High Resolution MRR: Direct Quantitation of Three Regioisomer Impurities in a Pharmaceutical Raw Material in One Measurement

Introduction

Even though regioisomers (also known as 'positional isomers') have the same molecular formula, their specific functional groups are located at different positions within a parent molecular structure or chain. This difference in molecular geometry may significantly impact both efficiency and safety of a drug product.[1] Therefore, identification and quantitation of regioisomer impurities is a very important task for pharmaceutical industry at essentially all stages of drug discovery and production: from raw materials and intermediates to drug candidates and final drug products. Yet, this task poses serious challenges for conventional analytical techniques including chromatography and mass spectrometry (MS).

Since regioisomers are MS-equivalent, their identification and quantitation generally rely entirely on their chromatographic separation. The elution properties of the regioisomers can be similar as well. Therefore, the development of appropriate analytical methods to enable reliable identification and quantitation of the regioisomer impurities at all stages of pharmaceutical synthesis workflow is typically a very challenging task requiring a significant investment. This is especially true in case of regioisomers of fluorinated pharmaceuticals that are known to be tough analytes for chromatography due to their high polarity.[2]

In contrast to chromatography, MRR is intrinsically well suited for quantitative analysis of highly polar, structurally similar molecules and is not dependent on the availability of pure reference materials.[3] In this case study, we have demonstrated the extraordinary resolving power of MRR for analysis of regioisomer impurities in fluorinated benzylamines. MRR was not only able to easily resolve individual contributions of regioisomer impurities present at trace level, but also was able to resolve and quantitate, with equal ease, the trace-level regioisomer of the main component.

iso*MRR*™ Instrument

Impurities in complex chemical mixtures can be identified and quantified using an iso*MRR*™ instrument (Figure 1). iso*MRR* can analyze volatilizable molecules with molecular weight of up to ~400 amu. Due to its extraordinary chemical specificity, iso*MRR* can directly resolve and quantitate individual contributions of structurally similar chemicals including regioisomers, diastereomers, and isotopologues without chemical separation or chemometrics. Enantiomer analysis with chiral tagging is also possible.

iso*MRR*'s sampling interface enables either direct syringe injection or can be connected to a chemical reactor to enable continuous near real-time sampling. The typical analysis cycle time is between 5 and 15 minutes including MRR measurements themselves (seconds to minutes, depending on number of analytes and desired detection limits) and about 5 minutes for automated sampling lines and chamber purge and cleaning between measurements.

Figure 1. A picture of a commercial iso*MRR*™ unit capable of quantitative analysis of impurities in complex chemical mixtures including regioismers, diastereomers, enantiomers, and isotopomers. Instrument is equipped with online-capable sampling manifold.

Method Development and Validation

Preparation of Validation Standards. Essentially pure 2,4-difluorobenzylamine (2,4-DFBA), 2-fluorobenzylamine (2-FBA), 4-fluorobenzylamine (4-FBA), and 2,6-difluorobenzylamine (2,6-DFBA) have been purchased from a well-known chemical vendor. Five validation standards containing different levels of three impurities in 2,4-DFBA have been prepared: 0% (blank 2,4-DFBA), 0.05%, 0.2%, 1%, and 5%. All samples have been injected into Iso*MRR* instrument 'as is' without any further pre-treatment.

Broadband MRR Measurements. A high signal-to-noise broadband MRR spectrum of a complex regioisomer mixture can be collected to enable identification of its individual constituents using BrightSpec MRR library followed by the selection of individual analyte peaks that work best for a particular analysis.

Targeted MRR Measurements. Once all analytes of interest are identified and their analysis peaks selected, the iso*MRR* instrument can be run in a highly sensitive, fast, and online-capable targeted mode. In this mode, analysis sensitivity for each analyte can be selectively adjusted independently from other analytes to optimize analysis cycle time and detection limits, as needed.

The top plots of Figure 2 show well isolated peaks of 2-FBA, 4-FBA, and 2,6-FBA measured directly in the 2,4-DFBA matrix to demonstrate MRR selectivity to these regioisomer impurities. Bottom plots of Figure 2 demonstrate linearity and repeatability of MRR response for each of these three impurities.

Table 1 shows basic MRR method validation parameters for analysis of these three regioisomer impurities in 2,4-DFBA. Along with excellent linearity and good repeatability, it should be emphasized that MRR detection limits for all these impurities are significantly below 0.1% threshold level that is set by FDA for characterization of impurities in drug substances and new drug products.[4]

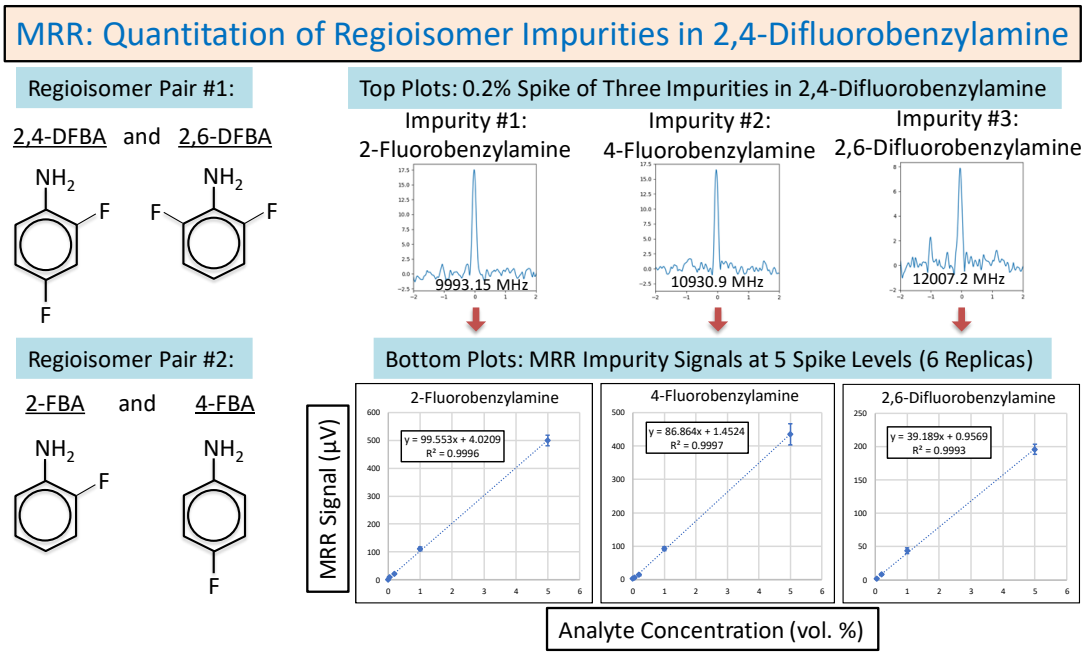

Figure 2. Selectivity, linearity, and repeatability of targeted MRR for quantitative analysis of regioisomer impurities in 2,4-Difluorobenzylamine. Top plots show well isolated MRR lines of three impurities introduced into the main component at 0.2 vol% level to demonstrate selectivity. Bottom plots demonstrate linearity and repeatability of MRR response for five spike levels: blank, 0.05%, 0.2%, 1% and 5%. MRR measurement times were 200 seconds for blank and 0.05% concentration points, and 80 seconds for other concentration points. Error bars represent standard deviation of six independent determinations.

Table 1. MRR method linearity, low detection limits, and repeatability for simultaneous analysis of three regioisomer impurities in 2,4-DFBA.

Analyte	MRR Linearity, R^2 (0 – 5 vol%)	Low Detection Limit * (vol%)	Repeatability ** at 1% Spike (n=6)
2-FBA	0.998	0.015%	5.7%
4-FBA	0.994	0.019%	5.9%
2,6-DFBA	0.997	0.031%	7.6%

* Low detection limits (LDL) are estimated from measured detector noise and MRR response slopes using a formula: LDL = 3 x Detector Noise (blank, n=6, 1σ) / Slope.
** Repeatability is estimated for n=6 independent determinations with MRR measurement times of 80 seconds per analyte.

Table 2. Concentrations of three impurities in 2,4-difluorobenzylamine (2,4-DFBA) measured by IsoMRR unit vs their nominal concentrations.

Nominal Spike Level (vol%)	Measured Spike Level (vol %) 2-FBA	4-FBA	2,6-DFBA
Commercial 2,4-DFBA	below LDL	0.043 *	below LDL
0.05	0.056	0.062	0.031 *
0.2	0.18	0.17	0.19
1	1.07	1.06	1.09
5	4.98	4.97	4.97

* Above the detection limit but below the quantitation limit for this analyte.

Results

Analysis of the pure and spiked commercial 2,4-DFBA samples directly demonstrates power of the targeted MRR for quantitation of trace-level regioisomer impurities in fluorinated benzylamines (Table 2). MRR was able to reliably detect about 0.04 vol% of 4-FBA impurity present in the 'pure' commercial 2,4-DFBA sample (Table 2, first row). In addition, MRR analysis confirmed that the other two impurities, 2-FBA and 2,6-DFBA, are not present in the commercial sample at levels above their detection limits (Table 1).

As for the spiked samples, there is a good agreement between the impurity concentrations measured by MRR and the nominal concentrations of these impurities in these samples (Table 2). It should also be noticed that longer MRR measurements and/or further method optimization can significantly improve analysis accuracy.

Conclusions

A highly selective and free of any separation challenges MRR method, that is capable of reliable discrimination and quantitation of several regioisomer impurities in 2,4-difluorobenzylamine in one measurement, has been demonstrated for the first time. This method enables not only analysis of trace-level impurities that are regioisomers of each other, but also the unambiguous identification and direct quantitation of trace-level regioisomers of the main component present at concentration levels down to less than 0.1%, with analysis cycle time of between 5 and 15 minutes.

The major benefits of MRR implementation are the significantly improved analysis throughput, no consumables, and online-capability. In addition, MRR can also serve as an orthogonal method for analysis verification or certification purpose, or as a fast and convenient screening tool for identification and quantitation of unexpected analytes in complex chemical mixtures including regioisomers, diastereomers, and enantiomers. Furthermore, MRR can be directly utilized for quantitative analysis of 'hard-to-separate' chemical mixtures such as those containing analytes with multiple chiral centers and/or multiple isomeric impurities.

References

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Please visit us on the Web at http://www.brightspec.com/ or contact us at sales@brightspec.com or +1 (434) 202-2391.