

# Bioanalytical Method Intraday Validation for the Quantitation of Paroxetine in Bovine Plasma Using the TSQ Quantum Mass Spectrometer

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## Introduction

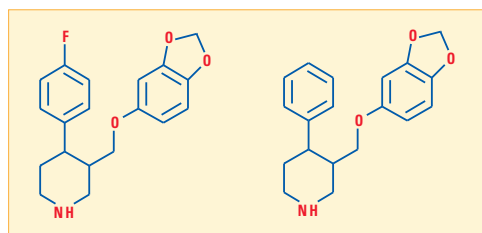
Guidelines on Bioanalytical Method Validation acceptance criteria were established by the US Food and Drug Administration (FDA) from a conference on “Analytical Methods Validation – Bioavailability, Bioequivalence and Pharmacokinetic Studies” held in Arlington, VA, December 3-5, 1990! These acceptance criteria were reviewed ten years on at “Bioanalytical Methods Validation—A Revisit with a Decade of Progress”, Arlington, VA, January 12-14, 2000<sup>2</sup>; a meeting sponsored by the American Association of Pharmaceutical Scientists and the FDA. This led to the issue of the FDA Guidance for Industry document on Bioanalytical Method Validation in May 2001<sup>3</sup>. A full method validation should assess accuracy, precision, selectivity, sensitivity, reproducibility and stability of an analyte in matrix samples.

Paroxetine is an orally administered antidepressant which acts as a selective serotonin reuptake inhibitor (SSRI) but has only minimal effects on the neuronal reuptake of norepinephrine and dopamine. Paroxetine is administered for the treatment of depression, social anxiety disorder, obsessive compulsive disorder, panic disorder and generalized anxiety disorder.

## Goal

This report describes the ease of use of the Thermo Scientific TSQ Quantum to validate bioanalytical methods in accordance with the guidelines issued by the US Food and Drug Administration.

The aim of this investigation was to demonstrate the sensitivity, linearity, and robustness of the TSQ Quantum to allow researchers to achieve excellent accuracy and precision for high-throughput quantitation of analytes in biological matrices. Paroxetine spiked into bovine plasma was injected as an “intra-day” batch consisting of blanks, calibration standards, and QC’s at eight replicates.



Paroxetine (left), De-Fluoro Paroxetine

## TSQ Quantum Tune View Optimization of Paroxetine

Paroxetine (C<sub>19</sub>H<sub>20</sub>FNO<sub>3</sub>, molecular weight 329.37) was infused, 0.1 ng/μL, into the ESI source and the four most abundant product ions for the MS/MS breakdown were determined using the automated compound optimization procedure in the TSQ Quantum (Figure 1).

The transition yielding the most abundant product ion (*m/z* 192.1) was used for the analysis of Paroxetine.

## Experimental Conditions

**Sample Preparation:** Aliquots (1 milliliter) of Bovine Plasma (Sigma P4639) were spiked with Paroxetine in the concentration range of 0.1, 0.2, 0.5, 1, 2, 5, 10, 50, 100, 200, 500 and 1000 pg/μL for the calibration standards and at levels of 0.3, 25 and 750 pg/μL for quality control samples. De-Fluoro-Paroxetine (Internal Standard) was spiked at a concentration of 25 pg/μL. Blank Bovine 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 250 μL of Acetonitrile, 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:** The plasma extracts were chromatographed (Thermo Scientific Surveyor™ System) on a Thermo Scientific Hypersil™ Elite C18 100 mm × 2.1 mm column at a flow rate of 250 μL/min with a linear gradient of 10% Solvent B (methanol containing 0.1% acetic acid) to 95% B over five minutes. Solvent A was water containing 0.1% acetic acid. Injection volumes of 10 μL were used.

## Mass Spectrometry

Mass spectrometer: TSQ Quantum

Ionization mode: Electrospray (ESI), positive ion

Resolution: 0.7 Dalton Full Width Half Maximum on Q1 and Q3

Selected Reaction Monitoring:

Paroxetine 330.20 > 192.1 +/- 0.3 Da, 22 V collision energy

De-Fluoro-Paroxetine 312.20 > 174.1 +/- 0.3 Da, 20 V collision energy

Argon collision gas pressure 1.5 mTorr

## Results

The chromatography (Figure 2a–2d and 3a–3b) was capable of resolving Paroxetine and De-Fluoro-Paroxetine from any matrix peaks and was suitable for the quantitation of unknowns.

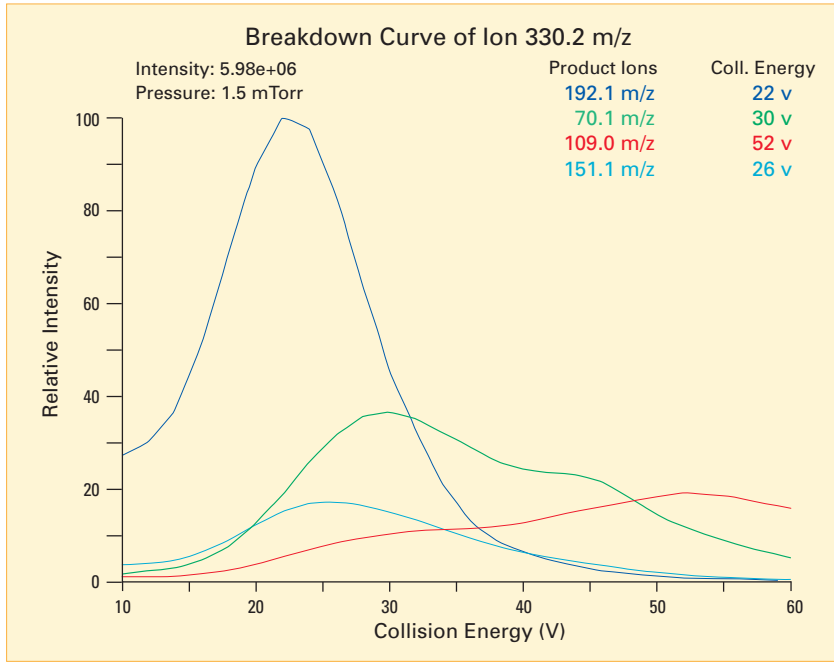


Figure 1. Automated optimization of MS/MS parameters for Paroxetine

## LC/MS/MS Chromatograms of Bovine Plasma Spiked with Paroxetine and Internal Standard

Blank Plasma, Plasma containing Internal Standard, Calibration Standards at 0.1 and 0.5 pg/ $\mu$ L

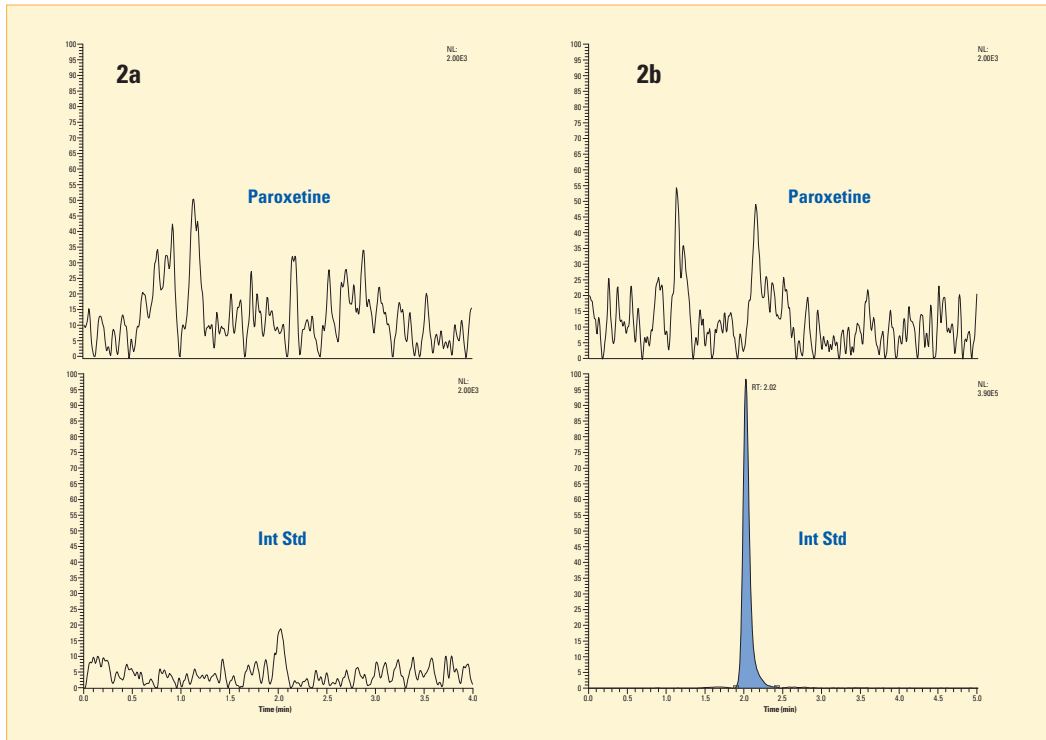


Figure 2a. Blank Plasma

Figure 2b. Plasma containing Int Std

A calibration curve was constructed from the first and eighth replicate set of calibration standards (Figure 4). The linear fit calibration curve, weighted 1/X, of area ratio

plotted against concentration was used to calculate the concentrations of all calibration standards and quality control samples throughout the analytical batch.

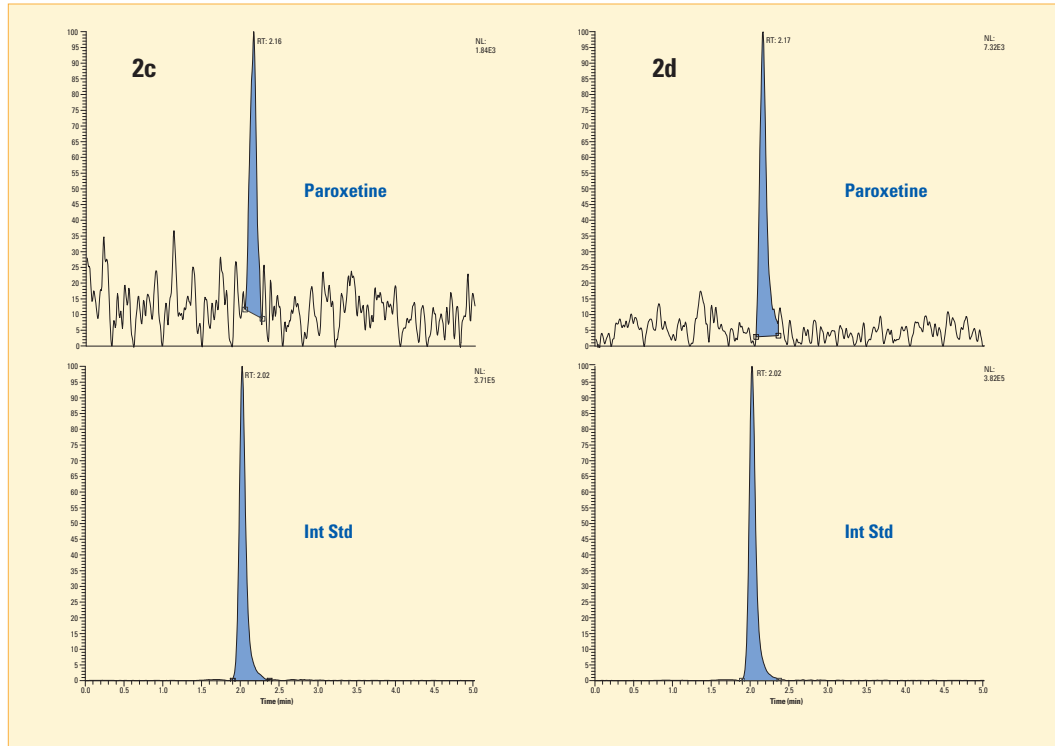


Figure 2c. Plasma spiked at 0.1 pg/ $\mu$ L  
Figure 2d. Plasma spiked at 0.5 pg/ $\mu$ L

### LC/MS/MS Chromatograms of Bovine Plasma Spiked with Paroxetine and Internal Standard

Quality Control samples at 0.3 and 25 pg/ $\mu$ L

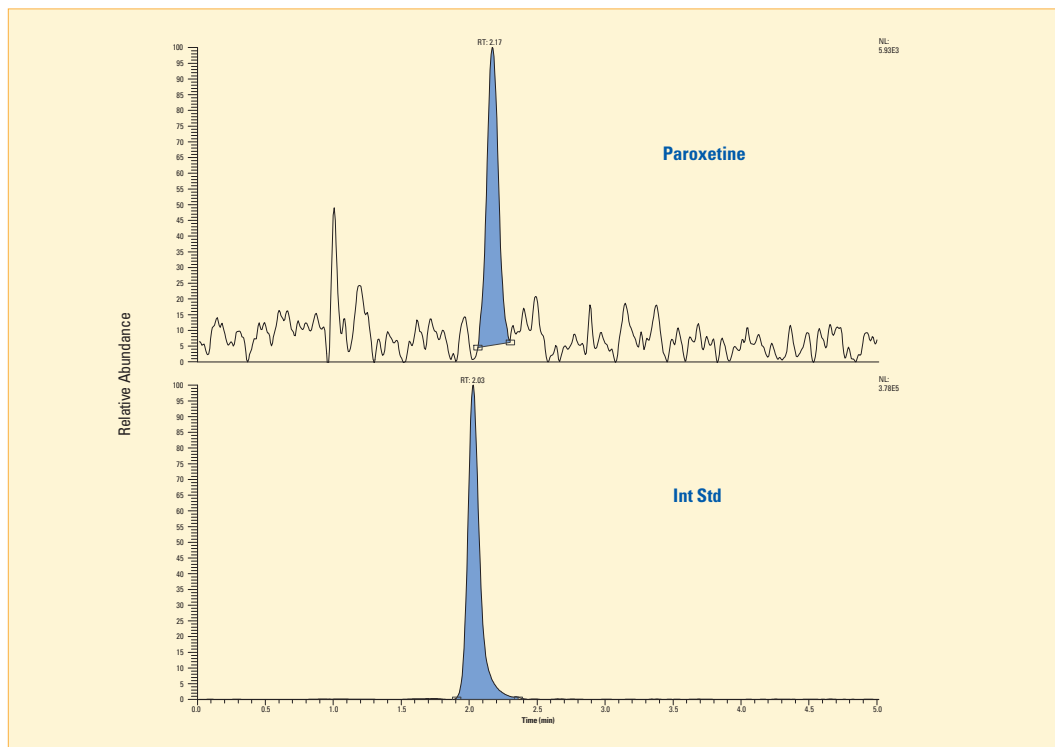


Figure 3a. Quality Control at 0.3 pg/ $\mu$ L

A correlation coefficient of  $r^2=0.9994$  with an equation of  $Y=0.00346891 + 0.0312025 * x$  was obtained for the curve.

The internal standard peak area response was 3.1% cv for 121 injections (Figure 5).

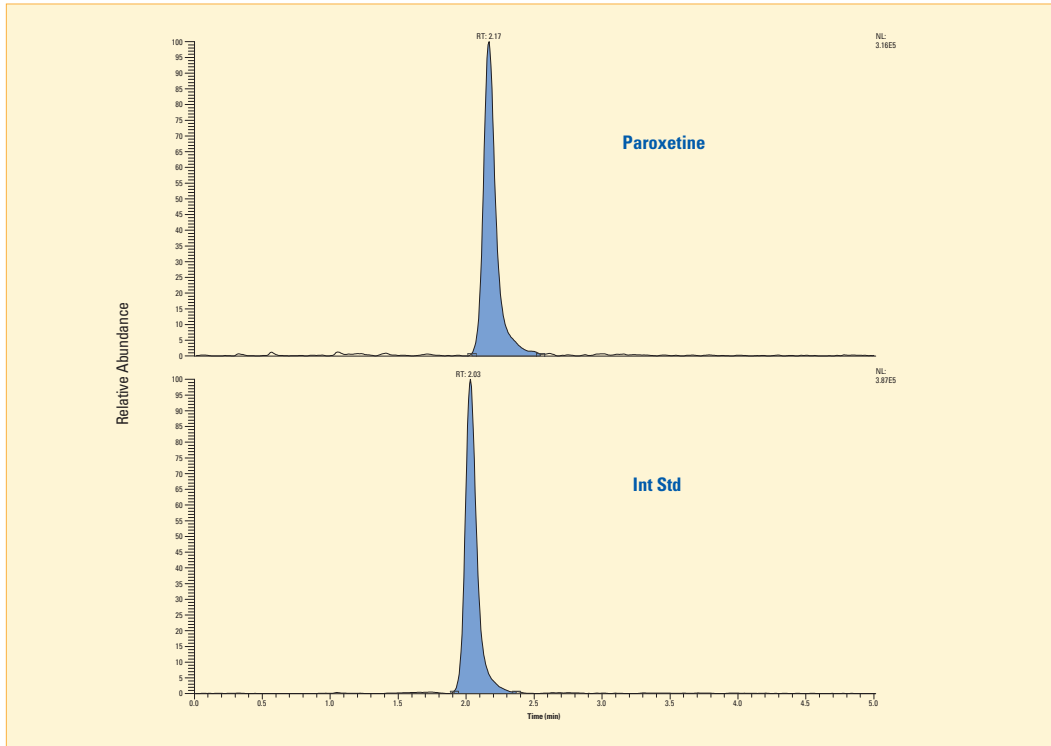


Figure 3b. Quality Control at 25 pg/ $\mu$ L

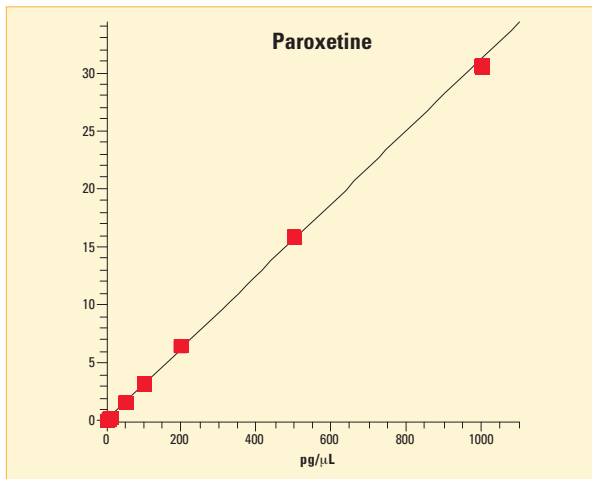


Figure 4. Paroxetine/Int Std area ratio

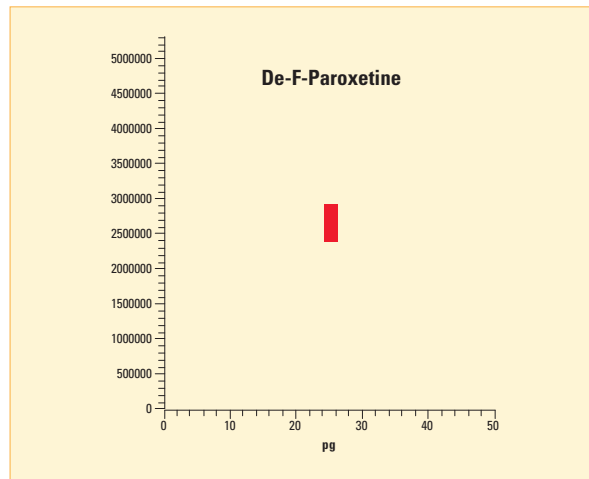


Figure 5. Internal Standard area response

The calibration curve was used to calculate the values of the eight replicates at each level of quality control sample (Table 1) and of the remaining six replicates of standards (Table 2).

QC Replicate Number	Actual Concentration (pg/ $\mu$ L)		
	0.3	25.07	50.0
Back Calculated Concentration (pg/ $\mu$ L)			
1	0.30	26.15	745.42
2	0.31	26.17	752.82
3	0.31	26.05	743.09
4	0.31	26.03	747.07
5	0.32	25.82	746.66
6	0.32	25.92	754.03
7	0.31	26.15	742.24
8	0.30	25.74	749.04
Mean	0.31	26.00	747.55
SD (n-1)	0.007	0.165	4.237
%CV	2.2	0.6	0.6
% Accuracy	103.6	104.0	99.7

Table 1. Intra-batch accuracy and precision of quality control data for Paroxetine in Bovine plasma

Curve Number	Actual Concentration (pg/ $\mu$ L)											
	0.1	0.2	0.5	1.0	2.0	5.0	10.0	50.0	100.0	200.0	500.0	1000.0
Back Calculated Concentration (pg/ $\mu$ L)												
1*	0.097	0.19	0.48	0.94	2.01	5.02	10.08	53.23	103.89	207.82	507.37	983.99
2	0.097	0.20	0.50	0.96	2.01	4.95	10.12	51.56	103.02	208.77	508.51	981.64
3	0.098	0.19	0.49	0.95	2.02	4.94	10.17	53.24	102.40	210.02	504.85	979.63
4	0.105	0.19	0.48	0.95	2.01	5.07	10.05	52.76	103.37	205.95	510.11	976.85
5	0.101	0.19	0.49	0.95	2.03	4.98	10.12	52.94	102.95	205.59	507.31	982.34
6	0.104	0.19	0.48	0.96	2.01	5.03	9.96	52.46	102.65	207.19	510.25	978.97
7	0.102	0.19	0.50	0.96	2.01	5.03	10.06	52.51	103.13	205.11	504.85	982.97
8*	0.102	0.19	0.49	0.96	2.02	5.00	10.20	52.46	102.66	207.74	504.75	975.90
Mean	0.101	0.19	0.49	0.95	2.02	5.00	10.10	52.64	103.01	207.27	507.25	980.29
SD (N-1)	0.003	0.004	0.009	0.008	0.005	0.043	0.075	0.544	0.469	1.672	2.285	2.934
%CV	3.0	1.9	1.9	0.8	0.2	0.9	0.7	1.0	0.5	0.8	0.5	0.3
% Accuracy	100.8	94.7	97.6	95.4	100.8	100.1	101.0	105.3	103.0	103.6	101.5	98.0

Table 2. Intra-batch accuracy and precision of calibration data for Paroxetine in Bovine Plasma

\*Calibration data sets 1 and 8 used to construct curve

## Discussion

The lowest calibration standard selected for this assay at 0.1 pg/ $\mu$ L had a minimum signal-to-noise of greater than 5:1 and variability of 3% from eight determinations. This was well within the acceptance criteria set by the guidelines which defines that the Limit of Quantitation (LOQ) of an assay should have less than 20% variability.

The Intra-batch variability of back-calculated concentrations of Paroxetine in Bovine Plasma was less than 3% across the whole calibration range. This falls well within the expected method validation acceptance criteria as set by Shah et al of 20% variability at the LOQ and less than 15% variability at all other concentrations of the calibration standards.

The validated method accuracy and precision were determined from the back-calculated values of the Quality Control samples. The intra-batch accuracy was 103.6%, 104.0% and 99.7% at concentrations of 0.3, 25 and 750 pg/ $\mu$ L respectively. The intra-batch precision was 2.2%, 0.6% and 0.6% at concentrations of 0.3, 25 and 750 pg/ $\mu$ L respectively.

The validation data indicate that the TSQ Quantum is sensitive, robust and linear (four orders of dynamic range demonstrated here) and is an ideal instrument for the quantitation of analytical substances in Biological matrices.

## References

- <sup>1</sup> Shah, V.P., et al, *Pharmaceutical Research*, Vol 9, No 4, 588-592, 1992
- <sup>2</sup> Shah, V.P., et al, *Pharmaceutical Research*, Vol 17, No 12, 1551-1557, 2000
- <sup>3</sup> Guidance for Industry, Bioanalytical Method Validation, U.S. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.

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