Application Note: 426

Enhanced Reproducibility Performance of the TSQ Vantage: Bioanalysis of Paroxetine in Rat Plasma

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Key Words

- TSQ Vantage™
- Inter-Day Validation
- Paroxetine
- Reproducibility
- Robustness

Introduction

In May 2001, the FDA promulgated its first official guidance for bioanalytical methods.[1] This document is titled *Guidance for Industry Bioanalytical Method Validation,* and serves as a guide for bioanalytical methods supporting clinical and non-clinical studies. Recently, the reproducibility and accuracy of the concentration determined in incurred samples was subject of significant debate, outlined in the American Association of Pharmaceutical Scientists (AAPS) journal paper titled *Workshop/Conference Report – Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays.*[2] Recommendations were put forth to randomly re-assay a subset of previously assayed incurred samples and compare them to the original value to ensure reproducibility and accuracy. Significant deviation (>15-20%) from the original measurement would then require re-evaluation of the bioanalytical method.

The availability of a sensitive and reproducible validated bioanalytical method is a prerequisite for the generation of reliable data on pharmacokinetics (PK), bioavailability, and bioequivalence of drugs. Method validation demonstrates that a bioanalytical method will successfully meet the minimum standards recommended by the Guidance for Industry Bioanalytical Method Validation as it pertains to accuracy, precision, selectivity, sensitivity, reproducibility, and stability. However, after repeated exposure to biological matrices, ensuring precision and accuracy at the lower limit of quantitation (LLOQ) of a validated bioanalytical method is a challenge faced by every bioanalytical laboratory.

Goal

The aim of this study was to demonstrate the robustness and reproducibility of the Thermo Scientific TSQ Vantage for highly sensitive bioanalysis of paroxetine in rat plasma. Paroxetine was spiked into rat plasma and was injected as a batch consisting of blanks, calibration standards, and QCs. The total batch consisted of 1000 plasma samples with the intent of overlaying the calibration curves (five), which were run every 200 injections as a measure of reproducibility and ion source robustness.

Experimental Conditions

Sample Preparation

Aliquots (5 mL) of rat plasma were spiked with paroxetine in the concentration range of 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, and 500.0 pg/mL for the calibration standards and at levels of 2.5, 25.0 and 250.0 pg/mL for quality control samples. Alprazolam (analogue Internal Standard) was spiked at a concentration of 50.0 pg/mL. Blank rat plasma and plasma containing internal standard were also prepared. Eight replicate plasma aliquots of 100 µL were taken from each spiked level and protein precipitated using 500 µL of Acetonitrile (5:1, v/v protein precipitate), centrifuged at 13,000 rpm for 10 minutes and the supernatants decanted. The extracts were evaporated under a stream of nitrogen gas and reconstituted in 100 µL of Water/Methanol/Acetic acid (80/20/0.1 v/v/v).

Sample Analysis:

HPLC pump: Thermo Scientific Accela™ pump (Thermo Fisher Scientific, San Jose, CA)
Auto-sampler: CTC PAL (CTC Analytics, Basel, Switzerland)
Column: Thermo Scientific Hypersil GOLD™ C18 50 mm × 2.1 mm (3 µm) column (Thermo Fisher Scientific, Bellefonte, PA)

HPLC Method: A linear gradient of 10% Solvent B (acetonitrile containing 0.1% formic acid) to 95% B over five minutes was used to chromatograph paroxetine. Solvent A was water containing 0.1% formic acid. The flow rate was 1mL/min. Injection volumes of 10 µL were used.

MS Conditions

Mass spectrometer: TSQ Vantage (Thermo Fisher Scientific, San Jose, CA)
Ionization mode: HESI-II in positive ion mode
Vaporizer Temperature: 400 °C
Ion Sweep Gas: 5 au
Ion Transfer Tube Temp: 300 °C
Sheath Gas: 60 au
Aux Gas: 30 au
Resolution: 0.7 Da (FWHM) on Q1 and Q3
Scan Time: 0.2s
Scan Width: 0.002 Da
Chrom Filter: 5s

Selected Reaction Monitoring:
Paroxetine *m/z* 330.20 → 192.1 Da
Alprazolam *m/z* 309.1 → 281.0 Da
Collision Energy: 22V
Collision Gas Pressure: 1.5mTorr

Results and Discussion

Reproducibility is an important parameter for a bioanalytical method. A validated bioanalytical method is often tested to the limit due to continual build-up of proteins, salts, sugars, phospholipids, and other components that constitute biological matrix. A key component in ensuring accurate and precise measurements over time is a robust ion source which ultimately bears the brunt of non-volatile components present in bioanalytical samples.[3] The TSQ Vantage was designed to perform reproducibly at high levels of sensitivity after repeated exposure to samples containing biological matrix, as shown by Figure 1. This figure shows 5 standard curves (1-500 pg/mL) run once a day during the analysis of 1000 rat plasma samples over a 5-day period (200 samples per day). The 5 curves are overlaid to demonstrate the robustness of the ion source.

Figure 2 shows the chromatograms at the LLOQ level on day 1, 3, and 5. There is essentially no difference in signal to noise (S/N) between the sample run on day 1 and the sample run on day 5, despite the system being exposed

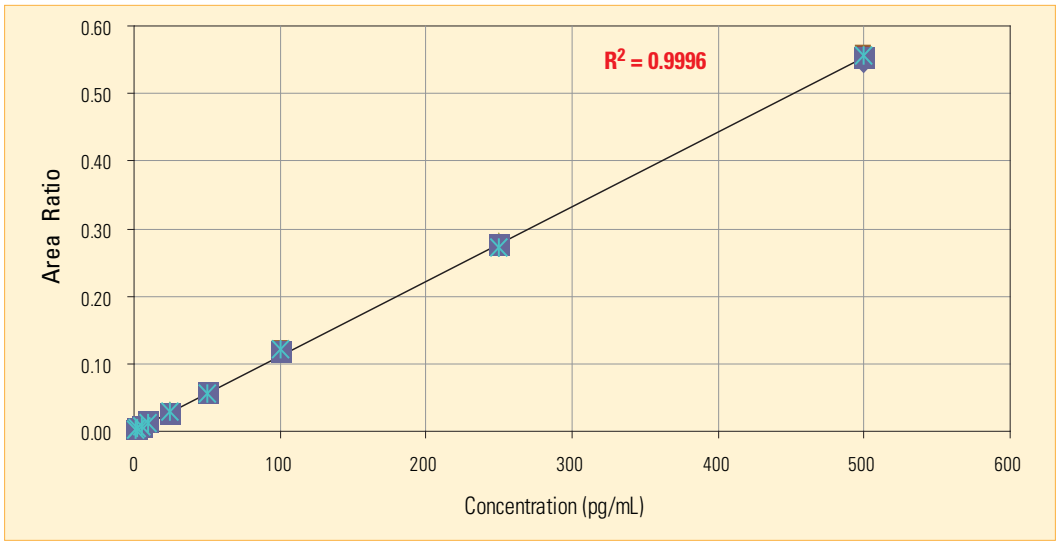

Figure 1: An overlay of 5 standard curves in spiked rat plasma, sampled during the analysis of 1000 rat plasma samples over a 5-day period.

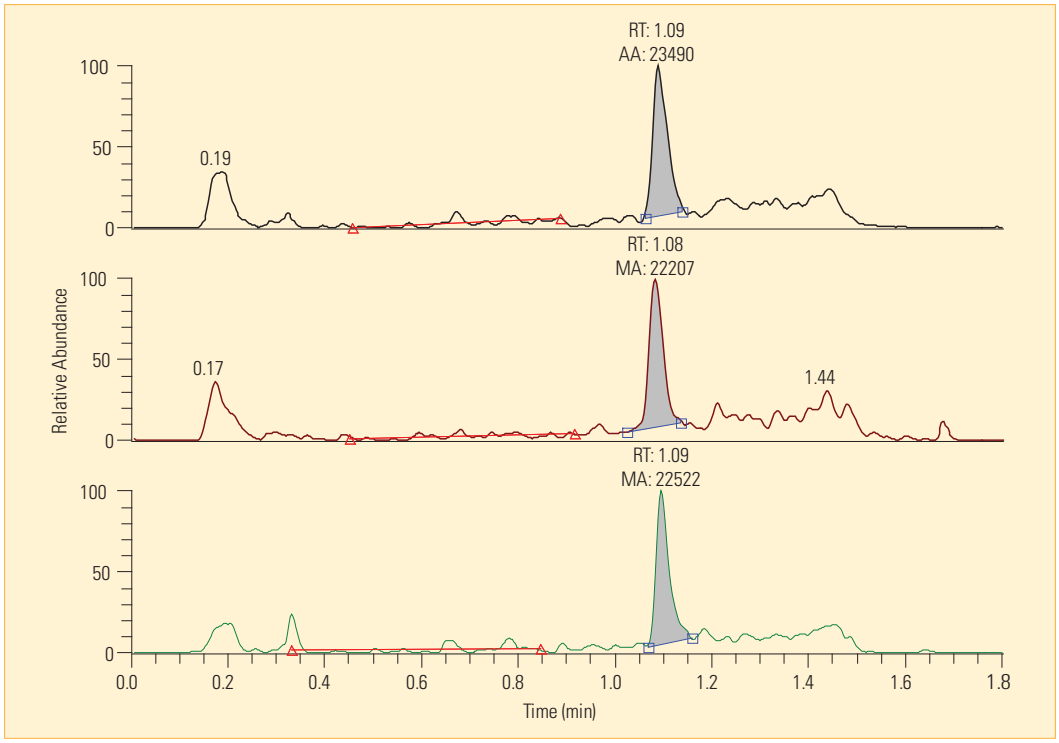

Figure 2: Chromatograms at the LLOQ (1 pg/mL) level of paroxetine spiked in rat plasma on day 1, 3, and 5, sampled during the analysis of 1000 rat plasma samples

to 1000 rat plasma samples. The robust reproducibility is reflected in the accuracy (systematic bias) and precision (random error) of the measured area ratios (Table 1) over the 5-day period. The ability of the ion source to continue to reproducibly perform at high sensitivity is indicated by a %CV of 5.44% at the LLOQ and a %CV of 1.00% at the upper limit of quantitation (ULOQ). The calculated concentration (Table 2) values thus become important as it is these values that will eventually influence the study PK parameters.

	Area Ratio (Inter-Day Study)					
Conc (pg/mL)	Day 1	Day 2	Day 3	Day 4	Day 5	%CV over 5 Days
1.0	0.00255	0.00230	0.00263	0.00238	0.00253	5.44%
2.5	0.00401	0.00390	0.00382	0.00371	0.00417	4.47%
5.0	0.00738	0.00748	0.00704	0.00701	0.00769	3.99%
10.0	0.01348	0.01322	0.01412	0.01345	0.01327	2.68%
25.0	0.02767	0.02723	0.02850	0.02719	0.02906	2.96%
50.0	0.05715	0.05462	0.05676	0.05721	0.05674	1.90%
100.0	0.12082	0.12074	0.11716	0.11923	0.12030	1.28%
250.0	0.27194	0.27326	0.27828	0.27571	0.27121	1.06%
500.0	0.54463	0.55885	0.55534	0.55279	0.55686	1.00%

Table 1: Area ratio measurements of 5 standard curves in spiked rat plasma, run once a day over a 5-day period, during which the system was exposed to 1000 rat plasma samples (200 samples/day)

	Calculated Conc (pg/mL)					
Conc (pg/mL)	Day 1	Day 2	Day 3	Day 4	Day 5	Avg. Calc. Conc. Over 5 Days
1.0	1.163	1.170	1.063	0.925	0.962	1.057
2.5	2.521	2.689	2.423	2.571	2.435	2.528
5.0	5.084	5.062	5.194	5.249	4.977	5.113
10.0	10.377	10.675	10.087	10.470	10.632	10.448
25.0	25.540	24.929	24.831	24.982	24.627	24.982
50.0	50.275	49.038	50.199	50.052	49.801	49.873
100.0	108.333	105.863	104.106	108.246	105.643	106.438
250.0	252.346	248.579	249.451	251.288	248.419	250.016
500.0	498.107	499.085	497.290	503.946	499.066	499.499

Table 2: Calculated concentrations of standard curve in spiked rat plasma, run once a day over a 5-day period, during which the system was exposed to 1000 rat plasma samples (200 samples/day)

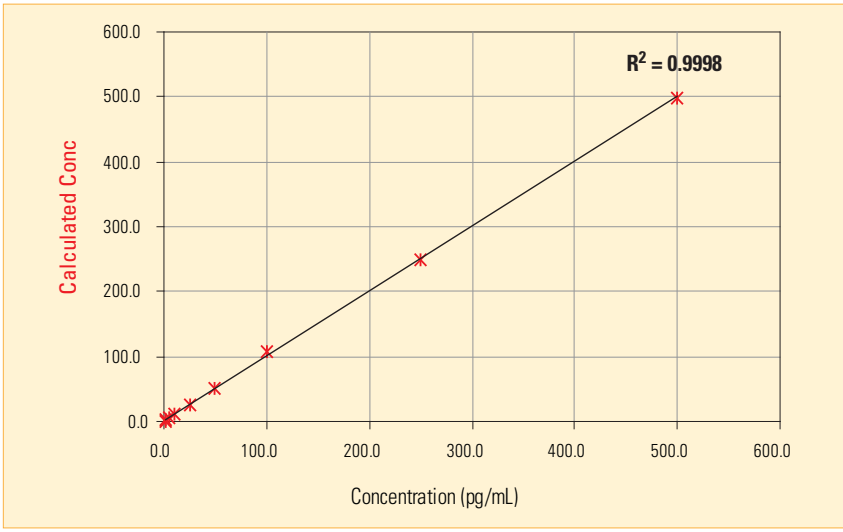

Figure 3: Correlation plot between the actual concentration and the calculated concentration of paroxetine standard curves in spiked rat plasma over a 5-day period demonstrating the reproducibility of the TSQ Vantage after being exposed to 1000 rat plasma samples

Figure 3 shows the linear correlation (R^2 of 0.999) between the actual spiked concentration (assumed) and the calculated concentration (measured) over a 5-day period. The R^2 value, which represents the goodness of fit, shows that there is no variation of the actual observations (measured) from the fitted values (assumed) regardless of repeated exposure to 1000 rat plasma samples. This strong correlation indicates that the total system error (accuracy + precision) can be minimized over a period of continuous exposure to samples containing biological matrix. This is further validated in Figure 4, which shows five randomly selected mid-QC levels (paroxetine) measured over a 5-day period. The data compiled in this application note substantiates the robust reproducibility of the TSQ Vantage for the bioanalysis experiment.

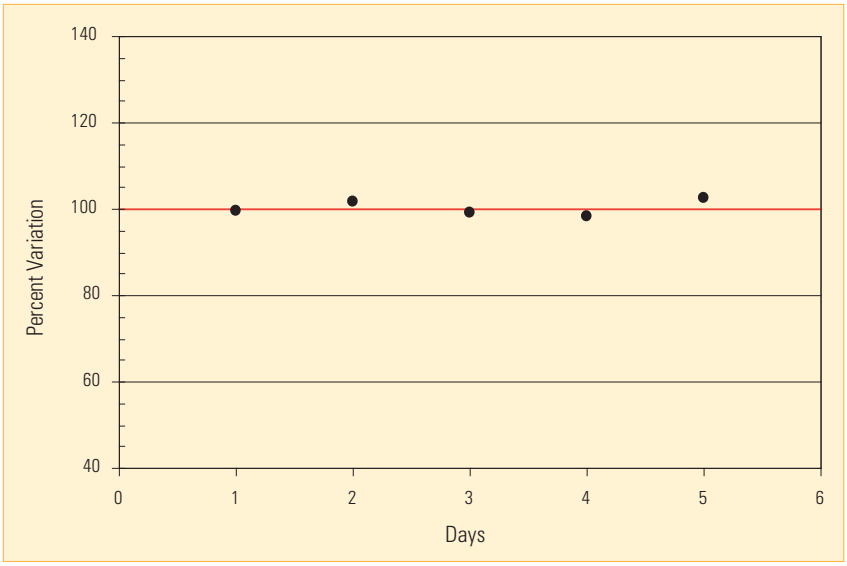

Figure 4: A representation of inter-day % accuracy of five randomly selected mid-QC levels (paroxetine) measured over a 5-day period after continuous exposure to over 1000 protein precipitated rat plasma samples.

Conclusion

Reproducibility is important because it predicates reliability or the ability of the method to yield similar concentration for a sample when measured on different occasions.[4] This is of particular importance to the summary guidance issued at the recent AAPS/FDA Crystal City meeting which requires demonstrating reproducibility of incurred samples. It also means longer time between preventive maintenance cycles, which means higher productivity. The data shown in this article clearly demonstrates the superior reproducibility performance of the TSQ Vantage for high sensitivity LC-MS/MS.

References

[1] Guidance for Industry, Bioanalytical Method Validation. Drug Information Branch (HFD-210), Center for Drug Evaluation and Research (CDER), 5600 Fishers Lane, Rockville, MD 20857 (Tel) 301-827-4573, http://www.fda.gov/cder/guidance/index.htm

[2] Workshop/Conference Report – Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. The *AAPS Journal 2007*; 9 (1) Article 4 (http://www.aapsj.org).

[3] The Effects of Sample Preparation Methods on the Variability of the Electrospray Ionization Response for Model Drug Compounds. *Rapid Commun. Mass Spectrom.* 13, 1175–1185 (1999).

[4] Key Elements of Bioanalytical Method Validation for Small Molecules. The *AAPS Journal 2007*; 9 (1) Article 11, http://www.aapsj.org.

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