Integrated workflow with quality control for large cohort and clinical metabolomics research using robust hardware and signal correction

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Sebastian Goetz[1]; Ulrike Schweiger-Hufnagel[1]; Matthias Szesny[1]; Aiko Barsch[1]; Sven W. Meyer[1]; Matthew R. Lewis[2]; Nikolas Kessler[1]
[1]Bruker Daltonik GmbH, Bremen, Germany [2]Imperial College London, London, United Kingdom

Introduction

Metabolomics research relies on precision measurement of statistically powered sets of hundreds or thousands of samples. First, this requires robust analytical hardware with long term stability, capable of generating high precision data. Second, processing of large datasets may require additional mathematical correction to compensate for systematic changes in observed signals as samples interact with the analytical system affecting its performance. We investigated the long-term stability of an LC-HR-QTOF system by measuring a batch of more than 1000 urine samples and monitoring the effect of data acquisition on MS ion source contamination and detector aging. To address the remaining within-batch intensity drift we present a software workflow which includes quality assessment and correction, further improving data precision and statistical reliability.

Methods

Six different human urine samples were diluted 1:3 with water and centrifuged. The supernatant was aliquoted for chromatographic separation using a linear reversed-phase gradient with a 15 minute cycle time. MS data (ESI positive mode) were acquired on an Impact II QTOF-MS (Bruker Daltonics). Every seventh injection corresponded to a quality control sample which was an equal-parts mixture of each sample (see Figure 4 for the experimental setup). The ion source region was cleaned before and after the sample batch, and detector tuning was performed directly after cleaning. Data processing for untargeted profiling was conducted in a new version of the MetaboScape software and additional targeted data evaluation was performed using the TASQ software (Bruker Daltonics).

Results

- Despite the ion source region of the impact II instrument appearing significantly contaminated, no decrease in peak areas was observed across several compounds (Figure 1).
- Broader investigation revealed intensity changes as a function of acquisition order in some analytes, potentially caused by sample degradation, aging of the LC column, or source contamination (see Figure 2).
- Figure 2 (top) shows Taurine (tentatively) as an example of the outstanding robustness of intensities across hundreds of measurements.

- Raw data (gray) shown in Figure 2 bottom highlights a downward intensity trend for a compound tentatively identified as 4-nitrophenyl sulfate. 40 consecutive sample injections from the same vials indicates a possible degradation of this analyte over time.
- MetaboScape 5.0 addresses these drifts by fully automated feature-wise intensity correction based on quality control samples.

Ion source after > 1100 continuous injections.

The automatic detector tuning set exactly the same voltages before and after the whole batch, i.e. no aging of the detector observed.

Uric acid

No decrease in peak areas observed: 3.8% RSD   3.8% RSD

Mass accuracies remained stable for all data at low levels

Isotopic patterns remained stable.
Quality of the isotopic patterns is represented by the so-called mSigma value. Numbers < 20 point out a very good matching of isotopic patterns while values < 10 depict excellent matchings.
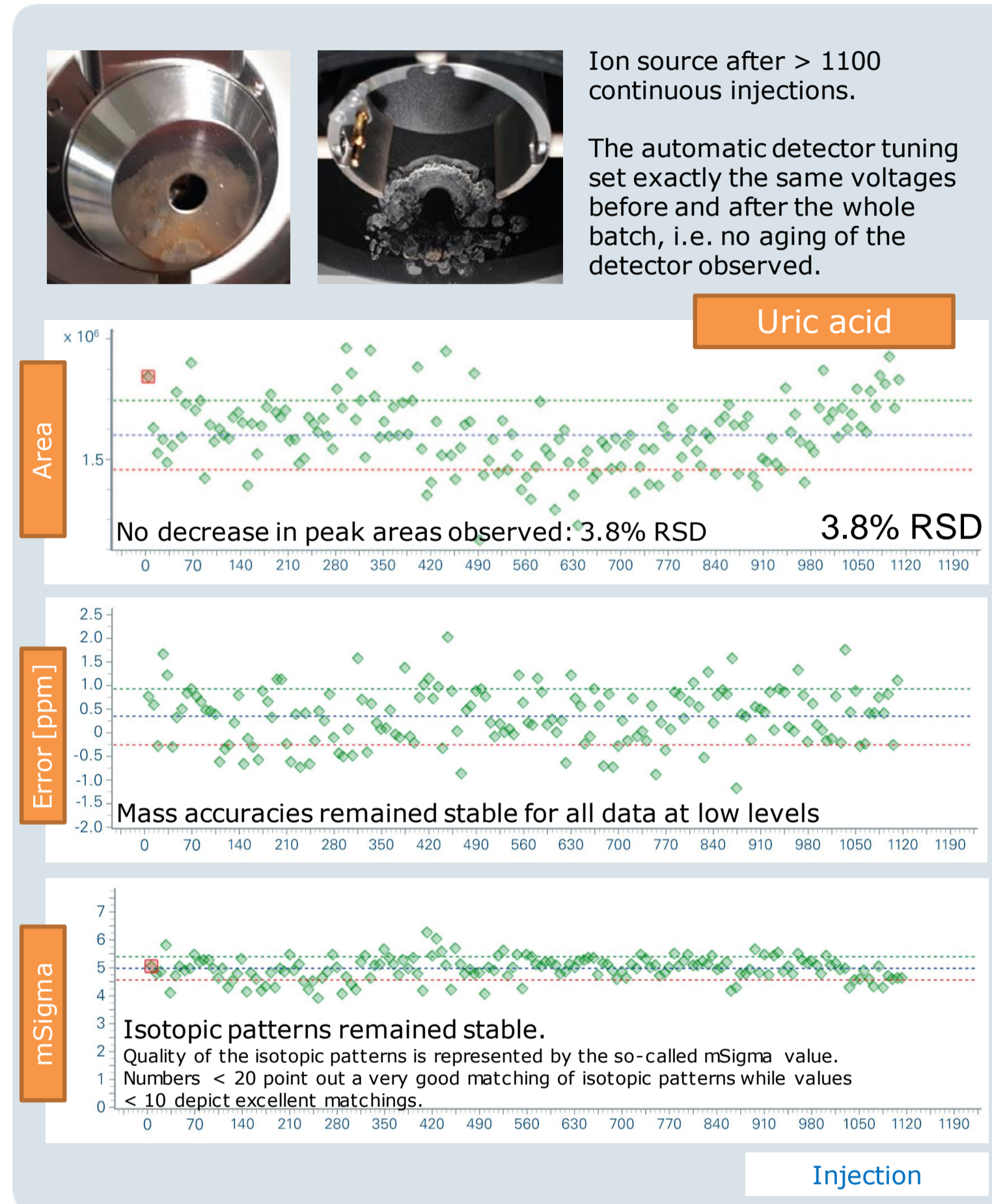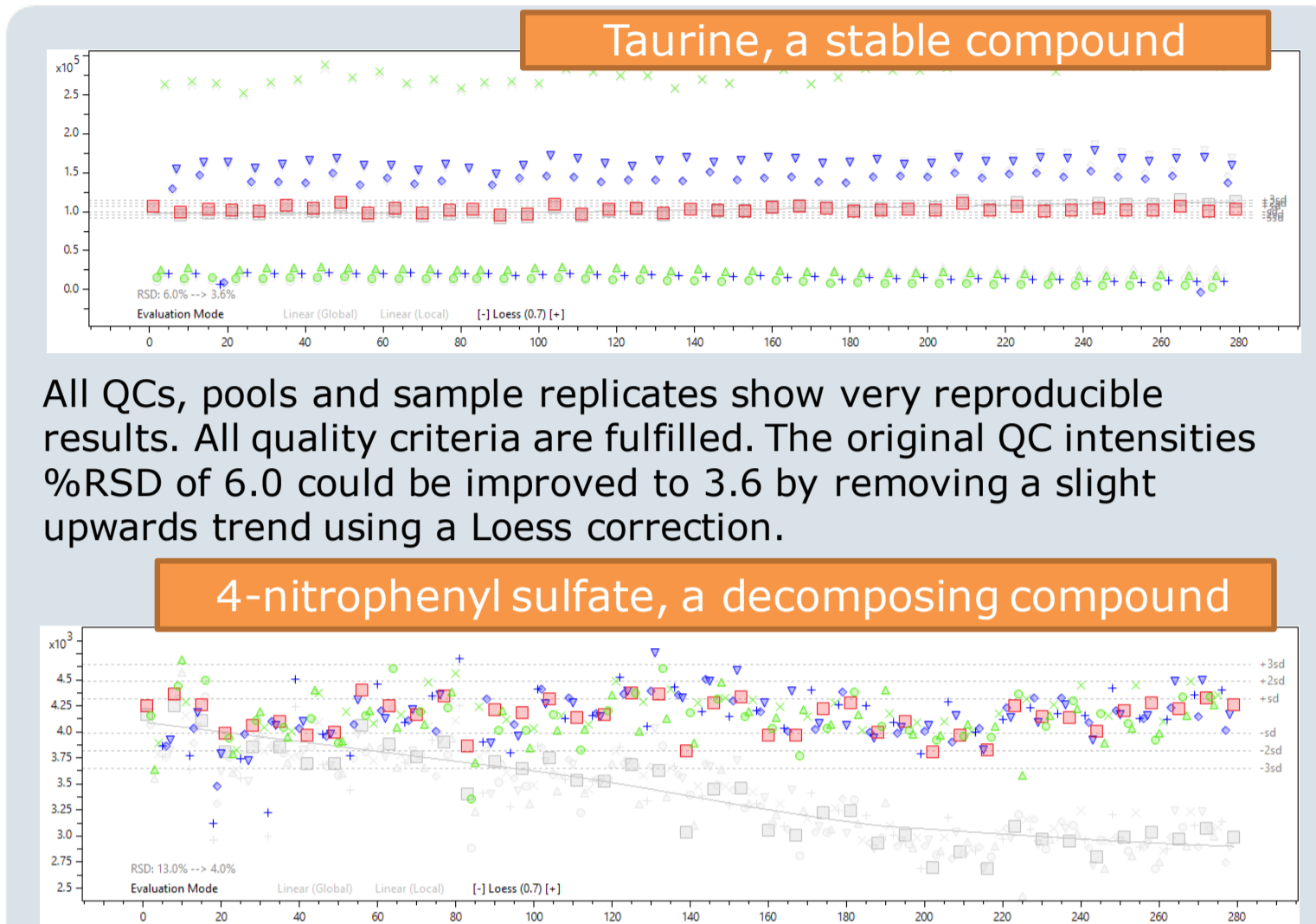
Injection

Fig. 1 Top: Ion Source region after 1100 urine injections shows heavy contamination.
Bottom: Remarkable quality and reproducibility of the impact II performance exemplified for a compound tentatively identified as uric acid.
Only each 7th sample is displayed from the sequence of more than 1100 injections.

Taurine, a stable compound

All QCs, pools and sample replicates show very reproducible results. All quality criteria are fulfilled. The original QC intensities %RSD of 6.0 could be improved to 3.6 by removing a slight upwards trend using a Loess correction.

4-nitrophenyl sulfate, a decomposing compound

In the raw data, the QC intensities show a clear downwards trend over time, likely caused by sample degradation. After correction with a soft Loess all QC intensities remain within two standard deviations and %RSD improved from 13.0 to 4.0.

Fig. 2 Quality control plots in MetaboScape 5.0 for two compounds in urine over time, and their behavior after within-batch correction.
X axis: For clearer visualization the example shown highlights the last 280 analyses of the long-term study. These comprise the last 40 injections for each of the 7 samples. The different measurements are ordered by acquisition time
Colour coding: gray for raw data. Corrected data is colored according the experimental setup in Fig. 4.
Lines: The soft Loess correction function is shown as a gray line. Horizontal dotted lines are plotted for the one, two, and three standard deviation marks, respective to intensities found in the quality control analyses.
Data shown: (Top) 126.0225 m/z, 0.52 min, $C_2H_7NO_3S$ (Taurine). (Bottom) 219.9890 m/z, 10.48 min, $C_8H_2N_3O_3P$ (4-nitrophenyl sulfate)

- Run-order signal drift correction effectively reduced the relative standard deviation (RSD) of feature intensities within sample groups measured across replicate quality control sample measurements to 4 % (see Figure 2).
- Signal drift correction also increased the number of analytes which meet the requirements of an RSD below 20%, a typical cut-off (see Figure 3).
- Visually this improvement was also observed in PCA, with more closely clustering of sample groups (see Figure 4).

Experimental setup

Urine 3
Urine 2+3   Urine 1+3
Urine pool QC
Urine 2   Urine 1
Urine 1+2

Within-Batch Correction

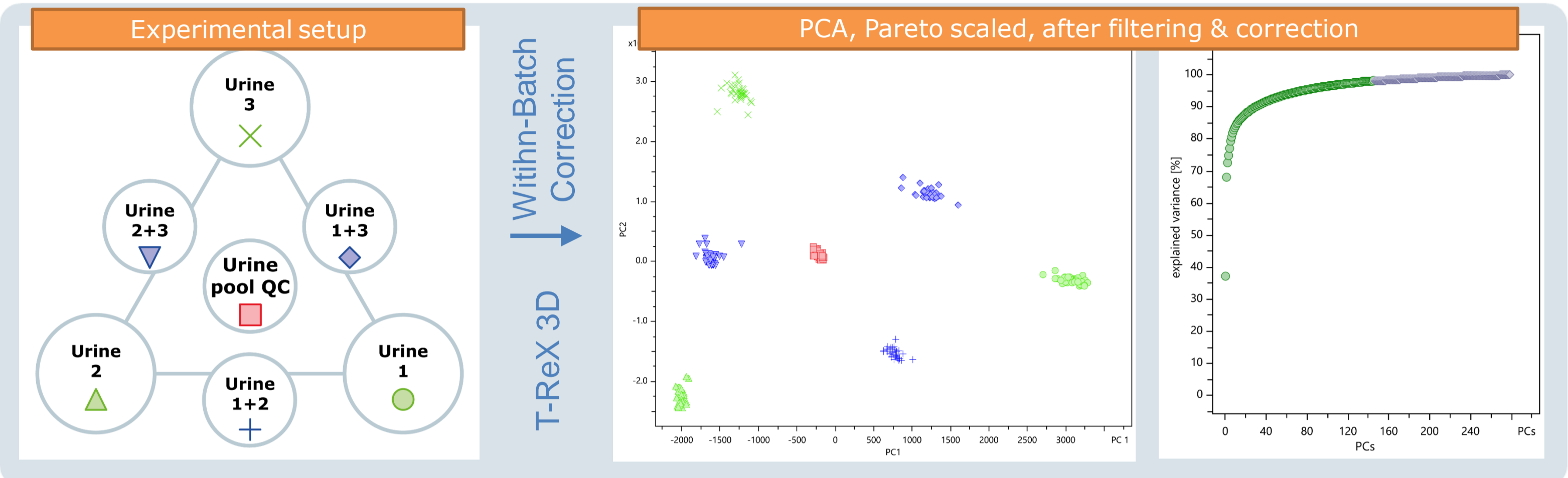T-ReX 3D

PCA, Pareto scaled, after filtering & correction

Fig. 4 (Left) Legend of original and pooled samples; (Center) The PCA clusters nicely according to the samples and pooled mixtures, illustrating the robustness of the entire system, including long-term hardware stability, feature detection, and within-batch correction. (T-ReX 3D = **T**ime aligned **R**egion complete e**X**traction algorithm); (Right) The first two principal components explained 68% of the variance.

Summary

We present a workflow for population and clinical metabolomics research enabled by robust LC-HRMS hardware and software allowing filtering and correction for signal drift effects.

CV limit 20%

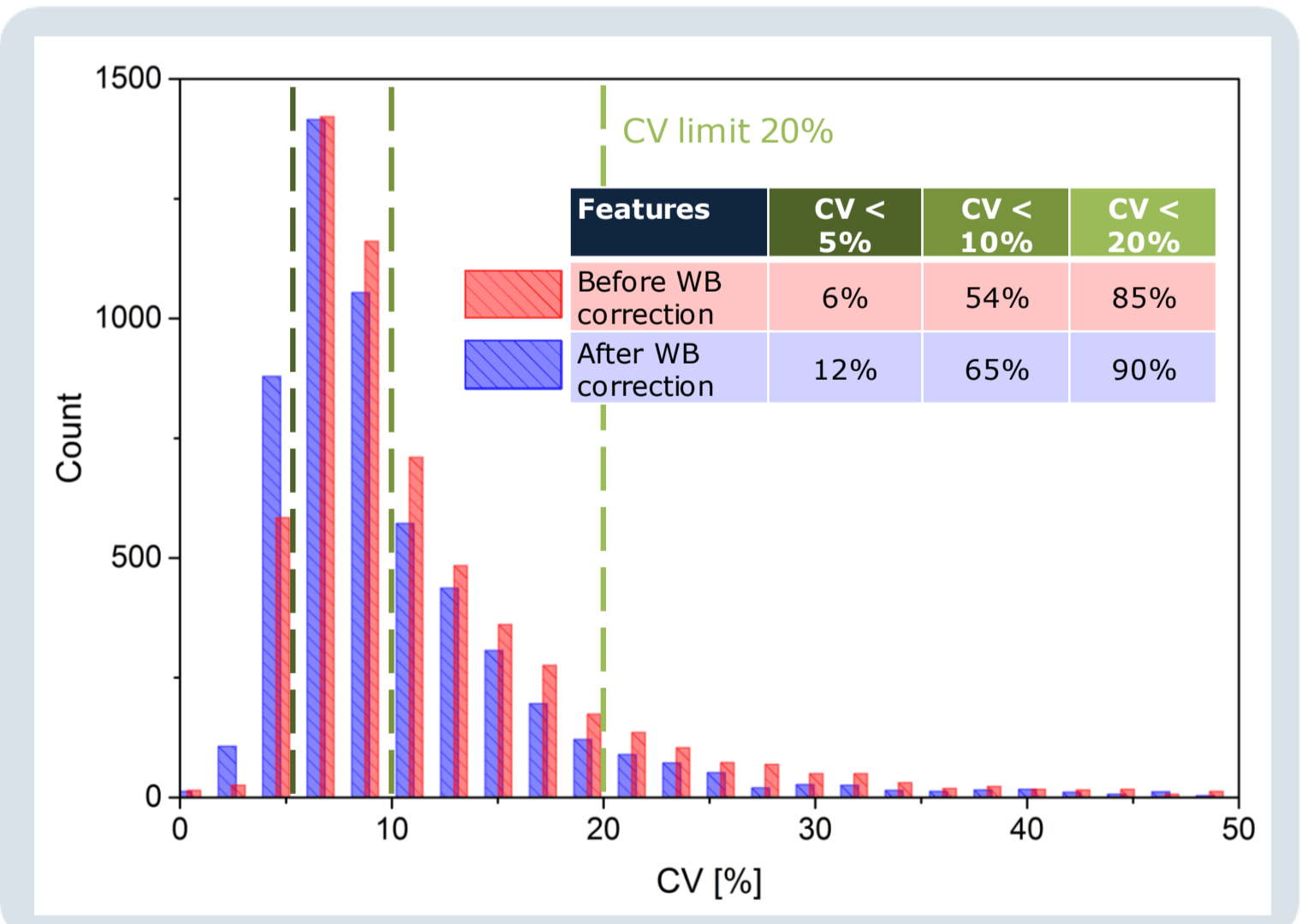| Features	CV < 5%	CV < 10%	CV < 20%
Before WB correction	6%	54%	85%
After WB correction	12%	65%	90%

Fig. 3: The histogram shows the coefficients of variance for the intensities of all features found in the forty replicates of each of the urine samples 1 to 3. The overall CV values of the sample replicates are clearly improved by the within-batch correction using the quality control replicates.

Conclusions

New workflow solution for population and clinical metabolomics research

- Outstanding robustness: acquisition of > 1100 injections with an impact II QTOF system, without detector tuning
  - No decrease in peak area
  - Mass accuracies stable <1.5 ppm
  - Isotopic pattern stable
- Intuitive and interactive visualization for rapid review of batch quality
- Automated correction of analytical variability helps to reveal subtle but significant metabolic differences: 90% of features with RSD below 20%

MetaboScape