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Direct Thermal Desorption Gas Chromatographic Determination of Toxicological Relevant Concentrations of Ethylene Glycol in Whole Blood

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Abstract

A simple and rapid method involving thermal-desorption gas chromatography with flame ionisation detection (TD-GC) has been successfully developed for the determination of ethylene glycol in whole blood. No sample extraction or derivatization steps were required. The conditions required for the direct determination of ethylene glycol in whole blood were optimised and require only the addition of the internal standard, 1,2-butanediol to the sample. A 1 µL aliquot of the sample was then introduced to the thermal desorption unit, dried, and thermal desorbed directly to the gas chromatograph. A calibration curve was constructed over the concentration range of 1.0 to 200 mM and was found to be linear over the range investigated with an R^2 value of 0.9997. The theoretical limit of detection, based on 3 σ was calculated to be 50.2 µM (3.11 mg/L). No issues with carryover were recorded. No interferences were recorded from endogenous blood components or a number of commonly occurring alcohols. The proposed method was evaluated by carrying out replicate ethylene glycol determinations on fortified whole blood samples at levels of 12.5 mM, 20.0 mM, 31.2 mM, 100 mM and 200 mM comparable with commonly reported blood levels in intoxications. Mean recoveries of between 84.8 % and 107 % were obtained with coefficients of variation of between 1.7 % and 5.8 %. This data suggests that the method holds promise for applications in toxicology and where a rapid, reliable method to confirm ethylene glycol poisoning is required.

Introduction

Ethylene glycol is a common organic solvent that has a wide range of uses, such as antifreeze in vehicles, brake fluid, and as a chemical feedstock for polymer manufacture. Ethylene glycol is an odourless, colourless chemical with a sweet taste that produces effects similar to that of ethanol [1] with a dose of 1.4–1.6 mg/kg [2] considered a lethal dose in adults. Due to the sweet tasting nature of ethylene glycol and its ease of availability it is a commonly seen poison within emergency medicine in both humans and animals. Cases of ethylene glycol poisonings are rare within the United Kingdom, in the NPIS report of the year 2015/16 a total of 68 cases of poisoning were recorded. [3] However, cases are more prevalent within the United States of America; in their annual report of 2015, the American Association of Poison Control Centers reported a total of 6204 cases of ethylene glycol exposure resulting in 22 deaths and 178 major injuries. [4]

Ethylene glycol is itself is sparingly toxic; once ingested into the body the minority (20 %) of ethylene glycol is excreted unchanged [5] and initial consumption results in symptoms of intoxication similar to that seen with ethanol. The remanding ethylene glycol is converted by the action of alcohol dehydrogenase into glycoaldehyde, which is then further metabolised to glycolic acid, glyoxylic acid and finally oxalic acid [5] resulting in acidosis and its associated adverse effects. Oxalic acid can then combine with calcium to form calcium oxalate crystals. These crystals precipitate in many organs, but are particularly toxic to the kidneys, resulting in renal failure. The average elimination half-life of ethylene glycol is about 3 hours, with metabolites having a biological half-life in the order of many days. [6]

The current accepted method for the treatment of ethylene glycol poisonings is the administration of either ethanol or fomepizole (4-methylpyrazole) once a patient is stabilized and acidosis corrected. These treatments work via interfering with the oxidation of ethylene glycol by alcohol dehydrogenase and preventing its metabolism. These treatments are usually continued until serum

ethylene glycol concentrations fall below 20 mg/dL and, coupled with haemodialysis, generally provide a positive outcome in cases where diagnosis is made early enough. [7]

Detection of ethylene glycol is very analytical challenging and possible poisoning is often diagnosed by analysis of serum osmol and anion gap. [5,8,9] However, these changes are not always easily detectable and as a result alternative methods for the determination of ethylene glycol poisoning have been sought. [8] Methods based on the detection of other common components of ethylene glycol antifreeze formulations, such as fluorescein, have been reported. [10] These offer a simple method for the detection of ethylene glycol consumption by measuring the fluorescence resulting from the dye in urine, but as a result can be non-specific. Enzyme-based assays have also been developed, but generally can only detect high concentrations (300 mg/dL). [11] The small molecular mass of ethylene glycol does not lend itself to LC/MS and other HPLC approaches suffer from the lack of a chromophore, requiring Refractive Index detection, [12] which lacks specificity and sensitivity.

Presently, gas chromatography (GC) is the most commonly employed laboratory based approach. However, methods are laborious and potentially problematic; based on headspace, direct aqueous injection, or requiring complex derivatisation steps (Table 1). Methods based on the derivatisation of ethylene glycol [13] can be lengthy [14] and complicated, requiring reagents to remove water for the derivatisation reaction to occur. Isolation by liquid or solid-phase extraction is hampered by the polar nature of ethylene glycol. To overcome these problems, direct injections of aqueous dilutions of serum or urine have been attempted. [15] However, water is a relatively poor solvent for gas chromatography and injections can result in poor peak shape and non-reproducible retention times. The co-introduction of serum or urine components can also severely limit the analytical life span of the column and lead to problems with carryover and sensitivity. Headspace analysis similarly suffers, as the high water content present in biological fluids can result in co-injection of water and its introduction to the GC.

However, studies have shown the possibility of determining other alcohols such as ethanol in blood by pyrolysis-gas chromatography. [16] We believe a similar approach such as thermal desorption-gas chromatography (TDC-GC) could be used to determine ethylene glycol. This would allow for the rapid, automated extraction, drying and introduction of sample overcoming the issues suffered by other GC methods. In this present investigation, we first explore this possibility and then optimise the conditions required for the TDC-GC determination of ethylene glycol in µL volumes of whole blood directly without the need for extraction or derivatization.

We believe it possible to thermally desorb ethylene glycol present in samples of both water and whole blood using a commercial pyrolysis thermal desorption unit connected to a gas chromatograph (TD-GC) with flame ionisation detection (FID) for quantification. Samples of whole blood or aqueous analytical standards are first dried at low temperature and then thermally desorbed and focused onto a Tenax® cartridge. The dried focused compounds can then be thermally transferred to the inlet of the GC and then separated and detected by FID. After analysis the remaining blood residues are effectively removed by pyrolysis at high temperature. In the following sections we have optimised the conditions required for this procedure and shown the possibility of determining forensically and toxicologically relevant concentrations of ethylene glycol in whole blood.

Technique	Sample	Detection limit, mg/L	Comment	Ref.
GC- DMS	Human saliva	100	Saliva volatiles collected on a polydimethylsiloxane coated titanium cylinder and thermally desorbed to the GC.	[17]
GC/MS	Human plasma	5.0	De-proteinated with acetic acid; vortex; centrifugation; supernatant spiked with internal standard; reaction with butylboronic acid; neutralize with NH_4OH, extraction with dichloromethane; concentration.	[18]
GC/MS	Human serum	10	Acetic acid/acetonitrile containing the internal standard, 1,3-propanediol add to the serum sample to precipitate proteins. Following centrifugation, 2,2-dimethoxypropane/dimethylformamide added to convert water to methanol, and the volume reduced to < 100 µL of dimethylformamide. Introduced to GC/MS after formation of tert-butyldimethylsilyl derivative.	[19]
GC/MS	Human plasma	50 (limit of quantitation).	Samples (50 µL) vortex mixed with internal standard and centrifuged. Supernatant extracted with DMF and derivatization with BSTFA.	[20]
GC/MS	Human blood	0.001 (estimated)	Whole blood was fortified with isotopically labelled ethylene glycol and extracted/deproteinated with acetonitrile and derivatised with heptafluorobutyric anhydride. The resulting derivatives extracted with n-hexane.	[21]
GC/MS	Human serum and urine	50	Samples of urine or serum mixed with borate buffer and the internal standards: 1,3-propylene glycol, IS2: (3-(4-chlorophenyl) propionic acid added. Isobutanol and pyridine added and the mixture and derivatization phenylboronic acid undertaken.	[22]
GC/FID	Human serum and urine	31 (limit of quantification)	Direct Injection of serum or urine samples diluted with water containing 2,3-butanediol as Internal standard.	[23]
GC/FID	Human serum and urine	25	Samples of urine or serum mixed with borate buffer and the internal standards: 1,3-propylene glycol, IS2: (3-(4-chlorophenyl) propionic acid added. Isobutanol and pyridine added and the mixture and derivatization phenylboronic acid undertaken.	[24]
GC/FID	Human serum		Internal standard (in acetonitrile) added to sample; centrifugation to remove protein precipitate; esterification with butylboronic acid and 2,2-dimethoxypropane; neutralization with NH_4OH in acetonitrile.	[25]
GC/FID	Human serum	25	Serum samples were deproteinated using ultrafiltration and 1 µL was injected directly to GC.	[15]
GC/FID	Human plasma and serum	10	Headspace GC using 1,3-propanediol as an internal standard following derivatization with phenylboronic acid.	[26]
GC/FID	Human plasma	20.8 (lower limit of quantification)	Sample extracted with 2:1 (v:v) acetonitrile containing internal standard 1,2 butanediol.	[27]
HPLC/UV	Human serum	3.1	Extraction from salted, acidified serum using methyl ethyl ketone followed by removal of organic	[28]

			phase and evaporation to dryness and derivatization with PNBDI.	
TLC	Urine		Acidification; extraction with $CHCl_3$; concentration; TLC.	[29]
HPLC/UV	Human plasma	0.15	Heparinized blood deproteinated by addition of acetonitrile and phosphate buffer (pH 7), centrifugation, removal of solvent and evaporation to dryness.	[30]
HPLC/UV and LC/MS	Water	1.0 (UV), 0.03 (MS)	Following derivatization with benzoyl chloride benzoyl esters of glycols are separated by microcolumn reversed-phase HPLC with either UV (237 nm) or electrospray ionization mass spectrometric (ESI-MS) detection using selected ion monitoring.	[31]
LC/MS	Human Serum	1.1	Derivatization of ethylene glycol with benzoyl chloride in serum sample. Mixtures then extracted with pentane and blown down to dryness. Extract re-constituted in mobile phase.	[32]
GC/FTIR	Water	120	Direct Injection.	[33]
GC-MS	Human Serum	5.0	Derivatization with perfluorooctyl.	[34]
GC/ECD	Human serum	0.38	Acetonitrile with internal standard added to sample; centrifugation; concentration; extraction with *p*-bromophenyl boric acid in ethyl acetate.	[35]
UV-Vis spectroscopy	Human serum	62.1	Colorimetric: precipitation of protein with trichloroacetic acid followed by centrifugation, addition of chromotropic acid, heating, and dilution. Absorbance at 580 nm.	[36]
GC/FID	Air		Sample adsorbed on Amberlite® XAD-2 with personal sampling pump; extraction with diethyl ether.	[37]
GC/FID	Hydrocarbon based or synthetic lubricants	1.0	Derivatization of ethylene glycol with phenyl boronic acid in lubricant sample followed by headspace GC analysis.	[38]
GC/FID	Beer	0.73	Addition of ammonium sulfate and then extracted with ethyl acetate.	[39]
GC/FID	Whole blood	3.11	Direct thermal desorption gas chromatography of 1 µL of whole blood.	This method

Gas chromatography/differential mobility spectrometry (GC/DMS); Gas chromatography/mass spectroscopy (GC/MS); Gas chromatography flame ionisation detection (GC/FID); High performance liquid chromatography UV detection (HPLC/UV); Gas chromatography/Fourier transformer infrared detection (GC/FTIR); Gas chromatography/electron capture detection (GC/ECD); Thin layer chromatography (TLC).

Table 1. Previously reported methods for the determination of ethylene glycol

Experimental

Chemical and Reagents

All reagents were obtained from Sigma-Aldrich (Gillingham, UK) unless otherwise stated. Deionised water was obtained from a Purite RO200–Stillplus HP System, (Purite Oxon, UK). Defibrinated horse blood was obtained from Fisher Scientific (Loughborough, UK). Blood collection was carried out by the supplier in stress-free conditions by qualified, trained personnel in accordance with the Animals (Scientific Procedures) Act 1986.

Instrumentation

The CDS 5200 pyrolysis thermal desorption instrument conditions were as follows: Drying temperature 100 °C for 20 s; desorption temperature 250 °C for 2 min. Transfer line 250 °C, cleaning temperature, 1100 °C for 10 s. Gas chromatography analysis was carried out using an Agilent 6890 instrument with flame ionisation detection. Sample extracts were introduced via a CDS 5200 pyrolysis thermal desorption instrument (CDS Analytical, Oxford, PA) on to a HP-5 capillary column (15 m x 0.25 mm ID, 0.25 µm film thickness, 5 % diphenyl–95 % dimethylsiloxane phase). The GC oven temperature was maintained at 100 °C for 2 min and then programmed to 250 °C at 20 °C/min and finally held isothermally for 10.5 min at this temperature. The injector and flame ionisation detector were held at 250 °C and 260 °C respectively.

Sample Analysis

A scheme for the analysis of blood samples is given in figure 1. Whole blood samples were diluted in ratio of one-to-one with deionised water containing 50 mM of internal standard, 1,2-butanediol. A suitable aliquot of this solution (0.5 – 1.0 µL) or aqueous analytical standard was then pipetted into a quartz pyrolysis tube containing a plug of salinized glass wool and placed into the pyrolysis probe. The remainder of the procedure was then carried out under the automated control of the CDS 5200 pyrolysis thermal desorption instrument. The tube was heated in the pyrolysis probe at 100 °C for

20 s to dry the sample. The probe was then inserted into the thermal desorption instrument and heated to 250 °C in an inert atmosphere (He) to thermally desorb the sample components from this residue. The resulting vapour was then focused onto an integrated Tenax® cartridge and thermally desorbed to the gas chromatograph and oven temperature program initiated. The pyrolysis probe was then removed and remaining extracted sample residue contained in the quartz pyrolysis tube removed by heating at 1100 °C for 10 s. Periodic cleaning of the quartz pyrolysis tube crucible was undertaken with acetone with the aid of ultrasonication.

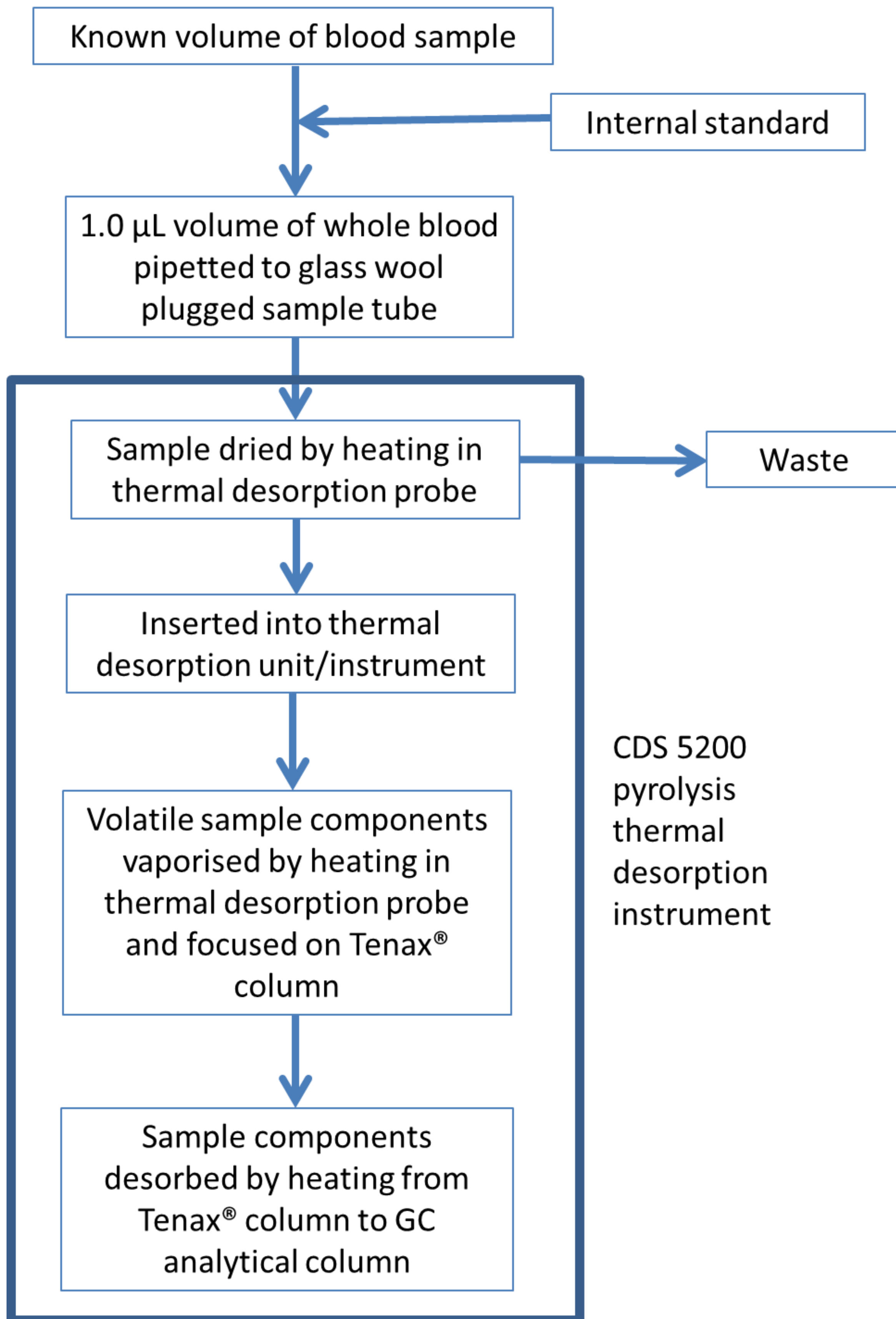

Figure 1. Flow diagram of ethylene glycol determination in whole blood sample by TD-GC.

Results and Discussion

Proposed Analytical Procedure

Optimisation of Thermal Desorption Conditions

The effect of drying time on aqueous solution of 25 mM ethylene glycol was first investigated. Figure 2 shows the resulting peak height of the ethylene glycol standard. As can be seen the maximum response was obtained using a drying time of 20 s. Consequently, further studies were made using these conditions. We next investigated the effect of desorption temperature of ethylene glycol from the quartz pyrolysis tube over the range 150 °C to 325 °C. Figure 3 shows the relative peak height responses for an aqueous 25 mM ethylene glycol standard. A temperature of 250 °C was found to be optimum, in terms of both sensitivity and precision and was used in further studies. Studies were then made on the effect of time on the desorption of ethylene glycol from the Tenax® to the GC. Figure 4 shows that the optimum time based on both precision and response was 2 minutes. This time was hence used in further investigations.

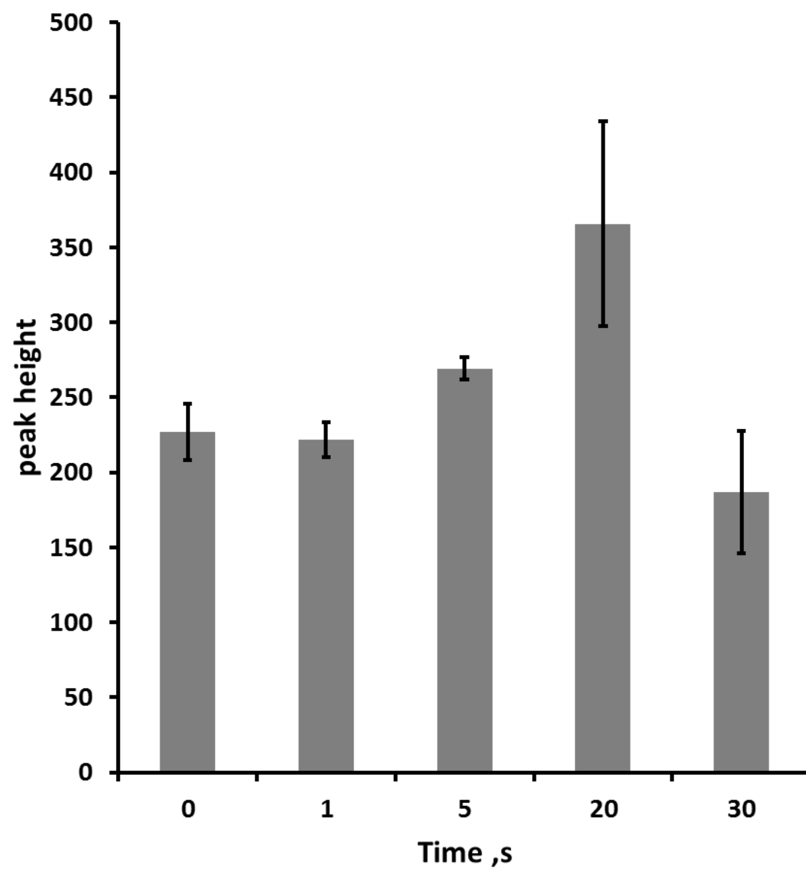

Figure 2. Effect of drying time on ethylene glycol chromatographic peak height.

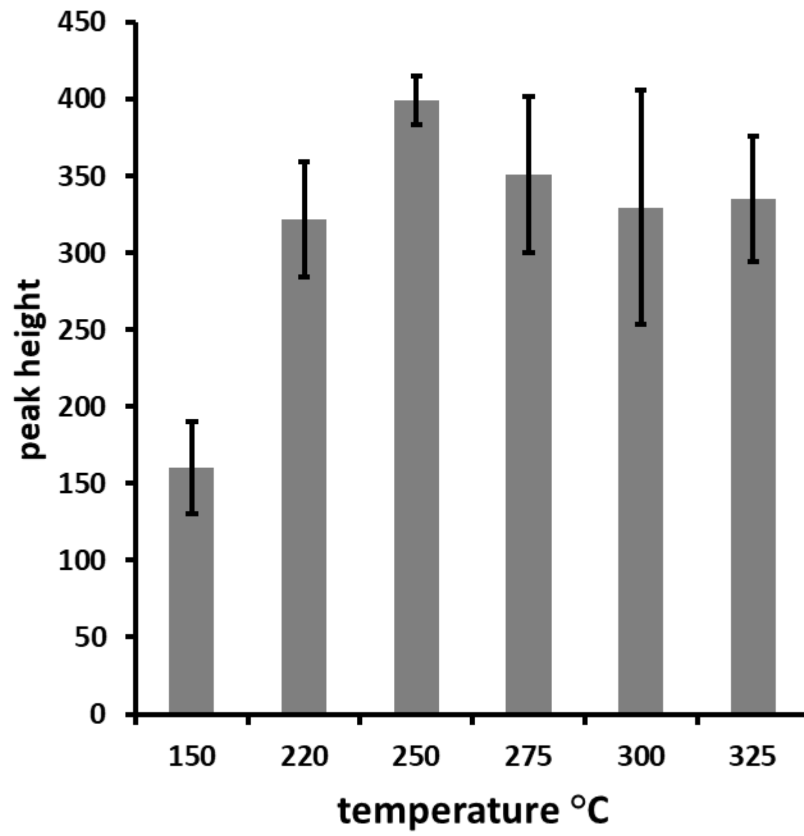

Figure 3. Effect of desorption temperature on ethylene glycol chromatographic peak height.

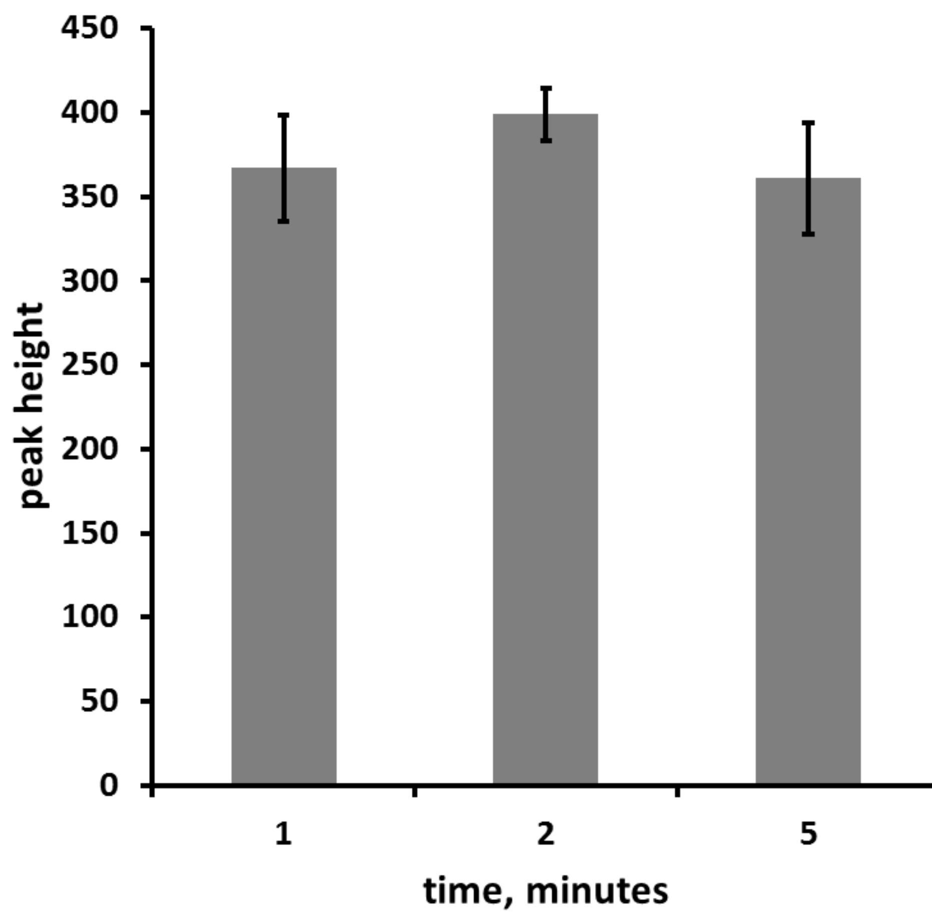

Figure 4. Effect of desorption time based on resulting ethylene glycol chromatographic peak height.

Precision, Calibration Plot, Limit of Detection and Quantification

Standard solutions containing ethylene glycol in the concentration range 0.05 - 500 mM were prepared in deionised water and determined by the optimized TD-GC procedure. This range was chosen as it covers the lower limit for antidote treatment through to severe toxic levels. [40] The calibration plot was found to be linear over the range 1.0 to 200 mM with an R^2 value of 0.9997. The theoretical limit of detection, based on 3 σ, was calculated as 50.2 µM (3.11 mg/L) of ethylene glycol for a 1 µL sample and the limit of quantification was defined to be 1.0 mM (62.1 mg/L). It should be noted that larger sample volumes can be utilised and hence greater sensitivity can be obtained.

Studies of Possible Interferences

Ethanol, methanol, lactic acid, fomepizole (4-methylpyrazole) and glycolic acid, as well as γ-butyrolactone and 1,4-butanediol were investigated at concentrations of 45 mM as possible interferences for the determination of ethylene glycol at a concentration of 45 mM. At this concentration; ethanol, methanol, lactic acid and glycolic acid were not detected and did not interfere with the determination of ethylene glycol. Fomepizole, γ-butyrolactone and 1,4-butanediol were found to co-elute with each other, but were all fully resolved from the chromatographic peaks for both ethylene glycol and the internal standard, 1,2-butanediol and hence did not interfere with the determination of ethylene glycol. Figure 5 shows a typical chromatogram for a solution containing: ethanol, methanol, fomepizole, lactic acid, γ-butyrolactone, 1,4-butanediol ethylene glycol all at concentrations of 45 mM along with the internal standard 1,2-butanediol. As can be clearly seen, both ethylene glycol and the internal standard are well-resolved and the quantification of ethylene is unaffected. Investigations of possible carryover for a 200 mM ethylene glycol standard showed less than 2 % for a subsequently injected blank.

Compound	RT, min	RRT, min
methanol	ND	ND
ethanol	ND	ND
1,4-butandiol	4.19	1.16
1,2-butandiol	3.60	1.00
γ-butyrolactone	4.06	1.13
fomepizole	4.17	1.16
lactic acid	4.86	1.35
ethylene glycol	3.20	0.89

Table 2. Retention times (RT) and relative retention times (RRT) (Analyte RT/Internal standard RT) for possible interfering compounds. ND = not detected.

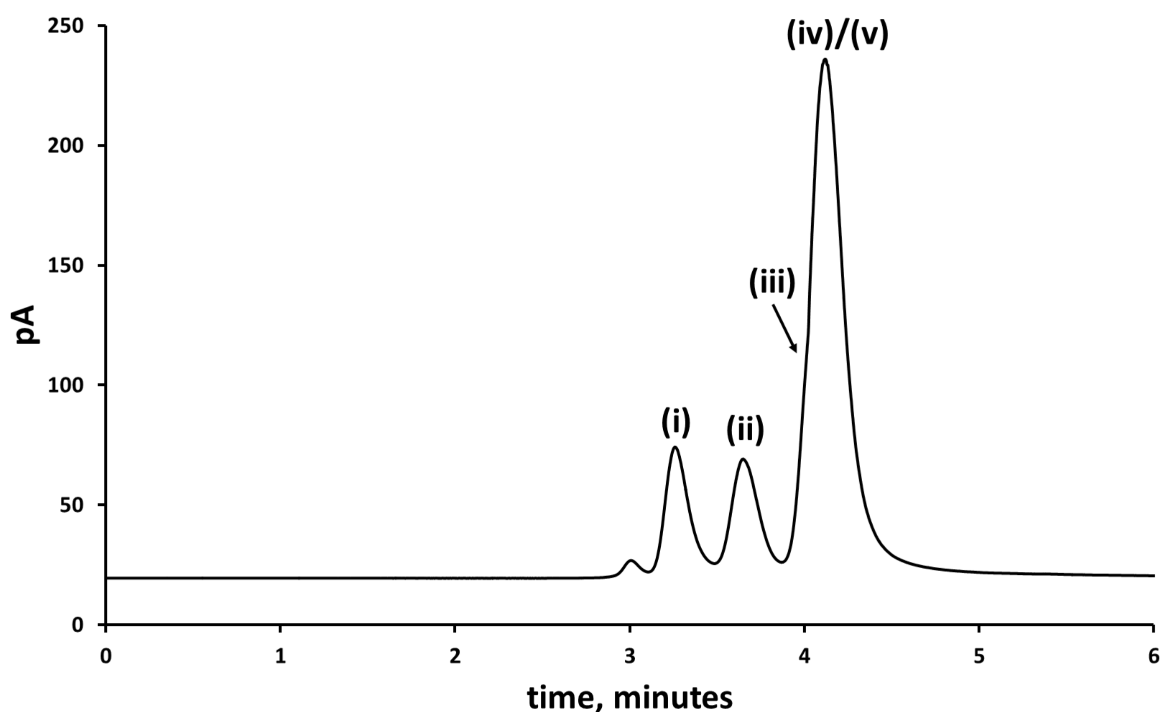

Figure 5. Gas chromatogram showing the separation of ethylene glycol (i) and the internal standard, 1,2-butanediol (ii) in presence of methanol (ND), ethanol (ND), 1,4-butanediol (iii), ɣ- butyrolactone (iv) and fomepizole (v); 45 mM of each compound. No further peaks were detected after 6 minutes. ND = not detected.

Analytical Application

To assess the performance of the optimised TD-GC method, replicate determinations of ethylene glycol in fortified and unfortified whole blood samples were undertaken. Blood samples were fortified to be 12.5 mM, 20.0 mM, 31.2 mM, 100 mM and 200 mM ethylene glycol. The appropriate volume of internal standard was added to the whole blood and quantification was achieved by

external calibration. Figure 6 shows representative chromatograms for samples of whole blood (a) fortified with 31.2 mM ethylene glycol and (b) unadulterated whole blood.

Recoveries and precision data gained for the blood samples fortified with ethylene glycol are summarized in Table 3. These levels were selected as previous investigations have shown blood samples taken from human and animal victims to contain levels of ethylene glycol over this range. [13,22,23,41] The extracts showed well defined signals for ethylene glycol under the conditions described. The method can be seen to give reliable data over the concentration ranges investigated. The use of blood sample volumes as small as a µL offers advantages in both health and safety and to areas such as forensic and biomedical sciences where large sample volumes may not be obtainable. Blood samples stored at 4° C were found to be stable for at least two weeks.

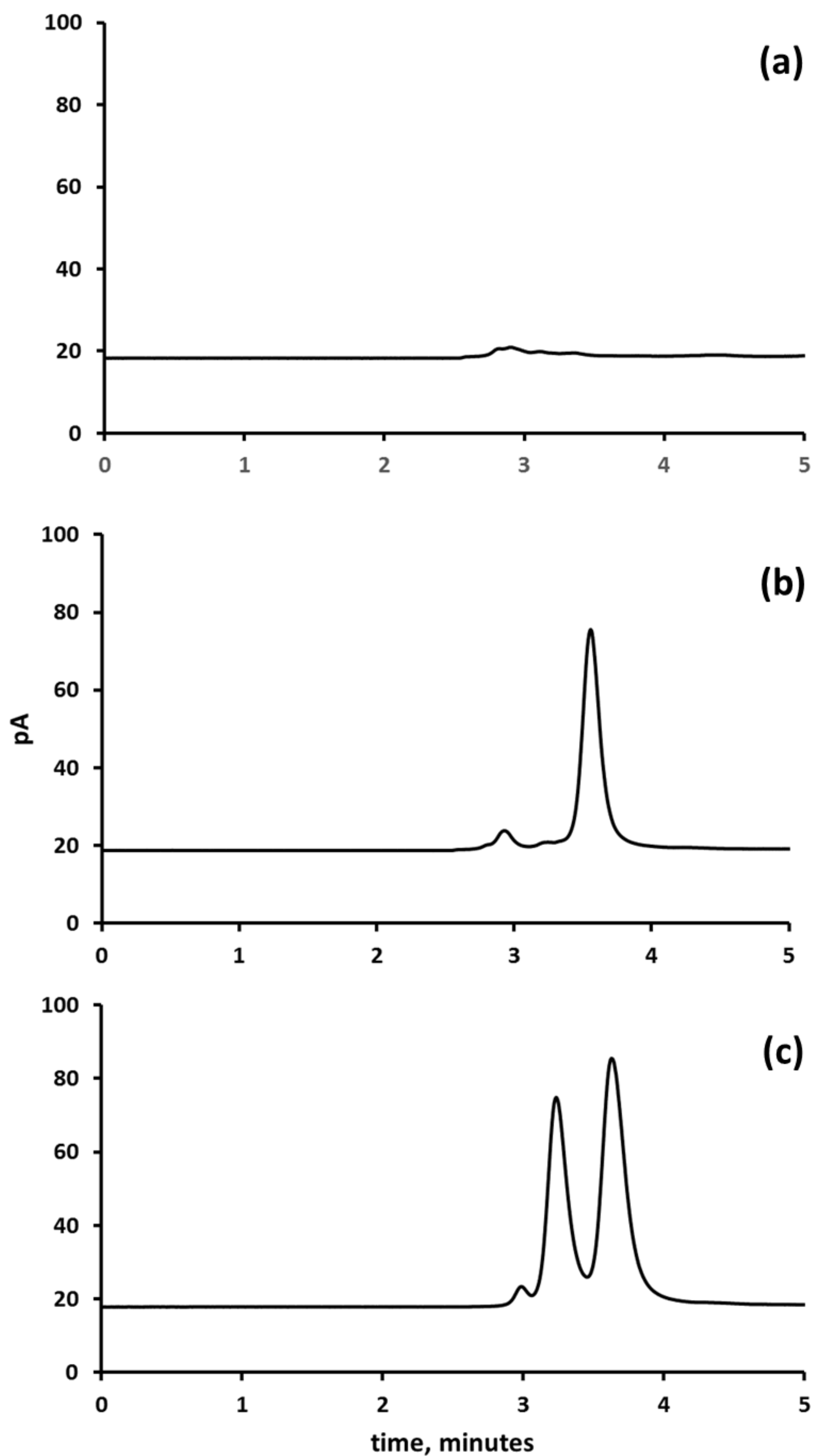

Figure 6. Representative chromatograms of whole blood samples obtained by TD-GC for (a) whole blood (b) whole blood with internal standard (1,2-butanediol) 3.6 minutes (c) whole blood with ethylene glycol (3.2 minutes) and internal standard.

Blood Sample	Native	Added, mM	Mean Found, mM	% Mean Recovery	%CV
1	ND	12.5	10.5	84.8	4.4
2	ND	20.0	19.5	96.7	2.3
3	ND	32.2	29.4	94.3	5.8
4	ND	100	107	107	3.9
5	ND	200	209	105	1.7

ND = not detected; %CV = percentage coefficient of variation

Table 3. Recovery and Precision Data for ethylene glycol obtained on whole blood

Conclusions

Our method requires very little sample preparation, requiring only the simple addition of internal standard to a whole blood sample. Other workers (table 1) have reported GC based assays for ethylene glycol, but these required rigorous extraction and derivatization procedures. Therefore, the overall time and skill required for these assays is much greater than our proposed assay, which is an important consideration in medical and forensic investigations. [7] In addition, a derivatization reagent itself and its reaction by-products might be expected to give responses with similar retention times to that of ethylene glycol itself.

Although other workers have also reported on the detection of ethylene glycol in biological fluids, it does not appear that they have the required speed and simplicity needed for rapid toxicological analysis. It would be readily simple to also determine ethylene glycol concentrations in dry blood samples important in forensic analysis as the TD-GC can be readily applied for solid samples. As far as we are aware, our report is the first to describe the use of a TD-GC assay for the detection of any glycol. However, we believe that the approach developed here could form the basis of a generic approach for the analysis of other alcohols, and in future studies, we plan to investigate this further. No extraction or derivatization steps were required and the assay was free from interference from common endogenous blood components or other structurally similar compounds. The small volumes of blood (μL) utilised offer advantages for health and safety of the analyst and in forensic and biomedical investigations where obtaining large samples can be a problem.

Acknowledgements

We would like to thank the support of the Analytical Chemistry Trust Fund. Kevin Sudlow and Sarah Almond are thanked for their technical assistance.

Conflicts of Interest

There are no conflicts of interest to declare.

References

[1] A. Vale, *Medicine*, **2012**, 40, 89–93.
[2] R. C. Baselt, *Disposition of Toxic Drugs and Chemicals in Man*, 9th edition. Biomedical Publications, Seal Beach, CA, **2011**, 637–640.
[3] National Poisons Information Service. *National Poisons Information Service Report 2015/2016*. National Poisons Information Service, 2016. 15 June 2017.
[4] J. B. Mowry, D. A. Spyker, D. E. Brooks, A. Zimmerman and J. L. Schauben, *Clin. Toxicol.* **2016**, 54, 924-1109.
[5] J. Brent, *Drugs,* **2001**, 61, 979-988.
[6] C. D. Peterson, A. J. Collins, J. M. Himes, M. L. Bullock and W. F. Keane, *N. Engl. J. Med.* **1981**, 304, 21-23.
[7] J. Latus, M. Kimmel, M. Dominik Alscher and N. Braun, *Clin. Kidney J.* **2012**, 5, 120–123.
[8] J. A. Kraut, *Clin. Toxicol.* **2015**, 53, 589–595.
[9] W. H. Porter, *Clin. Chim. Acta* **2012**, 413, 365–377.
[10] K. L.Wallace, J. R. Suchard, S. C. Curry and C. Reagan, *Ann. Emerg. Med.* **2001**, 38, 49–54.
[11] J. B. Hack, W. K. Chiang, M. A. Howland and H. Patel, *Acad. Emerg. Med.* **2000**, 7, 294–297.
[12] Z. Siroka, R. Bily, V. Polacek, J. Dvorak and M. Svoboda, *Vet. Med.-Czech.* **2014**, 59, 388–395.
[13] T. G. Rosano, T. A. Swift, C. J. Kranick and M. Sikirica, *J. Anal. Toxicol.* **2009**, 33, 508-513.
[14] P. Van hee, H. Neels, M. De Doncker, K. E. Maudens, W. Lambert and L. Patteet, *Clin. Chim. Acta,* **2013**, 415, 107-108.
[15] R. H. Williams, S. M. Shah, J. A. Maggiore and T. B. Erickson, *J. Anal. Toxicol.* **2000**, 24, 621-626.
[16] S. Yamamoto, K. Ueda, M. Noami and Y. Nokami, *Japn. J. Sci. Tech. Identif.* **2000**, 5, 55-60.
[17] L. Criado-García, D. M. Ruszkiewicz, G. A. Eiceman and C. L. P. Thomas, *J. Breath Res*. **2016**, 10, 017101.
[18] C. Giachetti, G. Zanolo, A. Assandri and P. Poletti, *Biomed. Environ. Mass Spectrom.* **1989,** 18, 592–597.
[19] W. H. Porter, P. W. Rutter and H. Ya, *J. Anal. Toxicol.* **1999**, 23, 591-597.
[20] A. F. Robson, A. J. Lawson, L. Lewis, A. Jones and S. George, *Ann. Clin. Biochem*. **2017**, 54, 481-489.
[21] A. Wurita, O. Suzuki, K. Hasegawa, K. Gonmori, K. Minakata, I. Yamagishi, H. Nozawa and K. Watanabe, *Forensic Toxicol.* **2013**, 31, 272–280.
[22] T. Hložek, M. Bursová and R. Čabala, *Clin. Biochem.* **2015**, 48, 189–191.
[23] K. Aarstad, O. Dale, O. Aakervik, S. Øvrebø and K. Zahlsen, *J. Anal. Toxicol.* **1993**, 17, 218-221.
[24] T. Hložek, M. Bursová and R. Čabala, *Talanta*, **2014**, 130, 470-474.
[25] N. B. Smith, *Clin. Chim. Acta*, **1984**, 162, 269–272.
[26] A. Ehlers, C. Morris and M. D. Krasowski, *SpringerPlus* **2013,** 2:203.

[27] D. J. Orton, J. M. Boyd, D. Affleck, D. Duce, W. Walsh and I. Seiden-Long, *Clin. Biochem.* **2016**, 49, 132–138.
[28] T. P. Hewlett, A. C. Ray and J. C. Reagor, *J. Assoc. Off. Anal. Chem.* **1983**, 66, 276–283.
[29] J. H. Riley, S. O'Brien and M. G. Riley, *Vet. Hum. Toxicol.* **1982,** 24, 331–334.
[30] A. A. Brega, P. Quadri, P. Villa, P. Prandini, J. – Q. Wei, and C. Lucarelli, *J. Liquid Chromatogr.* **1992**, 15, 501–511.
[31] M. Holcapek, H. Virelizier, J. Chamot-Rooke, P. Jandera and C. Moulin, *Anal. Chem.* **1999**, 71, 2288-2293.
[32] L. Imbert, E. Saussereau and C. Lacroix, *J. Anal. Toxicol.* **2014**, 38, 676–680.
[33] US EPA (1986) *Standard scenarios for estimating exposure to chemical substances during use of consumer products. Volumes I and II.* Prepared by Versar Inc. for Exposure Evaluation Division, Office of Toxic Substances, US Environmental Protection Agency ,Washington, DC (EPA Contract No. 68-02-3968).
[34] A. Dasgupt, W. Blackwell, J. Griego and S. Malikc, *J. Chromatogr. B* **1995***,* 666, 63-70.
[35] L. L. Needham, R. H. Hill, D. L. Orti, M. E. Felver and J. A. Liddle, *J. Chromatogr.* **1982**, 233, 9–17.
[36] A. D. Fraser and W. MacNeil, *J. Toxicol. Clin. Toxicol.* **1993**, 31, 397-405.
[37] K. Andersson, J. – O. Levin, R. Lindahl and C. – A. Nilsson, *Chemosphere* **1982**, 11, 1115-1119.
[38] K. Gras, K. Luong, M. Lin, M. Gras and R. A. Shellie, *Anal. Methods,* **2015**, 7, 5545–5550.
[39] S. A. Williamson and W. G. Verson, *J. Am. Soc. Brew. Chem.* **1993**, 51, 51:0114.
[40] R. Hess, M. J. Bartels and L. H. Pottenger, *Arch. Toxicol.* **2004**, 78, 671–680.
[41] R. D. Cox and W. J. Phillips, *Mil. Med.* **2004**, 169, 660-663.