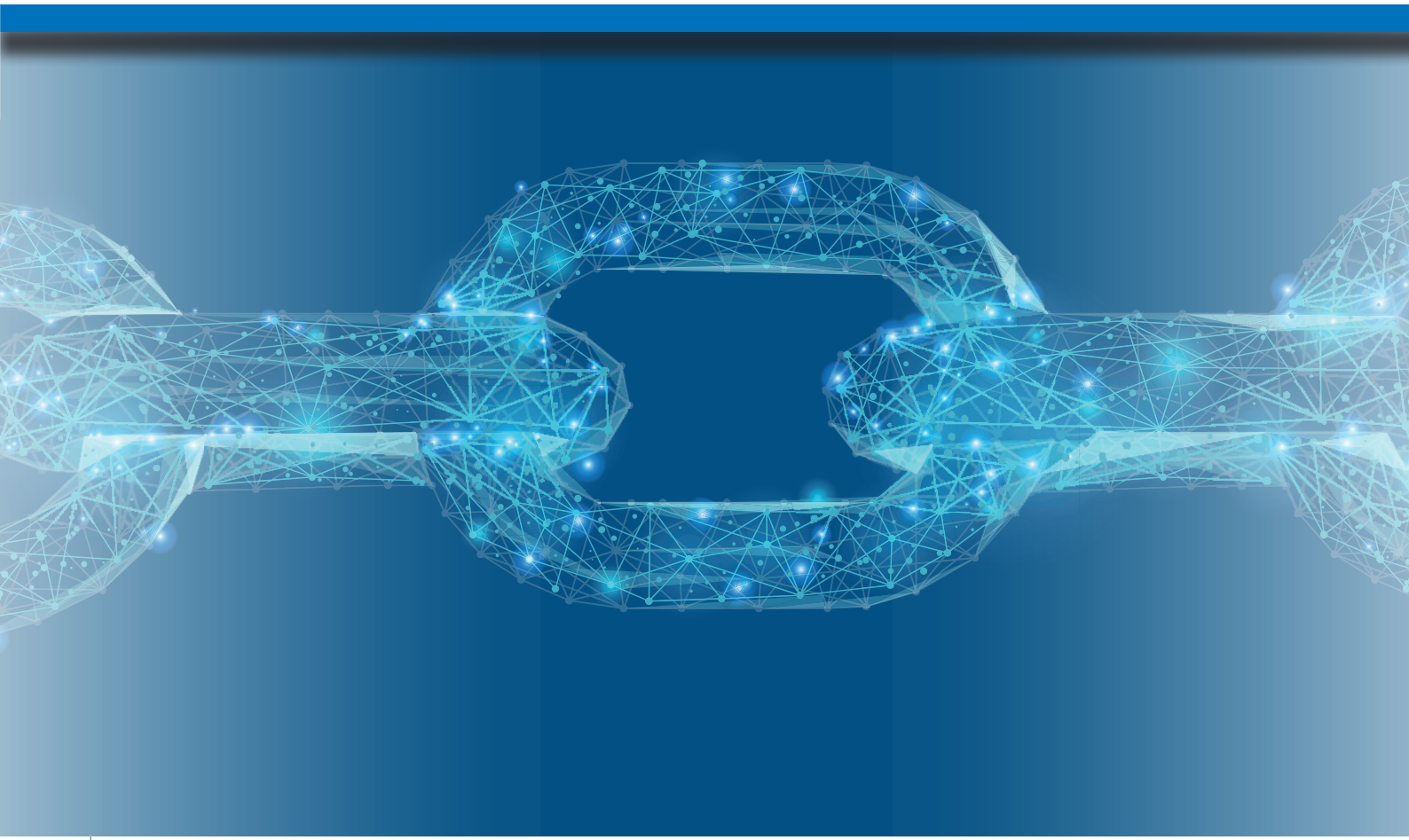

Proven robustness for large-scale metabolomics studies using the 'Phenomics Workhorse'

HRAM LC-MS profiling of > 1000 urine samples demonstrates the outstanding analytical skills of the impact II

Introduction

The metabolome is the final manifestation of biochemical pathways. The temporal and spatial changes of metabolites reflect the outcome (phenotype) of interactions at the genomic, transcriptomic and proteomic level. The immense diversity of the metabolome can be further influenced in virtually countless ways. In order to understand the answers or changes related to e.g. a disease or therapeutic interaction at the level of the metabolome, large profiling studies with generally hundreds

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or even thousands of samples are needed. This calls for integrated solutions and a robust long-term performance of the analytical devices.

Bruker offers a broad portfolio of mass spectrometers for application in metabolomics. The impact II is the workhorse QTOF-MS system coming with outstanding long-term robustness and a constantly high quality of the data. The present Technical Note demonstrates the quality of a sequence of > 1100 injections of urine. Special focus was put on the reproducibility of the data, the robustness of the system and the degree of detector aging during this sequence of > 14 days.

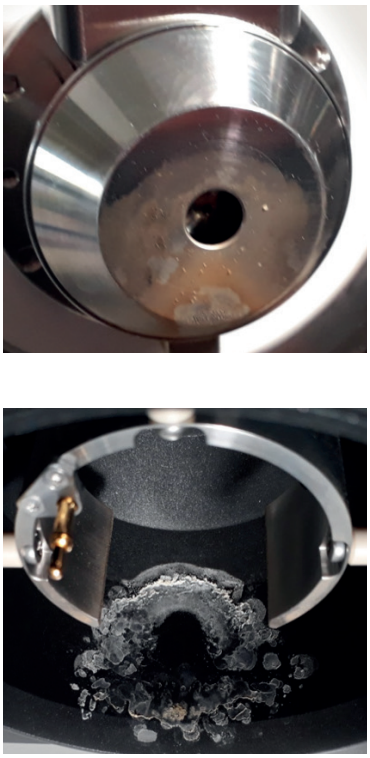

Figure 1: Ion source after > 1100 continuous injections.

Results

A diluted urine extract was used as basis for a batch of > 1100 LC-MS sample measurements (> 14 days). For such large sample cohorts, the reproducibility of the data is absolutely critical. Only data with a constant quality can deliver the guarantee for subsequent statistical experiments.

Heavy contamination of the ion source region can decrease the signal intensity due to ion suppression or increasing carry-over effects (Figure 1). Furthermore, all ions hitting the detector will contribute to the natural aging of the detector plate. In short, system contamination and detector aging can lead to decreasing signal intensities or degraded signal quality. As a result, the peak areas as well as the quality of mass accuracy and isotopic patterns would decrease.

The screenshots taken from TASQ, Brukers software solution for targeted screening workflows, demonstrate an outstanding quality and reproducibility of the QTOF data for the whole sequence. No decrease in peak areas was visible for compounds investigated (Figure 2). Furthermore, the mass accuracies stayed stable over all experiments at low levels of < 1.5 ppm (Figure 3). The quality of the isotopic patterns is another parameter essential for metabolite identifications. It 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. The observed mSigma values for compounds were almost all < 10 mSigma emphasizing again the distinguished quality of the data (Figure 4).

Finally, no detector aging was observed in this period of time. The automatic detector tuning set exactly the same voltages before and after the whole batch.

Figure 5 shows the data in a novel version of MetaboScape. A MS-based HMDB library was used to annotate some of the compounds by their masses and isotopic patterns. After the de-replication of knowns, the next step in a standard profiling experiment usually is the identification of unknowns. MetaboScape offers several workflows for this purpose but this is not subject of the present paper.

Altogether, the present data highlight the impact II system as predestined for large cohort analyses of many samples. The impact II deservedly bears the nickname 'Phenomics Workhorse' and it is perfectly fit to carry out large-scale Metabolomics profiling studies.

Table 1: Acquisition parameters

MS	Bruker impact II
Ion Source	Apollo II ESI source
Ion polarity	ESI(+)
Scan range	m/z 50-1300
Acquisition rate	10 Hz
HPLC	Waters UHPLC
Injection volume	2 µL, full loop
Gradient	See table 2
Mobile phases	A: H_2O + 0.1% FA B: ACN + 0.1% FA

RT [min]	Flow [mL/min]	%A (H_2O+0.1% FA)	%B (ACN + 0.1% FA)
0	0.6	99	1
0.1	0.6	99	1
10	0.6	45	55
10.15	0.61	35	65
10.3	0.63	25	75
10.45	0.67	15	85
10.6	0.75	5	95
10.7	0.8	0	100
11	1	0	100
11.55	1	0	100
11.65	1	99	1
11.7	0.9	99	1
11.8	0.8	99	1
12	0.7	99	1
12.1	0.65	99	1
12.15	0.61	99	1
12.65	0.6	99	1
14	0.6	99	1

Table 2: Solvent gradient used for chromatography

Methods

Following a standard operating procedure, the samples were prepared from human urine. These samples were diluted 1:3 with water and centrifuged. The supernatant was aliquoted for acquisition.

All data acquisition was done on an impact II QTOF-MS. The ion source region (incl. glas capillary) was cleaned before and after the sample batch. A detector tuning was performed directly after each of the cleaning procedures. The MS parameters were optimized using a mixed standards solution containing 10 analytes. Mass calibration was done by automatically injecting a 1 mM solution of sodium formate at the beginning of each run. The chromatographic separation was performed using a linear gradient with 15 minutes cycle time (see table 1). To condition the system after the cleaning, several initial runs were performed until the signal intensities stayed stable. These conditioning runs were excluded from the further data evaluation. The data processing was done using TASQ 1.4 and MetaboScape 4.0.

Matthew R. Lewis, COO, MRC-NIHR National Phenome Centre, Director of Metabolic Profiling, Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London.

"I was impressed with the data generated, with minimal configuration of the instrument. The sensitivity and precision were beyond what I expected, and robustness trials have yielded encouraging results for biofluid profiling applications. MetaboScape and TASQ software are practical and feature-rich - well suited to the extraction and analysis of this data. The total package looks like a real 'Phenomics Workhorse'!"

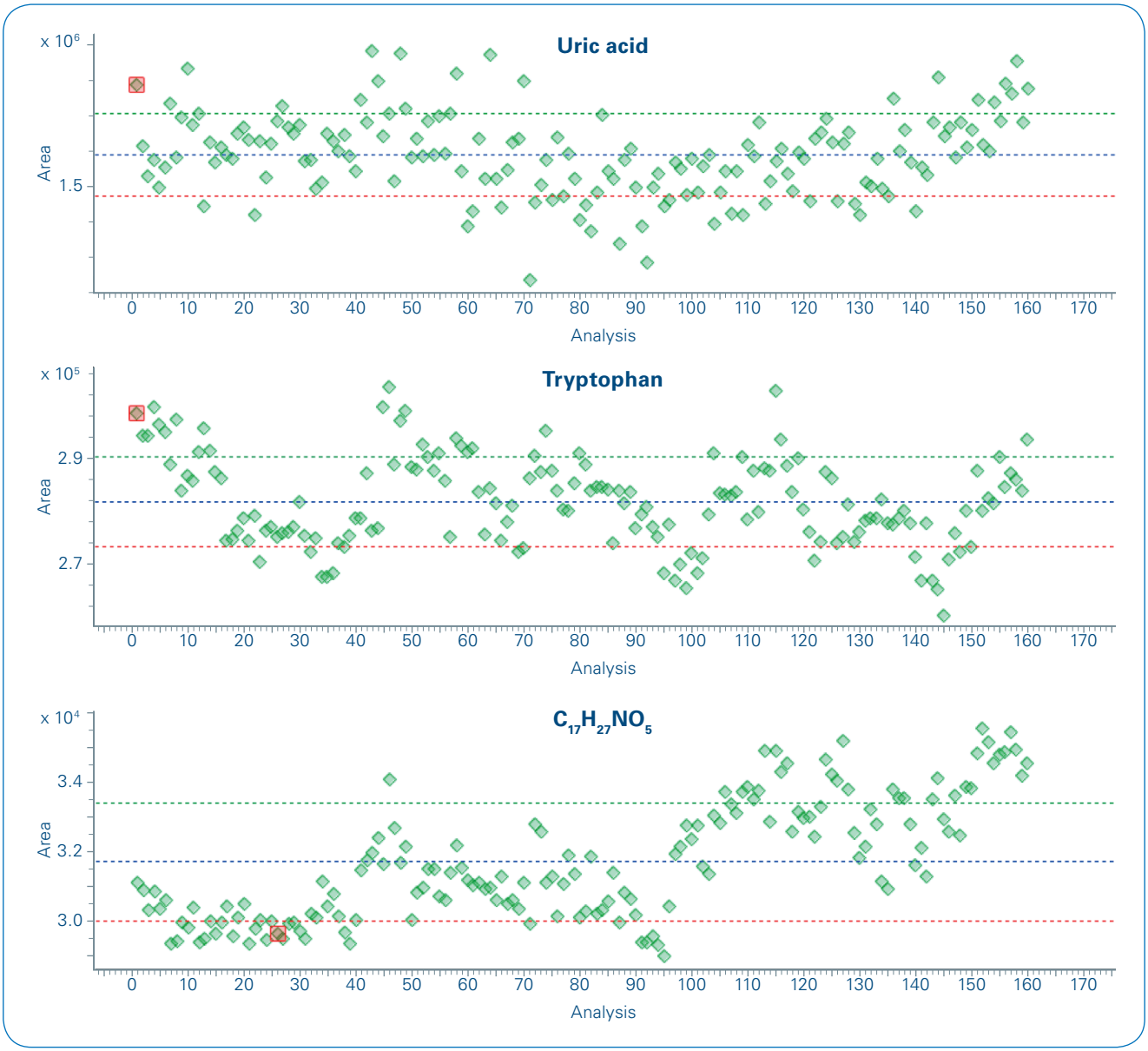

Figure 2: Peak areas for 3 compounds. Only each 7th sample is displayed. The coloured lines represent 1 sigma. The standard deviation was between 3% RSD to max. 5.4% RSD. No long-term trend can be observed but a pattern that seems to correlate with separate vials.

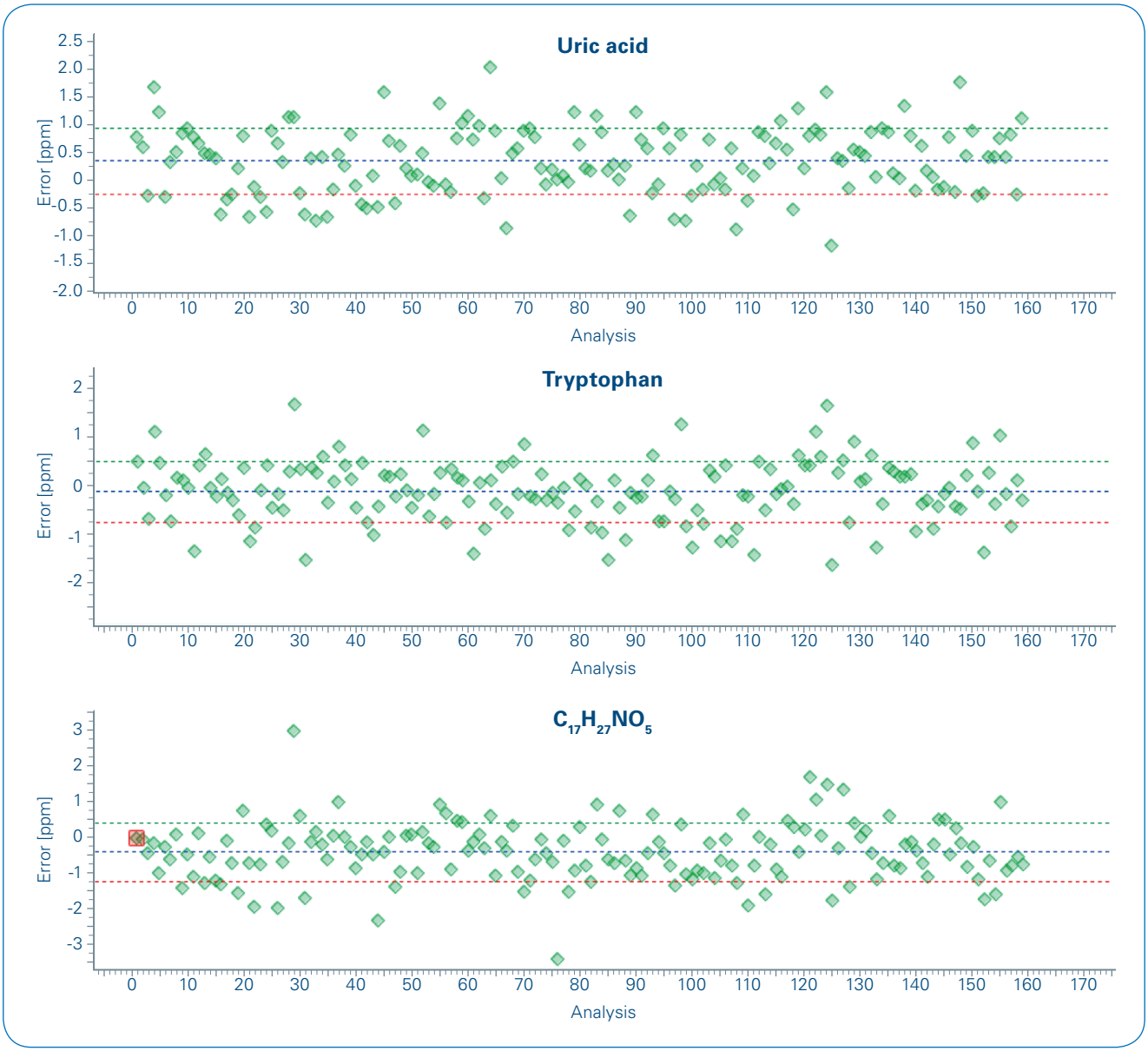

Figure 3: The mass accuracies for 3 compounds show a steady quality over time. Only each 7th sample is displayed. The coloured lines represent 1sigma. The standard deviation was between 3% RSD to max. 5.4% RSD for the shown compounds.

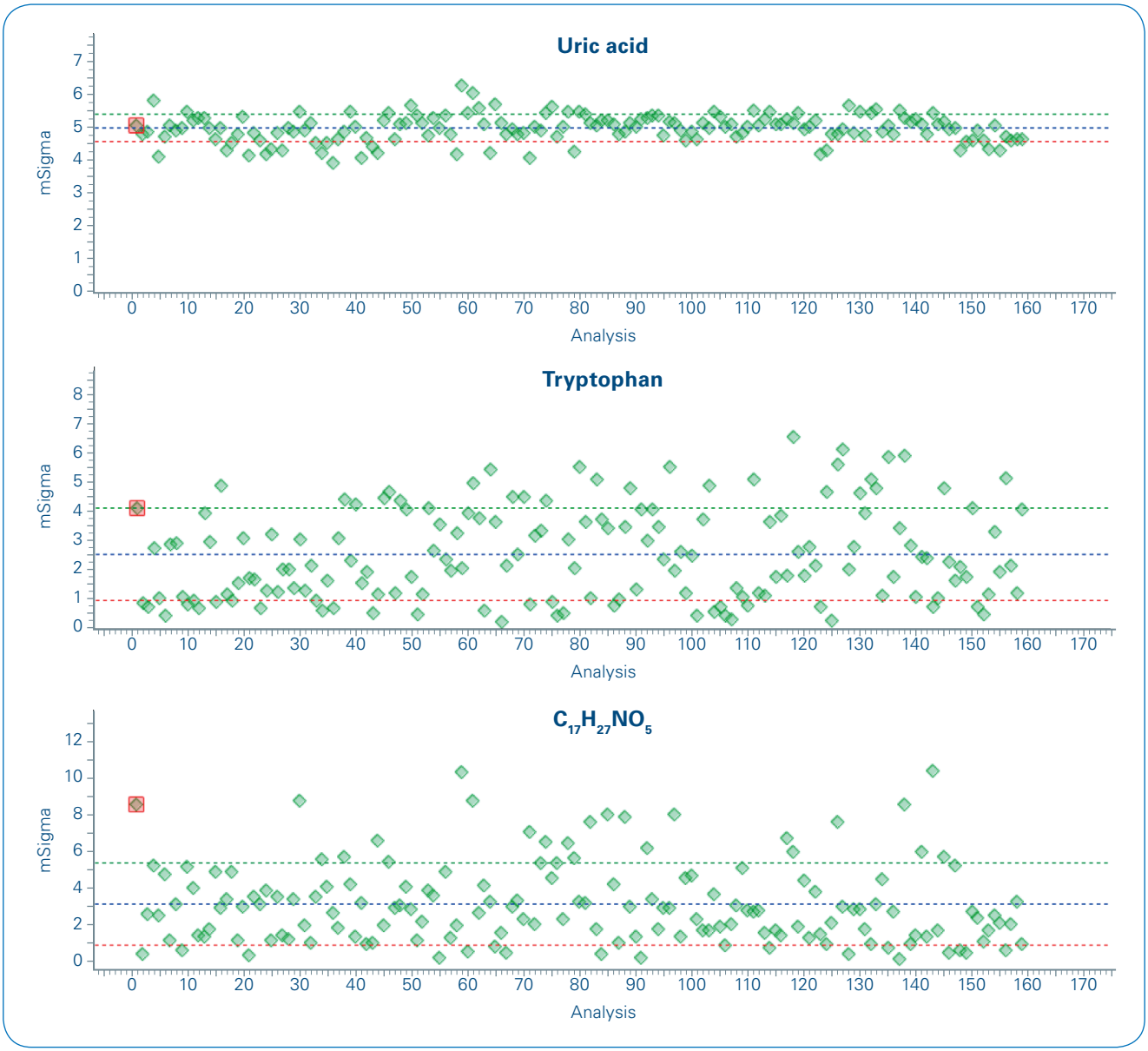

Figure 4: The matching of the isotopic patterns showed excellent performance (almost all < 10 mSigma) without a trend in time. Only each 7th sample is displayed.

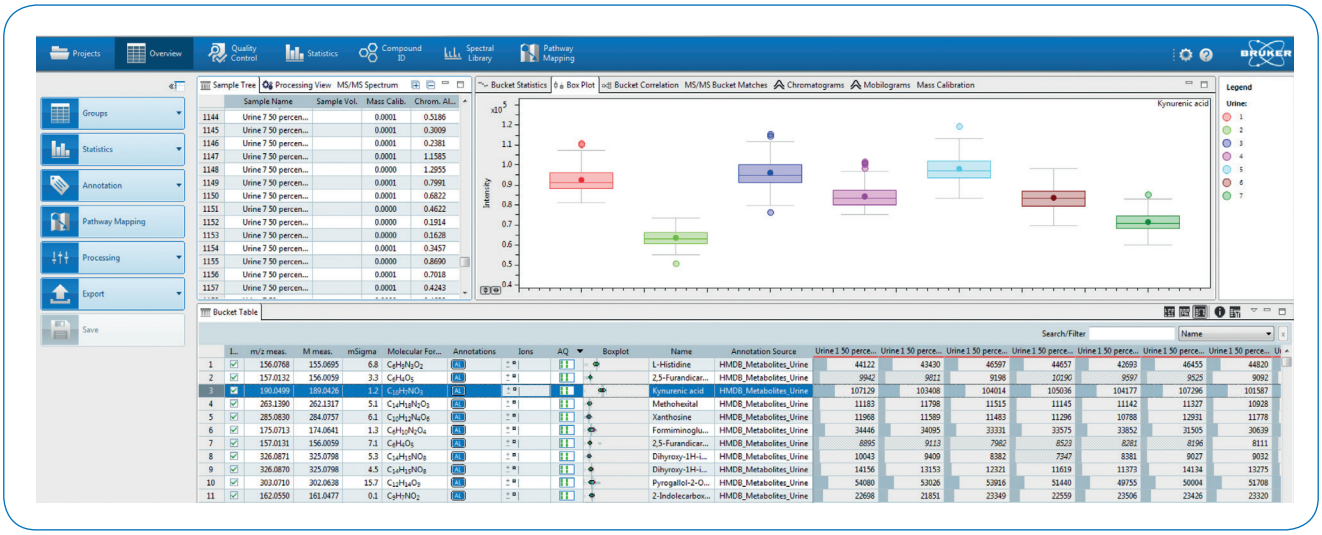

Figure 5: Data processed in a novel version of MetaboScape. Compounds shown were identified with a MS-based HMDB library by mass accuracy and isotopic pattern matching.

Acknowledgement

We would like to thank Jeremy Nicholson, Matt Lewis and Jake Pearce from Imperial College London, London, United Kingdom, for helpful feedback and for providing samples.

Conclusions

- The impact II showed an outstanding robustness in a long-term profiling study of > 1000 injections of human urine
- After 14 days of **24/7** operation, the detector needed no tuning in order to counterbalance contamination or aging. This provides excellent conditions for large-scale profiling studies
- The peak areas, mass accuracies and isotopic pattern quality of compounds exhibited a remarkable quality and reproducibility for the whole time

impact II, the
'Phenomics Workhorse'

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