Sreekanth Kakarla et al. / Journal of Pharmacy Research 2015,9(12),662-669

Research Article
ISSN: 0974-6943

Available online through
www.jprsolutions.info

Sensitive liquid chromatography tandem mass spectrometry method for the quantification of Ropinirole in human plasma using liquid-liquid extraction technique

Sreekanth Kakarla[1*] and Ganapaty Seru[2]

[1] Department of Pharmacy, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.

[2] Gitam Institute of Pharmacy, Gitam University, Visakhapatnam, Andhra Pradesh, India.

Received on:06-10-2015; Revised on: 17-10-2015; Accepted on: 20-12-2015

ABSTRACT

Background: A sensitive high-performance liquid chromatography – positive ion electroscopy tandem mass spectrometry method was developed and validated for the quantification of Ropinirole in human plasma. **Methods:** Following liquid-liquid extraction, the analyte and internal standard were separated using an isocratic mobile phase on a reverse phase C_{18} column and analyzed by MS/MS in the multiple reaction monitoring mode using the respective $[M+H]^+$ ions, *m/z is* 261 to 114 for Ropinirole and *m/z is* 325 to 262 for the internal standard. **Results and discussion:** The assay exhibited a linear dynamic range of 10.0 pg/mL to 1006.3 pg/mL for Ropinirole in human plasma. The lower limit of quantification was 10.0 pg/mL with a relative standard deviation of less than 7%. This LC-MS/MS method was validated with between-batch and within-batch precision of 5.5 to 8.6% and 4.3 to 10.9%, respectively. The between-batch and within-batch accuracy was 99.0 to 104.4% and 94.1 to 107.4%, respectively. **Conclusion:** This validated method is simple, rapid, cost effective and repeatable enough to be used in pharmacokinetic studies.

KEYWORDS: Ropinirole, liquid chromatography-tandem mass spectrometry, human plasma, isocratic and pharmacokinetic studies.

INTRODUCTION

Ropinirole is used to treat the symptoms of Parkinson's disease. It is a non-ergoline dopamine agonist with high relative *in vitro* specificity and full intrinsic activity at the D2 and D3 dopamine receptor subtypes, binding with higher affinity to D3 than to D2 or D4 receptor subtypes. Ropinirole was the first drug approved by the FDA in May 2005 for the indication of treatment of Restless Legs Syndrome[1, 2].

The empirical formula of Ropinirole is $C_{16}H_{24}N_2O$ and the molecular weight is 260 Dalton. The structure of Ropinirole is shown in Figure. 1.

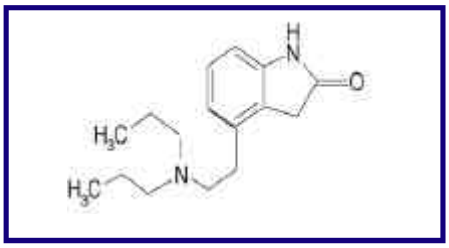


Figure 1: Structural representation of Ropinirole

***Corresponding author.**
Sreekanth Kakarla
Department of Pharmacy,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur,
Andhra Pradesh, India.

Ropinirole is absorbed rapidly after oral absorption and the bioavailability is approximately 50% and peak concentration is achieved at 1.5 hours of post dose. The pharmacokinetic parameters of Ropinirole have wide inter-individual variability[3, 4]. Due to relatively low dosing range it was necessary to develop simple and sensitive analytical method for the quantification of Ropinirole in human plasma at picogram level.

Quantification of drugs in biological matrices by LC-MS/MS is becoming more common due to the improved sensitivity and selectivity of this technique. The bioanalytical component of a pharmacokinetic study requires a drug assay with simplicity, high sensitivity, selectivity, reliability, small volume and rapid turnaround time. Few LC-MS (Liquid Chromatography – Mass Spectrometry) methods for the quantification of Ropinirole in human plasma have been developed using SPE (Solid Phase Extraction) technique with a sample volume of 0.5-1 ml and reported[5-7]. However, in this study we have developed LC-MS method with LLOQ (Lower Limit of Quantification) 10.0 pg/mL, using a simple and cost effective LLE (Liquid- Liquid Extraction) technique and will be applied in the bioequivalence study.

The purpose of the present experiment was to investigate the high selectivity and sensitivity of a triple-quadrupole MS system operated

in MS-MS mode with an electrospray interface for the development and validation of a robust reversed-phase LC-MS/MS method in multiple reaction monitoring (MRM) mode for the quantification of Ropinirole in human plasma. This manuscript depicts the development and validation of the specific and sensitive LC–MS/MS method for the determination of Ropinirole in human plasma with 10.0 pg/mL of LLOQ using less plasma sample volume.

EXPERIMENTAL

Chemicals

Ropinirole hydrochloride and Citalopram hydrobromide (internal standard, IS) were obtained from APL Research Centre Ltd (Hyderabad, India). Gradient grade LiChrosolv Methanol was purchased from Merck (Darmstadt, Germany). Ethyl acetate, n-Hexane, Ammonium acetate, Sodium carbonate and Formic acid were purchased from Merck (Worli, Mumbai, India). Ultrapure type-1 water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

LC-MS/MS instrument and conditions

The HPLC SIL HTC system (Shimadzu Corporation, Kyoto, Japan) is equipped with LC-AD VP binary pump, a DGU20A5 degasser, and a SIL-HTC auto sampler equipped with a CTO-10AS VP thermo stated column oven. The chromatography was performed using Discovery C_{18}, column with following specifications 50 mm x 4.6 mm, 5µ particle size, at 35°C temperature. The analyte was eluted by a isocratic mobile phase system consisting of a mixture of 2 mM ammonium acetate and 100 µl of formic acid (pH adjusted to 8 with dilute ammonia solution) / methanol (40/60, v/v) pumped at a flow-rate of 0.8 mL/min.

Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (MDS-SCIEX, Concord, Ontario, Canada) using MRM. A turbo ion spray interface operating in positive ionization mode was used. Typical source conditions were as follows: the turbo-gas temperature was set at 425°C, and the ion spray needle voltage was adjusted at 4000 V. The mass spectrometer was operated at low resolution for Q1 and unit resolution Q3 in the MRM mode, with a dwell time of 200 ms per MRM channel. The precursor/product ion pairs monitored were *m/z* 261 to 114 for Ropinirole and *m/z* 325 to 262 for the IS. GS1 and GS2 were at 35 and 40 (arbitrary units), respectively. Collision gas and curtain gas were set at 6 and 35 (arbitrary units), respectively. The declustering potential, collision energy, collision cell exit potential and entrance potential were set at 70, 28, 8 and 10 for Ropinirole and at 40, 28, 8 and 10 for IS, respectively. Data acquisition was performed with analyst 1.4.2 software (MDS-SCIEX, Concord, Ontario, Canada).

Sample preparation

Standard stock solutions of Ropinirole (1 mg/mL) and the IS (1 mg/mL) were separately prepared in methanol. Working solutions for calibration and controls were prepared by appropriate dilution in water-methanol (50:50 v/v; diluent). The IS working solution (1 µg/mL) was prepared by diluting its stock solution with diluent. Working solutions (1000 µL) were added to drug-free human plasma (50 mL) as a bulk, to obtain Ropinirole concentration levels of 10, 20, 40, 100, 200, 400, 800 and 1000 pg/mL as a single batch at each concentration. Quality control (QC) samples were also prepared as a bulk on an independent weighing of standard drug, at concentrations of 10 (LLOQ), 28 (low), 440 (medium) and 730 pg/mL (high) as a single batch at each concentration. The calibration and control bulk samples were divided into aliquots in ria vial tubes (Tarson, 4.0 mL) and stored in the freezer at below –70°C until analysis.

A plasma sample (300 µL) was pipetted into a 4.0 mL ria vial tube and then 50 µL of IS, working solution (1 µg/mL) and 100 µL of 1M sodium carbonate buffer were added. After vortex mixing for 10 sec, 2.5 mL aliquot of the extraction solvent, ethyl acetate: n-hexane (80:20, v/v), was added and the sample was vortex-mixed for 10 min. The organic layer (2 mL) was transferred to a ria vial tube and evaporated to dryness using an evaporator at 50°C under a stream of nitrogen. Then the dried extract was reconstituted in 300 µL of mobile phase and a 10-µL aliquot was injected into the chromatographic system.

Bioanalytical method validation

Selectivity

The selectivity of the method was determined by six different human blank plasma samples, which were pretreated and analyzed to test the potential interferences of endogenous compounds co-eluting with analyte and IS. Chromatographic peaks of analyte and IS were identified based on their retention times and MRM responses. The peak area of drug at the respective retention time in blank samples should not be more than 20% of the mean peak area of LOQ of drug. Similarly, the peak area of IS at the respective retention time in blank samples should not be more than 5% of the mean peak area of LOQ of ISTD.

Matrix effect

To predict the variability of matrix effect in samples from individual subjects, matrix effect was evaluated by determining the Normalized Matrix Factor (FMN), which was calculated as follows[8-12]:

$$FMN = \frac{\text{Response of the analyte in matrix/Response of the IS in matrix}}{\text{Response of the analyte in solution/Response of the IS in solution}}$$

Six lots of blank biological matrices were spiked each in triplicates at the low quality control (LQC), high quality control (HQC) levels, and compared with neat standards. The overall precision of the matrix factor is expressed as coefficient of variation (CV %) and it should be ≤15%.

Linearity

The analytical curves were constructed using concentrations ranging from 10 - 1000 pg/mL of drug in human plasma. Calibration curves were obtained by weighted $1/x^2$ (where x is concentration) to produce linear regression. The ratio of drug peak area to ISTD peak area was plotted against the ratio of drug concentration in pg/mL. One set of calibration curve standard samples and 6 replicates of quality control samples were prepared for analysis. The correlation coefficient was obtained >0.99 by using a simple linear regression model in the whole range of tested concentrations.

The acceptance criterion for a calibration curve was a correlation coefficient (r) of 0.99 or better, and that each back-calculated standard concentration must be within 15% deviation from the nominal value except at the LLOQ, for which the maximum acceptable deviation was set at 20%. At least 67% of non-zero standards were required to meet the above criteria, including acceptable LLOQ and upper limit of quantification.

Precision and accuracy

Precision and accuracy for the back calculated concentrations of the calibration points, should be ±15% of their nominal values. However, for (LLOQ) the precision and accuracy should be ±20%.

Dilution Effect

Dilution effect was investigated to ensure that samples could be diluted with blank matrix without affecting the final concentration. Ropinirole spiked plasma samples prepared two times more concentration of the highest calibration curve and analyzed 6 replicates by diluting with pooled plasma using dilution factor. As part of validation, the replicates had to comply to have both precision of < 15% and accuracy of 100 ± 15%[8-13]

Cross interference verification:

Cross interference was investigated to ensure that no interference was contributing from IS at the retention time of analyte and vice versa. Analyte was spiked in solution and plasma at LLOQ & ULOQ level independently and also spiked IS in solution and plasma independently and evaluated.

The % interference due to IS at Analyte retention time in blank with IS sample shall be = 20% of the analyte response in LLOQ and the % interference due to Analyte at IS retention time in ULOQ shall be = 5% of the IS response in blank with IS sample, in both extracted and un-extracted samples.

Stability

LQC and HQC samples (n=6) were retrieved from the deep freezer after five freeze–thaw cycles according to the clinical protocols. Samples were frozen at -70 °C in five cycles. In addition, the long-term stability of drug in quality control samples was also evaluated by analysis after 120 days at -70°C and at -20°C. Autosampler stability was studied following 23.92 hours storage period in the autosampler tray with control concentrations. The stability of Ropinirole processed samples (without Reconstitution), was successfully assessed by storing six replicates of processed stability samples at a temperature below 10°C for 23.25 hours at low and high concentrations and then analyzing after reconstitution with six replicates of freshly prepared quality control samples (Comparison samples) at low and high levels using freshly prepared calibration curve. Bench top stability was studied for 16.83 hours period with control concentrations. Stability samples were processed and extracted along with the freshly spiked calibration curve standards. The precision and accuracy for the stability samples must be within =15% and ±15% respectively of their nominal concentrations.

Recovery

The extraction recovery of analyte and IS from human plasma was determined by analyzing quality control samples. Recovery at three concentrations (28.1, 439.0, and 731.7 pg/mL) was determined by comparing peak areas obtained from the plasma sample with those from the standard solution spiked with the blank plasma residue. A recovery of more than 50% was considered adequate to obtain required sensitivity.

Limit of quantification (LOQ) and limit of detection (LOD)

The response (peak area) was determined in blank plasma samples (six replicates from different plasma), and spiked LOQ sample prepared from the same plasma was determined. The peak area of blank samples should not be more than 20% of the mean peak area of LOQ of drug and 5% of the mean peak area of ISTD. The precision and mean accuracy of the back calculated LOQ replicate concentrations must be =20% and ±20%, respectively.

The LOD is a parameter that provides the lowest concentration in a sample that can be detected from background noise but not quantitated. LOD was determined using the signal-to-noise ratio (S/N) of 3:1 by comparing test results from samples with known concentrations of analyte with those from the blank samples.

RESULTS AND DISCUSSIONS

Method development

LC–MS/MS has been used as one of the most powerful analytical tools in clinical pharmacokinetics for its selectivity, sensitivity and reproducibility[10- 13]. The goal of this study was to develop and validate a simple, rapid and sensitive assay method for the quantitative determination of drug from plasma samples. A simple extraction technique was utilized for the extraction of drug and IS from the plasma samples. Chromatographic conditions, especially the composition and nature of the mobile phase, were optimized through several trials to achieve better resolution between interference peaks and increase the signal of drug and IS. The MS optimization was performed by direct infusion of solutions of both drug and IS into the ESI (Electro Spay Ionization) source of the mass spectrometer. Along with other parameters, such as the nebulizer and the desolvation gases were optimized to obtain a better spray shape, resulting in better ionization and droplet drying. A protonated product ion spectrum for drug and IS yielded high-abundance fragment ions of m/z 261 to 114 for Ropinirole and m/z 325 to 262 for the IS. After the MRM channels were tuned, the mobile phase was changed from an organic phase to a more aqueous phase to obtain a fast and selective LC method. A good separation and elution were achieved using ethyl acetate: n-hexane (80:20, v/v) as the mobile phase, at a flow rate of 0.7 mL/min and injection volume is 10 µL.

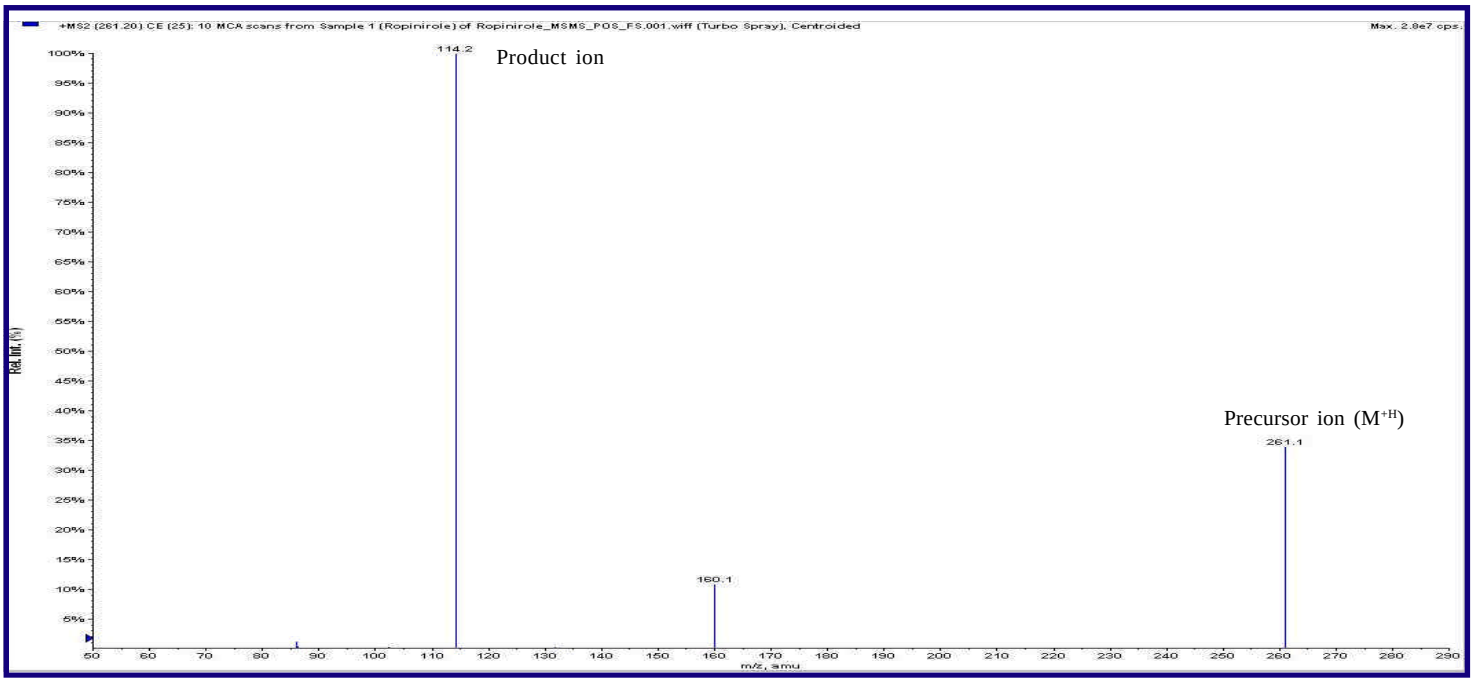


Figure: 2 Mass spectra of parent and product ion for drug

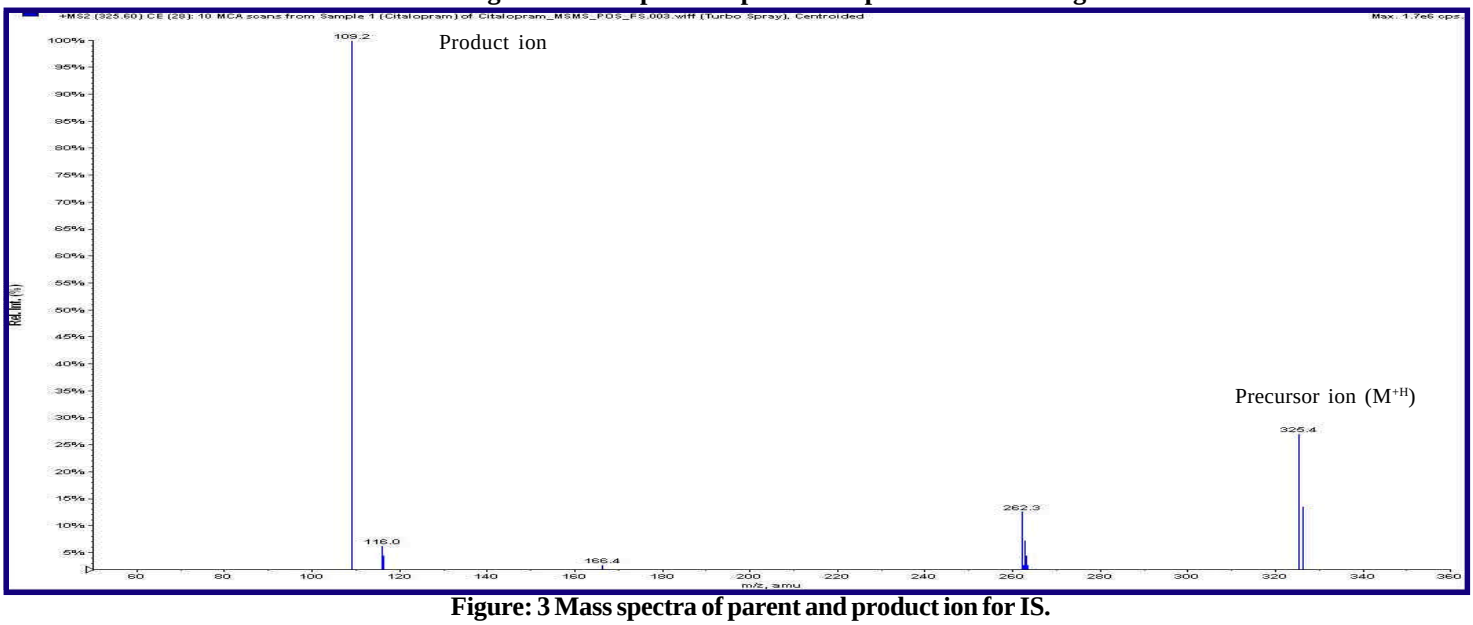


Figure: 3 Mass spectra of parent and product ion for IS.

Method validation

The developed method was validated over a linear concentration range of 10.0 pg/mL to 1006.3 pg/mL. The validation parameters, including selectivity and specificity, matrix effect, linearity, precision and accuracy, stability (freeze–thaw, auto sampler, bench top, long term), recovery, LOQ and LOD, were evaluated under validation section as per standard guidelines (i.e. EMEA, ANVISA, FDA etc.)

Selectivity

The analysis of drug and IS using MRM function was highly selective with no interfering compounds (Figure. 2 & 3). Selectivity was performed by using six different lots of human plasma. Plasma interference of one blank at RT (Retention Time) of drug and internal standard is shown in the Figure 4.

Response of interfering peaks at the retention time of analyte (s) must be =20% of the mean response of LOQ standard (STD 1). Response of interfering peaks at the retention time of IS must be =5% of the mean response of IS (Figure 5). However, 100 % of different volunteer matrix lots should meet above criteria along with one lipeamic and one haemolysed matrix lots.

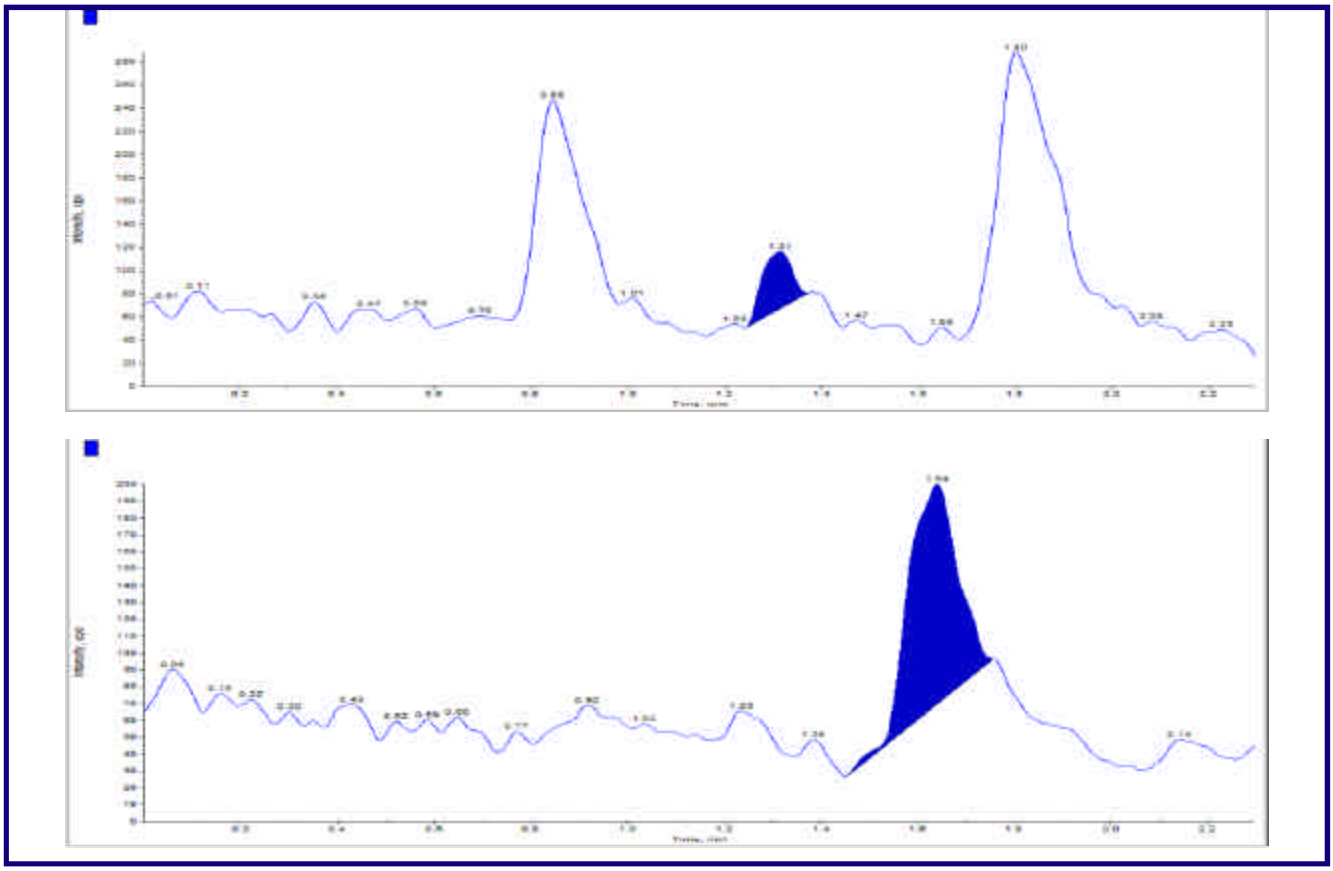
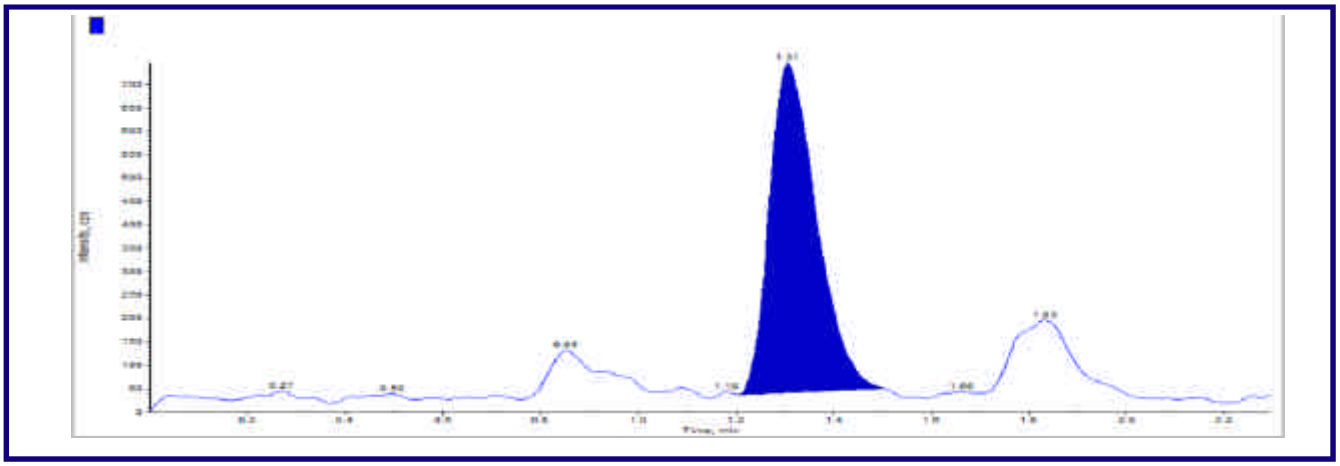


Figure 4 Blank of drug and IS



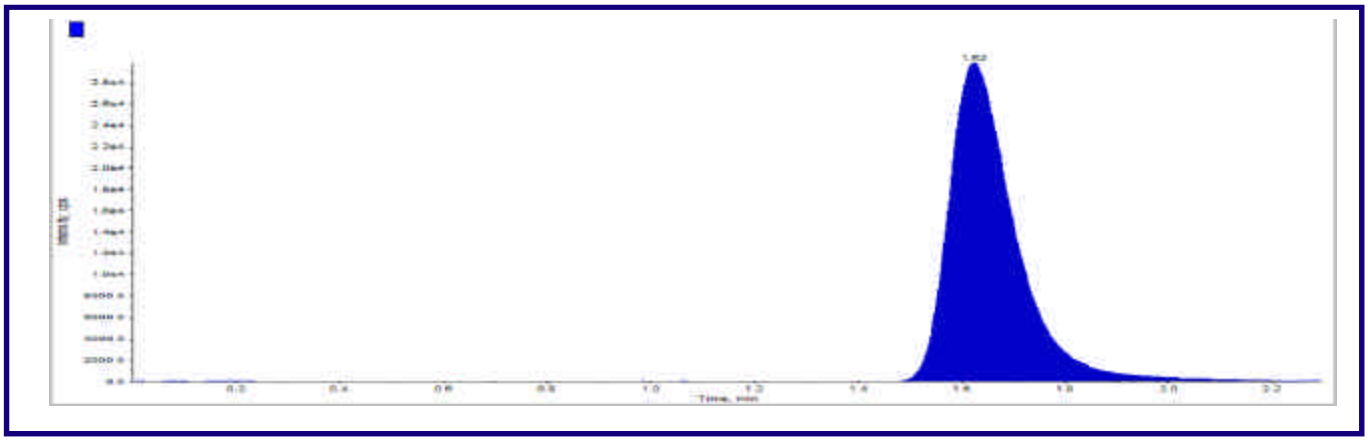


Figure 5: STD 1 of drug with IS

Matrix effect

Matrix Effect for Ropinirole was evaluated by analyzing six replicates of post extracted QC samples at low and high concentrations (prepared by spiking equivalent aqueous samples in to blank plasma extracts) in 6 different matrix lots along with two lipeamic and two haemolysed matrix lots. The %CV and % response ratios for Ropinirole in K2-EDTA plasma were in range from 1.2 to 3.4% and 105.3 to 105.7% respectively.

Linearity

Calibration curves were plotted as the peak area ratio (drug/IS) versus (drug) concentration. Calibration was found to be linear over the concentration range of 10.0 pg/mL to1006.3 pg/mL. The % CV was less than 15% and the accuracy ranged from 95.9 % to 107.3 %. The determination coefficients ($\mathbf{r}^2$) were greater than 0.99 for all curves (Table 1). The correlation coefficient (r) ranges from 0.9971 to 0.9991 for Ropinirole during the validation.

Table 1. Calibration curves details of drug from 04 batches of validation.

Sample ID	Spiked plasma Conc. (pg/mL)	Mean conc. (pg/mL) ± SD	RSD (%) (n=4)	Accuracy (%)
STD 1	10.0	9.70±0.200	2.1	97.0
STD 2	20.1	21.58±0.562	2.6	107.3
STD 3	40.3	38.65±2.412	6.2	95.9
STD 4	100.6	105.38±2.824	2.7	104.7
STD 5	201.3	199.40±4.243	2.1	99.1
STD 6	402.5	400.75±11.368	2.8	99.6
STD 7	805.1	782.85±14.122	1.8	97.2
STD 8	1006.3	997.03±34.263	3.4	99.1

%RSD = (Standard deviation/mean concentration measured) ×100.

Precision and accuracy

Precision and accuracy for this method was controlled by calculating the intra- and inter-batch variations at four concentrations (10.1, 28.1, 439.0 and 731.7 pg/mL) of QC samples in six replicates. As shown in Table 2, the intra-day RSD was less than 7.2 % and the accuracy ranged from 98.5 % to 107.4 %. Inter-day RSD was less than 8.6 % and the accuracy ranged from 99.0 % to 104.4 %. These results indicate the adequate reliability and reproducibility of this method within the analytical curve range.

Table 2. Precision and accuracy of Ropinirole from 4 batches of validation.

Sample ID	Spiked plasma conc. (pg/mL)	Intra-day (One batch) Concentration measured (n=6) (pg/mL) (mean±SD)	RSD (%)	Accuracy (%)
LLOQ QC	10.1	10.85±0.777	7.2	107.4
LQC	28.1	29.55±1.906	6.4	105.2
MQC	439.0	443.58±19.264	4.3	101.0
HQC	731.7	720.72±32.216	4.5	98.5
Sample ID	**Spiked plasma conc. (pg/mL)**	**Inter-day (4 batches) Concentration measured (n=24) (ng/mL) (mean±SD)**	**RSD (%)**	**Accuracy (%)**
LLOQ QC	10.1	10.54±0.907	8.6	104.4
LQC	28.1	28.98±1.585	5.5	103.1
MQC	439.0	442.26±32.970	7.5	100.7
HQC	731.7	724.28±50.122	6.9	99.0

%RSD = (Standard deviation/mean concentration measured) ×100

Dilution Effect

Standard curve can be extended up to 2012.6 pg/ml without affecting the final concentrations. The results have shown that the precision and accuracy for six replicates of diluted samples were within the acceptance range (data not shown).

Cross interference verification:

The % interference due to IS at Analyte RT in blank with IS sample shall be = 20% of the analyte response in LLOQ and the % interference due to Analyte at IS RT in ULOQ shall be = 5% of the IS response in blank with IS sample, in both extracted and un-extracted samples. The results have shown that there was no cross interference from each other.

Stability

Quantification of drug in plasma subjected to five freeze–thaw (-70°C to room temperature) cycles showed the stability of the analyte. No significant degradation of drug was observed even after 23.92 hrs storage period in the auto sampler tray, the in-injector stability (processed sample stability) of Ropinirole after 23.92 hours was 105.0% and 102.6% at low and high concentrations respectively. Dry extract stability of Ropinirole at a temperature of below 10°C for 23.25 hours was 105.6% and 100.4% at low and high concentrations respectively. In addition, the long-term stability of drug in QC samples after 120 days of storage at -70 °C and -20 °C was also evaluated. The long-term stability of Ropinirole in human plasma at a temperature of -70°C for 120 days was 98.4% and 107.5% at low and high concentrations respectively.

The Long-term stability of Ropinirole in human plasma at a temperature of -20°C for 120 days was 100.5% and 99.7% at low and high concentrations respectively. These results confirmed the stability of drug in human plasma for at least 120 days at -70°C & -20°C (Table 3).

Table 3. Stability data of Ropinirole in plasma samples.

Stability	Spiked plasma Conc. (pg/mL)	Conc. measured (pg/mL) (mean±SD ; n=6)	RSD (%) (n=6)
Room temperature	28.1	29.98±2.018	6.7
stability (16.83 hours)	731.7	726.55±35.567	4.9
Processed sample stability	28.1	28.10±2.030	7.2
(23.92 hours at 10°C)	731.7	711.28±28.895	4.1
Dry extracted stability	28.1	28.27±1.693	6.0
(23.25 hours at 10°C)	731.7	695.78±12.015	1.7
Long term stability	28.1	28.77±1.354	4.7
(120 days at -70.0°C)	731.7	740.06±39.850	5.4
Long term stability	28.1	29.39±1.461	5.0
(120 days at -20.0°C)	731.7	686.45±36.669	5.4
Freeze–thaw	28.1	28.08±2.764	9.8
stability (cycle 5)	731.7	688.08±42.289	6.1

%RSD = (Standard deviation/mean concentration measured) ×100

Stability in whole human Blood:

Evaluate the stability of analyte in whole human blood at room temperature. Spiked separate aliquots of whole human blood at low and high QC level (stability aliquots) and placed on the working bench at room temperature. After a period of approximately 2 hours spiked separate aliquots of whole human blood at low and high QC level (comparison aliquots) and separated the plasma from both the comparison and stability aliquots by centrifuging the blood at 3000 rpm at 4ºC for 20 min. Each 3 replicates of the comparison and stability samples were evaluated. The percent stability at both the levels using area or response ratios was calculated as follows,

$$\% \text{ Stability} = \frac{\text{Mean area ratio of stability samples}}{\text{Mean area ratio of comparison samples}} \times 100$$

The percent stability in whole human blood shall be within 85-115%. The %CV for the area ratios at both low and high QC levels in stability as well as comparison samples shall not exceed 15%. Whole human blood stability of Ropinirole at room temperature after 2 hours was 100.5% and 98.9% at low and high concentrations respectively. The %CV was found to be from 2.03 to 3.41.

Recovery

The recovery following the sample preparation using the liquid–liquid extraction method was calculated by comparing the peak area ratios of drug in plasma samples with the peak area ratios of solvent samples and estimated at control levels of drug. The recovery of drug was determined at three different concentrations 28.1, 439.0 and 731.7 pg/mL and found to be 101.6 %, 80.2% and 88.3%, respectively. The overall average recovery of drug and IS was found to be 82.7 %.

Sensitivity or Limit of quantification

The LOQ for this method was proven as the lowest concentration of the calibration curve which was proven as 10.0 pg/mL.

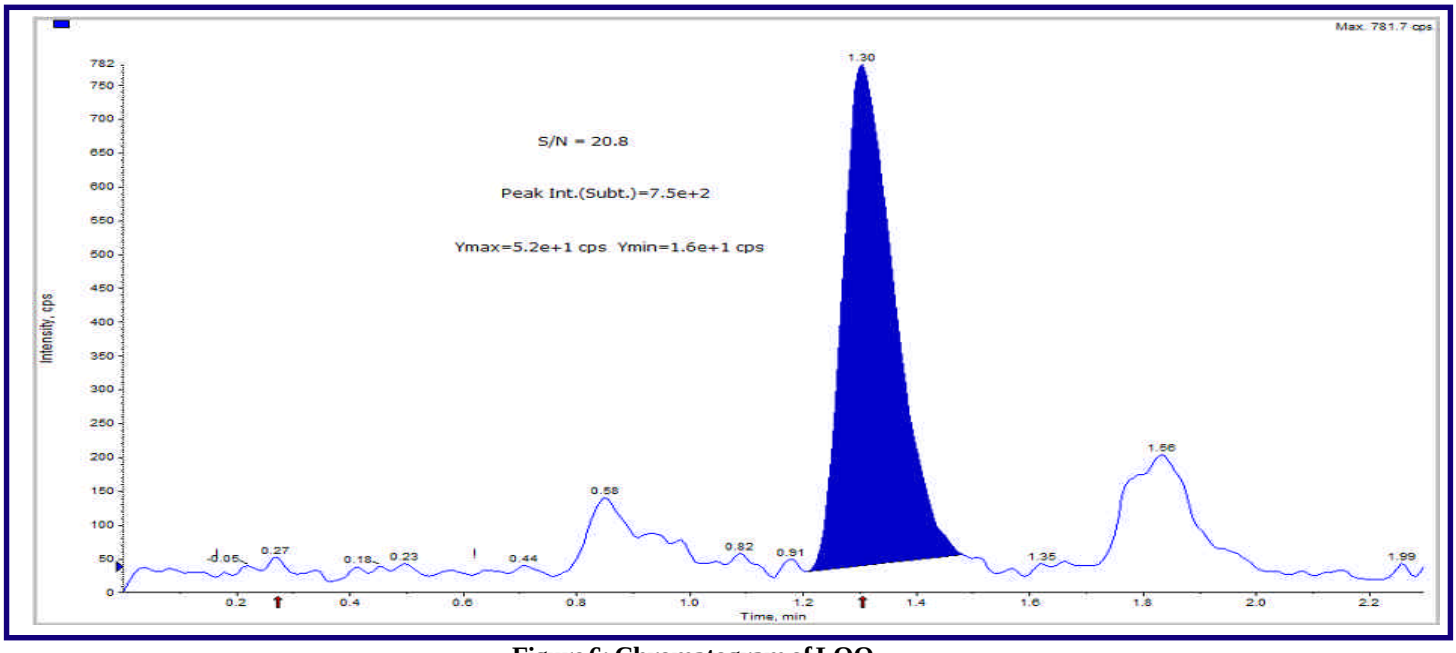


Figure 6: Chromatogram of LOQ

Application

The method can be applied to determine the plasma concentration of Ropinirole in human plasma in field of clinical trials and bioavailability and bioequivalence studies.

CONCLUSION

In summary, a method is described for the quantification of Ropinirole in human plasma by LC-MS/MS in positive electrospray ionization mode using multiple reaction monitoring and fully validated according to commonly accepted criteria. The advantage of this method is preventing the contamination and interference peaks from various unknown sources which were well separated in liquid-liquid extraction. It is rugged and robust technique to attain incurred sample reanalysis. The current method has shown acceptable precision and adequate sensitivity for the quantification of Ropinirole in human plasma samples obtained for pharmacokinetic studies. The sensitivity of Ropinirole was achieved with an LLOQ of 10 pg/mL, which has a within- and between- batch CV of 6.2 and 8.6% respectively. Many variables related to the electrospray reproducibility were optimized for both precision and sensitivity to obtain these results. The method can be successfully applied to quantify the concentrations of Ropinirole in pharmacokinetic studies.

REFERENCES

1. Matheson AJ and Spencer CM. Ropinirole: A review of its use in the management of Parkinson's disease. Drugs 2000; 60: 115–137.
2. Kaye CM and Nicholls B. Clinical pharmacokinetics of ropinirole. Clinical Pharmacokinetics 2000; 39: 243–254.
3. http://www.mhra.gov.uk/home/groups/par/documents/websiteresources/con111727.pdf
4. Wen AD, Jia YY, Luo XX, Bi LL, Chen XY, Zhong DF, The effect of Madopar on the pharmacokinetics of ropinirole in healthy Chinese volunteers, Journal of Pharmaceutical and Biomedical Analysis, 2007, 43, 774-778.
5. D. Vijaya Bharathi, B. Jagadeesh, S. Sirish Kumar, Revathi Naga Lakshmi, Kishore Kumar Hotha, A. Naidu, Ramesh Mullangi Highly sensitive method for the determination of ropinirole with a lower limit of quantitation of 3.45 pg/mL in human plasma by LC-ESI-MS/MS: application to a clinical pharmacokinetic study Journal of Biomedical Chromatography 2009: 23: 557.
6. Jignesh Bhatt, Arvind Jangid, Raghavendra Shetty, Bhavin Shah, Sandeep Kambli, Gunta Subbaiah, Sadhana Singh. Rapid and sensitive liquid chromatography-mass spectrometry method for determination of Ropinirole in human plasma. J. Pharm. and Bio.med. Anal 2006; 40(5): 1202-1208.
7. Azeemi A, Iqbal Z, Ahmadi FJ, Khari RK, Talegaonkar S, A sensitive method for the determination of ropinirole in human plasma by LC-MS-MS, Acta Chromatographica, 2008, 20, 95-107.
8. Liusheng Huang, Xiaohua Li, Florence Marzan, Patricia S Lizak, and Francesca T Aweeka Determination of Lumefantrine in small-volume human plasma by LC–MS/MS: using a deuterated Lumefantrine to overcome matrix effect and ionization saturation. Bioanalysis. 2012; 4(2): 157–166.
9. Dams R, Huestis MA, Lambert WE and Murphy CM. Matrix effect in bioanalysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. Journal of American Society for Mass Spectrometry 2003; 14: 1290–1294.
10. Guidance for Industry: "Bioanalytical Method Validation", U.S. Department of Health and Human Services, Food and Drug Administration, May 2001, Rockville, MD, USA
11. ANVISA Resolution- RDC no 27 May 17, 2012.
12. ANVISA Resolution – RE n. 899 of May 29, 2003
13. EMEA guidelines on Validation of bio analytical methods, July 2011.

Source of support: Nil, Conflict of interest: None Declared