

On global profiling accuracy and the effect of instrument sensitivity

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Introduction

The observed quantitation of a given analyte introduced for mass spectrometry via electrospray ionization is commonly influenced (suppressed or enhanced) by the simultaneous presence of other, potentially biologically unrelated, analytes. This so-called “matrix effect” may be measured and corrected in targeted analyses using isotopically labeled internal standards. However, application of the same principles to discovery-oriented global profiling studies is impractical owing to both the broad scope of analytes measured and a lack of *a priori* knowledge of their identity. Consequently, the accuracy of metabolite measurement is easily compromised. Strategies are emerging to mitigate this effect involving large mixtures of internal standards and extrapolation from proxy standards. Here, the role and fundamental value of instrument sensitivity in mass spectrometry are explored.

Methods

A mixture of Metabolite Yeast Extract Kit (U-¹³C) (Cambridge Isotope Labs) and flavonoids was spiked at constant levels into a dilution series of human urine (NIST Standard Reference Material 3672). The resulting sample set was analyzed by a 12.65 min reversed-phase chromatography using an Agilent 1290 Infinity II LC and a Bruker Bio-AQ column (150 x 2.1mm) coupled to a novel high sensitivity TIMS-MS (timsMetabo; Bruker) and a conventional QTOF MS system. Analyte ionization suppression was measured across the retention time range, representing varying matrix conditions, and at each urine sample dilution. The urine metabolic profiles themselves were evaluated for depth and data richness.

Results & Discussion 1:

Novel, high sensitivity HR-TIMS MS allows for working at ~ten-fold higher sample dilution compared to conventional QTOFs

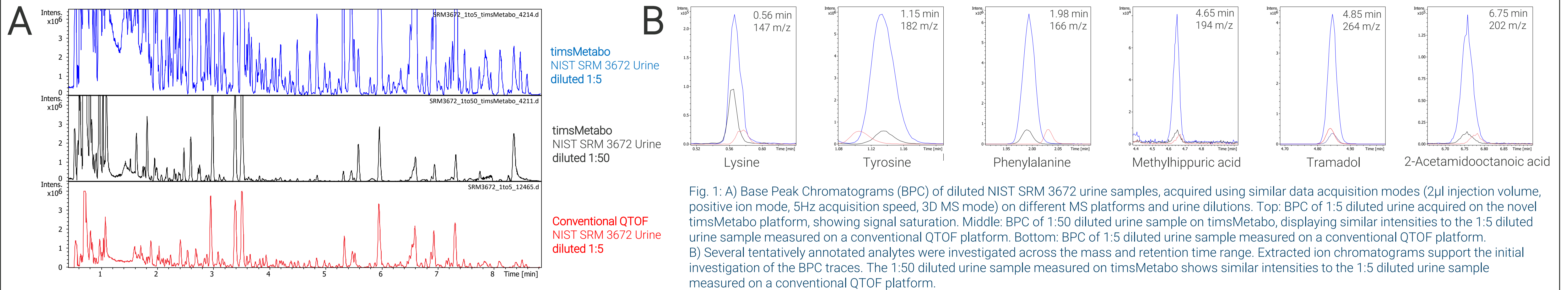


Fig. 1: A) Base Peak Chromatograms (BPC) of diluted NIST SRM 3672 urine samples, acquired using similar data acquisition modes (2µl injection volume, positive ion mode, 5Hz acquisition speed, 3D MS mode) on different MS platforms and urine dilutions. Top: BPC of 1:5 diluted urine acquired on the novel timsMetabo platform, showing signal saturation. Middle: BPC of 1:50 diluted urine sample on timsMetabo, displaying similar intensities to the 1:5 diluted urine sample measured on a conventional QTOF platform. Bottom: BPC of 1:5 diluted urine sample measured on a conventional QTOF platform. B) Several tentatively annotated analytes were investigated across the mass and retention time range. Extracted ion chromatograms support the initial investigation of the BPC traces. The 1:50 diluted urine sample measured on timsMetabo shows similar intensities to the 1:5 diluted urine sample measured on a conventional QTOF platform.

Results & Discussion 2:

Greater sample dilutions yield more accurate profiles

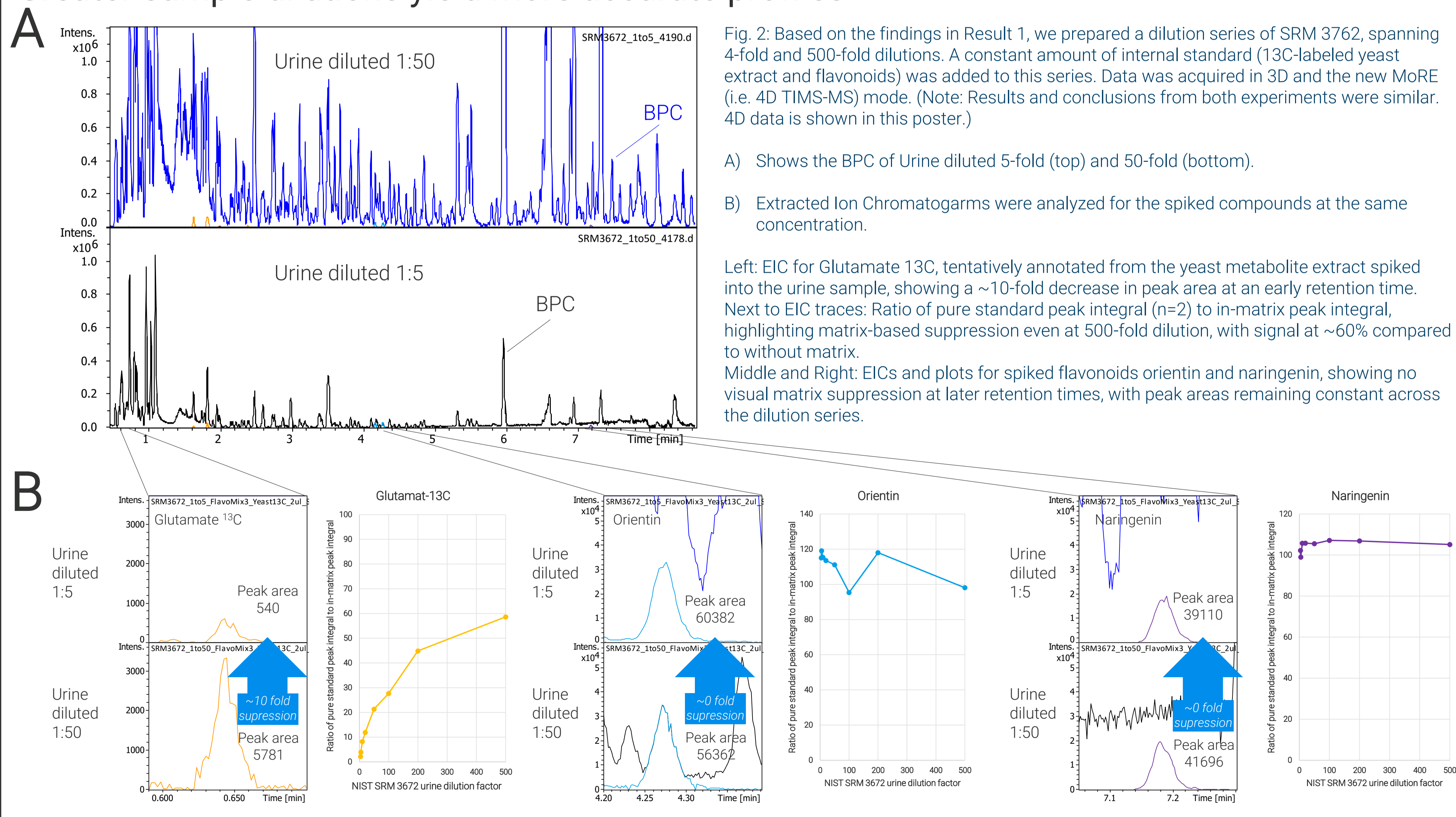


Fig. 2: Based on the findings in Result 1, we prepared a dilution series of SRM 3762, spanning 4-fold and 500-fold dilutions. A constant amount of internal standard (¹³C-labeled yeast extract and flavonoids) was added to this series. Data was acquired in 3D and the new MoRE (i.e. 4D TIMS-MS) mode. (Note: Results and conclusions from both experiments were similar. 4D data is shown in this poster.)

A) Shows the BPC of Urine diluted 5-fold (top) and 50-fold (bottom).

B) Extracted Ion Chromatograms were analyzed for the spiked compounds at the same concentration.

Left: EIC for Glutamate ¹³C, tentatively annotated from the yeast metabolite extract spiked into the urine sample, showing a ~10-fold decrease in peak area at an early retention time. Next to EIC traces: Ratio of pure standard peak integral (n=2) to in-matrix peak integral, highlighting matrix-based suppression even at 500-fold dilution, with signal at ~60% compared to without matrix. Middle and Right: EICs and plots for spiked flavonoids orientin and naringenin, showing no visual matrix suppression at later retention times, with peak areas remaining constant across the dilution series.

Note and Disclaimer:

- Authors Aiko Barsch, Aiko Barsch; Sven W. Meyer; Claudia Martelli; Cristian De Gobba; Nikolas Kessler; Matthew R Lewis are employees of Bruker Corporation or one of its subsidiaries (“Bruker”). Bruker manufactures and sells analytical instruments including mass spectrometers and software. Bruker mass spectrometers and software were used in this study.

Conclusion

- Higher sensitivity MS allows for working at greater sample dilution while still producing the same overall signal from the sample (i.e. without losing information)
- Greater dilutions yield more accurate profiles, as they mitigate ionization competition (i.e. suppression)
- This effect is variable, depending on the local co-elution environment. i.e. greater suppression at earlier more crowded elution times, highlighting the continued importance of chromatographic separation prior to ESI-MS.

Metabolomics by LC-TIMS-MS/MS