Direct In-Process Enantiopurity Monitoring Using MRR

OVERVIEW

Chiral purity of pharmaceutical, natural, and other consumer products is important for their effectiveness and safety. This is because the enantiomers may have different physiological activity, as their metabolic pathways within chiral biological systems may differ dramatically. As such, monitoring and controlling the chirality or enantiopurity during all stages of product manufacturing is a highly important task. However, most enantioselective techniques are not readily applicable for online monitoring as they are either too slow or laborious for routine online monitoring. In addition, many methods require enantiopure standards that can be expensive or not available. Therefore, analytical techniques capable of rapid enantiopurity measurements directly on crude process mixtures without standards are of high interest.

Molecular Rotational Resonance (MRR) technology can monitor enantiopurity of individual components in crude process mixtures directly, without any analyte purification prior to analysis. There is no need for producing or purchasing enantiopure standards – BrightSpec's chiral tagging method enables direct quantitation of each enantiomer in a mixture. Furthermore, chiral and achiral, i.e. optical and chemical purity analyses, can be performed in one measurement.

MRR ANALYSIS EXAMPLE: PANTOLACTONE

(R)-pantolactone is an important chiral intermediate that is used in the synthesis of (R)-pantothenic acid (vitamin B_5) and its prodrug, (R)-panthenol. At process setting, the pantolactone enantiopurity is conventionally monitored using polarimetry. A major downside of polarimetry is that the matrix interferences can lead to analysis errors. As such, time and labor consuming sample purification is required prior to the polarimetry analysis, delaying the feedback and handicapping the process control. In contrast to polarimetry, MRR is capable of reliable and fully automated near time enantiopurity measurements without enantiopure standards, analyte purification, or human intervention.

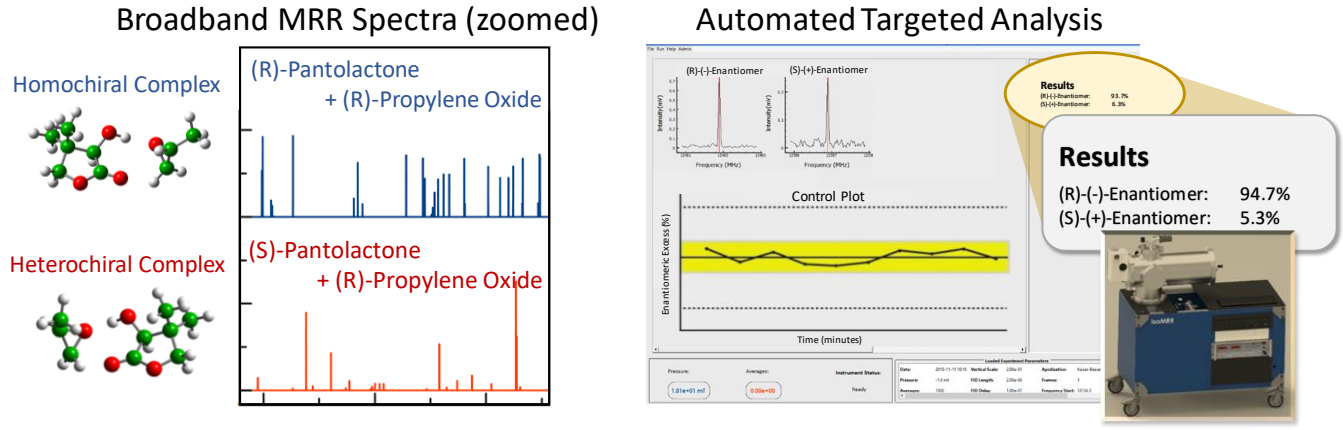

Figure 1. Left Panel. Broadband MRR spectra of homochiral (blue) and heterochiral (red) complexes between the analyte (pantolactone) and the low-cost gaseous chiral agent ((R)-propylene oxide). Right Panel. Example of fully automated analysis performed by a BrightSpec's targeted isoMRR instrument. The analysis can be executed every ~10 minutes and requires no enantiopure standards or sample purification.

Chiral Tagging Method. To enable direct quantitation of enantiomers in crude mixtures without separation and enantiopure standards, BrightSpec utilizes a chiral tagging method.[1] In this method, a chiral complexing agent (tag) is introduced into a carrier gas to form stable weakly-bounded complexes with an analyte in a gas phase. Different enantiomers of an analyte form different type of complexes with the tag, that can be readily discriminated and quantified by MRR to enable a reliable analyte enantiopurity determination in minutes (Figure 1). A few low-cost commercial tags can cover a wide range of analytes.

Targeted MRR Analysis. BrightSpec's targeted IsoMRR™ instrument (Figure 1) can be utilized for in-process enantiopurity monitoring. This instrument measures narrow-band rotational spectra around pre-selected MRR

transitions (Figure 1, right panel) and can be directly interfaced with a process[2] to achieve automated operation and fast sample-to-sample cycle time. Targeted MRR frequencies to monitor each enantiomer can be chosen either by using the BrightSpec spectral library or by measuring the relevant broadband spectra (Figure 1, left panel).

Figure 1 (right panel) demonstrates an example of quantitative pantolactone enantiopurity analysis, performed by IsoMRR™ instrument directly out of the process mixture. These analyses can be performed continuously about every 10 minutes and without human intervention. BrightSpec's Edgar software is 21 CFR Part 11-compliant and is capable of not only automated determination of enantiopurity but also generating process control plots (Figure 1, right panel).

Targeted MRR Method Validation. To validate the developed targeted MRR enantiopurity determination method, we have purchased commercial (R)- and (S)-pantolactone from a major chemical company and prepared several mixtures of varying but known enantiopurity. To estimate the analysis repeatability, we have performed 3 individual measurements of each sample. As evident from Figure 2, targeted MRR analysis demonstrates excellent sensitivity, linearity, range and repeatability that are comparable or better than those of conventional stereoselective methods.

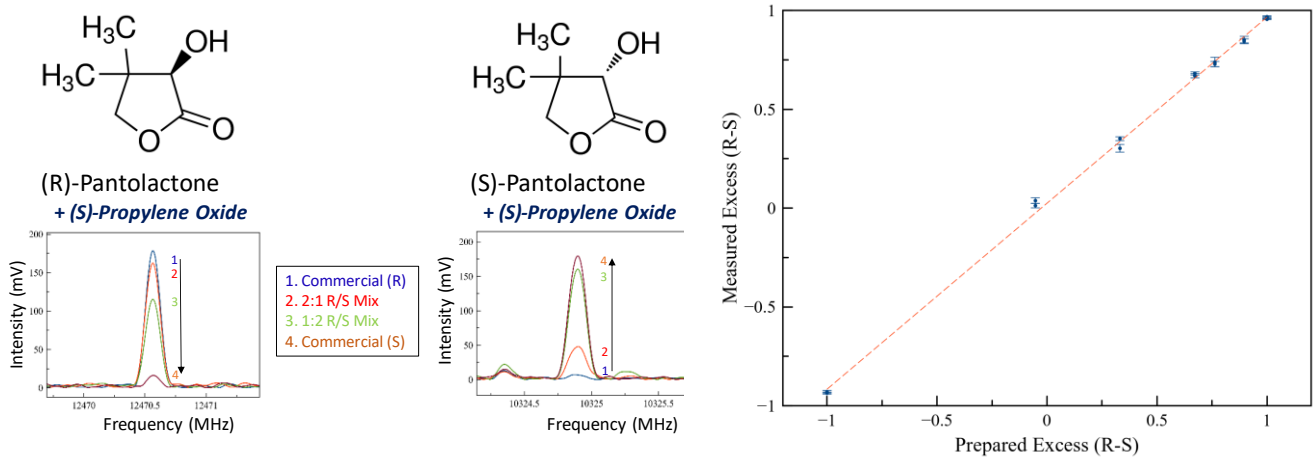

Figure 2. Targeted MRR method validation using prepared mixtures of commercial (R)- and (S)-pantolactones. MRR shows excellent analysis sensitivity, linearity, range, and repeatability.

CONCLUDING REMARKS

MRR enables fast, reliable, and fully automated in-process chiral purity measurements without separation, chemometrics, or enantiopure standards. Additional MRR benefits include the ability to perform chiral and achiral analyses in one run, the ability to easily discriminate between all types of isomers and isotopologues, and robustness of MRR methods to process variations due to its interference-free nature. As a result, MRR implementation can likely improve control of existing chiral processes, accelerate the development of new processes; as well as contribute to developing of next generation QbD-based quality control strategies to improve product quality and reduce manufacturing costs.

REFERENCES

1. B.H. Pate, et al. *"Quantitative Chiral Analysis by Molecular Rotational Spectroscopy"*, in *"Chiral Analysis: Advances in Spectroscopy, Chromatography, and Emerging Methods"*, 2nd Ed: P. Polavarapu, Editor; Elsevier, **2018**.

2. J.L. Neill et al., *"Online Stereochemical Process Monitoring by Molecular Rotational Resonance Spectroscopy"*, Org. Process Res. Dev., **2019**, *23 (5)*, pp. 1046-1051.

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