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# Rapid, Sensitive and Direct Quantitation of Tiotropium at sub-pg/mL in Plasma using Shimadzu LCMS-8060NX

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## 1. Introduction

Tiotropium is an inhaled long-acting anti-cholinergic for the maintenance treatment of COPD (chronic obstructive pulmonary disease).The long duration of action with tiotropium is owing to prolonged, competitive binding to M (3) muscarinic receptors (refer fig.1 for Structure of Tiotropium). Tiotropium is administered in the form of dry powder inhalation which results in very low systemic bioavailability. This translates into significant challenges to develop a sensitive and reproducible bioanalytical method that can reliably measure plasma levels of tiotropium at very low expected levels. The required LLOQ for most inhalation dosed medications is typically in the range of pg/mL to sub pg/mL. In this work, we present a rapid, sensitive and simple method for the accurate quantification of tiotropium in human plasma using Shimadzu LCMS-8060NX triple quadrupole mass spectrometer coupled with Nexera X2 UHPLC.

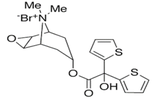

**Fig. 1** Structure of Tiotropium [(1)]

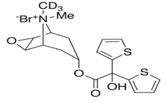

**Fig. 2** Structure of Tiotropium-D3 Bromide[(2)]

## 2. Materials and methods

### 2-1. Sample Preparation

Calibration standards and quality control samples were prepared in K2 ETDA human plasma with sample concentrations ranging between 0.20-200.00 pg/mL and 0.20-150.00 pg/mL respectively.

Plasma samples were extracted using solid phase extraction. SPE cartridges were conditioned with 1 mL methanol and equilibrated with 1 mL water. Plasma samples were added to the cartridges and allowed to pass under gravity. 1 mL of milli Q water and 5% methanol in water effectively removed the interferences from SPE cartridges. Tiotropium was eluted using 0.5 mL of 50 % methanol in water and was loaded on prelabelled HPLC vials before analysis on LC-MS/MS system

## 3. LC-MS/MS analysis

LCMS-8060NX coupled with Nexera™ X2 UHPLC system (Shimadzu Corporation), was used to acquire the data in MRM mode. The instrumental conditions used during the analysis are presented below in Table 1

**Table 1** Instrument Parameters for analysis of Tiotropium

| | |
|---|---|
| **UHPLC condition (Nexera™ X2)** | |
| Column | Shim-pack VelocTM C18 100 × 2.1 mm, 2.7 µm (P/N: 227-32015-03) |
| Mobile phase | A: 0.1% formic acid in water, B: 0.1% formic acid in Methanol |
| Flow rate | 0.20 mL/min |
| Elution mode | Gradient |
| Column temp | 50 °C |
| **MS parameters (LCMS-8060NX)** | |
| MS interface | Electro Spray Ionization (ESI) |
| Nitrogen gas flow | Nebulizing gas- 3 L/min; Drying gas- 10 L/min |
| Zero air flow | 1kV |
| MS temp | Desolvation line- 200 °C; Heating block- 500 °C; Interface- 400 °C |

## 4. Results

### 4-1. Selectivity

The selectivity of the method was assessed in 6 different lots of blank human plasma. Results are presented in Table 2. Representative chromatogram is shown in fig.3.

**Table 2** Selectivity

| | Tiotropium | | |
|---|---|---|---|
| **Lot no.** | Area in blank matrix | LLOQ area | % Interference |
| **V3071** | 159 | 1,362 | 11.67 |
| **V1889** | 267 | 1,416 | 18.86 |
| **V1789** | 208 | 1,912 | 10.88 |
| **V1166** | 0 | 1,371 | 0.00 |
| **V3074** | 374 | 2,051 | 18.24 |
| **V3077** | 168 | 1,267 | 13.26 |

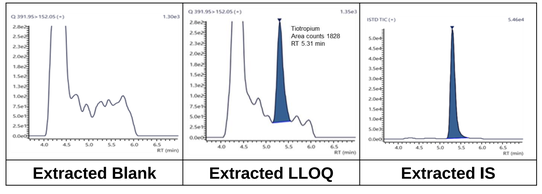


**Fig. 3** Chromatograms of extracted blank, extracted LLOQ (0.20 pg/mL) and extracted IS

### 4-2. Linearity

Calibration curve of tiotropium was found linear from 0.20-200.00 pg/mL (refer figure 6) The goodness of fit was consistently greater than 0.99 during validation with a 1/x^2 weighting factor (refer fig.4).

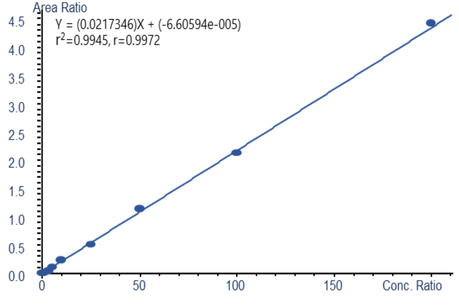


**Fig. 4** Representative calibration curve of Tiotropium

### 4-3. Intra-day and Inter-day Precision and accuracy

Intra-day and inter-day precision and accuracy results in plasma quality control samples are summarized in table 3 and table 4 and were found within the acceptance criteria

**Table 3** Intra-day Precision and Accuracy

| QC level (n=6) | Mean Conc. | % Accuracy | %RSD |
|---|---|---|---|
| LLOQ QC (0.20 pg/mL) | 0.22 | 112.96 | 10.03 |
| LQC (0.60 pg/mL) | 0.65 | 109.00 | 4.34 |
| MQC (10.00 pg/mL) | 10.39 | 103.89 | 1.78 |
| HQC (150.00 pg/mL) | 154.32 | 102.88 | 1.35 |

**Table 4** Global Precision and Accuracy

| QC level (n=30) | Mean Conc. | % Accuracy | %RSD |
|---|---|---|---|
| LLOQ QC (0.20 pg/mL) | 0.21 | 106.92 | 18.63 |
| LQC (0.60 pg/mL) | 0.63 | 105.35 | 10.40 |
| MQC (10.00 pg/mL) | 10.31 | 103.13 | 6.48 |
| HQC (150.00 pg/mL) | 154.26 | 102.84 | 6.35 |

### 4-4. Recovery

Recovery experiments were conducted to evaluate precision, reproducibility and consistency of the analyte(s) at LQC, MQC and HQC level. Global recovery for tiotropium was found 57.61 % with precision less than 15%.The recovery of tiotropium was found precise, consistent and reproducible at all QC levels. Results of Recovery statistics and global recovery is presented in Table 5 and Table 6 respectively

**Table 5** Recovery

| Sr.No. | Ext- Sample | PE-Sample | Ext- Sample | PE-Sample | Ext- Sample | PE-Sample |
|---|---|---|---|---|---|---|
| | LQC | | MQC | | HQC | |
| 1 | 5,218 | 8,199 | 91,965 | 1,37,018 | 16,25,420 | 19,84,533 |
| 2 | 5,468 | 11,927 | 70,290 | 1,41,066 | 12,88,221 | 20,27,336 |
| 3 | 4,866 | 9,257 | 88,104 | 1,46,521 | 15,93,512 | 21,50,800 |
| 4 | 5,600 | 10,649 | 95,595 | 1,48,279 | 11,75,451 | 22,13,041 |
| 5 | 5,245 | 10,188 | 92,714 | 1,54,339 | 12,29,835 | 22,96,291 |
| 6 | 5,112 | 10,273 | 84,364 | 1,60,024 | 11,44,293 | 23,66,370 |
| AVERAGE | 4,320 | 5,861 | 20,601 | 27,238 | 82,821 | 1,02,467 |
| STD DEV | 443.14 | 264.00 | 665.54 | 2239.08 | 1500.87 | 7953.60 |
| % RSD | 10.26 | 4.50 | 3.23 | 8.22 | 1.81 | 7.76 |
| % Recovery | 52.09 | | 58.95 | | 61.79 | |
| Note: Read Ext-Sample as extracted sample and PE-Sample as post extracted sample | | | | | | |

**Table 6** Global Recovery

| **QC level** | **Recovery** |
|---|---|
| LQC (n=6) | 52.09 |
| MQC (n=6) | 58.95 |
| HQC (n=6) | 61.79 |
| **Mean** | **57.61** |
| **SD** | **4.99** |
| **% RSD** | **8.66** |

### 4-5. Matrix effect

Matrix factor was evaluated by comparing mean peak area ratio of QC sample in presence of matrix compared with mean peak area ratio of QC samples in absence of matrix. Post-extracted QC samples were prepared by extracting blank plasma from six different human plasma lots, followed by reconstitution with aqueous LQC and HQC samples. Post-extracted quality control samples and neat solution of quality control samples at LQC and HQC levels were analyzed on LCMS system. The IS normalized matrix factor at LQC and HQC levels were found 0.85 and 0.87 respectively, which is within acceptance criteria. Results of matrix effect are presented in Table 7.

**Table 7** Matrix Factor

| **Tiotropium** | **Response ratio in AQ- sample** | **Response ratio in PE- sample** | **Matrix factor** | **Tiotropium** | **Response ratio in AQ- sample** | **Response ratio in PE- sample** | **Matrix factor** |
|---|---|---|---|---|---|---|---|
| **LQC** | 0.013 | 0.012 | 0.87 | **HQC** | 3.140 | 2.724 | 0.87 |
| | 0.013 | 0.010 | 0.77 | | 3.150 | 2.750 | 0.87 |
| | 0.012 | 0.011 | 0.89 | | 3.162 | 2.715 | 0.86 |
| | 0.014 | 0.011 | 0.80 | | 3.113 | 2.737 | 0.88 |
| | 0.014 | 0.012 | 0.82 | | 3.249 | 2.818 | 0.87 |
| | 0.013 | 0.012 | 0.93 | | 3.120 | 2.798 | 0.90 |
| **Mean** | | | **0.85** | | | | **0.87** |
| **SD** | | | **0.06** | | | | **0.01** |
| **% RSD** | | | **7.12** | | | | **1.51** |
| Note: Read AQ-Sample as aqueous sample and PE-Sample as post extracted sample | | | | | | | |

### 4-6. Carry-over effect

Carry-over effect was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was observed at the retention time and MRM transition of tiotropium in the extracted blank sample following the highest standard calibrator.

## 5. Conclusion

LCMS-8060NX, along with special sample preparation method, optimized chromatography provides a very selective and sensitive method for bioanalysis of Tiotropium in human plasma. Ultra-high speed and high-separation analysis was achieved on NexeraTM X2 UHPLC by using a simple mobile phase at a minimal gradient flow rate of 0.2 mL/min. By providing these ready to use solutions, we partner with your labs to achieve desired results in your scientific endeavors.

**Reference**

1) https://www.drugbank.ca/drugs/DB01409 (accessed Jan 02, 2020)
2) https://en.wikipedia.org/wiki/Tiotropium_bromide (accessed Jan 02, 2020)