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Rising above helium: A hydrogen carrier gas chromatography flame ionization detection (GC-FID) method for the simultaneous quantification of toxic alcohols and ethylene glycol in human plasma specimens

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ABSTRACT

Here we validate a GC, Flame Ionization Detection (GC-FID), liquid injection method using hydrogen as a carrier gas combining analysis of toxic volatile alcohols (VA): methanol, ethanol, isopropanol, acetone, as well as glycols, ethylene glycol (EG) and propylene glycol (PG), in a single method.

Methodology: 200 µL of calibrator, QC, or patient specimen were deproteinized with 400 µL of acetonitrile containing internal standards (10 mmol/L N-propyl alcohol for VA and 2.5 mmol/L 1,2-butanediol for glycols). GC-FID analysis using hydrogen carrier gas and nitrogen makeup gas utilized an Agilent 7890 system equipped with Agilent 7683 liquid autosampler on a 30 m × 530 µm RTX-200 fused silica column. Method validation included repeatability, recovery, carryover, linearity, lower limit of quantification (LLOQ), accuracy, selectivity and measurement uncertainty.

Results: The 8.3 min from injection to injection reduced time of analysis by 45% over a previously reported method using Helium carrier gas with no loss in resolution. Within-run and Between-run variability were ≤1.4% and ≤6.8% respectively. Recovery was 100% within a 95% confidence interval. Carryover was negligible for all but EG. LLOQ was < 1 mmol/L for all analytes. The upper range of linearity was 120 mmol/L for methanol, ethanol and isopropanol, 100 mmol/L for acetone and 50 mmol/L for EG. Analytes demonstrated acceptable accuracy and measurement uncertainty using College of American Pathologists (CAP) criteria. Toluene can cause a false positive EG, while benzene, xylene and 1,3 butanediol can cause false negative EG.

Conclusions: Converting from Helium to Hydrogen carrier gas benefits patient care through a reduction in turnaround time and provides a cost savings to the laboratory.

1. Introduction

Accidental or intentional poisoning of patients with methanol, isopropanol and ethylene glycol are all medical emergencies with similar toxidromes resembling ethanol poisoning [1]. However, it is the downstream metabolic end-products of these compounds that are responsible for their toxicity. Expeditious clinical and laboratory assessment is critical for patient intervention and treatment. Once identified, levels in serum continue to be monitored until toxic concentrations have been reduced to safe levels and metabolic acidosis is resolved. Unfortunately, on-site availability of testing for these toxins is largely limited to referral centres and academic hospitals. For example, our laboratory is responsible for reporting these analytes for a catchment area of 85,000 km^2 that services 1.5 million people and six additional tertiary care centres. Therefore, the provision of both accurate results and rapid turnaround time is paramount.

Quantification of alcohols and glycols can be achieved by either enzymatic or gas chromatography (GC) assays [2,3]. Enzymatic assays lack specificity [4], while GC methods using with flame-ionization (GC-FID) or mass-spectrometry detection are the gold standard. GC-FID offers multi-analyte reporting with a single injection because it is possible to chromatographically resolve methanol, ethanol, isopropanol, acetone, propylene glycol and ethylene glycol using a single extraction and liquid injection. Therefore, GC-FID continues to be the preferred method due to cost-effectiveness and excellent sensitivity, despite requiring full chromatographic separation and technical expertise.

Our group has previously published a method for combined detection of toxic alcohols and glycols in a single liquid injection using an Agilent 6890 GC with FID using a Helium carrier gas [5]. Not long after we had implemented this method, there was a world shortage of Helium [6] that caused a significant increase in helium gas prices. Although new helium reserves have been identified, the non-renewable

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and limited ressource has not resulted in a price plateau or decline; a recent shortage was announced during the preparation of this manuscript [7].

Hydrogen gas offers a number of advantages compared with helium, including lower viscosity at high temperature that results in excellent resolution at increased flow rates; emerging as a renewable, cost-effective alternative. The benefit of hydrogen's lower viscosity allows for superior chromatographic separation efficiency and shorter run time [8]. Among published clinical diagnostic methods for toxic alcohols and glycols, use of hydrogen as a carrier gas is a novel concept. Historically, hydrogen gas stored in tanks posed safety concerns, however hydrogen generators that produce small quantities of hydrogen are very safe. Furthermore, hydrogen is a more sustainable and less expensive carrier gas than helium, because it can be generated from water on-demand. We report a rapid GC-FID application with hydrogen carrier gas and nitrogen make-up gas to simultaneously quantify methanol, ethanol, isopropanol, acetone, ethylene glycol and propylene glycol with a single extraction and injection followed by GC-FID chromatographic separation in patient plasma.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (MX0486), ethanol (EX0278), isopropanol (PX1834), acetone (AX0116), n-propyl alcohol (CAPX1824-6), and acetonitrile (CAAX0142-6) and 1,4 butanediol (34078) were purchased from VWR (Edmonton, Canada). Ethylene glycol (85978), propylene glycol (398039), 1,2 butanediol (177652), 2,3 butanediol (177652), and diethylene glycol (H26456) were purchased from Sigma (Oakville, Canada). Drug free serum (DFS; 456) and Liquicheck Serum Volatiles L1 (383) and L2 (384) Quality Control (QC) material was from Biorad (Mississauga, Canada). Calibration standards for methanol, ethanol, isopropanol and acetone (A-057, A-071) and Gamma Hydroxybutyrate (G-001) were purchased from Cerilliant (Round Rock, USA).

2.2. Ethics and patient specimens

This project was approved by the University of Calgary Research Ethics Board (REB17-1241). Leftover plasma specimens collected in lithium-heparin plasma gel separator tubes (BD Biosciences, 367,962) were stored at −20 °C. When necessary, the specimens that were negative for volatile alcohols and ethylene glycol were pooled and spiked with VA and EG and PG prior to analysis. PG at very low levels is common in patient specimens and samples with < 0.5 mmol/L propylene glycol were considered negative.

2.3. Sample preparation and extraction

A 200 μL aliquot of calibrator, QC or patient plasma was combined with 400 μL of acetonitrile containing internal standards (10 mmol/L N-propyl alcohol for volatile alcohols, and 2.5 mmol/L 1,2-butanediol for glycols). Specimens were briefly vortexed and centrifuged for 2 min at 10,000 ×*g*. The deproteinized supernatant was transferred into a GC sample vials that was immediately crimped and analyzed to prevent evaporation of volatiles.

2.4. Instrument calibration

Instrument calibration procedures were conducted as previously described [5]. Briefly, six point calibration curves for methanol, ethanol, isopropanol and acetone used N-propyl alcohol internal standard, while ethylene glycol and propylene glycol used 1,2-butanediol internal standard. Calibration was performed prior to the commencement of method validation and following major instrument maintenance. Two calibrations were performed over the course of the method validation phase of this study. Calibration for this method is required every 6 months, with major instrument maintenance (e.g. guard column change, main column change) and/or new stock solution of internal standards in acetonitrile. While considered minimally-toxic, Propylene Glycol was included in the calibrators as it is a common additive in food and drug formulations, and contributes to an increased osmolal gap [9].

2.5. Quality control (QC) materials

QC materials for this assay included an in-house 10 mmol/L ethylene glycol/propylene glycol in 5% albumin (EG10) as well as the Biorad Liquicheck L1 and L2. These materials were for routine QC monitoring throughout this study as well as to assess intra- and inter-day method variability. EG10 control material was prepared, aliquoted and stored at −20 °C.

2.6. Gas chromatography

GC analysis was conducted using an Agilent 7890 system with flame ionization detection (FID) and an Agilent 7683 liquid autosampler using hydrogen carrier gas and nitrogen makeup gas. The GC was equipped with a Restek 30 m × 530 μm RTX-200 fused silica column (Chromatographic specialties, 15,085, Brockville, Canada) with a deactivated fused silica (10 m × 530 μm) guard column (Agilent, 160–2535-10). The inlet liner was a split/splitless single taper, glass wool, deactivated, low pressure drop from Agilent (5183–4701).

A 1 μL sample injection with 6:1 split flow was preceded by 5 water, and 3 sample pre-injection needle washes. Injection port temperature was 250 °C and the needle dwell time was 0.25 min. Initial flow rate was 6.49 mL/min for 0.62 min, which was ramped to 18 mL/min at 20 mL/min. Oven temperature was held at 50 °C for 1.6 min and was ramped at 117.9 °C/min and held for 0.3 min at 75 °C. Finally, the oven ramped to 250 °C at 120 °C/min where it was held for 1.42 min to clear the column of residual sample constituents. Total run time was 8.3 min and data analysis was performed using Agilent Chemstation software (Version 6.9).

Analyte quantification was performed using peak area ratios of analyte to internal standard with comparison to a 6-point standard curve for quantitative analysis of each analyte.

2.7. Method validation

The method validation followed guidelines from the Clinical and Laboratory Standards Institute (CLSI), [10–12].

2.7.1. Linearity and lower limit of quantitation (LLQ)

Linearity was confirmed by spiking drug free serum with methanol (120 mmol/L), ethanol (120 mmol/L), isopropanol (120 mmol/L), acetone (100 mmol/L), and ethylene glycol (50 mmol/L). Standards were further diluted with drug free serum at ratios of 4:0, 3:1, 1:1, 1:3, and 0:4 and analyzed in quadruplicate. Instrument response was plotted relative to mmol/L expected concentration and analyzed by least squares linear regression. The lower limit of quantification (LLOQ) was performed through dilution of 2.5 mmol/L methanol, ethanol, isopropanol, acetone and ethylene glycol with drug free serum at 4:0, 1:1, 1:3 and 0:4 ratios. The LLOQ was calculated as the standard deviation (using 5 separate extracts) of the raw chromatographic counts per second (CPS), multiplying by 10, and dividing by the slope of the line between the lowest calibrator and drug free serum, as previously described [5].

2.7.2. Imprecision and accuracy assessment

Repeatability was assessed using twenty consecutive injections of the following sequence: water, DFS, L1, L2, and EG10 QC materials performed within-day. The between-day precision was assessed by

injecting the same sequence 20 times over the course of 6 weeks by different medical laboratory technologists. Accuracy assessment was performed using College of American Pathologists (CAP) survey materials from 2017 to 2018. A minimum of four samples were used to confirm accuracy of each analyte. Percent deviation was calculated relative to the All Methods Mean. Comparison studies were conducted using leftover patient plasma spiked with Alcohols and Glycols across the analytical measurement range. Slope and Intercept were calculated using Deming linear regression (Graphpad Prism 6.0, San Diego, USA).

2.7.3. Carryover and recovery

Carryover was assessed by triplicate sequential injections of water, low level sample, high level calibrator, low level sample, and water. The average concentration change in the low level sample after calibrator injection was used to calculate percent carryover.

Recovery was assessed by the standard addition method described previously [13]. Briefly, a patient sample was spiked with a calibrator containing each analyte as a baseline. Three aliquots of the baseline sample were mixed with additional calibrator and analyzed in parallel to three aliquots at baseline with a volume of water added equal to the volume of additional calibrator. The resulting difference between the expected and calculated concentrations of each of the samples was used to assess recovery.

2.7.4. Interferences

Method specificity and interferences were assessed based on relative retention times (RRT) and the presence of additional peaks. Briefly, glycerol, 2,3 butanediol, 1,4 butanediol, diethylene glycol, Gamma hydroxybutyrate, benzene and toluene were spiked into blank patient samples and patient samples positive for VA, EG, and PG. Additionally, samples of diabetic ketoacidosis (DKA), methylmalonic acidemia (MMA), and propionic academia (PA) patients were tested for additional or interfering peaks.

The potential for hemolysis, icterus, and lipemia to affect extraction efficiency were tested as previously described [5]. Patient samples were spiked with different amounts of red cell hemolysate, bilirubin ditaurate (Calbiochem, 201,102), and intralipid (Sigma, I141) to assess hemolysis, icterus and lipemia interferences, respectively.

2.7.5. Measurement uncertainty

Measurement Uncertainty has been calculated using the Uncertainty Estimation process described in EURACHEM/CITAC Guide CG 4 [14] and ISO TS 20914:2019 [15]. Maximum Allowable Uncertainty was set to the College of American Pathologists (CAP) criteria of ± 25%. In brief, there are 5 steps involved in developing the MU: Specification of the measurand; Identifying uncertainty sources; Quantifying uncertainty and converting components to standard deviations; Calculating the combined uncertainty by the sum of squares method; Calculating the expanded uncertainty (using k = 2). The measurand(s) were defined as Amount of Methanol/Isopropanol/Acetone/Ethylene Glycol concentration in plasma by GC-FID in mmol/L. The major uncertainty components identified for this method were the long term imprecision of the method (u_{Rw}), which over the course of 1 year of routine operation of the method captured the majority of variables affecting method performance, including: sample matrix effects; between-technologist pipetting variability; lot-to-lot variability for internal standard; guard column changes; 4 calibration events; daily, weekly and monthly instrument maintenance events; and 2 lot numbers of Quality Control material (Supplementary Fig. 1–3). Long term imprecision of the method (u_{Rw}) was calculated by the "top down" approach as described in ISO TS20914:2019 Medical laboratories—Practical guide for the estimation of measurement uncertainty [15]. The calibrators for the alcohols for this method purchased from Cerilliant are fully traceable to the SI through NIST, however they were modified from their original values by the addition of EG, PG and Albumin matrix which necessitated calculation of u_{cal} as described in EURACHEM/CITAC Guide CG 4. EG and PG were analytical standard grade reagents with stated % impurities on the certificate of analysis for the lot# used. Calibrator uncertainty (u_{cal}) included the following identified major contributors: u_{cal} assigned by Cerilliant, uncertainty from the pipetting events (includes: tolerance, temperature and repeatability), and uncertainty of the EG and PG standard solution used to prepare calibrator (Supplementary Fig. 2). Fresh calibrators are prepared for each calibration event, thus extraction and instrument variability from calibration events has been captured in u_{Rw}. The calculation of measurement uncertainty for the additional pipetting events and glycols purity required a "bottom up" approach and this additional uncertainty was combined into a u_{cal} estimate for this method using the sum of squares method. The Measurement Uncertainty calculation at each level of QC is presented in Supplementary Figs. 1–3 and Supplementary Table 1.

Table 1
Elution and quantification summary of solvents, internal standards, and analytes.

Analyte	Retention time (min)	Internal Standard	Relative retention time[a]
Acetonitrile[b]	2.178	N/A	N/A
N-Propanol[c]	1.89	N/A	N/A
1,2 Butanediol[c]	3.132	N/A	N/A
Methanol	1.506	N-Propanol	0.797
Ethanol	1.619	N-Propanol	0.857
Isopropanol	1.727	N-Propanol	0.914
Acetone	2.03	N-Propanol	1.074
Ethylene Glycol	2.573	1,2-Butanediol	0.822
Propylene Glycol	2.75	1,2-Butanediol	0.878

[a] Elution time of analyte relative to IS (e.g. methanol/N-propanol).
[b] Acetonitrile is necessary extraction component.
[c] Internal standards.

3. Results

The retention times of analytes and internal standards are presented in Table 1, and typical chromatograms obtained using the Hydrogen GC-FID method are presented in Fig. 1. The most significant difference between the Hydrogen GC-FID and a Helium GC-FID method was the dramatic improvement in analysis time between injections. Indeed, the Hydrogen GC-FID method was able to achieve complete baseline separation of all compounds analyzed method (Fig. 1) in 8.3 min from injection to injection versus 15 min as previously reported [5], which represents an overall 47% time savings for each injection. The reduced viscosity of hydrogen allowed both an shortened elution of analytes, with 1,2 Butanediol internal standard for Glycols eluting at 3.1 min rather than 4.5 min, as well as faster re-equilibration of the column.

Hydrogen carrier gas provided stable FID background with a low baseline signal and an excellent precision profile (Table 2). Within-day imprecision was 0.3–3.2% and between run imprecision was 4.1–8.4%. Between-run precision studies occurred over 6 weeks allowing for environmental variation, inter-operator pipetting variability, and instrument maintenance events to capture "true" imprecision. The recovery of the Hydrogen GC-FID method was well within the 25% cut-off limit for the CAP proficiency testing survey program (Supplemental Table 2), and the mean percent deviation from all methods mean was −5.5, −5.1, −4.2, −0.3, −12.7% for methanol, ethanol, isopropanol, acetone, and ethylene glycol, respectively. Comparison of patient samples with the previously published Helium method showed excellent correlation across the AMR and no clinically significant bias (Fig. 2). Measurement Uncertainty for all analytes was within CAP criteria of ± 25%, and included data for the long term performance of this method for > 1 year of routine operation on two instruments (Supplementary Table 1).

The linear range and LLOQ of each analyte is shown in Table 3.

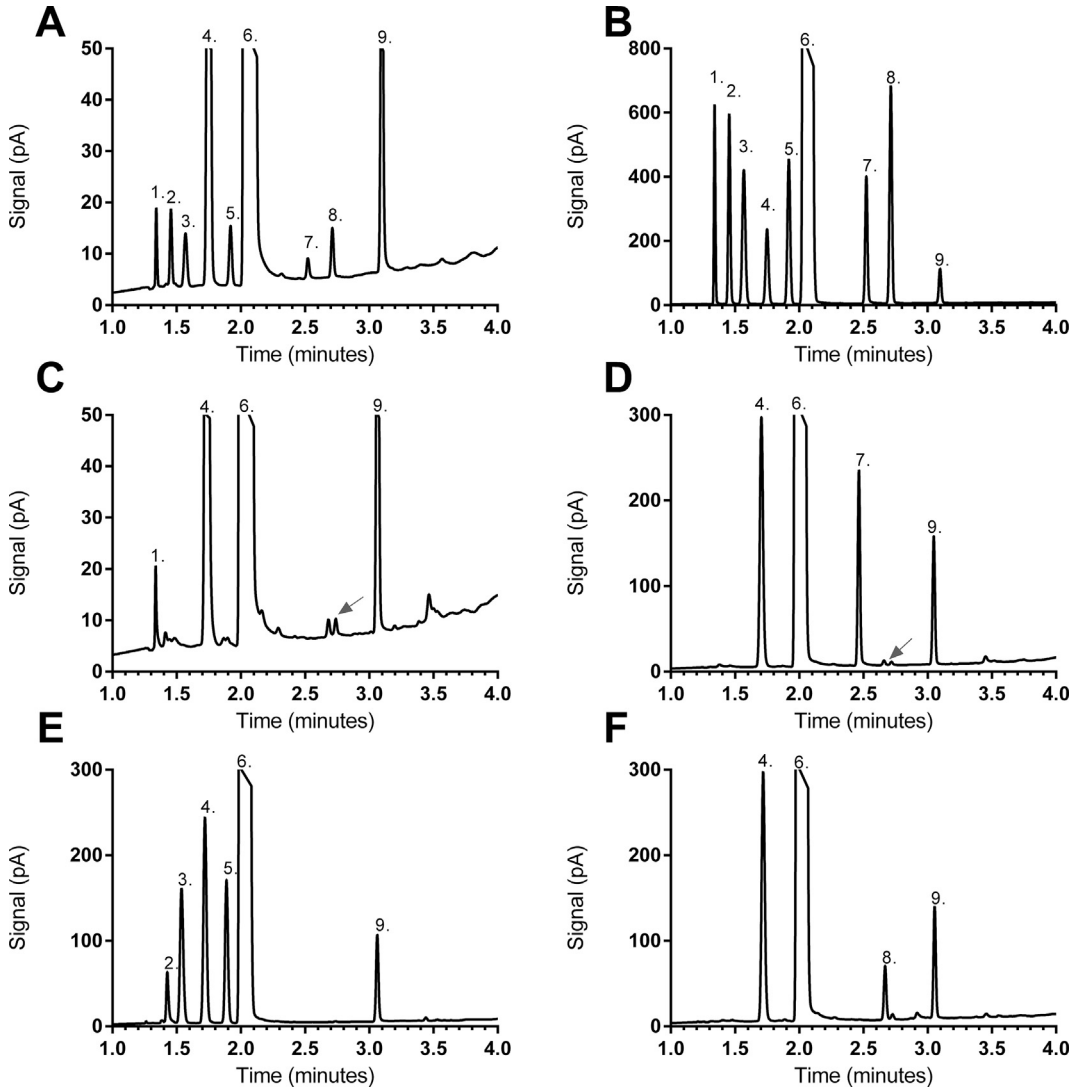

Fig. 1. Example chromatograms of (A) the lowest concentration and (B) the highest concentration calibrators. (C) Patient Sample positive for methanol (1.8 mmol/L), (D) Patient Sample Positive for Ethylene Glycol (19 mmol/L), (E) CAP Proficiency Testing Sample Positive for Ethanol (6.0 mmol/L), Isopropanol (15 mmol/L) and Acetone (16 mmol/L), (F) Patient sample positive for Propylene Glycol (4 mmol/L). Peak identities are: 1. Methanol, 2. Ethanol, 3. Isopropanol, 4. N-propanol (internal standard), 5. Acetone, 6. Acetonitrile (extraction solvent), 7. Ethylene glycol, 8. Propylene glycol, and 9. 1,2 Butanediol (internal standard). Note the acetonitrile (solvent) peak elutes at approximately 2.2 min. The volatile alcohols elute prior to the solvent peak, while the glycols elute after it. In patient samples, occasional additional peaks elute throughout the chromatogram, however these unverified peaks are only reported once they reach a height of > 100 pA, where they are reported as "Unverified Peaks", Propylene glycol is quantified and reported if > 3 mmol/L. Note the gray arrows (retention time 2.75 min) indicating the presence of PG at a concentration < 1 mmol/L in samples C and D. From 0 to 1.0 min chromatograms are blank with baseline approximately 5 pA.

While the technical LLOQ was < 0.5 mmol/L for all analytes, for practical purposes the lower end of the AMR was set to 1 mmol/L since lower values are not relevant for clinical reporting [16,17]. Analyte recovery was not statistically different from 100%, and sample carry-over was insignificant for all analytes except ethylene glycol which was 10–15% when levels > 10 mmol/L were used as the high sample (Table 3).

The evaluation of possible interfering substances in the Hydrogen GC-FID method is presented in Table 4. Toluene, benzene, xylene, 1,3-butanediol would interfere with this method because these co-elute with peaks of interest. In the serum of Diabetic Ketoacidosis (DKA) patients, acetone is physiologically present and was detected in these samples. No interferences were observed in serum from the methylmalonic acidemia (MMA) or propionic acidemia (PA) patients. Hemolysis, lipemia, and icterus did not interfere with the Hydrogen GC-FID method. Diethylene Glycol and Gamma Hydroxybutyrate do not interfere with Glycols analysis but are both detectable by this method and elute after the glycol internal standard at 4.1 and 4.15 min, making them indistinguishable chromatographically. If unidentified peaks are seen in this region of the chromatogram, the sample should be sent for Mass Spectrometry identification by an alternate method. 1,4 Butanediol was not detected by this method.

4. Discussion

This study validates a combined toxic alcohol and glycol testing GC-FID method using hydrogen carrier gas. The analytical characteristics of this method were as good or superior to contemporary one-step extraction assays using helium carrier gas [5] with a 47% reduction in injection to injection time. Moreover, the method is robust and has been implemented in a Regional tertiary care hospital clinical laboratory with > 30 individual operators offering analyses 24 h a day. The Measurement Uncertainty of the method calculated using a long-term precision estimate over 1 year of routine operation meets CAP acceptability criteria, providing evidence that this method is suitable for use in a clinical laboratory reporting patient results.

Table 2
Summary table of inter- and intraday variability of the hydrogen carrier gas GC-FID method.

Analyte	Within run (Intraday)		Between run (Interday)	
	Measured concentration	Coefficient of variation	Measured concentration	Coefficient of variation
	mmol/L		mmol/L	
Methanol	3.23	0.60%	3.07	4.40%
	24.66	0.30%	23.68	4.20%
Ethanol	12.13	0.60%	11.42	4.50%
	32.74	0.30%	31.54	4.30%
Isopropanol	5.16	0.60%	4.93	4.10%
	13.59	0.40%	13.11	4.10%
Acetone	1.86	1.30%	1.76	4.10%
	14.73	0.70%	13.91	4.50%
Ethylene glycol	1.66	1.40%	1.62	6.20%
	4.33	1.10%	4.23	6.80%
	10.73	3.20%	9.89	8.40%

Table 3
Parameters of the validated hydrogen GC-FID method.

Analyte	Tested linear range	LLOQ	Average recovery	Average carryover
	mmol/L		%	%
Methanol	1–120	0.286	97.8 ± 9.9	0
Ethanol	1–120	0.421	102.0 ± 4.8	0
Isopropanol	1–120	0.034	107.1 ± 9.4	0
Acetone	1–100	0.046	97.0 ± 9.3	0
Ethylene glycol	1–50	0.109	109.3 ± 7.7	10–15

4.1. Converting from helium to hydrogen

Hydrogen gas has lower viscosity at higher temperatures than helium, which offers greater chromatographic separation efficiency due to a more constant linear velocity across a large range of GC oven temperatures [8]. This characteristic led to a reduction in our method run time from 15 min to 8.3 min. We also achieved greater analytical sensitivity for hydrocarbon compound analysis by using Nitrogen as a makeup gas as evidenced by reduced LLOQ values for each analyte target relative to the previously reported helium method [5]. Sensitivity improvements were not accompanied by additional non-specific peaks in patient serum and the low baselines achieved with hydrogen carrier gas had very little noise.

Hydrogen carrier gas is also an attractive option to helium from an operational and sustainability perspective. Global helium gas shortages had approximately doubled the costs of helium supply for our laboratory, whereas hydrogen gas can be generated in house from water and Nitrogen gas can be extracted from compressed air. The Hydrogen/Nitrogen combination leads to reduced supply costs overall for the GC-FID.

4.2. Method optimization

The instrument software includes a conversion calculator, which provided initial conversion parameters to transition from helium to hydrogen carrier gas. These suggested parameters were further optimized to reduce the run time by adjustment of both flow rate and oven temperature gradients. Users aiming to operate this method into their laboratory must be aware that inter-instrument differences may require additional method development prior to implementation.

Column separation of glycols requires careful consideration because the presence of active hydroxyl groups make gas phase transitioning difficult. Therefore, special considerations were required to optimize EG quantitation and carryover. Holding the needle in the injection port for a 0.25 min dwell time and initially slowing the gas flow rate (2 mL/

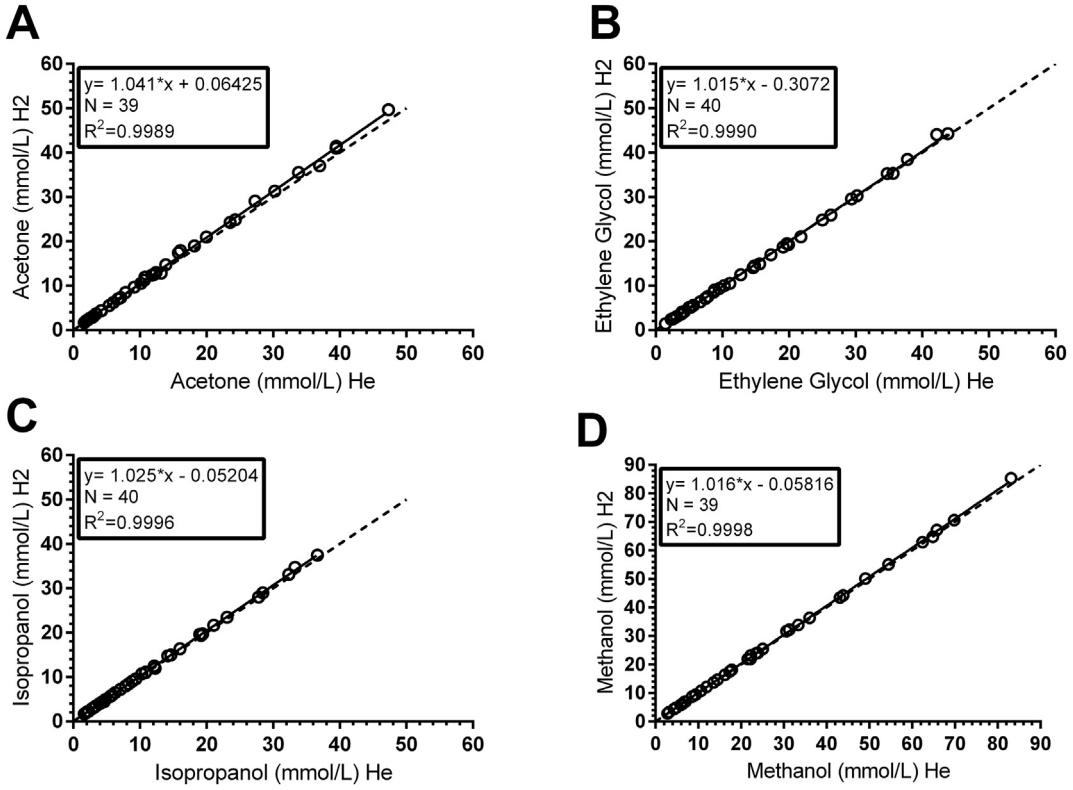

Fig. 2. Comparison of measured levels of acetone (A), ethylene glycol (B), isopropanol (C), methanol (D), in spiked patient plasma using the Hydrogen GC-FID method (y-axis) and a Helium GC-FID method (x-axis).

Table 4
Summary of interferences and specimens tested during method development.

Interference	Retention time (min)	Relative retention time[a]	Interference?
Toluene	2.6	0.83	Yes - EG
Benzene/Xylene	2.932, 2.973, 3.102[b]	0.936, 0.949, 0.990[b]	Yes - 1,2 Butanediol (IS)[c]
1,3 Butanediol	3.14	1	Yes - 1,2 Butanediol (IS)
2,3 Butanediol	2.936	0.94	No
Diethylene Glycol	4.114	1.31	No
Gamma hydroxybutyrate	4.15	1.36	No
1,4 Butanediol	N/D	N/A	
Diabetic Ketoacidosis	1.89	1.1	Acetone Peak
MMA Patient[d]	N/D	N/A	No
PA Patient[e]	N/D	N/A	No
Lipemia (559)[f]	N/D	N/A	No
Icterus (> 92)[f]	N/D	N/A	No
Hemolysis (> 892)[f]	N/D	N/A	No

[a] Relative retention time was calculated for the interfering substance retention time relative to the 1,2 butanediol internal standard.
[b] Three interfering peaks were identified. Relative retention times were calculated respectively.
[c] IS, internal standard.
[d] MMA, methylmalonic academia.
[e] PA, propionic academia.
[f] Serum lipemia, icterus, hemolysis indices are in brackets and were measured using cobas 8000 serum indices measurements.

min for 1 min) improved peak shapes for all analytes. We assert the additional time permitted "sticky" glycols to vaporize, enter the column, and elute as a uniform glycol peak.

Sample deproteinization using acetonitrile was necessary for this method. Other published methods for Ethylene Glycol analysis have used acetone as a deproteinizing agent [4], however, this would not allow for measurement of alcohols and glycols in a single injection method as Acetone is frequently present in patient specimens on a physiological level at our institution. Unfortunately, acetonitrile elutes as a large peak, and a temperature pause was required to enhance acetonitrile separation from the target analytes in patient serum and standards. The 530 µm column bore size is tolerant to the acetonitrile solvent injection with each specimen. It may be possible to reduce the total run time by using a smaller bore size, shorter column, however the authors have chosen this configuration as the wider bore columns have a service life of > 2 years in a lab running > 1000 injections/month with a guard column replacement at 6 month intervals. Dilution of standards with water rather than acetonitrile resulted in lower signal responses due to the higher expansion volume of aqueous solvent.

Ethylene glycol carryover could be due to non-specific interaction with the column material and persistence in the injection space due to poor volatilization. To eliminate EG carryover interferences, any injection with EG > 10 mmol/L was followed by a single water injection to clean the injection port. Additionally, changing the split-vent trap line and trap filter are performed as yearly maintenance on these instruments.

4.3. Interferences and limitations

Interference testing was an important part of this investigation. Poisoning of any cause is a medical emergency and thus it is critical that reported results are both accurate and specific. The most prominent interference was from toluene which co-eluted with ethylene glycol. Toluene is a found in household and industrial products and is extremely toxic at levels > 2 mmol/L in blood. However, as previously argued, toluenes rapid metabolism and urinary excretion as hippuric acid and ortho-cresol means that toluene poisoning is unlikely to cause appreciable levels of ethylene glycol interference physiologically [5]. Benzene and xylene were found to interfere with the 1,2 butanediol internal standard but only at concentrations deemed not physiologically reasonable to encounter in cases of xylene and benzene poisoning. Co-elution of 1,3 butanediol with 1,2 butanediol internal standard was expected here based on structural and boiling point similarities. 1,3 butanediol is more commonly known as a solvent and flavouring agent, although 1,3 butanediol may have use as a nutritional supplement because its metabolized to beta-hydroxybutyrate as a means of inducing ketones as the primary metabolic fuel [18]. This may cause false low detection of ethylene glycol and propylene glycol.

Hypothetical patient conditions that might lead to accumulation of organic acids such as DKA, methylmalonic academia, and propionic academia were assessed in this study. As expected, DKA patient serum contained only acetone, but MMA and PA patient serum did not contain any detectable peaks nor did it interfere with the internal standards. Although these interferences have been previously reported [19], they do not appear to affect this method.

Diethylene Glycol is detectable by this method and elutes after the glycol internal standard at 4.11 min. Since, Gamma Hydroxybutyrate (GHB) and 1,4 Butanediol (metabolic precursor to GHB) have been reported in our region, we also checked these compounds for interference. GHB elutes at 4.15 min (similar to Diethylene glycol and chromatographically indistinguishable). Since this method is unable to resolve these compounds chromatographically, Mass Spectrometry would be required to provide a definitive identification. This is an advantage of a liquid injection with no derivatization step for these compounds, although rare, they would not be detectable by headspace or derivatization-based methods. 1,4 Butanediol is not detectable by this method and it is possible that it does not elute from the column efficiently as the boiling point for this compound is 235 °C and the program does not hold the column oven for a sufficiently long time at this temperature to allow the compound to elute.

An additional limitation of this method is that urine specimens contain too much salt for liquid injection and headspace injection would be recommended for this sample type.

5. Conclusions

The analysis of VA and EG by GC-FID using hydrogen carrier gas enhances the speed, sensitivity, and sustainability of the previously reported helium carrier gas method. The added benefit of a 45% reduction in the time of analysis will support patient care, and reduced laboratory costs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.08.007.

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