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Note d'application

A Sensitive and Specific Method for the Estimation of Bromocriptine Using ACQUITY UPLC and Xevo TQ-S

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Abstract

This work demonstrates the capability of Xevo TQ-S for monitoring compounds with different parent masses arising out of isotopic distribution. Oasis Ion-Exchange cartridges, UPLC chromatography, and an advanced tandem quadrupole mass spectrometer for the development of a picogram-sensitivity method with a high degree of specificity for the quantification of bromocriptine in plasma were employed. In addition to addressing one of the key challenges of achieving desired sensitivity, which is often faced by the bioanalytical scientist, this application note addresses other challenges such as reproducibility and robustness.

Introduction

Bromocriptine, 2-bromoergocriptine, is a semi-synthetic derivative of a natural ergot alkaloid, ergocriptin (a derivative of lysergic acid), that is synthesized by bromination of ergocriptin using N-bromosuccinimide. Bromocriptine is a dopamine agonist that is used in the treatment of pituitary tumors, Parkinson's disease (PD), hyperprolactinaemia, neuroleptic malignant syndrome, and type II diabetes. Bromocriptine is classified as an ergopeptide, comprising ergoline derivatives that contain a tripeptide structure attached to the basic ergoline ring in the same location as that of the amide group of the lysergic acid derivatives. Typically, the ergopeptides comprise two other α-amino acids linked in an unusual cyclol formation >N-C(OH)< with the carboxyl carbon of proline, at the juncture between the two lactam rings.

Bromocriptine has been available for clinical use for many years and has vasoconstrictive properties similar to ergotamine and ergonovine, due to the unsaturated bond in the D ring of the lysergide ring system. This contrasts with the vasodilating properties of ergoline-based compounds, such as pergolide and dihydroergotoxine alkaloids, which have a saturated bond in the same position in the D ring. This class of compounds, including Bromocriptine exhibits high lipophilicity, and is also extensively metabolized prior to excretion. Traditional screening methods, such as enzyme linked immunosorbent assays (ELISA) and gas chromatography-mass spectrometry (GC-MS) lack the sensitivity to screen blood or plasma at the very low concentrations that Bromocriptine is likely to be found following intake. Therefore, a sensitive and specific liquid chromatographic-mass spectrometric (LC-MS) method is needed.

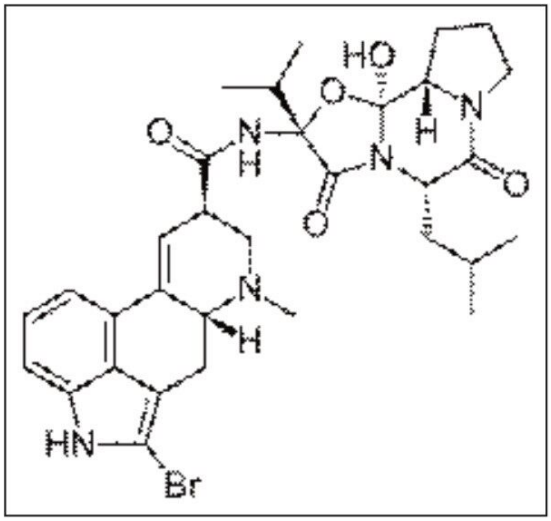


Figure 1. Molecular structure of Bromocriptine.

Experimental

LC conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC HSS C_{18} 1.8 µm, 2.1 x 150 mm
LC Column elution:	70% aqueous buffer over 2.0 minutes, followed by a 95% organic elution till 4.2 min; then change back to initial conditions.
Column temp.:	40 °C
Flow rate:	0.200 mL/min

Injection volume:	20 µL

MS Conditions

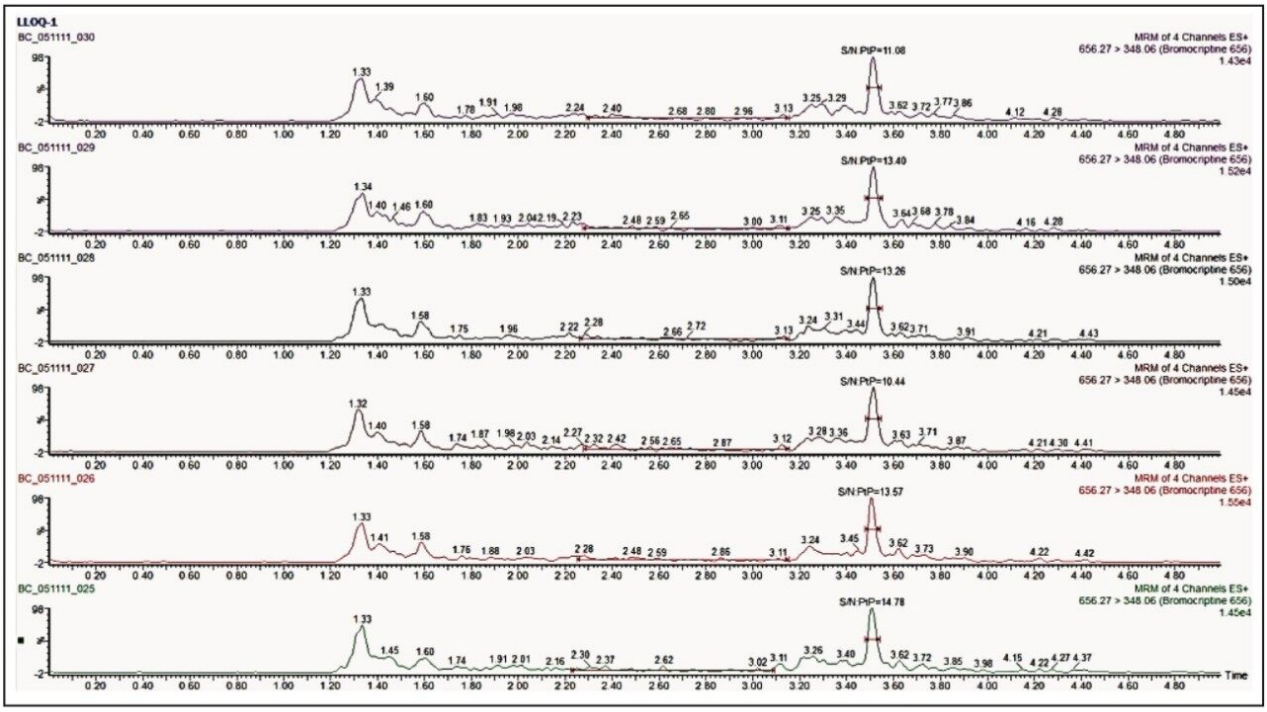
MS system:	Xevo TQ-S
MS mode:	ESI positive MS/MS method
MRM transition:	656.27 → 348.06

The selectivity of the isotope in Bromocriptine due to isotopic abundance is essential since the compound exhibits two different peaks of parent masses – a feature typical of molecules containing bromine. Both parent masses were monitored for method development. This molecule also exhibits strong protein binding ability, which is an essential requirement for the estimation in the pg/mL levels.

Samples were isolated using a solid phase extraction (SPE) method, employing Waters Oasis MCX (1 CC, 30 mg cartridges). A 500 µL aliquot of plasma was diluted with 5% o-Phosphoric Acid, and loaded onto the SPE cartridges that were previously conditioned with organic solvent and water. The plasma solution was then washed with acidified water followed by an organic wash, and then eluted with basic organic elution solvent. The eluted samples were evaporated and reconstituted prior to injection. Aliskiren (MW 551.76; retention time, 3.44 mins), typically employed as a renin inhibitor, was used as an internal standard (IS).

Results and Discussion

The chromatographic method using ACQUITY UPLC and the ACQUITY UPLC HSS C_{18} Column provided excellent resolution for the Bromocriptine analyte. Bromocriptine eluted at 3.51 minutes with a peak width of 12 s at the base. The data shown in Figure 2 illustrate the signal obtained from the lower limit of quantification (LLOQ) of Bromocriptine in human plasma, and the blank signal, shown in Figure 3. Analysis of aliskiren exhibited chromatographic and extraction conditions similar to those observed for Bromocriptine; therefore, aliskiren was selected as an internal standard (IS).



Signal to Noise Ratio of 6 replicates of Bromocriptine in LLOQ						
LLOQ1	LLOQ2	LLOQ3	LLOQ4	LLOQ5	LLOQ6	AVERAGE
11.08	13.40	13.26	10.44	13.57	14.78	12.75

Figure 2. Chromatogram of LLOQ concentration (2.0 pg/mL) of Bromocriptine with a signal-to-noise ratio of 12.75 (data for six replicates shown in the table) obtained using ACQUITY UPLC and Xevo TQ-S.

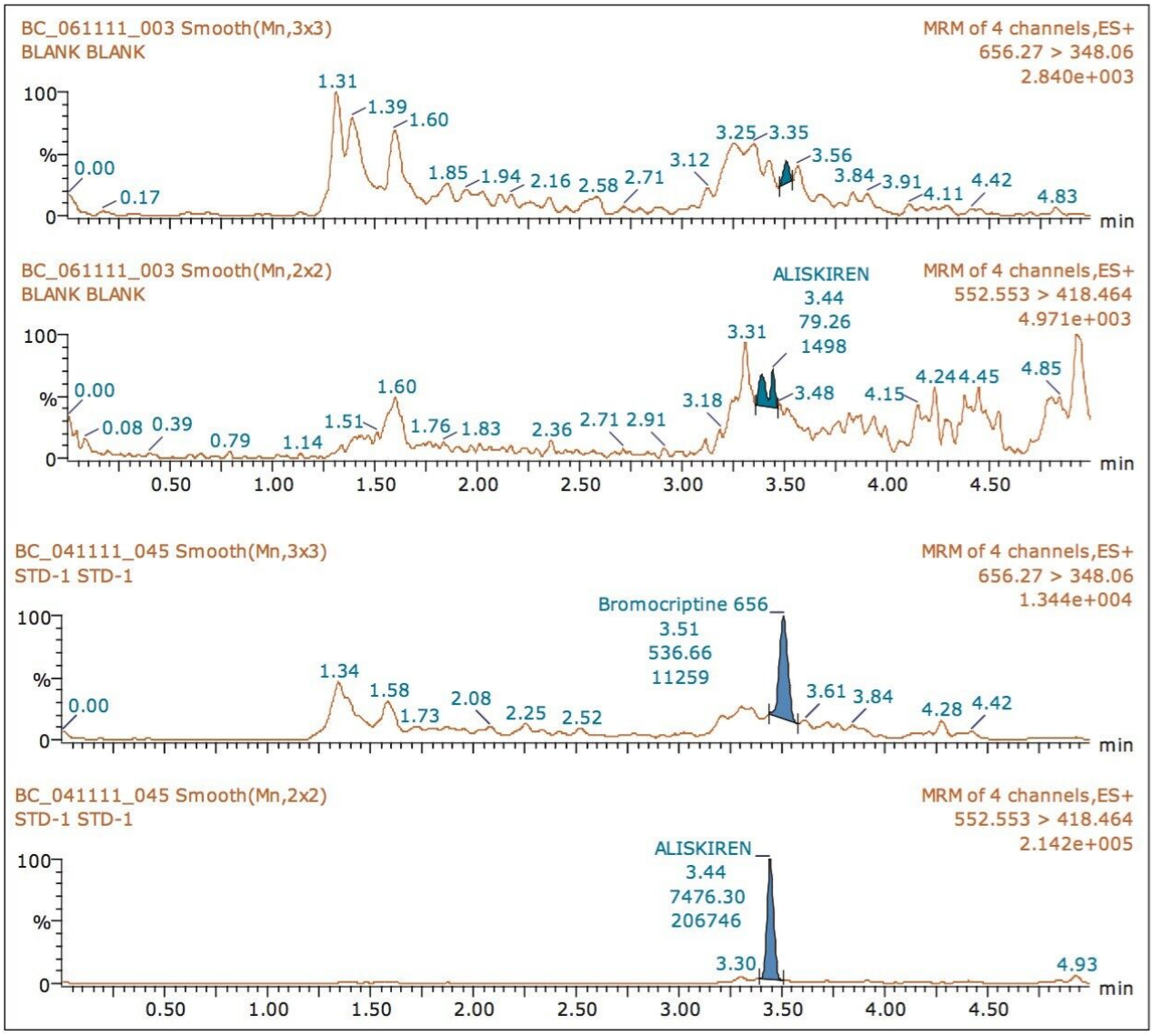


Figure 3. Chromatogram of blank and LLOQ concentration (2.0 pg/mL) of Bromocriptine with aliskiren (bottom chromatogram) as an IS.

As can be observed in Figure 2, the retention time of Bromocriptine does not interfere with any endogenous signals, and the signal corresponding to the analyte of interest can be easily observed at the LLOQ level. The Xevo TQ-S MS is equipped with a novel StepWave ion guide, which when combined with the high-resolution chromatography obtained from the ACQUITY UPLC System, results in the successful completion of extremely sensitive applications that can be performed with high reproducibility. Bromocriptine was detected at a concentration of 2.0 pg/mL, shown in Figure 2, with a signal-to-noise ratio of 12.75:1.00 for an average of six replicate injections.

The assay in this report showed linear calibration over the range of 2 to 256 pg/mL with an excellent r^2 value of 0.997, as shown in Table 1. The back-calculated concentration of the standard was found to be within 6% of the nominal concentration, as shown in Table 1. As observed from the data in Table 1, an excellent degree of accuracy was achieved for each sample. Although both parent masses, showed acceptable results owing to higher specificity and the chromatographic separation, shown in Figure 4, the parent mass of 656 was chosen for the estimation of Bromocriptine in matrix. This assay was performed with a 5-minute injection-to-injection time scale, highlighting the capability of Waters Regulated Bioanalysis System solution to deliver fast, accurate, and highly sensitive results while maintaining desired precision, and high throughput value.

Sample	Type	Nominal (pg/mL)	Analyte area	ISTD area	Area ratio	Calculated (pg/mL)	Accuracy
EXT_BLK	BLANK		20	49	0.4172		
EXT_BLK_IS	ZERO		47	7588	0.0063		
EXT_CC_1	STD-1	2	537	7476	0.0718	2.081	104.04
EXT_CC_2	STD-2	4	933	7138	0.1307	3.783	94.57
EXT_CC_3	STD-3	8	2020	7593	0.2660	7.688	96.10
EXT_CC_4	STD-4	16	3843	7324	0.5247	15.157	94.73
EXT_CC_5	STD-5	32	7140	6282	1.1367	32.827	102.58
EXT_CC_6	STD-6	64	15633	6847	2.2833	65.929	103.01
EXT_CC_7	STD-7	128	32043	7142	4.4866	129.543	101.21
EXT_CC_8	STD-8	256	54700	5946	9.1989	265.593	103.75

Table 1. Calibration data of Bromocriptine over the range of 2 to 256 pg/mL.

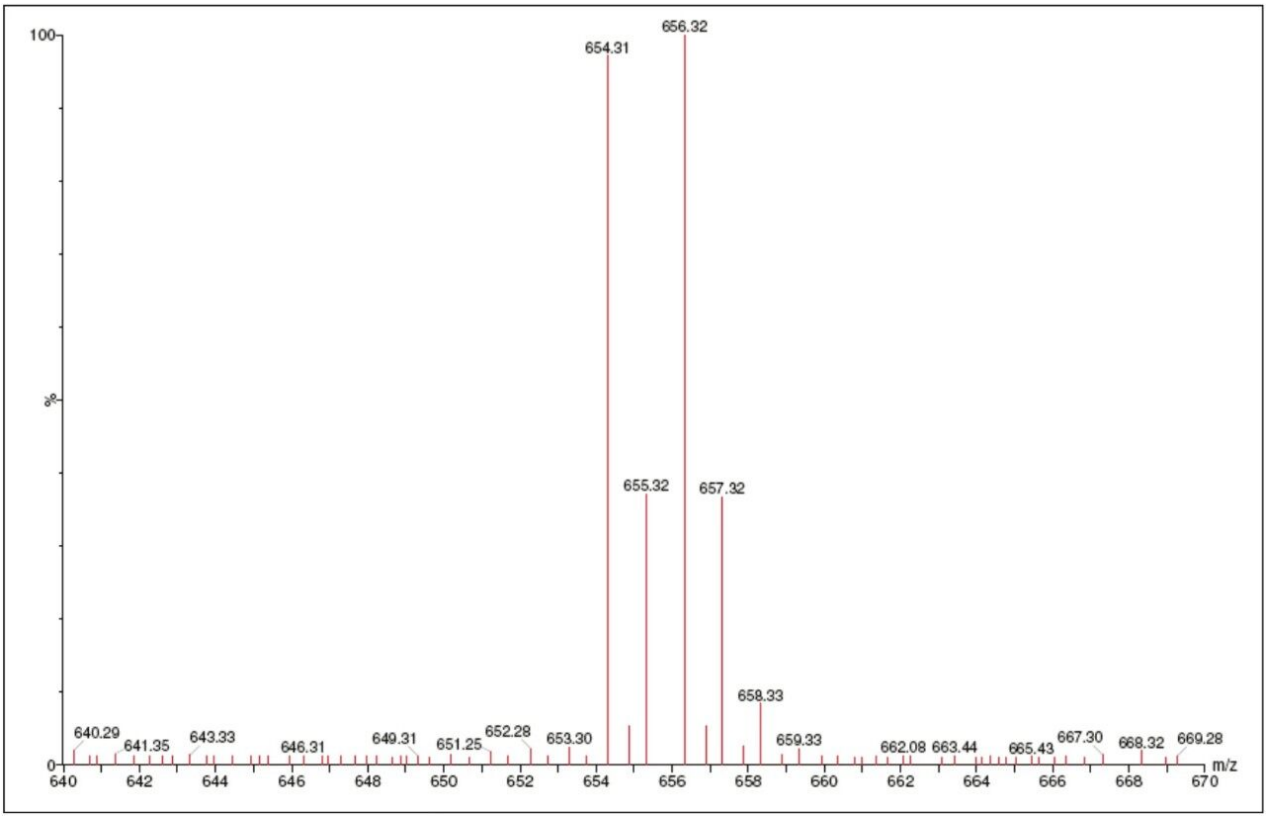


Figure 4. Isotopic distribution of the parent mass of Bromocriptine.

Recovery of the analyte and internal standard (IS) were performed by comparing the extracted QC samples against six post-extracted samples which were found to be approximately 84% at LQC, MQC, and HQC levels for both analyte and IS, as shown in Figure 4 and Table 2. The %CV for repeat batches were found to be within 10% of LLOQQC and varied between 1% to 3% for all QC levels.

The data in Figures 4.1, 4.2, and 4.3 exhibit analyte recovery values for the six samples in all three concentration levels (LQC, MQC, and HQC). As observed from Figures 4.1, 4.2, and 4.3, the recovery values for Bromocriptine did not vary significantly between the three concentration levels. In addition, the mean analyte recovery for all three concentration ranges was well within acceptable limits, as provided in Table 2.

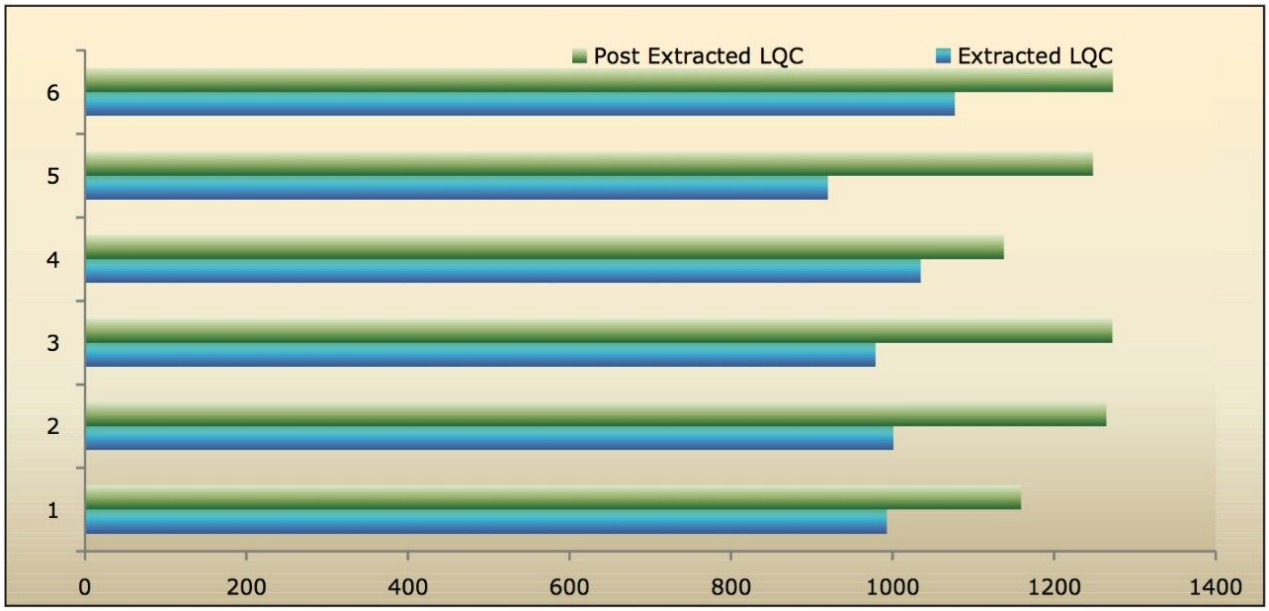


Figure 4.1. Analyte recovery (area under the curve) from six samples of Bromocriptine at LQC.

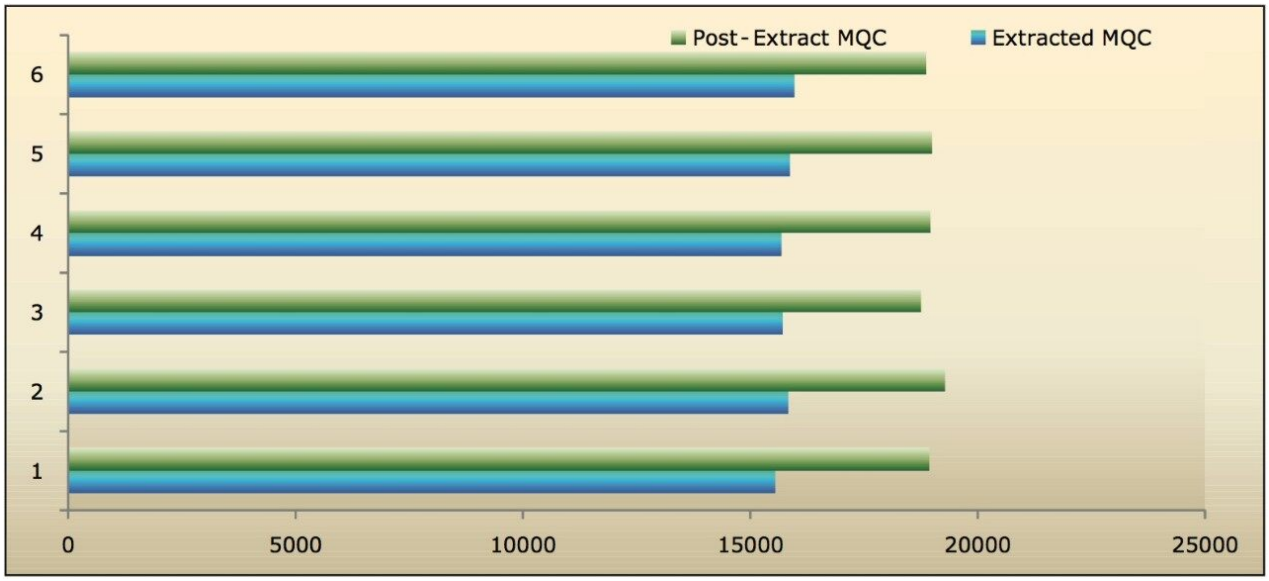


Figure 4.2. Analyte recovery (area under the curve) from six samples of Bromocriptine at MQC.

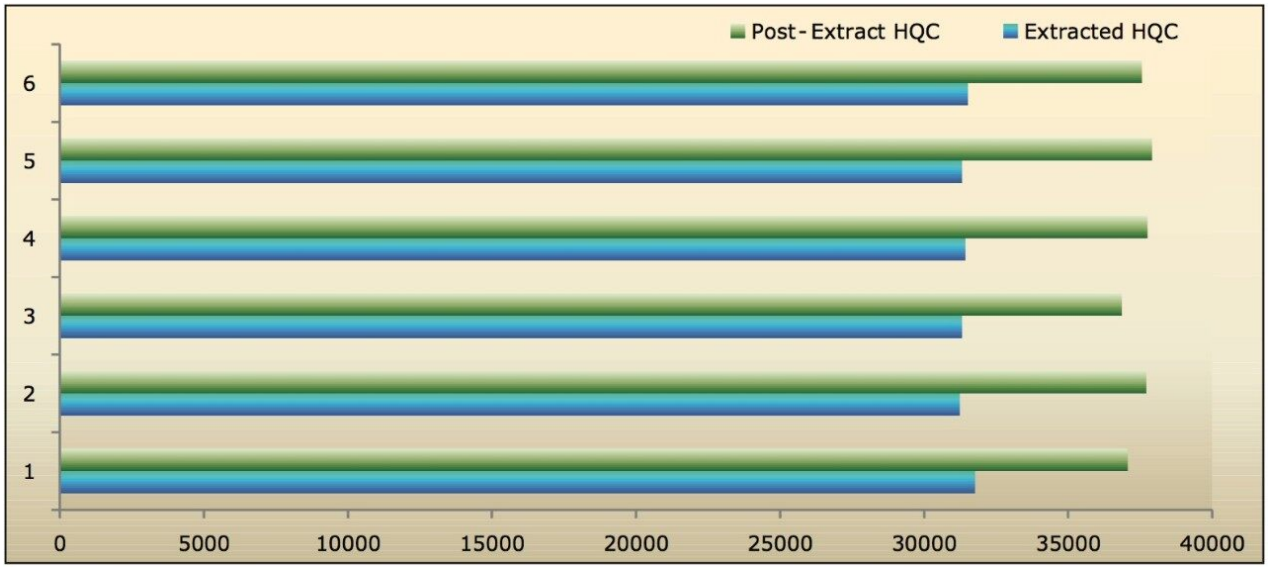


Figure 4.3. Analyte recoveries (area under the curve) from six samples of Bromocriptine at HQC.

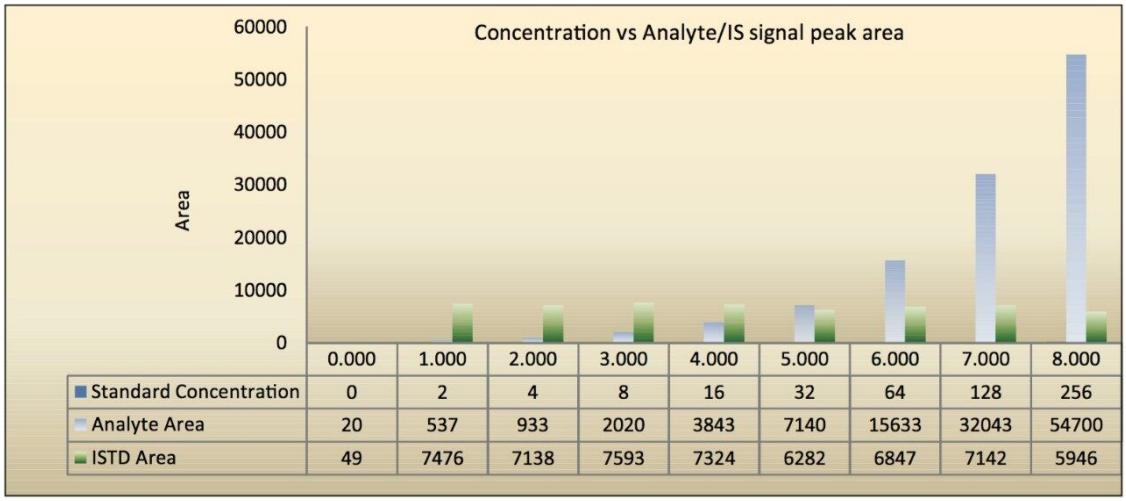
LQC	MQC	HQC
81.6	83.1	83.9
Mean Analyte Recovery (%) = 82.9		

Table 2. Mean analyte recovery (%) of Bromocriptine at LQC, MQC, and HQC levels.

For a comparison of samples within the global batches, three separate batches were prepared in three different concentration levels with six samples in each batch. These batches were categorized as LLOQQC (Lower Limit of Quantification Quality Control), LQC, MQC, and HQC. The data showed excellent agreement between the six samples for all three batches, as shown in Table 3. The mean accuracy obtained for all the samples was found to be >94% for every concentration, shown in Table 3.

P-A Batch-Global												
P-A-Batch-01	LLOQQC-1	2.000	2.153	LQC-1	4.000	4.123	MQC-1	64.000	70.856	HQC-1	128.000	141.730
	LLOQQC-2	2.000	2.131	LQC-2	4.000	4.427	MQC-2	64.000	63.347	HQC-2	128.000	141.175
	LLOQQC-3	2.000	1.935	LQC-3	4.000	4.531	MQC-3	64.000	67.146	HQC-3	128.000	135.465
	LLOQQC-4	2.000	2.016	LQC-4	4.000	4.313	MQC-4	64.000	68.635	HQC-4	128.000	138.904
	LLOQQC-5	2.000	1.818	LQC-5	4.000	4.162	MQC-5	64.000	68.517	HQC-5	128.000	139.271
	LLOQQC-6	2.000	2.054	LQC-6	4.000	4.194	MQC-6	64.000	67.945	HQC-6	128.000	139.981
P-A-Batch-02	LLOQQC-1	2.000	2.148	LQC-1	4.000	4.114	MQC-1	64.000	63.453	HQC-1	128.000	127.834
	LLOQQC-2	2.000	1.944	LQC-2	4.000	4.202	MQC-2	64.000	63.964	HQC-2	128.000	128.15
	LLOQQC-3	2.000	2.122	LQC-3	4.000	4.104	MQC-3	64.000	63.236	HQC-3	128.000	129.078
	LLOQQC-4	2.000	2.091	LQC-4	4.000	4.355	MQC-4	64.000	64.035	HQC-4	128.000	128.486
	LLOQQC-5	2.000	2.218	LQC-5	4.000	3.919	MQC-5	64.000	62.954	HQC-5	128.000	124.786
	LLOQQC-6	2.000	1.982	LQC-6	4.000	4.565	MQC-6	64.000	66.103	HQC-6	128.000	128.928
P-A-Batch-03	LLOQQC-1	2.000	1.903	LQC-1	4.000	3.834	MQC-1	64.000	64.823	HQC-1	128.000	133.081
	LLOQQC-2	2.000	1.602	LQC-2	4.000	3.735	MQC-2	64.000	65.374	HQC-2	128.000	135.066
	LLOQQC-3	2.000	1.915	LQC-3	4.000	3.993	MQC-3	64.000	67.298	HQC-3	128.000	131.327
	LLOQQC-4	2.000	1.972	LQC-4	4.000	3.911	MQC-4	64.000	66.926	HQC-4	128.000	132.167
	LLOQQC-5	2.000	2.070	LQC-6	4.000	3.995	MQC-6	64.000	66.164	HQC-6	128.000	139.426
	LLOQQC-6	2.000	1.662	LQC-5	4.000	3.466	MQC-5	64.000	65.554	HQC-5	128.000	131.819
	Mean		1.985			4.108			65.907			133.704
	SD		0.1660			0.2806			2.2335			5.3335
	%CV		8.36			6.83			3.39			3.99
	Accuracy		99.27			102.70			102.98			104.46

Table 3. Comparison of the three separate batches each containing six Bromocriptine samples at the LLOQQC, LQC, MQC, and HQC concentration.



	0.000	1.000	2.000	3.000	4.000	5.000	6.000	7.000	8.000
Standard Concentration	0	2	4	8	16	32	64	128	256
Analyte Area	20	537	933	2020	3843	7140	15633	32043	54700
ISTD Area	49	7476	7138	7593	7324	6282	6847	7142	5946

Figure 5. Comparison of area under the curve for Bromocriptine (analyte) and IS for the concentration range of 2.000 to 256.000 pg/mL.

Conclusion

LC-MS analysis of Bromocriptine was highly challenging due to the isotopic distribution for parent masses. It was a challenge to identify and quantify Bromocriptine with such distribution pattern for isotopic abundance and specificity issues. The combination of all the components of Waters Regulated Bioanalysis System solution (best-in-class chemistry solutions for sample preparation and column chemistries, UPLC Technology, and Xevo TQ-S) was able to achieve a highly sensitive, specific, and robust LC-MS method for Bromocriptine.

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