packed-column injection port modified for use
with a capillary column. For the inlet conversion we
used a deactivated fused-silica Uniliner® injection-port
liner and sleeve adapter (Restek, Bellafonte, PA). The
carrier gas was helium at a linear velocity of ~100 cm/s
(256 mL/min at 250°C, column head-pressure ~345
kPa). Nitrogen make-up gas (30 mL/min) was used at
the detector, with FID sensitivity set at 4 × 10⁻⁹ A
full-scale, and temperature set at 250°C. Detector output
was monitored with a Varian 4290 integrator
(Varian Canada) or an ACI chromatography worksta-
tion ( Dionex, Mississauga, ON).

All three columns tested were 30 m × 0.53 mm (i.d.),
obtained from Chromatographic Specialties (Brock-
ville, ON): (a) 5 µm DB-1; (b) 3 µm DB-624 (both from
J & W Scientific, Folsom, CA); and (c) 3 µm Rtx-200
(Restek). A 5-m length of 0.53 mm (i.d.) deactivated
fused-silica tubing was used as a retention gap/guard
column in each case.

Direct injection of 0.1-µL samples was performed
manually with a Model 1801RN (Hamilton) gas-tight
syringe with a 5-cm blunt-tip needle, through Thermo-
lite® (Restek) high-temperature septa. For automated
injections (0.1 µL) we used an HP 7673B (Hewlett-
Packard) autoinjector with nanoliter adapter through a
5-µL syringe with a 23/26-gauge tapered, removable
needle (Hewlett-Packard).

The optimized oven temperature program for the
Rtx-200 column was as follows: 40°C for 1.0 min,
increase to 250°C at 70°/min, and hold for 2 min. The
run time was 5.0 min, and the total time between
injections was ~10 min, including column cool-down
and a 1-min equilibration at 40°C. For the DB-1 and
DB624 columns, the conditions were as follows: 125°C
for 2 min, increase to 250°C at 50°/min, and hold for 2
min.

Sample preparation. To deproteinize serum/plasma
samples and the fortified serum standards, we pipetted
100 µL of serum plus 25 µL of internal standards (for
alcohols, n-propanol; for ethylene glycol, 1,2-butane-
diol; final concentrations, ~25 mmol/L) into Ultra-
free®-MC (Millipore Canada, Mississauga, ON) ultra-
filtration devices with 10 000-Da-cutoff regenerated
metabolites in serum preserved with triethylene glycol
(not glycerol). The samples were centrifuged for 5 min
t at 13 000g in a microcentrifuge, and the protein-free
filtrates were retained for analysis. For automated
injections, we transferred the filtrate to 100-µL
capillary polypropylene SnapCap GC® autoinjector vials (SRi;
Scientific Products and Equipment, Concord, ON).

Interference Studies

Interference by common therapeutic drugs was
tested by analyzing a commercial therapeutic drug
monitoring control serum, Lyphochek® Therapeutic
Drug Monitoring (TDM) Control Serum (Human) (Lev-
el 3; Bio-Rad, Anaheim, CA). Other alcohols and diols
were investigated by analyzing serum supplemented
with each compound (at 5 mL/L).

Results

Representative chromatograms are shown in Fig. 1.
The packed column and the Rtx-200 capillary column
attained baseline resolution between all the low-boil-
ing-point alcohols and acetone. All compounds of inter-
est were eluted within 3 min. There were no coelution
problems with compounds previously reported (10, 11)
to interfere with ethylene glycol. In contrast, when we
used the oven temperatures required for accurate measure-
ment of diols, the DB-1 and DB-624 capillary columns
were unable to separate acetone from isopropanol,
and the low-boiling-point alcohols were not base-
line-resolved (chromatogram not shown). Retention
times are presented in Table 1.

Detection limits for all analytes were 0.1 mmol/L on

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Fig. 1. (A-C) Capillary GC analyses of alcohols and diols on a
Rtx-200 column, 0.1-µL injection volume; (D) GC analysis on
HaysSep 80/100 packed column, of alcohols and diols in 1.0 µL
of the calibrator shown in (A).

Chromatographic conditions as described in Materials and Methods. (A)
Serum calibrator, with analytes at 1 mL/L. Peak identities and concentrations:
1, methanol 24.7 mmol/L; 2, ethanol 17.0 mmol/L; 3, isopropanol 13.1
mmol/L; 4, n-propanol 21.4 mmol/L (internal standard for alcohols and
acetone), 5, acetone 13.6 mmol/L; 6, ethylene glycol 17.9 mmol/L; 7,
propylene glycol 13.6 mmol/L; 8, 2,3-butanediol 11.0 mmol/L; 9, 1,2-butane-
diol 17.9 mmol/L (internal standard for diols). (B) Serum from patient positive
for methanol (14.1 mmol/L) and ethylene glycol (3.0 mmol/L). (C) Serum from
patient positive for ethanol (30.1 mmol/L), isopropanol (4.3 mmol/L), and
acetone (17.8 mmol/L).
Table 1. Retention times of alcohols and diols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rtx-200 wide-bore capillary</th>
<th>HayeSep R packed column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.59</td>
<td>2.2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.72</td>
<td>4.6</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0.85</td>
<td>6.8</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>1.09</td>
<td>8.0</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.32</td>
<td>6.0</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>1.82</td>
<td>-</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>2.01</td>
<td>11.2</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>2.17</td>
<td></td>
</tr>
<tr>
<td>2,3-Butanediol</td>
<td>2.35</td>
<td></td>
</tr>
<tr>
<td>1,2-Butanediol</td>
<td>2.51</td>
<td></td>
</tr>
<tr>
<td>1,3-Propanediol</td>
<td>2.58</td>
<td></td>
</tr>
</tbody>
</table>

The Rtx-200 column and 1 mmol/L on the HayeSep R column. Day-to-day precision (CV) was as follows: For the HayeSep R (n = 52), methanol = 8.9% at 26.51 mmol/L and ethylene glycol = 13.6% at 19.6 mmol/L; for the Rtx-200 (n = 30), methanol = 6.4% at 9.8 mmol/L, ethanol = 5.0% at 6.9 mmol/L, 2-propanol = 4.7% at 5.2 mmol/L, and ethylene glycol = 6.2% at 6.8 mmol/L. The HayeSep R system was linear (r > 0.99 with deproteinized serum samples) for concentrations of methanol, ethanol, 2-propanol, and ethylene glycol over the range 0–40 mmol/L. Results of least-squares regression for the Rtx-200 system are summarized in Table 2 (note: linearity is dependent on FID response and will vary with instrumentation). Recovery studies were conducted by standard addition technique; results for the capillary method are shown in Table 3. Table 4 lists the therapeutic drugs and metabolites tested and found not to interfere with the wide-bore capillary method. Table 5 lists retention times on the wide-bore system for various other organic compounds. None of these compounds (except for acetaldehyde; see Discussion) coeluted with the target analytes.

Compared with the performance of the packed-column method, the wide-bore capillary column provided roughly 10-fold lower detection limits for alcohols and diols and required less than half the analysis time. Within-run and between-day CVs were also lower by the capillary GC method.

Discussion

Traditionally, most laboratories have used separate chromatographic conditions for analyzing alcohols and diols. To avoid the system contamination seen with direct injection of whole blood (12) or deproteinized serum (4), researchers have used headspace GC for analysis of volatile alcohols (13, 14). Less-volatile compounds such as ethylene glycol usually require derivatization (1, 5, 6) before analysis. Aarstad et al. (15) quantitated ethylene glycol without derivatization by using direct injection onto a packed column; however, their high initial oven temperature did not allow quantitation of low-boiling alcohols. For laboratories receiving specimens that might contain alcohols and diols, a two-system approach is too time-consuming and labor-intensive. We therefore developed a single packed-column chromatographic system capable of analyzing alcohols and diols without sample derivatization.

The packed-column method we developed (16) for the analysis of serum alcohols and ethylene glycol uses a mid-polarity, acid-resistant porous polymer packing. The HayeSep R column gave good separation of low-boiling alcohols, was fairly robust, and involved relatively simple sample preparation. However, declining accuracy at medical decision thresholds (e.g., ∼2 mmol/L for ethylene glycol) and excessive run times warranted development of a more rapid and sensitive assay. Therefore, we decided to update the method to a wide-bore capillary system.

Several authors have described capillary GC methods for the analysis of alcohols or ethylene glycol

![Image](Image 33x15 to 579x777)

Table 2. Least-squares statistics for wide-bore capillary system.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc range, mmol/L</th>
<th>Slope</th>
<th>r²</th>
<th>Intercept</th>
<th>SE, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0–50</td>
<td>1.01</td>
<td>0.9995</td>
<td>0.02</td>
<td>0.562</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0–35</td>
<td>1.01</td>
<td>0.9988</td>
<td>0.07</td>
<td>0.265</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>0–35</td>
<td>1.00</td>
<td>0.9977</td>
<td>−0.31</td>
<td>0.318</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0–27</td>
<td>1.00</td>
<td>0.9999</td>
<td>0.00</td>
<td>0.149</td>
</tr>
<tr>
<td>Acetone</td>
<td>0–27</td>
<td>0.87</td>
<td>0.9907</td>
<td>0.06</td>
<td>0.318</td>
</tr>
</tbody>
</table>

Table 3. Recovery of alcohols and diols from supplemented plasma.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Added</th>
<th>Measured</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>49.40</td>
<td>45.21</td>
<td>91.52</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9.88</td>
<td>9.58</td>
<td>96.96</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>34.26</td>
<td>32.09</td>
<td>93.87</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>35.86</td>
<td>35.90</td>
<td>100.1</td>
</tr>
<tr>
<td>2,3-Butanediol</td>
<td>22.08</td>
<td>21.52</td>
<td>97.46</td>
</tr>
</tbody>
</table>

Table 4. Compounds that do not interfere with alcohol or diol analysis at the stated concentrations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc</th>
<th>Compound</th>
<th>Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>795 μmol/L</td>
<td>Phenytoin</td>
<td>99 μmol/L</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>835 nmoVL</td>
<td>Primidone</td>
<td>53 μmol/L</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>60 μmol/L</td>
<td>Procarbamide</td>
<td>53 μmol/L</td>
</tr>
<tr>
<td>Desipramine</td>
<td>791 nmoVL</td>
<td>Propanolol</td>
<td>855 μmol/L</td>
</tr>
<tr>
<td>Digoxin</td>
<td>3.8 nmoVL</td>
<td>Quinidine</td>
<td>20 μmol/L</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16.1 μmol/L</td>
<td>Salicylate</td>
<td>3.1 μmol/L</td>
</tr>
<tr>
<td>N-Acetylpromazineamide</td>
<td>34 μmol/L</td>
<td>Theophylline</td>
<td>164 μmol/L</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>207 μmol/L</td>
<td>Trobamcin</td>
<td>16.1 μmol/L</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>891 μmol/L</td>
<td>Vancomycin</td>
<td>39 μmol/L</td>
</tr>
</tbody>
</table>
(2, 5, 17–19). However, we were unable to find any reports of a single system for analyzing both alcohols and diols. Despite the fact that a mega-bore column is recommended as the first step in converting a packed-column method to a capillary system (20), we encountered several problems in transferring our packed-column method to a wide-bore column. These included column stationary-phase selection, sample preparation, choices of injection mode and technique, injection port septa, and type of syringe and needle style used for injection.

**Column selection.** A nonpolar phase such as dimethyl-polysiloxane (used in e.g., DB-1, HP-1, Rtx-1) may be appropriate for analysis of polar compounds (e.g., alcohols and diols) in nonpolar solvents, as is commonly depicted in column manufacturers’ literature. However, a 0.53-mm (i.d.) column of reasonable length (30 m) cannot separate these solutes when they are injected in water or a water-based matrix such as serum or plasma. At the low (30–40°C) initial oven temperature required for analysis of alcohols, the diols remain in the water bolus as it moves down the column. The result is extensive peak broadening and flattening, sometimes so severe that it is impossible to distinguish from baseline an ethylene glycol peak in fortified samples known to contain contaminants as great as 5 mmol/L. Increasing the initial oven temperature to 125°C (21) facilitates cold-trapping of ethylene glycol while allowing the water vapor to proceed down the column. However, with injection temperatures 25°C above the solvent’s boiling point, there is no solvent-effect focusing of peaks, which is necessary for baseline resolution of low-boiling alcohol. Also, acetone coelutes with isopropanol. Similar results were obtained with a more polar DB-624 column (6% cyanopropylphenyl/94% dimethyl-polysiloxane). Even with a retention gap installed to facilitate concentration of the alcohols, neither column was suitable for separating aqueous alcohols and diols in a single run.

Highly polar polyethylene glycol (PEG) phases (such as DB-Wax, HP-20M, Stabilwax) have been used to analyze alcohols or diols (3, 13). However, we did not attempt our analysis on a PEG column for two reasons: First, the phase cannot separate ethanol from isopropanol in short, wide-bore columns; second, PEG phases are more susceptible to damage induced by water, oxygen, and the higher temperatures required to elute some of the less-volatile constituents of serum. This translates into increased column bleed, with its attendant decrease in sensitivity, and shorter column lifetime.

Of many phases of intermediate polarity that might provide the desired separation, we chose the Rtx-200 column for the unique selectivity towards lone pair electrons provided by its trifluoropropylmethyl-polysiloxane phase. The highly electronegative fluorine atoms have a strong affinity for the hydroxyl groups in alcohols and diols, for ketones, and for chlorofluorocarbons. The elution order of the alcohols may be predicted by boiling point, and by the number of hydroxyl groups present. Ketones elute later than their boiling point would predict (Table 1), so acetone is more strongly retained than the group of monohydroxyl alcohols that are of interest here. This selective retention provides additional flexibility in terms of usable temperatures at the beginning of the chromatographic run, there being fewer weakly retained compounds to be resolved. The only compounds found to coelute were acetaldie-
hyde and ethanol; however, because serum acetaldehyde concentrations do not reach mmol/L levels, this problem was not judged to be clinically significant.

Sample cleanup. Acid precipitation is a commonly used method for deproteinizing serum samples. However, bonded-phase columns may be ruined by exposure to even small amounts of acidic solutions. To avoid precipitation of samples with large amounts of salts (which are deposited in the chromatographic inlet, and serve as active adsorption sites), we tested filters of various molecular mass cutoffs from several manufacturers. All but the UFC3-LGC type (Millipore) use glycerol as a membrane preservative, which is eluted as a very large peak (~100-fold the internal standard peak area) late in the chromatographic run. The LGC units use triethylene glycol, which, while eluting later than glycerol, is leached into the sample in amounts usually less than the concentrations of the internal standards (25 mmol/L). The 10 000-Da cutoff membrane removes >99.5% of albumin and >95% of cytochrome c (mass ~13 000 Da). The flow rate through the membrane is high enough to allow sufficient recovery (~50 μL) of filtrate after 5 min in a laboratory microcentrifuge. The regenerated cellulose membrane can be used with most organic solvents, including acetone, at concentrations likely to be found in serum. We have validated this method for use with serum or plasma; however, whole-blood analysis may be possible with alternative sample preparation.

Internal standards. n-Propanol is widely used as an internal standard for GC analysis of methanol (4, 14, 22, 23). Packaged-column/derivatization methods for ethylene glycol have used various internal standards, including 1,3-propanediol (7) and 1,2-propanediol (11); recent wide-bore capillary techniques (19) have used 2,3-butanediol. We have found 2,3-butanediol to be an occasional component of serum samples analyzed in our laboratory, and Porter et al. (24) recently suggested that this compound may be a metabolite of 2-butane, a denaturant in alcohol preparations. We elected to use a dual internal standard approach: n-propanol for alcohols, and 1,2-butanediol for diols, neither compound being a natural component of serum. We have also found 1,3-butanediol to be an acceptable internal standard for ethylene glycol.

Injection mode. The injection mode was limited by the hardware available: Our GC is equipped with an unpurged, packed-column injection port. Instead of making an expensive modification such as installing a dedicated on-column capillary port, or a split-injection port, we obtained capillary-column adaptors and injection-port liners of several types from two manufacturers. The best results were obtained by using a 5-mm (o.d.) Uniliner inside a metal adaptor (to reduce the likelihood of crushing the glass liner while making a gas-tight seal).

Direct injection, in which near-instantaneous vaporization of injected samples is the goal, is suitable for a large proportion of GC analyses. After vaporization of the sample in an expansion chamber within the port liner, the analytes of interest are moved to the column head by the carrier gas. Nonvolatile, high-boiling components of the sample are deposited in the port liner. These deposits eventually accumulate in sufficient quantities to affect subsequent analyses, but cleaning (with methanol:dichloromethane:water, 3:1:1 by vol) and deactivation (with dimethyldichlorosilane) of the liners is an accepted part of regular maintenance.

Careful sample cleanup will extend the scheduled maintenance interval. Ultrafiltration of the serum samples removes most of the nonvolatile residues, including proteins >10 000 Da, which might otherwise foul the column. Any contaminants that do accumulate may be removed by cutting ~1 m from the head of the guard column (retention gap). If this does not restore acceptable chromatography, the capillary column may be further cleaned by rinsing with methanol:dichloromethane:water (3:1:1, by vol). In our laboratory, solvent rinsing has not been required more than once every 1000 injections.

Hot on-column injection was attempted to avoid both discrimination and flashback problems. This mode is made possible by inverting the Uniliner to allow insertion of a 26-gauge needle directly into the head of the column. On-column injection yielded well-formed peaks with no tailing evident as long as the column head was clean. However, as nonvolatile sample residues accumulated, active solutes such as alcohols and diols, including ethylene glycol, begin to adsorb to the column, again resulting in underestimation of the sample concentrations of these analytes. Chromatographic deterioration was significantly more rapid with this method than with direct injection.

Choice of septum. The choice of injection-port septum is important with both on-column and direct vaporizing modes of injection. Because septum particles deposited directly into the column or trapped in a vaporizing chamber act as adsorption sites for alcohols and diols, a septum resistant to coring will reduce system maintenance requirements. We found the Thermolite septa to exhibit only minimal coring, even after ~100 manual injections. Further, these septa produced very low "bleed" signals at high column temperatures. This contributes to better quantitation of high-boiling solutes, which may coelute with some of the bleed peaks observed with other septa.

Needle. Septum coring was further reduced by using a tapered 26/23-gauge needle for automated injections, or, for manual injections, a blunt-tipped needle rather than the 22°-beveled needle usually recommended. Syringes with removable needles were used to minimize replacement costs when needles were bent or became plugged from repeated septum penetration. Modification to a septumless injection port (e.g., by installation of a Merlin Microseal) is not possible with our instrument, but for those whose inlet configuration is compatible, this option might further prolong the intervals between maintenance.

Syringe. The choice of syringe for injections also greatly affected assay reproducibility. For manual in-
jections with a standard microliter syringe, injecting water after the sample injection revealed that repeated syringe rinsing (>25×) was usually insufficient to remove ethylene glycol and glycerol or triethylene glycol from the syringe. To avoid this carryover of viscous solutes between injections, we used a gas-tight (Hamilton 1801RN) syringe. Without the sealing action of a Teflon tip on the plunger, septum penetration allows venting of carrier gas, so that sample is forced around the plunger and up the syringe barrel by the column headpressure. In contrast, the rapid plunger action of the autoinjector sometimes failed to aspirate sample when used with a gas-tight syringe. We therefore used a standard syringe for the automated injections. The “blowback” seen in manual injections was not a problem when we used the injector’s “fast” mode, and no carryover was seen between injects.

In summary, we have developed a rapid, sensitive capillary GC technique for simultaneous quantitation of alcohols and diols in serum. Several recent papers (25–29) have suggested that low concentrations of methanol may be found in alcohol abusers as well as in occupationally exposed workers and may be a useful marker of alcohol abuse (29). The recent “St. Louis” case of misidentification of propionic acid as ethylene glycol in a patient with methylmalonic acidaemia (10) emphasizes the advantages of our wide-bore system. Our method offers significant improvements in specificity, sensitivity, and turnaround time over existing techniques. In experienced hands, a sample, calibrator, and quality-control material can be analyzed and results reported in <40 min.

References