Where Precision Meets Throughput: Molecular Rotational Resonance Spectroscopy for Ethylene Glycol and Diethylene Glycol Excipient Safety Testing

Overview

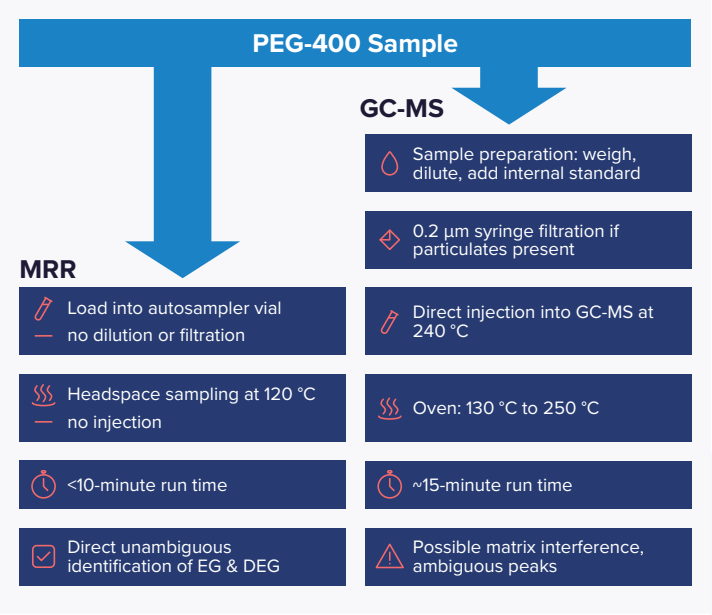
Ensuring excipient safety is among the most critical—and challenging—responsibilities in pharmaceutical manufacturing. Despite clear guidance and repeated warning letters urging independent verification, many companies still rely on supplier certificates of analysis. The stakes couldn't be higher. Contamination events involving ethylene glycol (EG) and diethylene glycol (DEG) have led to tragic outcomes worldwide, reminding us that every batch needs true container-by-container testing.[1-3]

Traditional gas chromatography-mass spectroscopy (GC-MS) methods meet regulatory standards but create real-world bottlenecks: heavy sample prep, long run times, and constant expert oversight. Those challenges only multiply with viscous materials like PEG-400. The result is an analytical setup stretched thin, where accuracy and throughput compete when you could be achieving both.

Molecular rotational resonance (MRR) spectroscopy offers a smarter path forward. By analyzing vapors from sealed headspace vials, MRR quantifies EG and DEG directly—no dilution, filtration, or derivatization required. MRR replaces complex chromatography with fast, matrix-independent analysis that delivers clear results in minutes instead of hours. This application note shows sensitive detection and reliable quantitation of EG and DEG in PEG-400, achieving low ppm-level limits of quantitation and strong linearity without the usual bottlenecks of traditional GC methods.

A Simpler, Smarter Alternative

BrightSpec's isoMRR™ platform integrates high-resolution rotational spectroscopy into routine workflows with fully automated headspace sampling and intuitive Edgar software for method setup and batch queuing. Compared with GC-MS, which relies on manual dilution, filtration, and temperature ramping, MRR eliminates variability and downtime (**Figure 1**). Samples load directly into 20 mL headspace vials, equilibrate in the autosampler oven, and produce unambiguous results in under 15 minutes, with typical MRR acquisition times of less than 10 minutes. It's a streamlined path that avoids the very steps most prone to bottlenecks and variability.



Inside the Workflow: Developing the MRR Method

Method setup with MRR is intuitive and straightforward. Analysts begin by selecting EG and DEG from the molecular library, then fine-tune temperature and analysis time to achieve optimal signal response.

Figure 1. **Workflow comparison of MRR and GC-MS for analyzing DEG and EG in PEG-400.** MRR bypasses traditional sample preparation steps like dilution and filtration, using headspace sampling to deliver fast analysis times and confident identification, even in injection-challenging matrices.

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Each analyte's frequency and averaging parameters are automatically prefilled from validated spectral data, minimizing manual input (**Figure 2**).

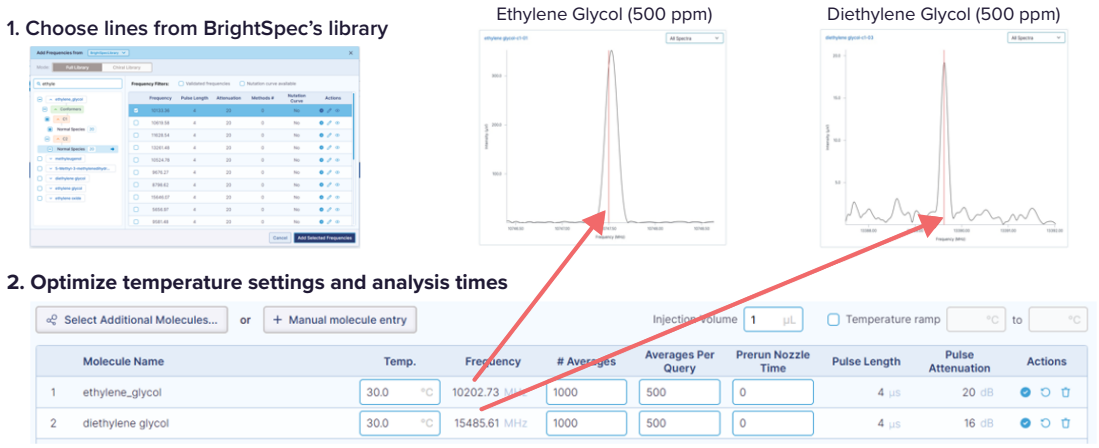


Figure 2. Simplified method development using the isoMRR platform. Analysts select EG and DEG from the molecular library (top left) and fine-tune temperature and analysis times to optimize signal strength. Representative spectra for EG (500 ppm) and DEG (500 ppm) show clear, distinct peaks (center and right). The workflow allows direct parameter control (e.g., frequency, averaging, pulse attenuation) within the Edgar software interface, enabling quick setup and reproducible results without extensive method development.

Once the method is defined, samples are queued in Edgar software, where experiment names, vial positions, and injection volumes can be assigned in a few clicks (**Figure 3**). The system supports both new and existing projects, allowing methods to be reused or modified without revalidation. With automated equilibration, sampling, and data collection, MRR and its associated software make what was once a complex setup now a streamlined process ready for routine use.

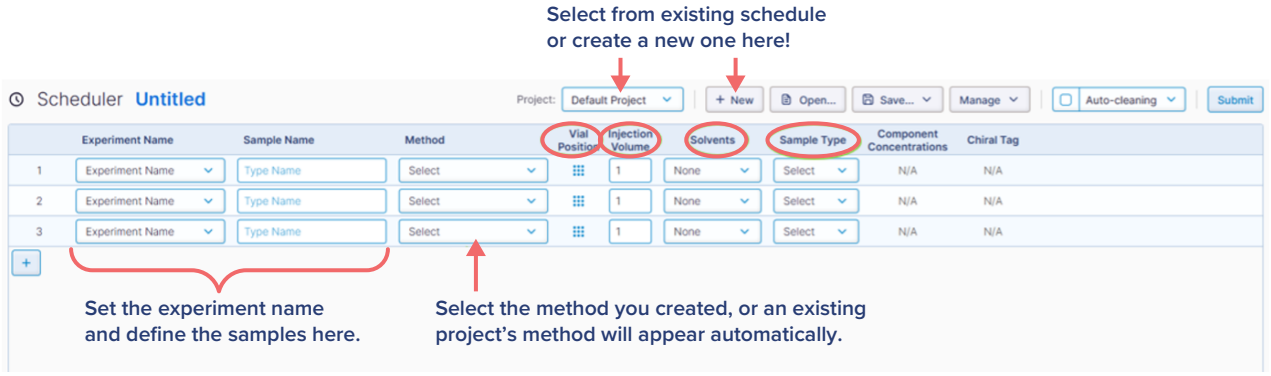


Figure 3. Automated sample scheduling and queue setup in Edgar software. The Scheduler interface allows users to define experiment names, assign sample identifiers, and select a pre-created method for each run. Newly created or existing methods can be applied automatically, making setup for multi-sample batches a breeze. Key parameters (e.g., vial position, injection volume, solvents, sample type) can be easily configured from a single screen. Automated scheduling supports continuous, unattended operation, improving throughput and consistency across excipient testing workflows.

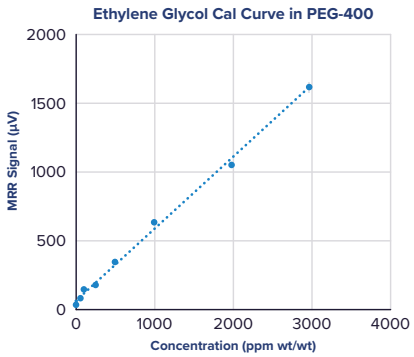
With MRR, method development no longer slows down the science. Every step, from parameter selection to batch scheduling, is designed for speed, precision, and confidence. That means more than improved efficiency. It means empowerment for analytical teams to deliver verified, reproducible results in every run.

Results

Calibration standards of PEG-400 were prepared by spiking samples with known concentrations of EG and DEG. Each sample was transferred into a 20 mL headspace vial, equilibrated, and analyzed using the isoMRR platform under fully

automated conditions. MRR demonstrated reliable quantitation of EG and DEG in PEG-400 with strong signal linearity and excellent reproducibility (**Figures 4 and 5**). Compared with GC workflows that typically require 45–60 minutes per sample, MRR achieves equivalent ppm-level sensitivity in less than 15 minutes—a time reduction of roughly 75%. The results that follow confirm that MRR meets the analytical confidence required for excipient safety testing, with dramatically improved efficiency and throughput.

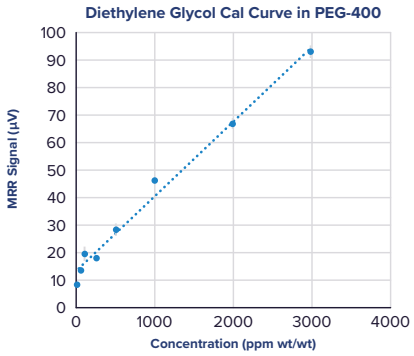
Calibration results for EG in PEG-400 demonstrate both the analytical precision and operational efficiency of MRR. As shown in the calibration plot (**Figure 4**), the MRR signal intensity increases linearly with concentration, yielding a strong correlation ($R^2 > 0.99$) over a wide dynamic range of 10–2960 ppm by weight (wt/wt). This performance reflects the instrument's ability to maintain quantitative accuracy even in viscous excipient matrices that typically challenge GC-based workflows. The method achieved a limit of detection (LOD) of 3 ppm and a limit of quantitation (LOQ) of 10 ppm. Repeatability only showed less than 3.1% variation across replicates, and each measurement required just 200 seconds. These validation parameters align with ICH Q2(R2) guidance for analytical procedures.[4]



ICH Q2 R2 Method Parameter	EG
LOD (ppm)	3
LOQ (ppm)	10
Linear Range (ppm)	10-2960
Linearity (R^2)	0.9967
Response factor, slope (µV / ppm)	0.5229
Repeatability (%)	3.02
Measurement time (s)	200

Figure 4. Calibration performance of EG in PEG-400 using MRR. Calibration curve illustrating the linear response of MRR signal (µV) versus EG concentration (ppm wt/wt). The method demonstrated high linearity ($R^2 = 0.9967$) across a 10–2960 ppm range, with a LOD of 3 ppm and a LOQ of 10 ppm.

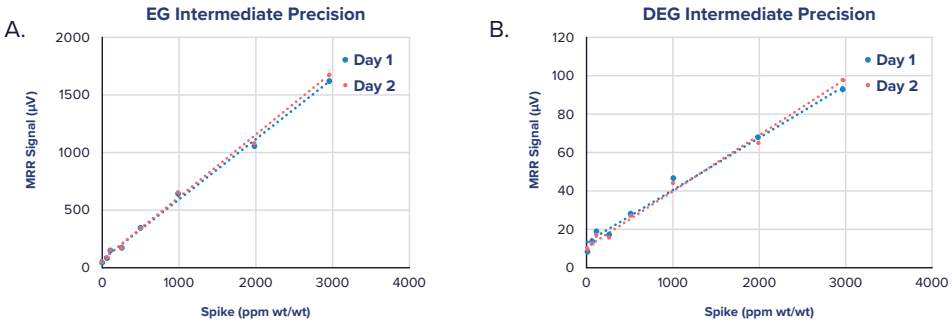
Building on the EG data, analysis of DEG in PEG-400 reinforces the precision and versatility of MRR for excipient safety testing. The calibration curve in **Figure 5** shows a clear, linear response across the concentration range of 182–2973 ppm by weight ($R^2 > 0.98$). Despite DEG's lower volatility compared to EG, MRR maintained excellent signal integrity and quantitation accuracy, achieving a LOD of 60 ppm and a LOQ of 182 ppm. This performance meets the linearity and precision expectations described in ICH Q2(R2) for quantitative methods.[4] Repeatability remained strong at less than 4%, confirming method robustness even for challenging analytes. Each measurement required only 600 seconds, highlighting the efficiency of MRR's automated workflow.



ICH Q2 R2 Method Parameter	EG
LOD (ppm)	60
LOQ (ppm)	182
Linear Range (ppm)	182-2973
Linearity (R^2)	0.9868
Response factor, slope (µV / ppm)	0.0274
Repeatability (%)	3.98
Measurement time (s)	600

Figure 5. Calibration performance of DEG in PEG-400 using MRR. Calibration curve illustrating MRR signal (µV) versus DEG concentration (ppm wt/wt). The method demonstrated strong linearity ($R^2 = 0.9868$) across a 182–2973 ppm range, with a LOD of 60 ppm and a LOQ of 182 ppm.

To further evaluate the robustness of the MRR method, intermediate precision and recovery were assessed for both EG and DEG over two independent days. The calibration plots in **Figure 6** show consistent linear responses across replicates, with nearly identical slopes on days 1 and 2 for each analyte. For EG, intermediate precision was measured at 3.7%, with recoveries ranging from 82% to 112% across concentration levels. DEG exhibited slightly higher variability, with 5.0% intermediate precision and recoveries between 96% and 111%. All results fall within acceptable ranges consistent with ICH Q2(R2) criteria for intermediate precision and recovery.[4]



ICH Q2 R2 Method Validation Parameter	EG (%)	DEG (%)
Intermediate precision	3.73	4.95
% Recovery, level 1 *	82	101
% Recovery, level 2 *	104	111
% Recovery, level 3 *	112	96

Figure 6. Intermediate precision and recovery of EG and DEG in PEG-400 using MRR. Calibration plots compare day 1 (blue) and day 2 (red) results, showing consistent signal response across concentration ranges. Table summarizes intermediate precision and percent recovery at three spike levels, demonstrating reproducible quantitation of both EG and DEG.

These results confirm that MRR quantitation remains stable and reproducible over time, even when testing is repeated under variable conditions. The data reinforce MRR's reliability not only for sensitivity and speed, but also for the long-term consistency required in regulated excipient analysis.

Conclusion

Headspace-MRR delivers a new level of simplicity and reliability to excipient testing. By eliminating manual preparation and long run times, it increases testing capacity and reduces operational burden. For laboratories tasked with ensuring product quality and patient safety, MRR provides an analytical pathway that is both scientifically rigorous and operationally modern. In an era where compliance and efficiency can no longer be traded off, MRR bridges the gap, upgrading excipient analysis into a faster, smarter, and more confident process.

References

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4. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). *ICH Harmonised Guideline: Validation of Analytical Procedures Q2(R2).* March 2024. **fda.gov/regulatory-information/search-fda-guidance-documents/q2r2-validation-analytical-procedures** (accessed Oct 15, 2025).

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