Direct Quantitation of Ethylene Glycol in mAb Formulation Matrix by MRR

Introduction

Ethylene glycol (EG) and diethylene glycol (DEG) contamination is a recognized pharmaceutical safety concern. Efforts to address this risk have focused on testing high-risk drug components, including glycerin and polyethylene glycols, as outlined in FDA guidance. More recently, USP highlighted the need to expand EG and DEG testing to finished dosage forms.

EG can enter biologic formulations through raw materials such as polyethylene glycols and polysorbates. EG may also be introduced during downstream processing, for example as an elution modifier in chromatographic purification. Subsequent buffer exchange or diafiltration removes EG, making confirmation of residual levels important.

A previous BrightSpec application note demonstrated MRR identification and quantitation of EG and DEG in raw materials, including glycerin, PEG-400, and polysorbate 80. This application note extends MRR-based EG analysis to biologic drug formulations, using a representative mAb formulation matrix.

The Analytical Challenge

Published finished-product methods for EG and DEG target oral liquids such as cough and cold syrups, and biologic matrices fall outside their validated scope. Pharmaceutical formulations contain buffers, salts, and excipients that can complicate EG analysis. Conventional chromatographic methods may require matrix-specific sample preparation and method development to address these components and evaluate potential interferences. Additional preparation increases time, effort, and validation requirements.

Direct analysis with minimal sample preparation offers a simpler workflow for monitoring EG in formulation buffers.

The MRR Approach

Molecular Rotational Resonance (MRR) identifies molecules through their structure-specific rotational spectra. A characteristic EG transition supports identification and quantitation in a single measurement, without relying on chromatographic separation for molecular specificity.

The automated headspace-SpectrAline-MRR workflow (**Figure 1**) minimizes hands-on preparation:

- **Sample preparation:** Place 10 µL of buffer directly into a 20 mL headspace vial, without extraction, dilution, or derivatization.
- **Sampling:** Heat the vial to 140 °C and transfer the headspace to the SpectrAline cartridge. Controlled heating releases EG for MRR measurement.
- **Automation:** The autosampler accommodates up to 66 vials for unattended analysis.

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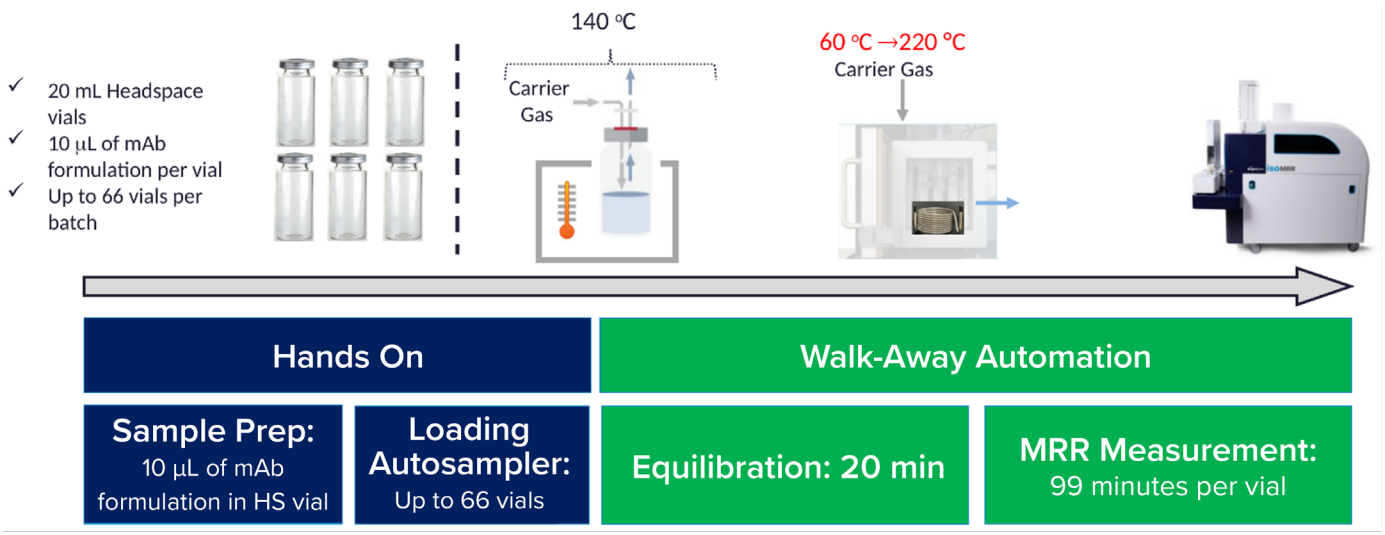


Figure 1. **Headspace-SpectrAline-MRR workflow for direct EG analysis in mAb buffers**

Method and Results

A representative mAb formulation matrix was prepared using Dulbecco's phosphate-buffered saline (DPBS), supplemented with 5% sucrose and 0.01% polysorbate 80. The matrix was analyzed without protein. Since proteins are non-volatile and remain in the vial during headspace sampling, the protein-free formulation represents the matrix the spectrometer analyzes. Calibration standards from 100 to 1,000 ppm were prepared for EG in this matrix and analyzed by headspace-SpectrAline-MRR. The upper level corresponds to the 0.10% limit the FDA applies to high-risk drug components, and the demonstrated LOQ falls well below the limit. **Table 1** summarizes the method conditions.

Parameter	Value
Sample volume	10 µL in 20 mL headspace vial
Headspace temperature	140 °C
SpectrAline Cartridge	SpectrAline Stabilwax, -2M
SpectrAline temperatures	60 °C (trap), 220 °C (elution)
Transfer line and nozzle	210 °C

Table 1. **Headspace-SpectrAline-MRR Method parameters**

ICH Q2(R2) Parameter	Result
Limit of detection	59 ppm
Limit of quantitation	179 ppm
Calibration range	100 to 1,000 ppm
Linearity (R^2)	0.99
Repeatability (RSD)	17%

Table 2. **Analytical performance for EG in DPBS**

The EG response increased with concentration across the tested calibration range, with an R^2 of 0.99 (**Figure 2**). **Table 2** summarizes analytical performance.

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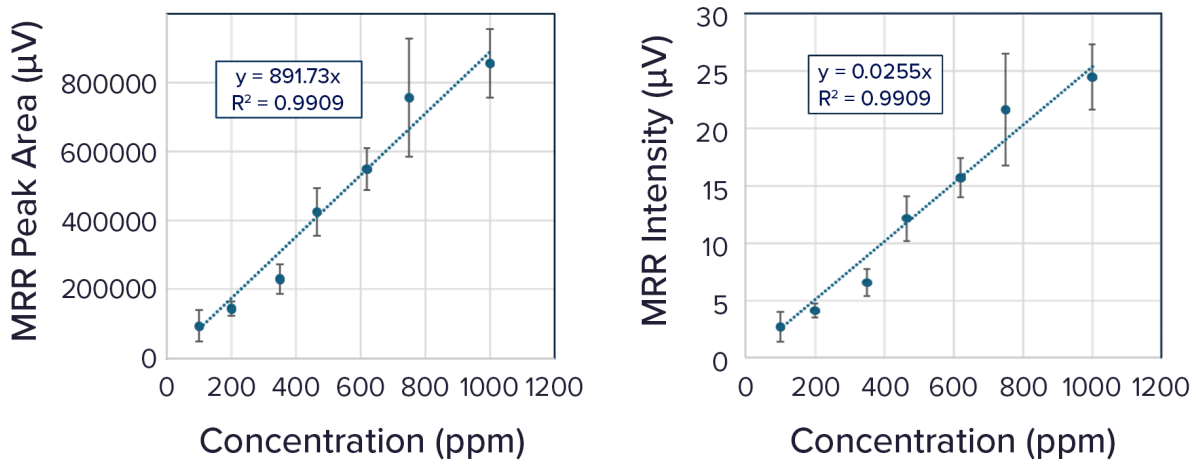


Figure 2. **Calibration curve for EG in DPBS**

Conclusion

MRR enables direct identification and quantitation of EG in representative mAb formulation matrix without extraction, dilution, or derivatization. Automated headspace sampling minimizes hands-on preparation, while structure-specific detection provides molecular identification alongside quantitation.

The approach could support residual EG monitoring in finished formulations and after downstream processing steps such as buffer exchange or diafiltration. It also complements raw-material testing as an additional check on product quality.

References

1. U.S. Food and Drug Administration. "Testing of Glycerin, Propylene Glycol, Maltitol Solution, Hydrogenated Starch Hydrolysate, Sorbitol Solution, and Other High-Risk Drug Components for Diethylene Glycol and Ethylene Glycol." **https://www.fda.gov/media/167974/download** Guidance for Industry, Revision 1, May 2023.

2. United States Pharmacopeia. "Diethylene Glycol and Ethylene Glycol Testing Method for Finished Dosage Forms." **https://www.uspnf.com/notices/deg-eg-method-for-finished-dosage-forms-gen-annc-20250228** General Announcement, February 28, 2025.

3. BrightSpec. "Identification and quantitation of EG and DEG in high-risk raw materials by MRR." Application Note.

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