Thorough and Practical Evaluation of the Use of QTOF in High Throughput Regulated Bioanalysis: Performance, Robustness and Return on Investment

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OVERVIEW

- **Purpose**
  - Practical demonstration of the advantages of using QTOF for quantification purposes in a high throughput regulated bioanalytical environment.
- **Method**
  - Alverine was extracted from human plasma by protein precipitation and injected on an API 5000™ (MRM) and TripleTOF5600™ (MRM^{HR}).
- **Results**
  - The high resolution mode of the TripleTOF™5600 instrument was able to increase the selectivity and throughput of the analytical method by fixing interference issues.

INTRODUCTION

High resolution accurate mass spectrometers (HRMS), such as QTOF, have recently demonstrated their ability to resolve complex qualitative problems while simultaneously performing reliable quantitation under strict regulatory guidance requirements. Hence, QTOF can be compared to triple quadrupole mass spectrometers, in terms of linearity, sensitivity and especially offering greater selectivity, since HRMS are able to increase the selectivity of an assay by using higher resolution.

Nonetheless important questions are still pending: Can QTOF produce reliable data overtime? Does its performance justify the extra expense for a regulated bioanalytical laboratory? Does the amount of data generated (file size) affect the data processing throughput? This work demonstrates the advantages of implementing QTOF for quantitative purposes.

METHODS

SAMPLE EXTRACTION

Alverine was extracted from human plasma by protein precipitation using a stable labeled internal standard for a range of 0.100 to 40.0 ng/mL.

CHROMATOGRAPHY

Samples were analyzed on a Waters ACQUITY UPLC with a mobile phase of 10mM NH_4HCO_3 pH10/MeOH/ACN at a flow rate of 600uL/min on an XBridge C18, 50x2.1mm, 5um.

DETECTION

Alverine samples were injected in ESI(+) on both API5000™ and TripleTOF™5600 system and operated with MRM mode and MRM^{HR} (High Resolution).

API 5000™ TRIPLE QUADRUPOLE

Alverine chromatography is affected by an endogenous interference that either needs to be chromatographically resolved or removed by the use of a cleaner extraction method. The **Figure 1** illustrates the injection of a reference sample with a chromatographic separation; Alverine and its interference elute respectively at 1.1 and 4.2 minutes.

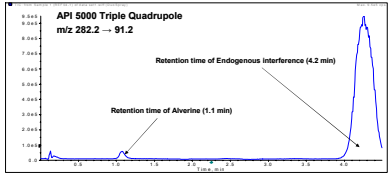

Figure 1 – Extracted Reference Sample 40 ng/mL of Alverine

HIGH RESOLUTION TRIPLE TOF™5600

The assay was transferred onto an TripleTOF™5600 using the MRM^{HR} mode. The **Figure 2** shows the scan obtain with the use of high resolution (30K) at the exact mass of Alverine 282.2217 amu.

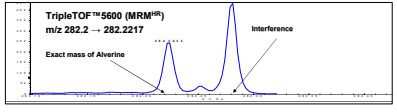

Figure 2 – QTOF Scan of an Extracted Reference Sample of 40 ng/m of Alverine in MRM^{HR}

Reconstructed chromatogram with exact mass of alverine (m/z 282.2217 ± 10mDa). **Figure 3** illustrates the absence of the interference at 4.2 minute.

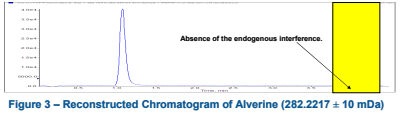

Figure 3 – Reconstructed Chromatogram of Alverine (282.2217 ± 10 mDa)

A blank sample (no drug) was injected to confirm the total removal of the interference in the Chromatogram (Figure 4).

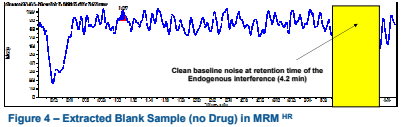

Figure 4 – Extracted Blank Sample (no Drug) in MRM ^{HR}

The ability to remove the chromatographic interference without additional sample cleaning or mobile phase gradient was allowed to increase the throughput of the method by decreasing the total runtime from 5 minutes to 2 minutes.

TRIPLETOF™5600 SENSITIVITY

Prior to submitting any calibration curve and QC's samples, the concentration of the lowest standard (LLOQ) was defined with a coefficients of variance calculated with the injection of 8 extracted samples. A %CV of 9.9% (**Figure 5**) was obtained with a concentration of 0.100 ng/mL (Criteria for the LLOQ evaluation is ≤ 20%). The result shows that this concentration was acceptable and reproducible for the LLOQ.

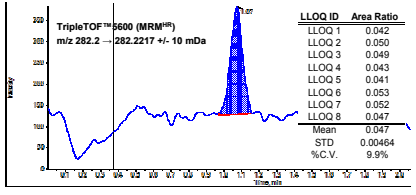

Figure 5: Example of Alverine at 0.100 ng/mL and %CV of the Replicate Injections

QUANTIFICATION RESULTS WITH TRIPLETOF™5600

The evaluation was completed with the injection of 3 different precision and accuracy batches onto the TripleTOF™5600 system to verify the quantitation performance of the instrument. **Figure 6** illustrates the linearity of the calibration curve with correlation coefficients (r^2) obtain for the between batch evaluation.

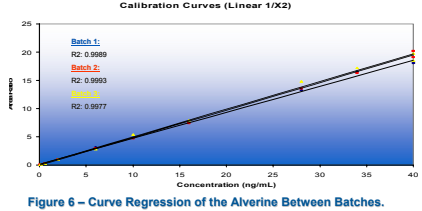

Figure 6 – Curve Regression of the Alverine Between Batches.

Precision of the LLOQ, Low, Mid and High QC's generated with the between evaluation were respectively 10.2%, 6.5%, 4.7% and 3.5% with an accuracy of 98.2%, 95.5%, 99.8% and 102.1%. Results are presented in **Table 1**.

Batch Number	LLOQ QC	Low QC	Medium QC	High QC
	0.100	0.300	8.000	30.000
	ng/mL	ng/mL	ng/mL	ng/mL
1	0.097	0.239	8.17	30.71
	0.095	0.289	7.81	30.87
	0.108	0.259	7.57	31.15
	0.105	0.248	8.63	28.82
	0.114	0.301	8.13	29.02
	0.102	0.309	8.40	31.43
2	0.096	0.308	7.87	32.18
	0.102	0.289	7.87	31.64
	0.099	0.281	8.07	29.39
	0.098	0.290	8.32	30.29
	0.094	0.299	5.10**	30.78
3	0.108	0.295	7.93	31.45
	0.107	0.292	8.08	30.67
	0.095	0.270	6.90	30.33
	0.099	0.301	7.99	30.60
	0.097	0.318	7.80	30.42
	0.109	0.281	8.17	31.91
	0.093	0.305	7.97	31.66
Mean	0.098	0.286	7.980	30.643
S.D.	0.010	0.019	0.376	1.071
N	18	18	18	18
%C.V.	10.2	6.5	4.7	3.5
%Nominal	98.2	95.5	99.8	102.1

** %Deviation unacceptable for QCs

Table 1: Precision & Accuracy Table of Alverine in MRM^{HR}

ROBUSTNESS PERFORMANCE OF THE TRIPLETOF™5600

System robustness and reliability of the instrument was monitored with 500 replicate injections over 21 hours with an extracted reference sample (ULOQ) of Alverine (40.00 ng/mL). A %CV of 5.4 % of the area ratio was obtained (**Figure 7**) .

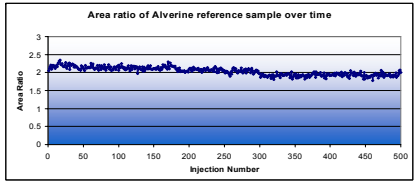

Figure 7 : Stability of the Area Ratio of a Reference Sample Over Time

Robustness and reliability of the instrument was also verify by monitoring the mass accuracy over time with several replicate injection (500). The average error found was 1.3 mDa. The quantification range of +/- 10 mDa was then considered suitable for the quantification of alverine. Figure 8

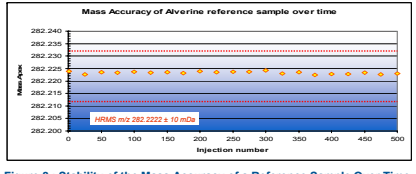

Figure 8 : Stability of the Mass Accuracy of a Reference Sample Over Time

DATA GENERATION, PROCESSING AND FILE SIZE OF THE TRIPLETOF™5600

The high resolution data of the TripleTOF™API5600 was compared to the data generated in MRM with the API 5000 to evaluate it's impact on laboratory throughput. **Table 2** shows that there is minimal difference for data processing and revision for a batch of 100 samples. It also demonstrates that HRMS data file is bigger (69.4 MB vs. 12.1 MB) but fairly manageable.

	SRM 282.1 ► 91.1	HRMS (exact Scan) 282.1 ► 278-283	HRMS (full scan) 282.1 ► 100-400
Batch size (MB)	12.1	69.4	73.8
Data Processing (minutes)	0.2	1.3	1.5
Data Revision (minutes)	1.2	3.3	3.6
Total time (minutes)	1.4	4.6	5.1

Table 2: Comparison Table of the Data File Size, Processing & Revision

RETURN ON INVESTMENT (ROI) TRIPLETOF™5600

Prior to investing in such high end instrumentation, the return on investment must be thoroughly verified. Based on the Alverine case, the HRMS instrument can:

- ✓ **Simplify the method of extraction.**
- ✓ **Reduce method development time.**
- ✓ **Increase the laboratory throughput.**

CONCLUSION

This study demonstrated that the TripleTOF™5600 System can be used for bioanalytical quantification into a regulated environment. In addition to good quantitation, the QTOF instrument was able to demonstrate good robustness, precision and accuracy of the exact mass throughout sample injection.

Furthermore, the benefits of HRMS in Regulated Bioanalysis exceed its ability to solely perform a reliable quantitation since it was also able to increase laboratory throughput. The outcome of this research showed that HRMS instrument can resolve specific issues without tedious extraction procedure or gradient chromatography to increase throughput with a positive impact on the ROI.

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