
Poster Note PN-32

# Quantitation of Ethylene Glycol in Postmortem Specimens by GC-MS/MS

## Introduction

Ethylene glycol (EG) is a colorless, odorless, and sweet-tasting industrial product typically found in antifreeze that may cause severe toxicity and/or death if ingested. The extensive metabolism of EG in the body can lead to metabolic acidosis, cardiopulmonary failure, and acute renal failure, resulting annually in thousands of poisonings nationwide, with dozens of untreated cases resulting in death. Previously published methods for the identification and/or quantitation of EG have used non-deuterated internal standards utilizing a gas chromatograph coupled to a flame ionization detector (GC-FID) or single-quadrupole mass spectrometer (GC-MS) using single ion monitoring (SIM). An updated approach using a deuterated internal standard utilizing a gas chromatograph coupled to a triple-quadrupole mass spectrometer (GC-MS/MS) with time-scheduled multiple reaction monitoring (MRM) to identify and quantify EG in postmortem specimens was desired.

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| Keywords | Instrumentation and Software |
|---|---|
| Ethylene glycol | EVOQ GC-TQ |
| Forensic Toxicology | MS WorkStation |
| Postmortem | |
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## Objective

The objective of this validation was to develop a method for the simultaneous identification and quantitation of EG in postmortem specimens by GC-MS/MS following SWGTOX guidelines.

## Materials & Methods

### Extraction Procedure

EG and a deuterated internal standard (EG-$d_4$) were extracted from diluted calibrators, controls, and biological specimens (200 µL) with cold acetonitrile (500 µL) followed by the addition of an 80:20 mix of 2,2-dimethoxypropane:DMF (1000 µL) to a 100-µL aliquot of the supernatant. Samples were dried to ~200 µL under a nitrogen stream on a heating block at 80°C. A derivatizing reagent (100 µL of MTBSTFA/1% t-BDMCS) was added at room temperature and allowed to sit for 30 minutes. Ethyl acetate (1500 µL) was added to the derivatized samples and a 1-µL aliquot was analyzed.

**Instrument Parameters:**

| | |
|---|---|
| **Bruker 436 GC** | |
| 30 m x 0.25 mm i.d. x 0.25 µm Agilent DB-5MS column | |
| Flow Rate: | 1 mL/min He |
| Injection Port: | Splitless @ 250°C |
| Oven Temp: | 80°C (1 min hold) |
| Oven Temp Ramp: | 20°C/min |
| Final Oven Temp: | 315°C (1.25 min hold) |
| **Bruker EVOQ GC-TQ** | |
| Detector Temp: | 200°C |
| CID Gas Pressure: | 1.8 mTorr |

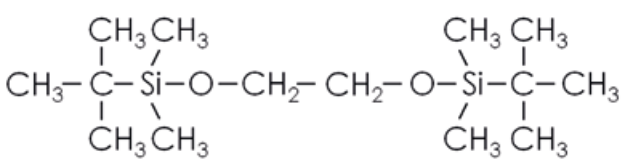

**Figure 1:** Ethylene Glycol di-t-BDMS (m/z 290)

| Analyte | Precursor (m/z) | Product (m/z) | CE (V) | Scan Time (ms) | Scan Time (%) |
|---|---|---|---|---|---|
| EG | 233.0 | 147.0 | 13.0 | 50 | 33.33 |
| EG | 233.0 | 149.0 | 13.0 | 50 | 33.33 |
| EG | 233.0 | 73.0 | 13.0 | 50 | 33.33 |
| EG-$d_4$ | 237.0 | 147.0 | 13.0 | 50 | 100 |

**Table 1:** EVOQ GC-TQ Acquisition Parameters

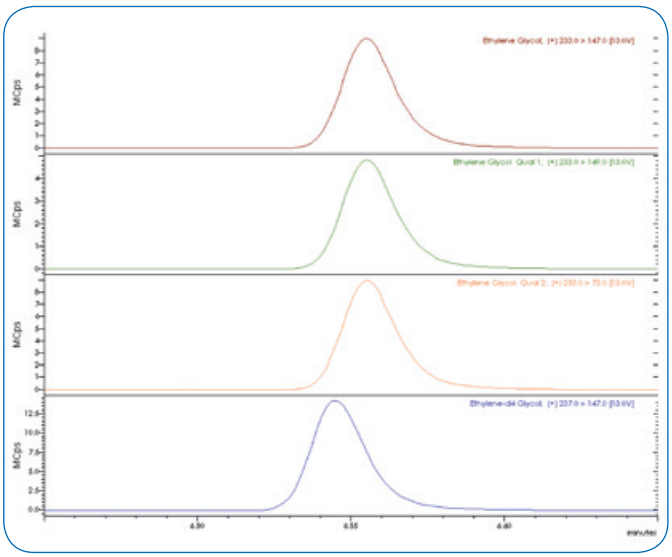

**Figure 2:** EG and EG-$d_4$ MRM Chromatograms

## Results

This method validation followed SWGTOX guidelines to evaluate a linear calibration model, limit of detection, limit of quantitation, precision, bias, carryover, dilution integrity, selectivity, and sample stability. Cross-talk effect was also evaluated; this was necessary due to the MRM daughter spectra of EG and EG-$d_4$ being similar, despite using different parent ions for the time-scheduled MRM transitions. Linearity was verified from 25 to 2000 mg/L using a 1/x2 weighing factor with an operating limit of detection of 10 mg/L and a limit of quantitation of 25 mg/L. Inter-day and intra-day precision at 50 mg/L and 1000 mg/L were both ≤1.0% with respective average biases of -2.0% and 2.6%. No carryover was observed in a blank sample following the injection of the 2000 mg/L calibrator. Dilution integrity was evaluated using specimens at a 1:1 dilution with deionized water; precision and bias remained within 5% for the 50 mg/L and 1000 mg/L controls. No interferences with EG or EG-$d_4$ were observed when analyzed in conjunction with 80+ compounds, including ethanol, propylene glycol, and glycolic acid. No cross-talk was observed between the m/z 237.0>147.0 (EG-$d_4$) and m/z 233.0>147.0, m/z 233.0>149.0, and m/z 233.0>73.0 (EG) MRM transitions. Samples remained stable over a 12-hour period at room temperature.

## Conclusions

A novel analytical method was developed and validated for the simultaneous identification and quantitation of EG by GC-MS/MS using EG-$d_4$ as an internal standard. This method has demonstrated to be highly effective for the identification and quantitation of EG in various postmortem and proficiency specimens. The technological advances in triplequadrupole and detector design over the last decade has allowed for faster scanning speeds and absence of crosstalk between MRM transitions, permitting further development of newer analytical methods. This method has been used by the Miami- Dade Medical Examiner Department to quantify EG in over a dozen postmortem and proficiency cases that had only been previously qualitatively identified since 2012.

## References


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