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Application News

High Performance Liquid Chromatograph Mass Spectrometer

LC-MS/MS Analysis of Ethylene Glycol in Blood Using a Simple Derivatization Method

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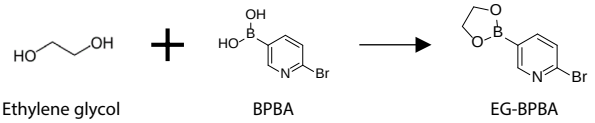
User Benefits

- A new quick and simple pretreatment allows for the measurement of ethylene glycol levels in blood by simultaneously removing proteins and derivatization.
- Since it uses the same mobile phase as the "LC/MS/MS Rapid Toxicology Screening System", it can be run on the same system with just a column change.
- This method does not use expensive reagents and isotope-labeled internal standards, slowing for low-cost running.

■ Introduction

Ethylene glycol is an industrial product used in antifreeze and other applications. However, it is also a toxic substance that causes metabolic acidosis and renal failure when absorbed by the body. As a highly polar, low molecular weight molecule, ethylene glycol is only weakly retained on reversed-phase columns and is not readily detected by LC-MS analysis without some form of pretreatment. It is also highly hydrophilic, difficult to isolate from blood, and not easily measured by GC without pretreatment. These issues led to the development of a GC-MS method that detects phenylboronic derivatives of ethylene glycol produced by derivatization reactions in aqueous solutions. However, the phenylboronic acid used by this method can accumulate in GC inserts and columns, affecting subsequent measurements.

This study investigated using a boronic acid derivatization reagent (2-bromopyridine-5-boronic acid [BPBA]) compatible with LC-MS analysis to develop a simple and rapid method for measuring ethylene glycol levels in blood. BPBA selectively reacts with the diol groups of ethylene glycol, forming a stable cyclic boronic ester (Fig. 1). The nitrogen atom in this cyclic boronic ester improves its electrospray ionization (ESI) efficiency, whereas BPBA reacts quickly with ethylene glycol in aqueous samples.[1)] Taking advantage of these attributes, a simple and rapid pretreatment process was developed that performs deproteinization and derivatization simultaneously.



Ethylene glycol　　BPBA　　EG-BPBA

Fig. 1 Derivatization Reaction

■ Sample Pretreatment

The pretreatment workflow is shown in Fig. 2. Deproteinization and derivatization were performed simultaneously by adding 390 µL of 0.1 % TFA-acetonitrile containing 2.5 mM of BPBA to the sample and mixing it in a vortex mixer. The mixture was then centrifuged at 12,000 *g* for 5 minutes and the supernatant was recovered for analysis. 1,2-Pentanediol (100 µg/mL) was used as an internal standard.

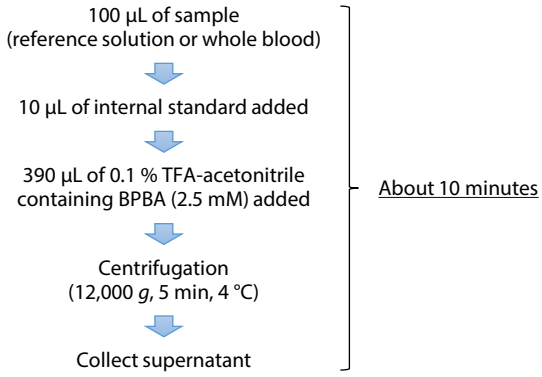


Fig. 2 Sample Pretreatment Workflow

■ Analysis Conditions

Analysis was performed using an LCMS-8050 RX (Fig. 3) using the conditions shown in Table 1. The derivative EG-BPBA contains bromine and is detected as a characteristic isotope ion. The MRM transitions below were chosen because the EG-BPBA isotope ions (*m/z* 228 and 230) are detected with about the same intensity, so they can be used for identification.

Table 1 Analysis Conditions

System	: Nexera™ XR + LCMS-8050 RX
Column	: Shim-pack Velox™ HILIC (Shimadzu, 2.1 mm i.d. 100 mm length, 2.7 µm)
Column Temp.	: 30 °C
Injection Volume	: 1 µL
Rinse Setting	: Sample interior/exterior cleaning
Rinse Solution	: MeOH
Mobile Phase	: A) 10 mM ammonium formate + 0.1 % formic acid (in water) : B) 10 mM ammonium formate + 0.1 % formic acid (in MeOH)
Flowrate	: 0.3 mL/min
Time Program (%B)	: 90 % (0 - 1.5 min)→20 % (1.5 - 4.0 min) → 90 % (4.0 - 7.0 min)
Nebulizing Gas	: 2 L/min
Drying Gas	: 10 L/min
Heating Gas	: 10 L/min
DL Temp.	: 150 °C
Heat Block Temp.	: 200 °C
Interface Temp.	: 300 °C

MRM Transitions

Compound Name	Quantitative Ion	Reference Ion
EG-BPBA	228.0 > 148.0 (CE -20 V)	230.0 > 148.0 (CE -20 V)
PD-BPBA (Internal Standard)	270.0 > 184.0 (CE -15 V)	272.0 > 186.0 (CE -15 V)

Fig. 3 Nexera™ with LCMS-8050 RX

■ Evaluation of Linearity and Sensitivity

Reference samples of aqueous solutions of ethylene glycol were analyzed to verify the linearity of the method. Given that ethylene glycol is toxic at blood concentrations of 200 µg/mL and above, reference samples of 10, 20, 50, 100, 200, and 500 µg/mL were prepared for analysis. Fig. 4 shows chromatograms of EG-BPBA (10 µg/mL) and PD-BPBA. The reference sample results show that the method is sensitive enough to detect ethylene glycol at 1/20 of the level that is toxic. The calibration curve is shown in Fig. 5. The results showed good linearity (R = 0.99996).

A solution of ethylene glycol (1000 µg/mL) without BPBA that was analyzed after verifying its linearity produced a chromatogram with no EG-BPBA peak (Fig. 6). This indicates that BPBA is unlikely to remain in equipment flow lines and affect subsequent analyses.

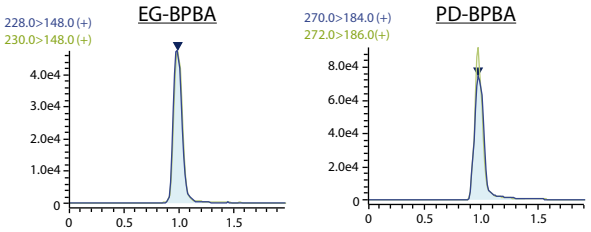


Fig. 4 MRM Chromatograms for EG-BPBA (10 µg/mL) and PD-BPBA

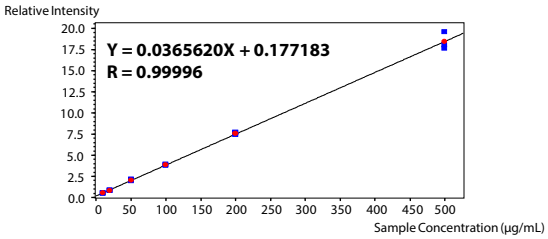


Fig. 5 Calibration Curve

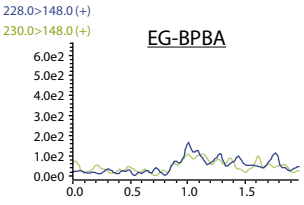


Fig. 6 High Concentration Ethylene Glycol Analysis after Multiple Successive Measurements

■ Validity Assessment with Spiked Whole Blood Sample

Ethylene glycol was added to whole blood to a concentration of 100 µg/mL and used to evaluate the repeatability and accuracy (n = 5) of the method. The results showed good accuracy (96.4 %) and repeatability (2.4 %) (Table 2). Analysis of a blank whole blood sample with no added ethylene glycol resulted in a peak with 1/20 the intensity of the 10 µg/mL reference solution (Fig. 7).

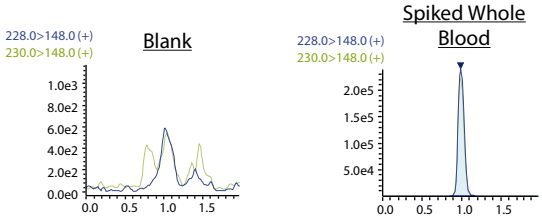


Fig. 7 MRM Chromatograms Showing EG-BPBA in Blank and Spiked Whole Blood (100 µg/mL)

Table 2 Accuracy and repeatability of spiked EG in blood

Concentration (µg/mL)						
1st Analysis	2nd Analysis	3rd Analysis	4th Analysis	5th Analysis	Accuracy %	RSD%
98.6	92.2	95.7	97.1	98.4	96.4	2.4

■ Conclusion

This article describes a simple and quick sample pretreatment procedure for quantifying levels of ethylene glycol in blood. When phenylboronic derivatives are used to measure ethylene glycol by GC-MS, the derivatization reagent can accumulate in the equipment, affecting subsequent measurements. However, with the new method, the LC flow lines (including the autosampler) can be cleaned with solvents that have a high affinity for BPBA, which eliminates derivatization reagent carryover.

The mobile phases used with this method are also used in Shimadzu's method package products, such as the "LC/MS/MS Rapid Toxicology Screening System". Therefore, with just a column change, toxicological drug screening and ethylene glycol measurements can both be performed with the same system.

<References>

1) S Shen, F Zhang, S Zeng, J Zheng, "An approach based on liquid chromatography/electrospray ionization–mass spectrometry to detect diol metabolites as biomarkers of exposure to styrene and 1,3-butadiene," Analytical Biochemistry, 386 (2009), p.186-193

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