

EXPLORING DIA DATA ANALYSIS FOR METABOLOMICS WITH SPECTRONAUT

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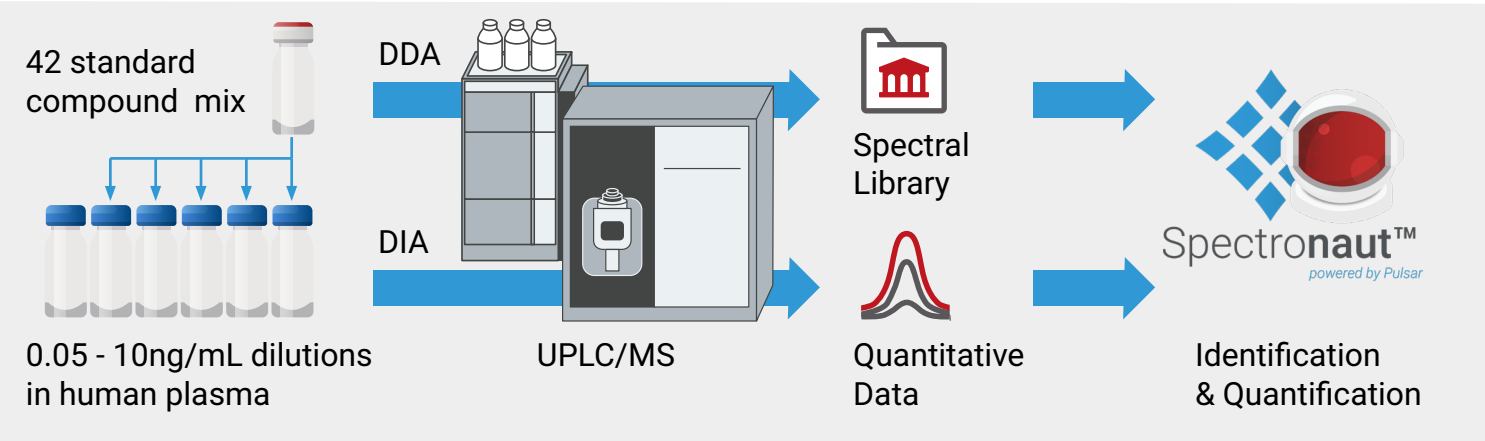
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INTRODUCTION

Data-independent acquisition (DIA) enables simultaneous identification and quantification with high dynamic range by providing MS2 data for all precursor ions across the full retention time range. The analysis of DIA data for metabolomics requires high quality spectral libraries and specialized tools to identify fragment ions belonging to the same precursor.

We investigated if the proteomics DIA data deconvolution workflow of Spectronaut can also be applied to metabolomics and used it to identify and quantify small molecules in a DIA dataset.
Figure 1: Workflow for this Study A mix of small molecules was spiked into human plasma and quantified from DIA data with Spectronaut.



CONCLUSIONS

- Spectronaut can be used to identify small molecules in DIA data
- Quantification performance is comparable to published manual quantification workflow
- Target/decoy- based FDR analysis enables automatic filtering but needs further testing for metabolomics
- Isomers with similar fragmentation patterns pose a challenge to address, e.g. by re-purposing post-translational modification localization algorithms

RESULTS

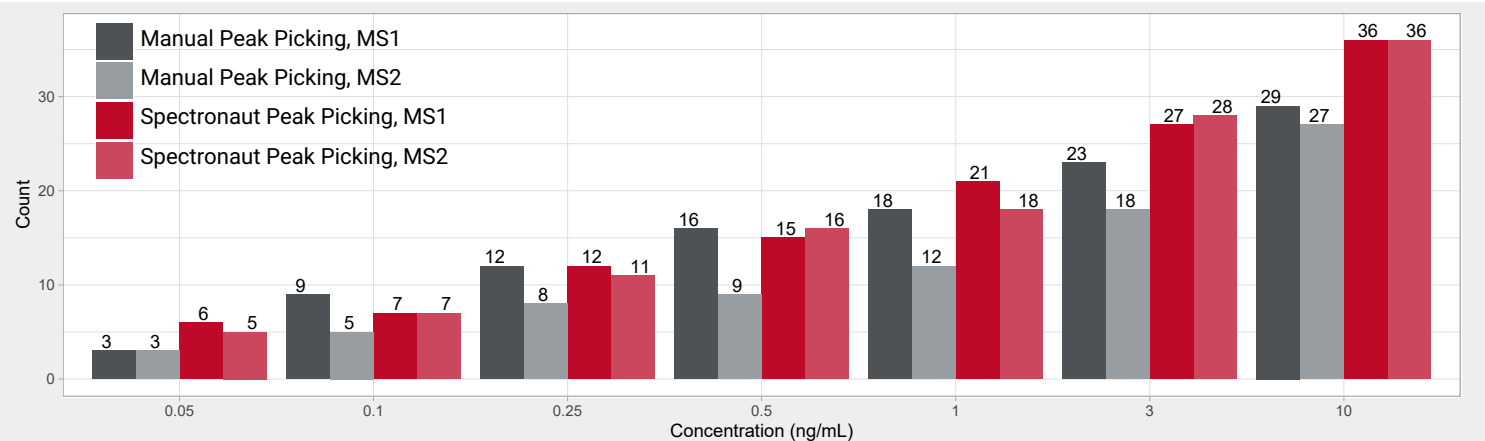


Figure 2: Number of compounds quantified with CV < 30% at different concentration levels Spectronaut automatically quantified a similar number of compounds as the manual workflow that used MS-DIAL for identification¹. At least 5 replicates had to pass a confidence filter (1% FDR filter in Spectronaut, or 5x blank intensity in manual workflow).

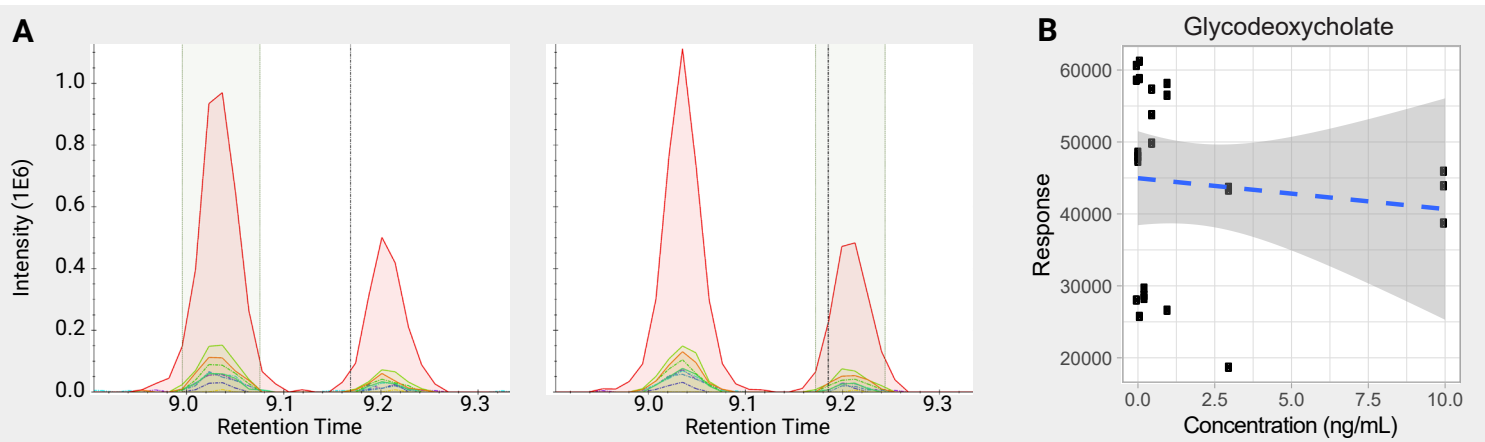


Figure 3: Isomers Are Challenging Isomeric metabolite peaks can cause peak picking issues. Spectronaut uses a broad set of scores at both MS1 and MS2 levels to match DIA data to spectral libraries, and some peaks can be close in total scores. The resulting picking of different isomers in different MS data files (A) can throw off quantification (B).

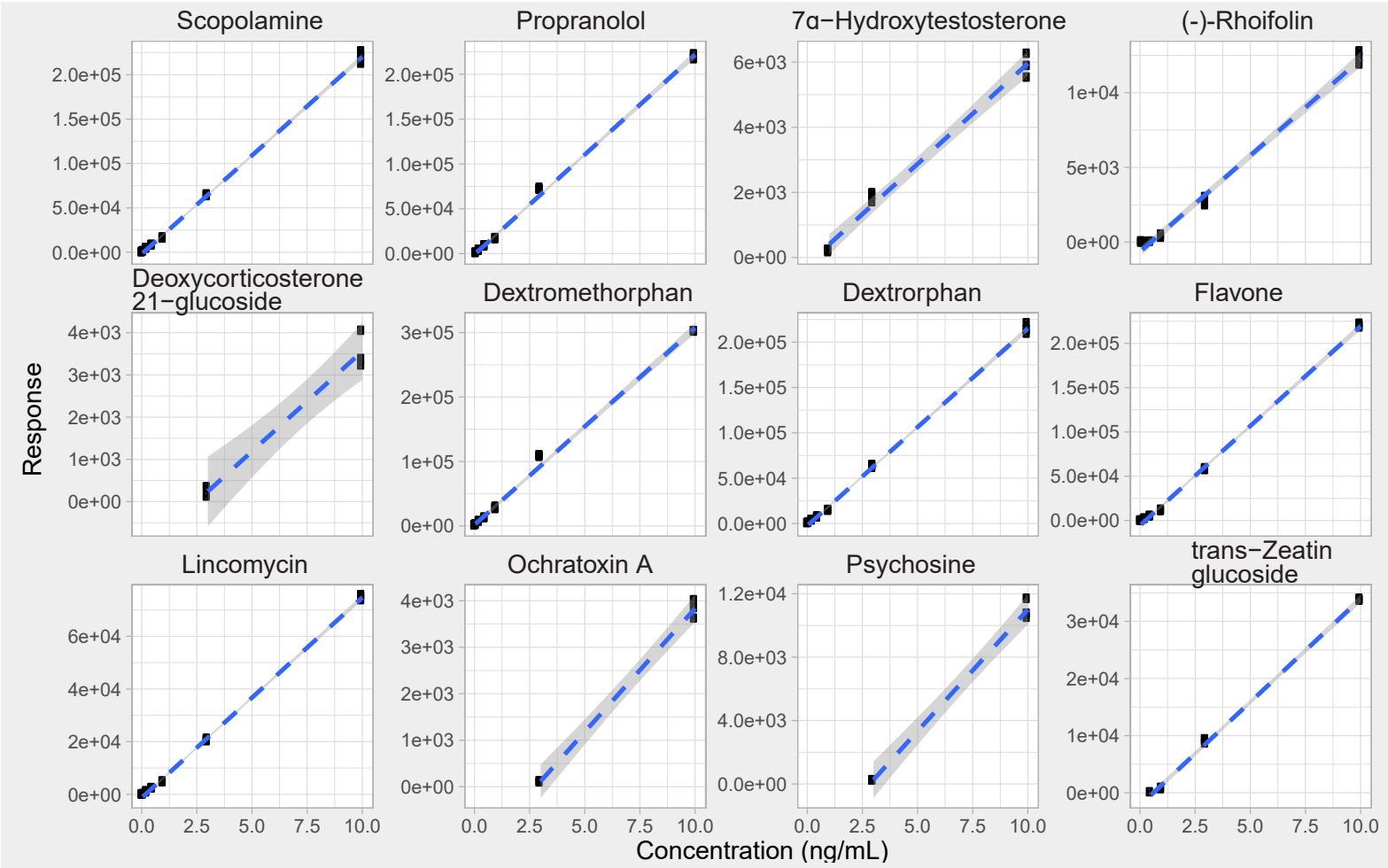


Figure 4: Selection of calibration curves for identified compounds (R-squared > 0.995) In total, 31 out of 42 compounds were reproducibly identified with R-squared > 0.9 after automatic target/decoy-based FDR filtering in Spectronaut. Two more compounds had an R-squared > 0.8. For the remaining compounds, no calibration curve could be fit, including three compounds with high scoring isomeric peaks resulting in inconsistent peak picking (Figure 3).

References:

1. Barbier Saint Hilaire, P.; Rousseau, K.; Seyer, A.; Dechaumet, S.; Damont, A.; Junot, C.; Fenaille, F. *Metabolites* **10**, 158 (2020).