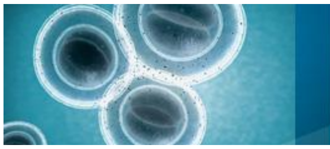

A Robust Bench Top QTOF System for Untargeted Metabolomics Analyses

Sensitivity and Reproducibility Evaluation of the X500R QTOF System

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Metabolomics focuses on the chemical processes central to cellular metabolism, giving scientists a snapshot of the cellular metabolic state, or metabolome at a given time. It consists of intermediates, conjugates, hormones, and even exogenous metabolites from food or the environment that have an impact on health. Mass spectrometry is the tool of choice for the measurements of these metabolites. However, they can be increasingly challenging workflows to setup for the novice metabolomics researcher or the technician. Therefore, a robust solution for screening metabolomics samples is of increased interest and the need for a more integrated and routine mass spectrometry system is in demand. The new X500R QTOF System was developed for routine, robust workflows and requires minimal MS expertise. The system integrates all data acquisition; processing and review as well as reporting into a single piece of easy to use, easy to learn software.

The X500R QTOF System was put through the paces to test its mass accuracy and reproducibility over time including assessment of the linear dynamic range in the MS1.

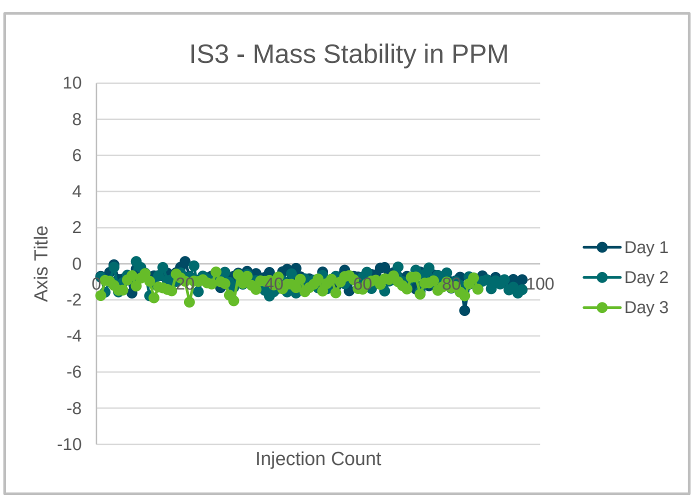

Figure 1: Mass Stability and accuracy of the X500R QTOF System . One internal standard (IS3) over three days resulting in over 300 injections (100 injections per day).

Key Benefits of the Untargeted Metabolomics Workflow on the X500R QTOF System

1. Simplified operation with new interface and workflow setup powered by SCIEX OS Software with minimal MS expertise.
2. Get to actionable results quickly with user-friendly processing software and libraries
3. Maximize your lab's efficiency using a robust and integrated system with automated calibration and a benchtop footprint

Methods

Sample Preparation: For assessment of linear dynamic range and limits of detection, triplicate injections of a neat calibration curve were prepared from 250,000ng/ml to 0.05ng/ml using a mixture of internal standards. These heavy labeled internal standards are typically spiked into every sample prepared for metabolomics analyses. A linear response was defined as points fitting a linear trend line to an R_2 of >0.98. Detection limit was defined as the concentration at which a peak could be discerned from background in triplicate injections. For the assessment of precision over time these same internal standards were spiked into extracted plasma and injected over several hundreds of injections (3 days) to assess the systems reproducibility and mass accuracy drift over time. Plasma was extracted using a simple protein crash using methanol. The supernatant was then dried and reconstituted in LC-MS/MS method appropriate solvents which contained the above described internal standards.

Chromatography: The reverse phase HPLC separation was performed using an Exion LC System, operating at a flow rate of 350 µL/min. The column used was an Waters BEH-C18 RP column (100 x 2.1mm, 1.8 µm) , maintained at 40 ºC. A standard reverse phase gradient was used employing mobile phase A as 0.1% formic acid in water and mobile phase B as 0.1% formic acid in methanol. The injection volume was 5 µL for all analyses performed.

Mass Spectrometry: The data was collected using information dependent acquisition (IDA) on the X500R QTOF System (SCIEX). Using optimized source conditions, the MS mass range analyzed was 80-1000 m/z and the MS/MS was acquired with a mass range of 50-1000 m/z with a 25 msec accumulation time. The instrument was set to automatically calibrate once per hour throughout the duration of the analyses.

Data Processing: The data was processed in MultiQuant™ Software and peak areas extracted of the spiked internal standards.

Platform Evaluation

Mass accuracy, mass precision and area precision were evaluated by monitoring the performance of nine isotopically labelled internal standards spiked into an extract of human plasma on the X500R over 300 injections over the course of 3 days. The X500R demonstrated excellent mass accuracy with 98% of the mass measurements being within three ppm mass error (Table 1) and very good mass precision with a mass error spread of between two and five ppm over the course of the three days for all standards (Figure 1 and 3). The three-day total percent relative standard deviation (%RSD) of the instrument area response ranged from 7.7 to 15 %, while individual days ranged from 4.4 to 16%. It should be noted that Day 1 had higher observed %RSDs than both Days 2 and 3 due to a slight upward trend in area response which resolved in the Day 2 and Day 3 analyses. The neat calibration curve of the internal standards demonstrated that the X500R was linear over a dynamic range of between three and four orders of magnitude (Figure 2) and limits of detection in the low ng/mL concentrations for the majority of standards (data not shown). Note that limits of detection are highly compound dependent and the internal standards tested here represent a very limited complement of chemical diversity.

N (out of 3059 measurements)	Error Range (ppm)
1524	0 to 1
1058	1 to 2
412	2 to 3
63	3 to 4
2	4 to 5
0	>5

Table 1. Mass Accuracy of Number of Measurements over 300 Injections over 3 Days. Number of measurements is the number of extracted compounds and their respective mass accuracies. Over 80% of measurements fall below 2ppm mass accuracy

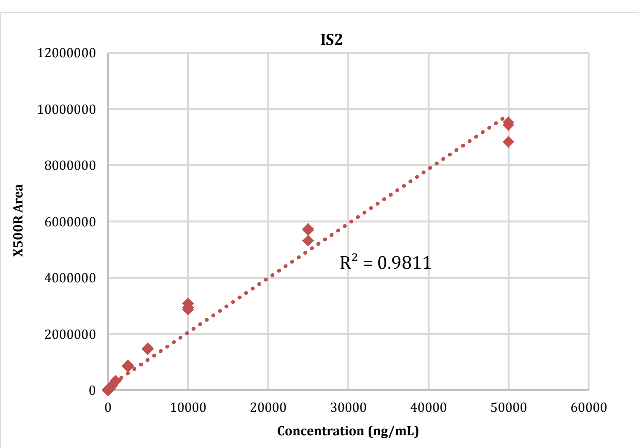

Figure 2: Linear Dynamic Range of the X500R QTOF System. Calibration curve of one internal standard highlighting 3-3.5 orders of linear dynamic range.

Standard	%RSD Total 3 days	%RSD Day 1	%RSD Day 2	%RSD Day 3
IS1	15	11	8.9	12
IS2	12	16	6.2	5.4
IS3	10	11	5.0	6.6
IS4	11	14	4.6	5.5
IS5	11	13	7.1	7.1
IS6	10	9.1	6.7	6.7
IS7	8.0	9.6	6.1	5.3
IS8	7.7	8.1	5.4	5.6
IS9	7.8	8.1	4.4	5.1

Table 2. The X500R QTOF System Reproducibility. The relative standard deviations (%RSDs) of 9 internal standards in extracted human plasma over 300 Injections over 3 days.

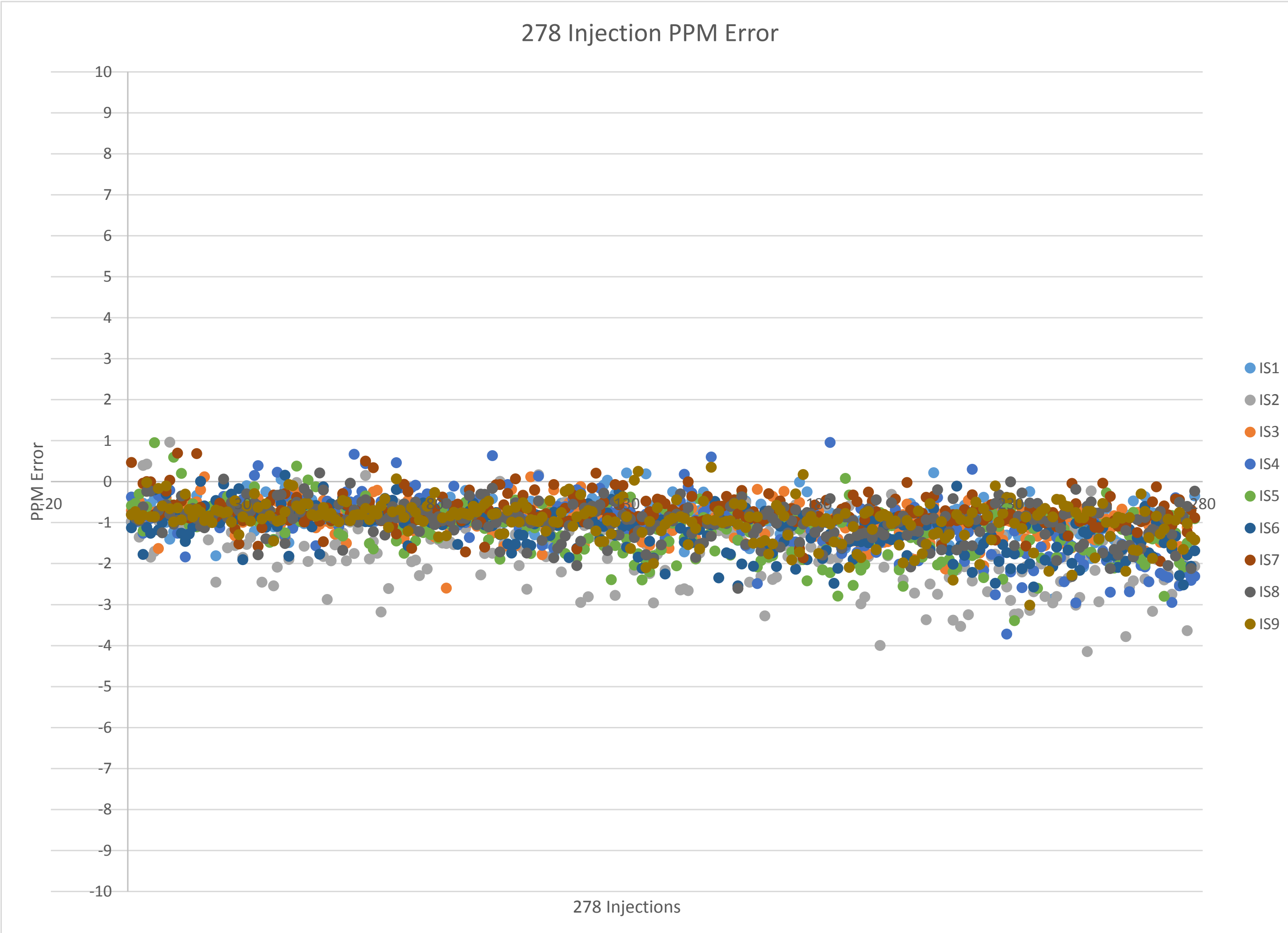

Figure 3: Mass Stability of the X500R QTOF System over Hundreds of Injections. Nine internal standards over 278 injections in Plasma Matrix.

Conclusions

The data presented here demonstrates the excellent analytical performance of the X500R. It demonstrated mass precision and accuracy critical for identification of molecular formula within the metabolomic space. It demonstrated linear performance over a competitive dynamic range and area reproducibility required for successful relative quantitation workflows.

Finally, the X500R QTOF system is a robust easy to operate, benchtop system that requires minimal MS expertise to perform untargeted metabolomics analyses.

As metabolomics continues to expand in disease research, robust easy-to-use solutions that provide quality answers will be increasingly important.

Document number: RUO-MKT-02-6945-A

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