Breaking the Standard: Alaninol Chirality Confirmed Without Chromatography

Overview

Chirality can make or break a molecule's safety and effectiveness. One enantiomer may be a safe and effective treatment—its mirror image inactive, or even harmful. One of the approaches to synthesize chiral drugs is the so-called "chiral pool," a strategy that uses naturally derived, enantiopure building blocks to lock in the correct stereochemistry from the start. Alaninol (2-aminopropan-1-ol), derived from alanine, is one such chiral raw material used to synthesize Cabotegravir (Vocabria®), an antiretroviral drug for HIV treatment. Ensuring its enantiomeric purity and correct absolute configuration (AC) is essential to guarantee product quality, efficacy, and safety.

Conventional techniques like chiral HPLC and GC often struggle with small, reactive amino alcohols like alaninol. These compounds lack UV chromophores for LC detection, and their sticky amine groups cause retention shifts and tailing in GC analysis—making derivatization a necessity. Even with derivatization, expensive standards are still required to calibrate the method. In this application note, we demonstrate how molecular rotational resonance (MRR) cuts through those challenges. For alaninol—and other amino alcohols like it—MRR delivers fast, accurate, and standard-free chirality confirmation. No derivatization or chromatography required.

Chiral Analysis Without the Hassle

Chiral chromatography comes with baggage: expensive column screens, derivatization steps, and the need to source enantiopure standards. And after all that, small, polar analytes like amino alcohols can still give poor resolution. That's where MRR comes in. Operating in the gas phase, MRR skips the columns and the standards, delivering AC and enantiomeric excess (EE) with minimal sample prep. It's fast, selective, and ideally suited for tricky molecules that don't behave well under traditional conditions. Whether you're validating raw materials or building out quality control methods, MRR offers a cleaner, more agile alternative (**Table 1**).

Feature	MRR	Chiral Chromatography
Chiral Columns	Not Required	Required
Derivatization	Not Required	Required
Enantiopure Standards	Not Required	Required
Spectral Overlaps	No	Often
Analysis Time	15-30 min	1-2 hr

Table 1. **Common barriers in chiral chromatography compared with MRR.**

Built-In Flexibility for Chiral Method Development

One of MRR's key advantages is its ability to adapt to what you already know about your analyte. If the molecule is already part of the BrightSpec chiral library, method development is as simple as selecting validated rotational lines, running a tag normalization measurement, and confirming the method (**Figure 1**). The software screenshot in **Figure 1B** shows this streamlined setup process within the BrightSpec Edgar software interface, where homochiral and heterochiral alaninol transitions, corresponding to the different enantiomers, respectively, are preloaded and ready for acquisition. If the analyte is new to the library, the workflow begins with spectral scans using racemic and chiral tags to capture the full rotational fingerprints of tag-analyte

diastereomeric complexes. Spectral fitting and quantum mechanical calculations identify enantiomer-specific transitions, which are then used to build out a targeted method. From there, it's the same final steps: tag normalization and validation. The software screenshot in **Figure 1C** shows the automated method validation queue, where replicate injections are easily scheduled and run with minimal manual input. Whether you're starting from scratch or analyzing a known molecule, MRR keeps method development simple and efficient.

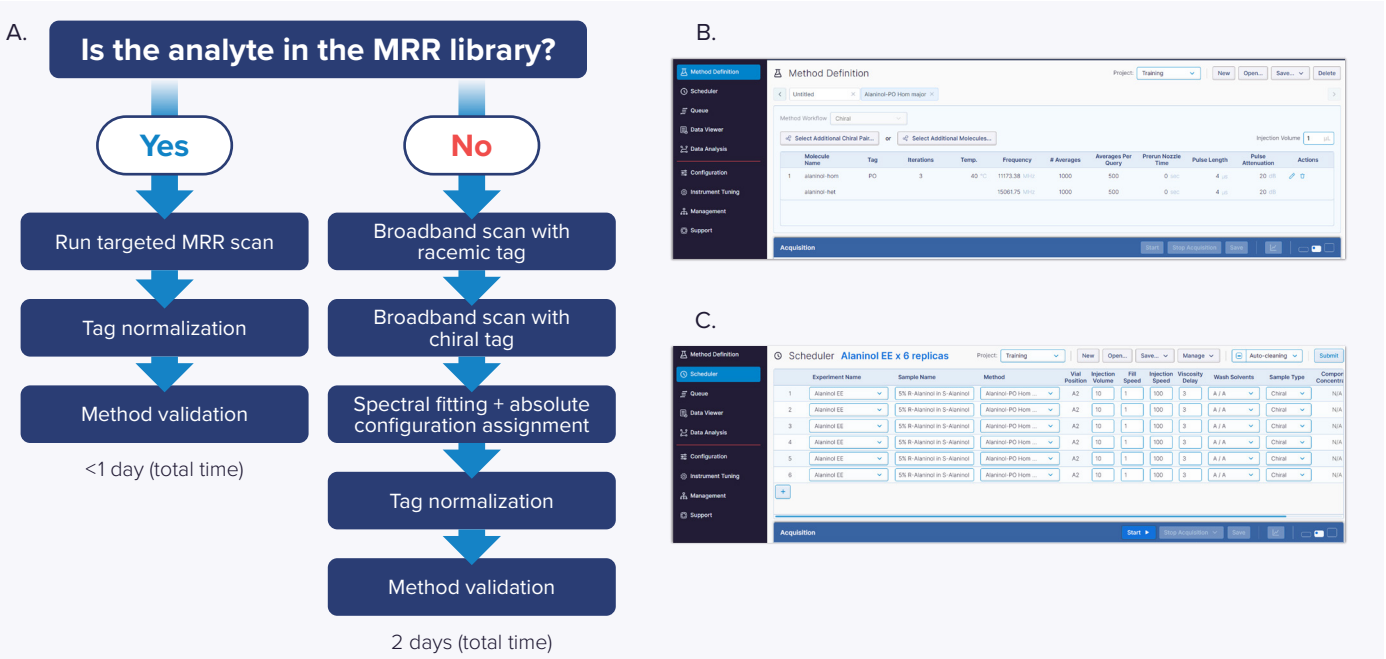


Figure 1. Method development workflows based on analyte library status. A. Whether you're starting with a known compound or something entirely new, the MRR workflow is flexible, with both paths leading to fast, reference-free enantiomeric analysis. **B.** Edgar software screenshot showing method definition using pre-validated homochiral and heterochiral transitions. **C.** Edgar software screenshot shows the sample queue for a validation run of alaninol %EE.

Results

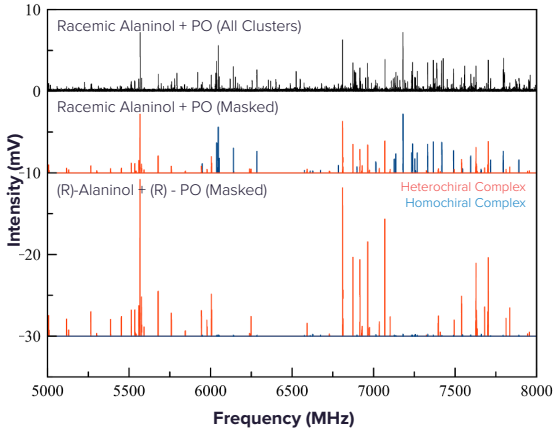
Absolute Configuration and Enantiomeric Purity

MRR spectroscopy relies on quantum chemical predictions to identify rotational frequencies of chiral complexes—no enantiopure reference standards required. These predictions, in conjunction with tags of known chirality, effectively serve as built-in standards. Chiral tags, like S-propylene oxide (PO), are automatically introduced with the carrier gas and analytes form gas-phase diastereomeric complexes that produce distinct rotational spectra, allowing direct assignment of both AC and EE. The result: faster, simpler chiral analysis, purpose-built for quality control. This approach has been previously validated for alaninol in pharmaceutical raw materials testing, including EE quantitation relevant to Cabotegravir synthesis.[1]

Alaninol is a primary amino-alcohol and is a representative challenge in pharmaceutical chiral analysis. Using MRR, both enantiomers of alaninol were characterized via their chiral-tagged spectra. First, the rotational spectra of alaninol tagged with S-(PO) was acquired, enabling its addition to the BrightSpec chiral library (**Figure 2**). Through this approach, two distinct diastereomeric complexes were resolved: a heterochiral complex formed between (R)-(-)-alaninol and (S)-PO, and a homochiral complex between (S)-(+)-alaninol and (S)-PO. These yielded distinct, color-coded spectral patterns: heterochiral in red, homochiral in blue (**Figure 2A**). A targeted scan of (R)-alaninol matched the heterochiral signature, confirming enantiomer identity.

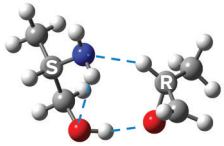
Theoretical predictions of rotational constants (A, B, and C), dipole moment components (μ), and quadrupole coupling components (χ) for each complex were calculated and matched closely with experimental values, making the spectral assignments unambiguous. With this validation, (S)-alaninol was confirmed as the major enantiomer and can now be

A. Broadband Measurements



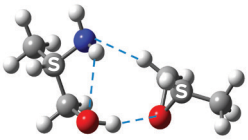
B. Unambiguous Determination of Absolute Configuration

Heterochiral Complex:
(R)-(-)-Alaninol / S-Propylene oxide



Parameter	Calculation	Experiment
A (MHz)	2274.5	2324.3
B (MHz)	754.3	728.7
C (MHz)	675.6	655.7
χ_{aa} (MHz)	-1.1	-0.87
χ_{bb}-χ_{cc} (MHz)	-1.4	-1.56
ΔE (cm^{-1})	0	

Homochiral Complex:
(S)-(+)-Alaninol / S-Propylene oxide



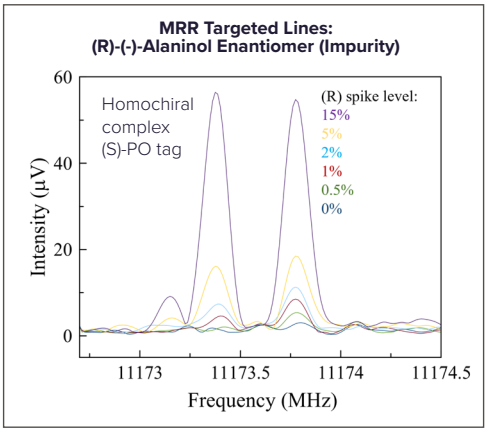
Parameter	Calculation	Experiment
A (MHz)	3084.8	3139.1
B (MHz)	641.7	623.8
C (MHz)	598.3	585.5
χ_{aa} (MHz)	0.6	0.35
χ_{bb}-χ_{cc} (MHz)	3.5	2.94
ΔE (cm^{-1})	0	

Figure 2. Broadband MRR spectra of alaninol complexed with S-(PO) demonstrates clear separation of heterochiral and homochiral diastereomeric complexes. A. Top: Broadband MRR scan of racemic alaninol + PO shows full cluster complexity. Middle: Masked trace highlights distinguishable spectral features of heterochiral (R-alaninol/S-PO, red) and homochiral (S-alaninol/S-PO, blue) complexes. Bottom: Targeted spectrum of (R)-alaninol + (S)-PO confirms match with heterochiral signals. **B.** Structural models and MRR parameters for both complexes are shown, with close agreement between theoretical predictions and experimental measurements, enabling the assignment of AC and %EE without reference standards.

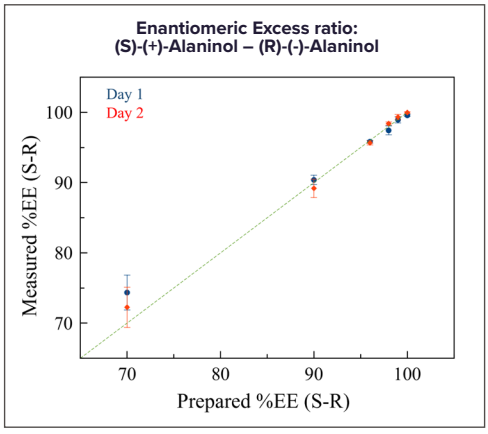
reliably tracked in future analyses. This method enables direct determination of both AC and %EE without the need for enantiopure standards—offering a fast, accurate solution for quality teams working with chiral amino-alcohols in pharmaceutical synthesis.

To validate the method's ability to quantify EE, (S)-alaninol was spiked with increasing levels of the (R)-enantiomer across a range of 0.5% to 15%. Distinct peaks corresponding to the (R)-alaninol signal were resolved within the homochiral complex spectrum, even at sub-percent levels (**Figure 3A**). Quantitative measurements across six replicates per level showed tight precision, with %EE values consistently aligning with expected spike levels. A linear correlation between

A.



B.



C.

Recovery Values for (S)-Alaninol Spiked with (R)-Alaninol

Spike Level (R)	Measured Spike (n=6)
0.5%	0.32% ± 0.22%
1%	1.14% ± 0.30%
2%	1.56% ± 0.33%
5%	4.34% ± 0.56%
15%	14.2% ± 1.9%

Figure 3. MRR-based quantification of EE in (S)-alaninol spiked with known levels of (R)-alaninol. A. Overlay of MRR spectral peaks showing increasing signal from the (R)-enantiomer within the homochiral complex region as spike levels rise from 0% to 15%. **B.** Correlation between prepared and measured %EE across two days demonstrates repeatability, intermediate precision, sensitivity, and linear response. **C.** Table of measured spike recovery across six replicates per level confirms method accuracy and sensitivity for low-level enantiomeric impurity detection.

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prepared and measured EE (**Figure 3B**) was observed across two independent days, underscoring the method's robustness and reproducibility.

This level of sensitivity and accuracy enables confident detection of low-level enantiomeric impurities—an essential requirement for pharmaceutical synthesis, where guidelines often demand control at the percent or sub-percent level. The ability to quantify enantiomeric purity without standards, columns, or derivatization simplifies workflows while maintaining analytical rigor.

Conclusion

MRR spectroscopy is a practical, streamlined alternative to traditional chiral analysis in pharmaceutical settings. It accurately determines both AC and EE—without the hassle of enantiopure standards, columns, or derivatization. In the case of alaninol, a small but critical building block for Cabotegravir, MRR delivered precise, reproducible results with minimal sample prep. For manufacturers leveraging the chiral pool strategy, this means faster validation of starting materials and greater confidence in the stereochemical fidelity of downstream products. With its speed, simplicity, and precision, MRR fits naturally into modern quality control workflows—especially for tricky, polar compounds that challenge conventional methods.

References

1. Justin L. Neill [a], Alexander V. Mikhonin [a], Ted Chen [b], Reilly E. Sonstrom [c], Brooks H. Pate [c]., Rapid Quantification of Isomeric and Dehalogenated Impurities in Pharmaceutical Raw Materials Using MRR Spectroscopy. *J. Pharm. Biomed. Anal.* 2020:189, 113474.
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BrightSpec, Inc.
770 Harris Street Suite 104b
Charlottesville, Virginia 22903