Rapid and Specific Quantification of Ethylene Glycol Levels

Adaptation of a Commercial Enzymatic Assay to Automated Chemistry Analyzers

JoEtta M. Juenke,1 Lindsay Hardy, MD,2,3 Gwendolyn A. McMillin, PhD,1,4 and Gary L. Horowitz, MD2,3

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Abstract

Ethylene glycol ingestion, accidental or intentional, can be a life-threatening emergency. Assays are not available from most clinical laboratories, and, thus, results often require many hours or days to obtain. Enzymatic assays, adaptable to automated chemistry analyzers, have been evaluated, but they have been plagued by analytic problems. With an alternative method of data analysis applied to an existing enzymatic assay, a modified assay was developed and validated on 2 different automated chemistry systems. Compared with a previously validated method based on gas chromatography with flame ionization detection, the modified enzymatic assay showed excellent agreement on patient samples (y = 1.0227x –1.24; r² = 0.9725), with a large analytic measuring range (2.5-300 mg/dL [0.4-48.4 mmol/L]). Interferences from propylene glycol, various butanediols, and other related compounds were almost entirely eliminated; when present, they generated error flags rather than falsely elevated ethylene glycol results. This modified assay should make it possible for more clinical laboratories to offer ethylene glycol measurements.

Ethylene glycol is a small organic molecule that is colorless, odorless, and sweet-tasting. It is a primary ingredient in many automobile antifreeze and brake fluid products owing to its ability to lower the freezing point of water. Ethylene glycol is sometimes ingested, accidentally or intentionally, leading to an intoxication that can be fatal if not treated. The life-threatening toxicity associated with ethylene glycol exposure is primarily a result of metabolism to glycolic acid, which is directly cytotoxic, and to oxalic acid and other acidic metabolites that chelate calcium. Ethylene glycol toxicity is characterized by an elevated osmolar gap and anion gap metabolic acidosis. Calcium oxalate can precipitate in the microtubules of the kidneys, contributing to renal failure. Glyoxylic acid may also contribute to toxicity. Ethylene glycol is a potential threat to humans and animals.

Ethylene glycol poisoning can be treated successfully by slowing or stopping the production of toxic metabolites. The most common mode of treatment is based on competitive inhibition of alcohol dehydrogenase, the enzyme that catalyzes the conversion of ethylene glycol to glycolaldehyde, a precursor to glycolic acid and oxalic acid. Competitive inhibitors used commonly include intravenous ethanol and fomepizole (4-methylpyrazole, Antizol, Paladin Labs, Dover, DE). Hemodialysis may be used to promote clearance of ethylene glycol, particularly when serum or plasma ethylene glycol concentrations exceed 50 mg/dL (8.0 mmol/L).

To minimize exposures to ethylene glycol, bittering agents have been added to some products. A closely related compound, propylene glycol, has also been used as a replacement for ethylene glycol because it shares

Upon completion of this activity you will be able to:
• describe the toxicity and therapy of ethylene glycol ingestion.
• explain the importance of reaction kinetics.
• describe 2 methods for measuring ethylene glycol and their respective limitations.

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Questions appear on p 322. Exam is located at www.ascp.org/ajpcme.
similar chemical properties but has minimal toxic potential. Propylene glycol is also used as a preservative, emollient, and a vehicle for oral and intravenous medications, with some containing as much as 80% propylene glycol,\(^{13}\) and it is a common ingredient in some pet foods.\(^ {14}\) Although propylene glycol is metabolized to relatively nontoxic compounds such as lactic and pyruvic acids, significant exposure can lead to some acidosis and elevated anion and osmolal gaps. To prevent unnecessary treatment, it is important that laboratory methods used to detect ethylene glycol poisoning are adequately specific to distinguish between ethylene glycol and propylene glycol.

Clinical findings can raise suspicion of ethylene glycol poisoning, but the diagnosis usually depends on the detection of the toxicant and/or toxic metabolites in serum or plasma. The most commonly used analytic methods for the detection and quantification of ethylene glycol are based on gas chromatography (GC) coupled to flame ionization detection (FID) or mass spectrometric detectors.\(^ {15-18}\) Unfortunately, these techniques are not automated and are not widely available in most clinical settings. Colorimetric and enzymatic methods, which could potentially be adapted for use at the point of care or with automated chemistry analyzers, have also been described.\(^ {19-21}\) One such assay, currently available for veterinary use through Catachem (Bridgeport, CT), was evaluated previously for human use and was found to exhibit poor specificity. Thus, false-positive results were observed in the presence of propylene glycol, 1,3-butanediol, 2,3-butanediol, 1-octanol, and 1,3-propanediol.\(^ {22}\)

The primary objective of the present study was to modify the Catachem assay parameters to eliminate or sufficiently minimize analytic interference to achieve adequate specificity for clinical use. By using a modified kinetic time interval and data analysis technique, all of the false-positive reactions observed previously with the Catachem assay were eliminated. Assay parameters were modified and adapted to 2 different automated chemistry systems, and results were compared with those generated by the GC-FID method.

## Materials and Methods

Ethylene glycol enzyme reaction reagents were obtained from Catachem. Ethylene glycol, propylene glycol, and 1,2-butanediol (internal standard for the GC-FID assay) were purchased from Sigma Aldrich (St Louis, MO). An internal standard solution was prepared with normal saline to contain 5 mg/dL (0.56 mmol/L) 1,2-butanediol. Methanol, ethanol, glycerol, glycolic acid, isopropanol, formic acid, n-propanol, acetone, polyethylene glycol, glycolic acid, oxalic acid, glyoxal solution, glyoxylic acid, 1,3-butandiol, 1,3-propanediol, 1-butanol, 1,2-butanediol, 1,4-butanediol, 2,3-butanediol, and 1-octanol, included in the interference studies, were also obtained from Sigma Aldrich. Supertech DOT 3 brake fluid was purchased from Wal-Mart (Bentonville, AR). Fresh frozen drug-free plasma was obtained through the ARUP blood donation center. Residual clinical plasma and serum samples were deidentified according to institutionally approved protocols.

The Catachem assay is based on a bacterial enzyme, glycerol dehydrogenase. This enzyme oxidizes ethylene glycol in the presence of NAD (nicotinamide adenine dinucleotide), generating NADH (the reduced form of NAD) and an increase in absorbance at 340 nm, which was detected spectrophotometrically in this study by using the Hitachi 917 (Roche Diagnostics, Indianapolis, IN) and Olympus AU400 (Beckman Coulter, Brea, CA) automated chemistry analyzers. A diagram of the reaction kinetics observed with ethylene glycol and propylene glycol is shown in **Figure 1**.

**Figure 1** Assay kinetics. With the reagents in this system, ethylene glycol (circles) exhibits a linear increase over time, whereas propylene glycol (squares) shows an initial increase (cycles 17-19) followed by a horizontal line. If one takes the absorbance difference between cycles 17 and 31 (original method), both compounds appear to be “positive.” In contrast, if one looks at the slope of the line between cycles 17 and 27 (new method), only ethylene glycol is positive. To convert ethylene glycol values to Système International units (mmol/L), multiply by 0.1611.
In the original method proposed by Catachem, the difference between the absorbance readings at 2 time points is used to determine the ethylene glycol concentration. In this study, the slope of the line was determined by measuring absorbance differences at several points, starting at a later time point than the original 2-point design. For samples containing ethylene glycol (indicated by the darker symbols), the difference between the 2 determinations is minimal because there is a reasonably constant increase in absorbance that is captured by the 2-point or slope approach. For samples containing compounds that mimic ethylene glycol (eg, propylene glycol, indicated by the lighter symbols), the difference between the 2 methods is substantial because the slope of the line flattens after an initial increase in absorbance. Listed in Table I are the modified instrument parameters used for the 2 separate instrument platforms.

Experiments designed to evaluate the performance of the Catachem assay with the modified parameters included accuracy, linearity, precision, and specificity. Agreement of results obtained with authentic clinical samples was evaluated for the 2 automated analyzers and a previously validated GC-FID method.

Samples for GC-FID analysis were prepared by transferring 0.10 mL of patient plasma or serum, controls, and calibrators and 0.10 mL of methanol to respective microcentrifuge tubes. After vortexing for 5 seconds, 0.4 mL of the internal standard solution was added to each tube. The sample was vortexed again and centrifuged for 5 minutes at 10,000 rotations per minute. The supernatant was then transferred to a glass autosampler vial and capped to await analysis.

The GC-FID assay was performed using an HP gas chromatograph model 5890 (Agilent Technologies, Palo Alto, CA) with a 15 m × 0.53 mm × 0.5 m capillary Supelco Nukol column (Sigma Aldrich) and a Perkin Elmer Nelson 1022 CA) with a 15 m × 0.53 mm × 0.5 m capillary Supelco Nukol chromatograph model 5890 (Agilent Technologies, Palo Alto, CA), and a Perkin Elmer Nelson 1022 CA) with a 15 m × 0.53 mm × 0.5 m capillary Supelco Nukol chromatograph model 5890 (Agilent Technologies, Palo Alto, CA). The injection volume was 2.0 μL, and the purge time was 0.2 minutes. The assay was calibrated for ethylene and propylene glycol from 5 to 100 mg/dL (0.81-16.1 mmol/L and 0.66-13.2 mmol/L, respectively). Samples with concentrations greater than 100 mg/dL were repeated at a dilution. Quantification was based on ratios of analyte and internal standard peak heights.

Results

As shown in Figure 1, the modified kinetic parameters applied to the Catachem reagent system accurately distinguished between propylene glycol and ethylene glycol.

### Table I

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<tr>
<th>Instrument/Parameter</th>
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<th>Modified Method</th>
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Thus, with the parameters recommended by Catachem, a sample that contained 20 mg/dL (2.6 mmol/L) of propylene glycol (squares) produced a result of 61 mg/dL (9.8 mmol/L) of ethylene glycol, whereas with the modified parameters, no ethylene glycol was detected. Both sets of parameters correctly quantified a sample containing 236 mg/dL (38.1 mmol/L) of ethylene glycol (circles).

### Precision

Precision and percentage of recovery were determined with 5 concentrations of ethylene glycol, each tested 20 times (5 times per day on 4 consecutive days) with the AU400. The sample set included 3 fresh frozen drug-free plasma–based samples, with target concentrations of 11, 40, and...
100 mg/dL (1.8, 6.5, and 16.1 mmol/L, respectively), and 2 quality control materials provided by Catachem, with target concentrations of 56 and 248 mg/dL (9.0 and 40.0 mmol/L, respectively). Imprecision was expressed as coefficient of variation for within-run (intra), between-run (inter), and total, as shown in Table 2. Similar results were observed with the Hitachi instrument.

Specificity

To determine if the modified assay eliminated false-positive results described previously, interferences studies were conducted for propylene glycol, 2-3-butanediol, and ethanol. Initially, 20 residual clinical samples for which ethanol testing was requested (ethanol concentrations between 0 and 261 mg/dL [0-56.7 mmol/L]) were tested with the original Catachem method (using Catachem parameters) and the modified method on the Hitachi 917 instrument. Of the 20 clinical samples, the original Catachem method produced 5 false-positive ethylene glycol results, whereas the modified method produced none. It is interesting that 2 of the false-positive results corresponded to the lowest (0 mg/dL [0 mmol/L]) and highest (261 mg/dL [56.7 mmol/L]) concentrations of ethanol tested and generated apparent ethylene glycol concentrations of 15 and 10 mg/dL (2.4 and 1.6 mmol/L), respectively. Thus, the extent of interference was not correlated with the concentration of ethanol.

This finding was investigated further by testing a 350-mg/dL (76.0-mmol/L) spiked standard of ethanol (in saline) diluted to 300, 250, 200, 150, 100, 75, 50, 25, 12.5, 6.25, and 3.125 mg/dL (65.1, 54.3, 43.3, 32.6, 21.7, 16.3, 10.9, 5.4, 2.7, 1.4, and 0.7, respectively). The spiked standard concentrations were checked for accuracy using an existing ethanol method (GC-FID). The standards were then tested on the AU400 using the Catachem assay, with both modified and original settings. All results were less than 5 mg/dL (0.81 mmol/L) ethylene glycol.

This information led us to suspect that the false-positive samples seen with the original assay were most likely due to the presence of 2,3-butanediol, which accumulates in some people with alcoholism.23 To test this hypothesis, a spiked 300 mg/dL (33.3 mmol/L) 2,3-butanediol standard (in saline), diluted to 250, 200, 150, 100, 75, 50, 25, 12.5, 6.25, 3.13, and 1.56 mg/dL, was tested on the original and modified Catachem assays on the AU400. The results were positive with both assays. On the original assay, all samples greater than or equal to 12.5 mg/dL (1.39 mmol/L) of 2,3-butanediol generated results greater than or equal to 10 mg/dL (1.61 mmol/L) apparent ethylene glycol. The modified assay triggered positive results at concentrations greater than or equal to a 25-mg/dL (2.78-mmol/L) spike, but each result was also flagged with a rate error and could, therefore, be easily identified as interference.

Propylene glycol was tested the same way, using a 300-mg/dL (39.5-mmol/L) spiked sample, diluted to 250, 200, 150, 100, 75, 38, 19, 9, 5, and 2.3 mg/dL. With the original
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Catachem assay, all spikes produced positive results (≥10 mg/dL [1.61 mmol/L]). In contrast, with the modified assay, no interferences were observed up to 100 mg/dL (13.2 mmol/L) of propylene glycol; at higher concentrations, apparently positive ethylene glycol results were generated but were easily identified as interference because the results were flagged by the rate error.

A series of 67 samples containing candidate interfering compounds were also tested with the original method and with the modified method. These samples contained combinations of the following: ethylene glycol, glycerol, propylene glycol, 2,3-butanediol, formic acid, n-propanol, isopropanol, acetone, methanol, ethanol, glycolic acid, polyethylene glycol, oxalic acid, glyoxal solution, glyoxylic acid, 1,2-butanediol, 1,4-butanediol, 1,3-propanediol, 1-butanol, 1,3-butanediol, DOT 3 brake fluid, and 1-octanol. All compounds were spiked to 300 mg/dL (varying molar concentrations), except the ethylene and propylene glycols, which were spiked to 100 mg/dL (16.1 and 13.2 mmol/L). Samples were produced by using the spikes directly and by diluting with other spikes to produce a mixture of concentrations and samples. Of these samples, 39 (58%) caused false-positive or falsely increased results using the original Catachem assay, whereas false-positives were observed with the modified method for only 4 samples, all of which contained more than 100 mg/dL (16.1 mmol/L) of ethylene glycol and required dilution for analysis by GC-FID. Only 8 samples required dilution for the modified Catachem assay.

**Discussion**

Notable for its absence from the repertoire of the vast majority of clinical laboratories is an assay for the determination of ethylene glycol levels. Although not a common intoxication, ethylene glycol ingestion is among the possible explanations of an anion gap metabolic acidosis, a frequent occurrence in emergency medicine, and, when present, it represents a life-threatening emergency, amenable to treatment. Enzymatic assays, adaptable to automated chemistry analyzers, have been reported in the literature for at least 30 years, but they have been plagued by analytic interference was likely. Samples with documented ethylene glycol concentrations greater than or equal to 5 mg/dL (0.81 mmol/L) were also tested (n = 33), spanning a range of 5 to 800 mg/dL (0.81-129.0 mmol/L) [Figure 3]. The regression statistics for these samples correlating the modified Catachem method with the GC-FID results were y = 1.0227x – 1.24; r² = 0.9725, and the Sy/x was 29.31. The relatively high standard error of the estimate is likely due to dilution error. That is, 19 of the samples contained more than 100 mg/dL (16.1 mmol/L) of ethylene glycol and required dilution for analysis by GC-FID. Only 8 samples required dilution for the modified Catachem assay.

**Patient Sample Correlations**

A total of 73 clinical samples were tested with the AU400 platform. Results obtained with the modified Catachem method were compared with results obtained by GC-FID. This study included 18 clinical samples that tested negative for ethylene glycol (<5 mg/dL [0.81 mmol/L]), 21 that tested negative for ethylene glycol but contained propylene glycol at concentrations less than 100 mg/dL (13.2 mmol/L), 1 sample that contained propylene glycol at more than 100 mg/dL (16.1 mmol/L), and 33 samples that were confirmed to contain ethylene glycol.

The results obtained with the 18 negative samples and the 21 samples containing propylene glycol at concentrations less than 100 mg/dL were negative for ethylene glycol (<5 mg/dL [0.81 mmol/L]) using the modified Catachem assay. The extremely high propylene glycol sample produced a linearity error. When this sample was analyzed with a dilution, a rate error was produced, notifying the user that analytic

![Figure 3](https://example.com/figure3.png) Ethylene glycol in patient samples. Of the 73 clinical patient samples run in parallel between the reference method and the modified enzymatic method on the Olympus AU400 automated analyzer (Beckman Coulter, Brea, CA), 33 had measurable concentrations of ethylene glycol and are shown. y = 1.0227x – 1.24; r² = 0.9725. To convert ethylene glycol values to Système International units (mmol/L), multiply by 0.1611. GC-FID, gas chromatography–flame ionization detection.
problems, largely precluding their use. The most recent such report was an evaluation of an assay currently available commercially for veterinary use.22

Careful examination of the kinetics of the reaction in that assay suggested an alternative method of data analysis. The results reported herein demonstrate that, using this method of analysis, all of the problems noted in a previous report are eliminated and the new method can be adapted to common automated clinical chemistry analyzers to facilitate rapid and accurate human testing. These modifications to data analysis programming were applied to 2 distinct open channel analyzers. Thus, our data were comparable, using 2 different instrument systems, physically located at different sites, suggesting that the assay could be modified in a similar manner for any open channel chemistry instrumentation to provide ethylene glycol testing in hospital laboratories without special instrumentation.

With the instrument parameters recommended by the kit manufacturer, clinically significant interferences (false-positives) were observed with propylene glycol, 2,3-butanediol, and 1,3-butanediol. (It is interesting that this particular glycerol dehydrogenase shows no reactivity with glycerol itself, with either set of parameters, under these specific conditions, a phenomenon that has been noted with other glycerol dehydrogenases previously.21) However, simple modifications to the assay parameters essentially eliminated such concerns. Results obtained with the modified method agreed well with those obtained by a reference method (GC-FID), suggesting that the automated assay could be used for accurate quantification of ethylene glycol. Successful application of the method to quantification of authentic patient samples containing ethylene glycol in combination with ethanol and fomepizole support use of the assay to manage decontamination testing as well. Because ethylene glycol is commonly considered as a potential poisoning, on-site testing would allow for the appropriate treatment and analytic follow-up as needed for the patient, independent of a reference laboratory. Another advantage of the automated assay is an extended analytic measurement range, allowing linearity to 300 mg/dL.

By using the assay clinically, we have observed calibration stability to at least 1 month and have experienced no issues in lot-to-lot consistency of the reagents. Quality control materials have also remained stable for at least 1 month. The labor savings over the GC-FID assay included an 85% decrease in labor time and an increase in the number of sample runs that can be performed in a day, reducing turnaround times for routine samples by approximately 10 hours. The ability to handle urgent samples has improved such that a positive result can be available within 30 minutes, instead of the 2 or 3 hours previously required by GC-FID. The assay has proven itself quite robust, and we believe it provides a practical approach to ethylene glycol testing. The Catachrem ethylene glycol reagents were successfully adapted to support testing on clinical samples from human beings.

References


