High Resolution Accurate Mass Quantitation of Iloperidone and Hydroxy Iloperidone Metabolites Using Full Scan and Selected Ion Monitoring Modes

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Overview

Purpose: To benchmark assay performance utilizing high resolution accurate mass for the quantitation of Iloperidone and Hydroxy Iloperidone.

Methods: Iloperidone and Hydroxy Iloperidone were analyzed in the presence of rat plasma sample matrix using Full Scan and Single Ion Monitoring (SIM) scanning functions. Data analysis were performed using TraceFinder software.

Results: The low limit of quantitation (LOQ) was determined to be 10 pg/mL for both Iloperidone and Hydroxy Iloperidone with an improvement observed in performance and sensitivity for SIM analysis relative to Full Scan analysis.

INTRODUCTION

Iloperidone is a member of the antipsychotic drug class and often prescribed for the treatment of schizophrenia. Many compounds in this class are metabolized by Cytochrome P450 enzymes, which are often used as a primary screen for drug-drug interaction profiling in the drug discovery process. [1] Additionally low serum concentrations are often observed for this compound class, requiring a sensitive and robust analytical method for accurate quantitation [2]. Here we investigate the advantages of high resolution accurate mass LCMS assay for quantitation of Iloperidone and the Hydroxy Iloperidone metabolite. A full scan experiment was performed to simultaneously monitor the drug compound and the metabolite over a large mass range. In additional a selected ion monitoring experiment was also performed to narrowly isolate each component and reduce interference from sample matrix.

METHODS

Sample Preparation

Lyophilized rat plasma (P2516) was obtained from Sigma Aldrich, St. Louis, MO and reconstituted in water at 1mg/mL. Protein precipitation (ppt) was performed for the reconstituted rat plasma solution using an Acetonitrile (ACN) at a ratio of 3:1, ACN to plasma. The resulting solution was centrifuged at 10,000rpm for 15 minutes. The supernatant was removed and split into two equal portions. One portion was diluted with an equivalent volume of water to make a crash solution containing approximately 35% ACN and 65% water, while the remaining portion was left unmodified containing 75% ACN and 25% water. Stock solutions of Iloperidone and Hydroxy Iloperidone (Figure 1) at 1mg/mL and 100ug/mL, respectively, were serial diluted using each of the crash solutions to produce two calibration curves, one with 35% ACN and one with 75% ACN. Both calibration curve preparations were produced with final concentrations ranging from 10pg/mL to 100 ng/mL. Isotopically labeled Iloperidone-D3 was added at each concentration level as an internal standard to produce a final internal standard concentration of 1ng/mL. All analyte stock solutions were certified standard solutions in methanol and obtained from Cerilliant Corporation, Round Rock, Texas.

Liquid Chromatography

Chromatographic separation was achieved using Thermo Scientific™ Vanquish™ UHPLC System. Samples were injected at both 1 uL and 5uL onto a 2.1 x 50mm, 2.2 um Thermo Scientific™ Acclaim™ RSLC C18 120 column. Gradient elution was accomplished using water + 10mM Ammonium Formate (A) and acetonitrile + 0.1% formic acid (FA) (B), with a 3 minute gradient at a flow rate of 500uL/min (Table 1). Total run time including column equilibration was approximately 4 minutes.

Mass Spectrometry

Compounds were analyzed utilizing a Thermo Scientific™ Q Exactive™ Focus MS with heated electrospray ionization (H-ESI II). Generic source conditions suitable for a 500uL/min LC flow rate were applied for all data collection (Table 2). Data was acquired at a resolution setting of 70,000 (FWHM) at *m/z* 200 utilizing both Full Scan and selected ion monitoring (SIM) mode with an external mass calibration.

Data Analysis

All data was collected and processed utilizing Thermo Scientific™ TraceFinder™ software . All chromatographic integration was accomplished using a 5 ppm mass extraction window and method defined processing settings. No manual integration or smoothing was applied to any chromatographic or spectral data.

Time (min)	Flow rate (uL/min)	%A	%B
0	500	80	20
0.3	500	80	20
2.3	500	60	40
2.3	500	5	95
2.8	500	5	95
2.8	500	80	20
3.7	500	80	20

Table 1. LC gradient method utilized for sample analysis.

HESI Source Settings	Value
Spray Voltage (V)	4000
Vaporizer temperature (°C)	450
Capillary Temperature (°C)	350
Sheath Gas Pressure (Arb)	45
Aux Gas Pressure (Arb)	15
Ion Sweep Gas Pressure (Arb)	1

MS Scan Settings	Value
Scan Type	SIM
Resolution	70,000
AGC Target	2.00E+04
IT Fill Time (ms)	260
Isolation Window	10

Table 2. Mass Spectrometer settings utilized for sample analysis.

Figure 1. Chemical structure of Hydroxy Iloperidone (Right) and Iloperidone (Left).

RESULTS

Assay performance and reproducibility were assessed using both Full Scan and SIM scanning experiments. Calibration curves ranging from 10 pg/mL to 100 ng/mL were evaluated and each concentration level analyzed with replicates of n=6. Linearity and reproducibility were calculated across the working range of the curve. The limit of quantitation (LOQ) was defined as the lowest concentration level that is both within <20% difference of the linear fit and <20% RSD for each group of replicate concentration points. The overall assay LOQ for the 3:1 ACN ppt with a 1:1 dilution of water was determined to be 10 pg/mL for both Iloperidone and Hydroxy Iloperidone utilizing the SIM scanning mode with a 5 uL injection volume. Full scan mode with 1 uL injection volumes produced LOQs of 50 pg/mL and 100 pg/mL for Hydroxy Iloperidone and Iloperidone respectively. The full results summary is listed Table 3.

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Hydroxy Iloperidone						
	Full Scan - 1 uL Injection		SIM - 1 uL Injection		SIM - 5 uL Injection	
Conc.Level (pg/mL)	Mean Calc Amt	% CV	Mean Calc Amt	% CV	Mean Calc Amt	% CV
10	--	--	--	--	10.5	9.17%
25	--	--	25.6	7.33%	23.4	3.82%
50	52.6	10.19%	49.56	3.50%	46	4.70%
100	90.9	8.90%	92.1	2.76%	85.4	2.11%
500	475	2.79%	499	2.48%	489	2.94%
1000	945	1.83%	939	1.86%	984	2.02%
5000	4886	0.55%	5116	2.50%	5371	0.27%
10000	9776	0.72%	10069	1.14%	10542	1.70%
50000	52979	1.96%	52889	1.71%	54401	3.55%
100000	103155	1.21%	103610	3.66%	105284	4.20%
Iloperidone						
	Full Scan - 1 uL Injection		SIM - 1 uL Injection		SIM - 5 uL Injection	
Conc.Level (pg/mL)	Mean Calc Amt	% CV	Mean Calc Amt	% CV	Mean Calc Amt	% CV
10	--	--	--	--	11.2	8.18%
25	--	--	--	--	23.1	3.65%
50	--	--	51.3	9.36%	46.7	1.62%
100	101	10.59%	94.9	6.69%	91.7	3.41%
500	493	1.20%	500	1.42%	504	1.63%
1000	933	2.59%	950	0.90%	974	0.88%
5000	4850	0.88%	5045	1.11%	5133	1.00%
10000	9643	0.27%	9894	1.53%	10086	1.11%
50000	51012	1.27%	52044	1.85%	53623	2.21%
100000	101159	1.12%	103194	1.79%	105857	1.91%

Table 3. Quantitative results for Hydroxy Iloperidone (Top) and Iloperidone (Bottom).

Full Scan Analysis

The Full Scan experiment type provides a simplified method setup that allows for the monitoring of multiple analytes across a wide mass range, with a minimal method development. Sample evaluation was performed using a 1 uL sample injection volume with a full scan mass range of 200 m/z to 800 m/z. The LOQ for the Full Scan experiment was determined to be 50 pg/mL and 100 pg/mL for Hydroxy Iloperidone and Iloperidone respectively. Although Iloperidone and Hydroxy Iloperidone were the two components analyzed in this assay, the LOQ results provide an indication of the sensitivity levels that are attainable for both know and unknown metabolites using Full Scan analysis. Extracted Ion Chromatograms (XIC) for Hydroxy Iloperidone and Iloperidone at the assay LOQ for the Full Scan experiment, as well as the calibrations curves and linear response show in Figures 2 and 3 below.

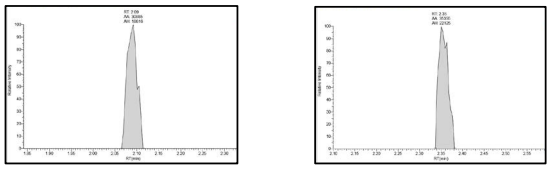

Figure 2. Full Scan XIC chromatogram for Hydroxy Iloperidone at 50 pg/mL (Left) and Iloperidone at 100 pg/mL (Right).

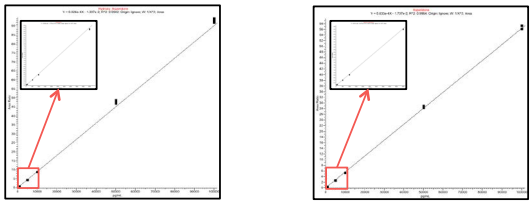

Figure 3. Calibration curve and linear response of Hydroxy Iloperidone (Left) and Iloperidone (Right) for the Full Scan experiment.

SIM Analysis – 1 uL injection Volume

The SIM Scan function provides an excellent balance between simplified method setup and low level analyte detection. By simply specifying in the method, the m/z for the target analyte and defining an appropriate quadrupole isolation window, interferences contained in the sample matrix are filtered out at the quadrupole allowing for analyte quantitation at lower concentration levels. The SIM scan experiment provides quantitative sensitivity comparable to a high performance triple quadrupole system, without the need for MS/MS method optimization.

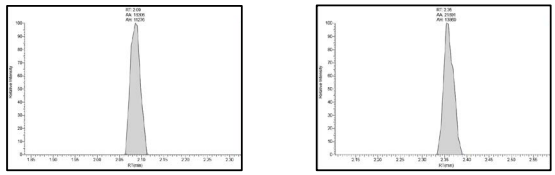

Figure 4. SIM Scan chromatogram XIC for Hydroxy Iloperidone at 25 pg/mL (Left) and Iloperidone at 50 pg/mL (Right).

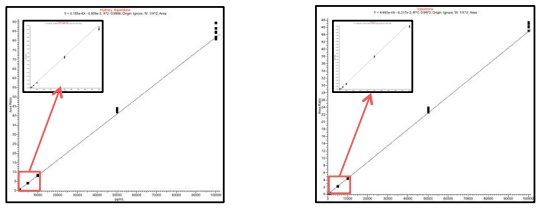

Figure 5. Calibration curve and linear response of Hydroxy Iloperidone (Left) and Iloperidone (Right) for the SIM Scan experiment.

The m/z differences for Hydroxy Iloperidone, Iloperidone, and Iloperidone-D3 are within 6 amu, allowing for both analytes as well as the internal standard to be collected continuously with a single 10 amu quadrupole isolation window. The LOQ for the 1 uL injection volume SIM analysis was determined to 25 pg/mL and 50 pg/mL for Hydroxy Iloperidone respectively (Figure 4), and the signal response was determined to be linear from the LOQ to 100 ng/mL (Figure 5).

SIM Analysis – 5 uL injection Volume

To further improve the overall assay LOQ a 5 uL injection volume was evaluated for the 3:1 ACN ppt with a 1:1 dilution of water. Chromatographic peak shape was maintained at the 5 uL injection volume and a 2.5x to 5x improvement in assay LOQ was observed. The LOQ for both Hydroxy Iloperidone and Iloperidone was determined to 10 pg/mL (Figure 6), and the signal response was determined to be linear from the LOQ to 100 ng/mL (Figure 7).

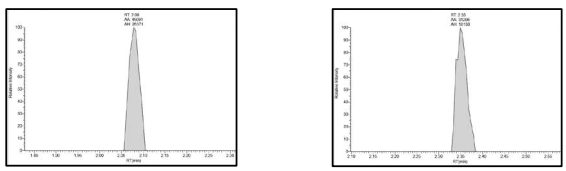

Figure 6. SIM Scan XIC chromatogram of Hydroxy Iloperidone at 10 pg/mL (Left) and Iloperidone at 10 pg/mL (Right).

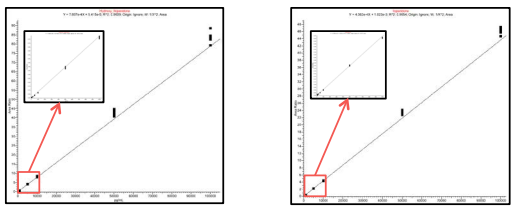

Figure 7. Calibration curve and linear response of Hydroxy Iloperidone (Left) and Iloperidone (Right) for the SIM Scan experiment.

Injection Volume Comparison

Previously reported results for the analysis of Hydroxy Iloperidone and Iloperidone describe the need for an evaporation and reconstitution step in the sample preparation procedure to avoid chromatographic peak broadening in the presence of a high percentage of ACN resulting from the ppt procedure. [2] Hence the need for the additional 1:1 water dilution described previously here. In an effort to evaluate the detector sensitivity and low the injection volume capabilities of the Vanquish autosampler, an additional calibration curve was prepared without the additional 1:1 water dilution step.

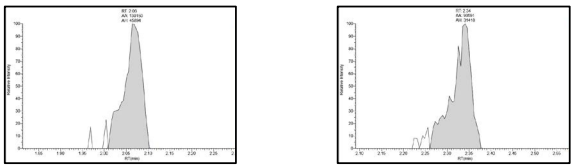

Figure 8. SIM Scan chromatogram of Hydroxy Iloperidone at 25 pg/mL (Left) and Iloperidone at 50 pg/mL (Right).

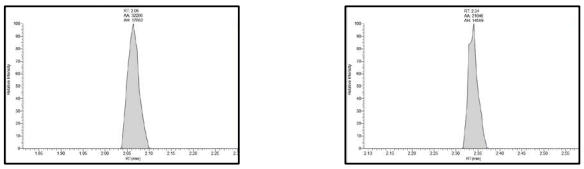

Figure 9. SIM Scan chromatogram of Hydroxy Iloperidone at 25 pg/mL (Left) and Iloperidone at 50 pg/mL (Right).

Mass Accuracy

Here we examine the scan to scan mass accuracy for Hydroxy Iloperidone and Iloperidone analysis at the assay LOQ of 10pg/mL (Figure 10). The mass accuracy for each scan across the analyte peaks was demonstrated to be less than 3ppm for both analytes. The high level of mass accuracy from scan to scan allows the utilization of a narrow mass extraction window providing robust and reproducible results at low analyte concentration levels while in the presence of a biological matrix. Evaluation of the scan to scan mass accuracy provides a potential method development and troubleshooting tool that is unique to HRAM and the Orbitrap mass analyzer. Additionally the spectral resolution setting of 70,000 was examined for each analyte to confirm adequate separation from sample interference. (Figure 11) Both analytes were observed to be fully resolved from interference at the assay LOQ of 10 pg/mL.

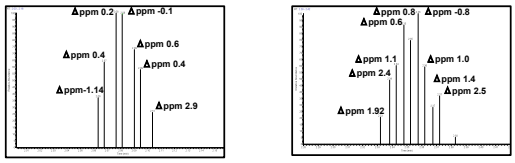

Figure 10. Scan to scan mass accuracy of Hydroxy Iloperidone (Left) and Iloperidone (Right) for the 5 uL injection SIM Scan experiment at 10 pg/mL.

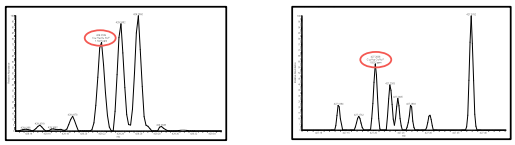

Figure 11. Mass spectra at the 70,000 resolution setting of Hydroxy Iloperidone (Left) and Iloperidone (Right) for the 5 uL injection SIM Scan experiment at 10 pg/mL.

CONCLUSIONS

Full Scan analysis provided a sensitive and easy to setup method that requires little method development. The LOQ was determined to be 50 pg/mL and 100 pg/mL for Hydroxy Iloperidone and Iloperidone respectively.

SIM Scan analysis with a 5 uL injection volume provided the lowest assay LOQ while maintaining method simplicity and minimal method development. The LOQ was determined to be 10 pg/mL for both Hydroxy Iloperidone and Iloperidone.

Detector sensitivity combined with the low volume injection capability of the Vanquish autosampler resulted in low level LOQs at 1uL injection volumes, providing the potential for a simplified sample preparation procedure.

The resolution setting of 70,000 provided adequate mass resolution from sample interferences and a scan to scan mass accuracy of less than 3 ppm was maintained for every scan at the 10 pg/mL assay LOQ.

REFERENCES

1. L. Patteet. et al., Clinica Chimica Acta, 2014, 429, 51–58
2. N. Ansermot. et al., Journal Chromatogr. A, 2013, 1292, 160–172

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