Beyond Mass:

Structure-Specific Quantitation of Closely Related Impurities in Pharmaceutical Raw Materials

Introduction

Structurally similar impurities can be difficult to identify and quantitate in pharmaceutical raw materials. Regioisomers are particularly challenging because they share the same molecular formula as the intended compound, differing only in the position of substituents on the molecular framework. They therefore cannot be distinguished by mass or fragmentation pattern alone and may also be difficult to separate chromatographically.

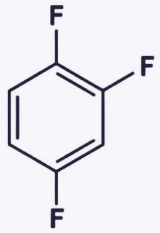
Because regioisomers often have chemical reaction pathways similar to those of the desired reagent, their reaction products may carry over into subsequent synthetic steps and contribute to related impurities in intermediates or the final drug substance. Structural analogs, including dehalogenated or partially substituted variants, present a similar challenge because they may retain closely related chemical and physical properties.

MRR distinguishes these compounds through their unique rotational spectra, collapsing hours of method development into a simple workflow. This study evaluates four structurally similar impurities in a commercial lot of 1,2,4-trifluorobenzene using two MRR sampling workflows.

Case Study: Impurity Quantitation in 1,2,4-Trifluorobenzene

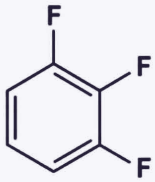
1,2,4-Trifluorobenzene was purchased from Oakwood Chemicals. The methods were developed to achieve LOQs of 100–500 ppm for each impurity, reflecting practical industry expectations for the targeted assessment of low-level, structurally related impurities in pharmaceutical raw materials.

Target Raw Material

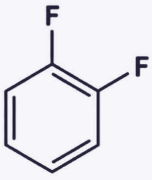


1,2,4-Trifluorobenzene
(1,2,4-TFB)

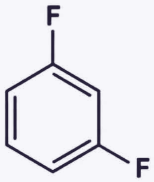
Targeted Impurities



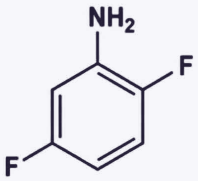
1,2,3-Trifluorobenzene
(1,2,3-TFB)



1,2-Difluorobenzene
(1,2-DFB)



1,3-Difluorobenzene
(1,3-DFB)

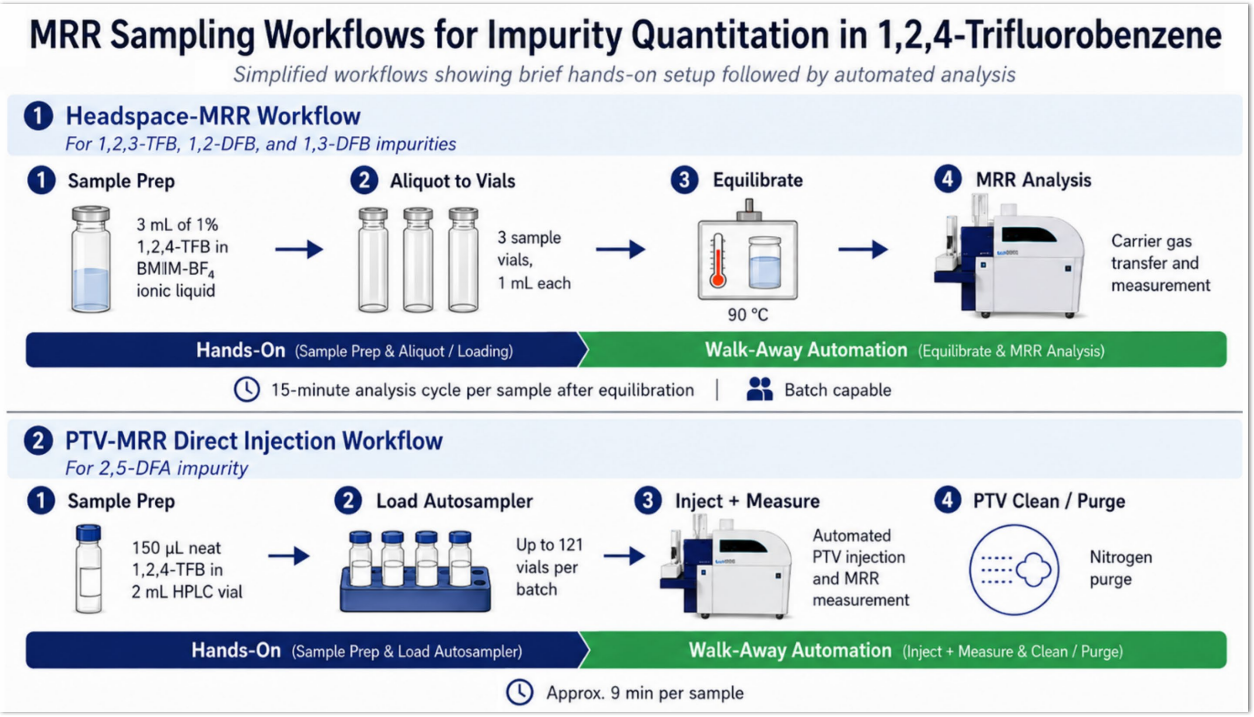


2,5-Difluoroaniline
(2,5-DFA)

Method

Two complementary MRR workflows were selected to accommodate the different volatility and sampling requirements of the four target impurities. The three fluorobenzene impurities were analyzed by headspace-MRR, while 2,5-difluoroaniline was analyzed by direct injection PTV-MRR. Five-point calibration curves were prepared for each impurity using spiked samples to evaluate linearity, repeatability, LOD, and LOQ before analysis of the commercial 1,2,4-trifluorobenzene lot.

BrightSpec



Results

MRR detected three of the four targeted impurities in the commercially acquired sample. All four methods met the target sensitivity, demonstrating confident differentiation and measurement of closely related impurities at low ppm levels.

Impurity	Measured Amount (ppm)	LOD (ppm)	LOQ (ppm)	Linearity (R^2)	Repeatability (% rsd)*
1,2,3-Trifluorobenzene	94 ± 15	62	187	>0.99	9%
1,3-Difluorobenzene	591 ± 158	162	487	>0.99	12%
1,2-Difluorobenzene	541 ± 15	80	241	>0.99	9%
2,5-Difluoroaniline	n.d., <71 ppm	71	210	>0.99	11%

*** Estimated using 18 determinations across the concentration range of the procedure (e.g., 6 concentrations / 3 replicas each).**

Table 1. **MRR Method Performance**

Conclusion

MRR methods were established for a regioisomer and three related structural analogs in 1,2,4-trifluorobenzene. All four methods achieved LOQs within the predefined 100–500 ppm range, meeting practical industry expectations for low-level impurity assessment. Two complementary sampling workflows accommodated differences in analyte volatility and sample introduction. Together, these results demonstrate a flexible, structure-specific approach for quantifying closely related impurities when molecular mass alone does not provide sufficient specificity, a challenge common across pharmaceutical building blocks and reagents.