CHAPTER 5

DETERMINATION OF PRAMIPEXOLE IN HUMAN PLASMA BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY (SIM)
1. ABSTRACT

A simple, rapid and sensitive method has been developed and validated for the
determination of pramipexole in human plasma by using gas chromatography mass
spectrometry (SIM). The LOD and LOQ are superior to the other reported LC-MS/MS
methods. After being made alkaline with NaOH, plasma samples (0.1 ml) were subjected to
LLE by using methyl-t-butyl ether (MTBE). The organic layer was separated and evaporated
and the residues are reconstituted by methanol. The reconstituted solution is directly injected
in to GC and separated by ZB-5ms capillary column (15 m X 0.25 mm X 0.25 μm). Analytes
were determined using electron impact (EI) ionization in a single quadrupole mass
spectrometer. GC–MS was performed in the selected-ion monitoring (SIM) mode using
target ions at \textit{m/z} 211,212 and 152 for pramipexole and, \textit{m/z} 194 and 165 for internal
standard (IS). A Linear calibration curve was plotted over the range of 20–1000 pg mL\(^{-1}\) for
pramipexole (\(r^2 > 0.996\)). The limit of detection and limit of quantification were 5 and 20.0 pg
mL\(^{-1}\), respectively, which offered high sensitivity and selectivity enough for bioanalytical
investigation. Inter- and intra-day precision ranged from 0.3 to 8.8% and from 0.9 to 11.33%,
respectively. The recovery of pramipexole from plasma ranged from 82.4±7.1%, to
87.8±5.7%. The method fulfill all standards required for bio analytical method and can be
successfully applied to a pharmacokinetic study of pramipexole.
2. INTRODUCTION

Pramipexole \((C_{10}H_{17}N_{3}S)\) is chemically (6R) - N' - propyl - 4, 5, 6, 7 - tetrahydro-1, 3-benzothiazole-2, 6-diamine, a non-ergot dopamine auto-receptor agonist. Pramipexole is a selective and specific non-ergot dopamine (DA) receptor agonist with high affinity and selectivity for the D2 receptor subfamily of dopamine receptors, particularly with highest affinity to dopamine D2 and D4 receptor subtype [1-3]. Pramipexole can protect dopaminergic neurons by a receptor-dependent pathway at nanomolar concentrations [4-6], and at higher than 10 micromolar concentrations it has shown to be neuroprotective in vitro independent of the dopaminergic agonism [7]. The drug has proved to be an effective agent for patients with Parkinson's disease and drug resistant tremor [8] and had a beneficial effect on mood and motivational symptoms in Parkinson's disease patients who did not have major depressive disorder. The clinical value of pramipexole in the treatment of depressive and apathetic syndromes is also valuable [9] and has proven a suitable alternative in patients with moderate to severe RLS, particularly when their therapy has to be switched to a dopamine agonist [10]. A single dose of 0.125-0.75 mg pramipexole (mean 0.3 ± 0.2 mg) in the evening resulted in a significant improvement of subjective restless legs syndrome RLS symptoms as rated by the International RLS Study Group Severity Scale [10-11].
Literature survey reveals the lack of good research done concerning analysis of pramipexole. Analysis of pramipexole in biological samples was performed using HPLC with atmospheric pressure chemical ionization tandem mass spectrometry [12] and HPLC with electrochemical and UV detection [13]. Both methods used 1ml of plasma samples, which require large quantity of sample. An enantiomeric separation of pramipexole was reported by LC [14]. Analysis of pramipexole and its impurities in bulk substances and pharmaceuticals is [15-17] also reported. There are studies on analysis of pramipexole [15-16], by experimental design and also to determine the dissociation constants (pKa) of pramipexole and its impurities [17]. Recently Nirogi developed a LC MS MS method for the determination of pramipexole in plasma with LQ of 200 pg ml$^{-1}$ [18]. A capillary electrophoresis with laser-induced fluorescence detection was reported with limit of detection and limit of quantitation of 10.0 and 25.0 ng mL$^{-1}$ respectively [19].
The dosing limit of pramipexole is very low between 125 to 750 µg per day [10-11]. Therefore, a highly sensitive and selective method for the determination of pramipexole in plasma is necessary to support pharmacokinetic evaluation in humans. The purpose of the present study is to develop a sensitive and selective method for the determination of pramipexole in human plasma.

The combination of gas chromatography coupled with mass spectrometry using selected ion monitoring (GC-MS-SIM) provides many benefits, including analytical ruggedness as well as enhanced sensitivity and selectivity. This paper presents a highly sensitive and selective GC-MS method using micro bore capillary column for the rapid quantitation of pramipexole in human plasma. The current method demonstrates a simple and rapid sample preparation method as well as significantly low volume of (0.1 ml) plasma samples required for the analysis. Lower quantification and detection limits were achieved compared to previously published methods [12-13]. One-step liquid–liquid extraction was used and a shorter analysis run time was achieved. The method was fully validated and fulfills all the requirements for using to a pharmacokinetic study of pramipexole.
3. EXPERIMENTAL

3.1 Reagents and Chemicals

Pramipexole (purity >98.6%) reference standard was gift samples from Claris Lifesciences Limited (Ahmedabad, India). Caffeine (purity >98.0%) was purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade methanol was purchased from Merck fine chemicals (Mumbai, India). HPLC grade dichloromethane (DCM) and methyl-t-butyl ether (MTBE) was obtained from Thomas baker (India). All other chemicals and reagents used were of HPLC grade and supplied by Merck (India). Purified water was prepared by Millipore (synergy) system and was used throughout the study. All other chemicals and reagents used were of HPLC grade and supplied by Merck (India).

3.2 GC-MS instrument and conditions

All analyses were performed using a Shimadzu GC-17A gas chromatograph interfaced with a Shimadzu QP-5050A quadrupole mass spectrometer (Shimadzu Corp., Kyoto, Japan). The GC–MS was operated with an interface temperature of 250°C, and an ionization source temperature of 300°C. The mass spectrometer was tuned everyday using PFTBA (perfluorotributylamine). The solvent delay before the MS filament turned on was set to 4 min to protect the filament from oxidation. Chromatographic separation was achieved by using a phenomenex make ZEBRON ZB-5ms (5% phenyl–methylsilicone, 15m × 0.25 mm i.d., 0.25μm film thickness) capillary column. Helium with a minimum purity of 99.9% was used as carrier gas at a flow rate of 4 ml/min. The gas chromatograph was equipped with a split/splitless injection port operated at 200°C. Samples were injected in the splitless mode
at a column temperature of 150°C, then the splitter was opened after 1 min sampling time. The gas chromatograph oven temperature was programmed as follows: initial temperature, 150°C for 1 min; from 150 to 300°C at a rate of 35°C /min and temperature was held for 1 min. The mass spectrometer was operated in the positive-ion electron impact (EI) mode. EI mass spectra were obtained at an ionizing energy of 70 eV, and at an emission current of 60 µA. Quantification was carried out by the selected ion monitoring (SIM) mode. In order to select the stable ion for monitoring, the mass spectra of pramipexole and IS were obtained by injecting 0.1µL of the analyte standards into the GC–MS.

3.3 Preparation of calibration standards and quality control (QC) samples

The standard stock solution of pramipexole was prepared by dissolving the accurately weighed compound in methanol to give a final concentration of 4000 ng mL⁻¹. This solution was sequentially diluted with methanol to obtain working solutions at concentrations over 1–40 ng mL⁻¹. A standard stock solution of caffeine (I.S.) was prepared by dissolving the drug in methanol to a final concentration of 100 ng mL⁻¹. This solution was diluted with methanol to a final concentration of 2 ng mL⁻¹. All the solutions were stored at 4°C until used.

The calibration standards were prepared by taking aliquot of working standard solutions and placed in to an Eppendorff tube, and the solvent was evaporated under a compressed nitrogen stream. The dried analyte was reconstituted using blank plasma to final desired concentrations of 20, 30, 40, 50, 100, 200, 300, 400,500,700 and 1000 pg mL⁻¹ for pramipexole, and the solution was then vortex mixed for 1 min. Quality control (QC) samples were prepared by spiking working standard in to drug free plasma at concentrations of 100, 250 and 500.0 pg mL⁻¹ for the analytes. For the determination of LLOQ of this
method, QC standard was made at concentrations of 20 pg mL\(^{-1}\) for analyte and tested for accuracy and precision. Aliquots of calibration standards, internal standard and quality control plasma samples were dispensed into labeled Eppendorf tubes and stored at -25°C until required for assay. In all determinations, calibration standards, QC samples were prepared together.

### 3.4 Sample preparation

Plasma samples were extracted employing a liquid–liquid extraction (LLE) technique. All frozen human plasma samples (i.e. calibration standard and QC sample) were allowed to thaw at room temperature. A 100 μL plasma sample was dispensed to the 1.5 ml polypropylene micro-centrifuge tube, the 10 μL of internal standard (IS) (2 ng mL\(^{-1}\) caffeine) solution and 10 μL of 1 M sodium hydroxide (0.1M) were added and vortex mixed for 30 s. 2 ml methyl tert-butyl ether was then added and vortexed for 3 min. 100 μL of methyl-t-butyl ether (MTBE) was then added to the tubes, and extracted by vortex-mixing for 1 min. The mixture was centrifuged at 13,000 rpm for 5 min at 4°C. The separated organic phase was transferred to another clean glass tube and evaporated to dryness under a compressed nitrogen stream at 35°C. The residue was reconstituted in 100 μL of methanol, followed by centrifugation at 13,000 rpm for 5 min. A 50 μL aliquot of the supernatant was transferred to a vial and 0.1 μL was injected into the GC-MS system.

### 3.5 Assay validation

The method validation was performed in accordance with FDA guideline [20] for bioanalytical method validation. The method was validated for specificity, linearity, precision, accuracy, recovery, matrix effects and stability.
3.5.1 Selectivity

The selectivity of the method was tested by analyzing blank human plasma samples from six different individuals. All blank samples were tested for interferences by following the proposed extraction procedure and analysing by proposed GC–MS conditions, and the results were compared with those obtained for an aqueous solution of the analyte at a concentration near the LLOQ. The area response of analytes in blank extract should not be greater than 20% compared to area response of LLOQ concentration. In addition the area response in extracted blank should not be greater than 5% of absolute area response of IS (200 pg mL$^{-1}$) in aqueous extract. Carry over was eliminated by rinsing injection system. This was demonstrated by analyzing blank samples immediately following the samples at highest concentration.

3.5.2 Linearity

The linearity was tested by analyzing calibration standard at 10 concentration levels from over the range of 20-1000 pg mL$^{-1}$ of the analyte. The samples were run in the order from low to high concentration. A blank plasma sample (without I.S.) and a zero sample (with I.S.) were also analyzed to confirm the absence of any interference; these data were not included to construct calibration plots. Standard curves based on peak area ratio of analytes to I.S. were prepared in. Linearity was assessed by least-squares regression analysis with a weighting index of 1/x$^2$. Standard deviations of the slope and intercept were calculated to make sure the reliability of calibration curve over a period of 1 week.
The LLOQ is defined as the lowest concentration on the calibration curve at which an acceptable accuracy (R.E.) within ±20% and a precision (R.S.D.) below 20% can be obtained. The lower limit of quantification (LLOQ) response should be ten times that of the average noise level in the chromatogram.

3.5.3 Precision and Accuracy

Intra and inter-day precision was calculated as coefficient of variation (CV) (or %RSD) and accuracy as relative error (R.E.) on the basis of five replicate sample analysis of each QC levels. The intra-day precision and accuracy of the assay were measured by analyzing replicate analysis of each QC (LLOQ, low, mid, and high concentration quality control samples) of analytes on the same day. Precision and accuracy is also determined near the LLOQ of method by preparing LLOQ (QC) sample. For determining the inter-day accuracy and precision, analysis of three batches of QC samples was performed on different days. Inter-day precision and accuracy were determined on three different days by analysis of three batches of QC samples at each QC levels (LLOQ, low, mid, and high concentration quality control samples). Calibration standards and QC samples were analyzed on three separate days. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV. The accuracy was required to be within ±15% relative error of the actual values.

3.5.4 Recovery and Effect of the Matrix

The recovery (extraction yield) of each analytes were determined by comparing the analytes and IS peak-area ratios obtained for each QC levels subjected to the extraction procedure with those obtained from post-extraction blank plasma samples spiked (spiked
after extraction) with equivalent amounts of each analyte at the same nominal concentrations (equivalent QC levels). The recovery of the IS was determined in the same way. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and that of the internal standard should be consistent, precise, and reproducible.

\[
\text{Absolute recovery} = \frac{\text{response of analyte spiked into matrix (processed)}}{\text{response of analyte of pure standard (unprocessed)}} \times 100 \quad \text{Ref [21]}
\]

Additionally, in order to avoid potential matrix effects on the detector response, in this method, the recovery was evaluated by the comparison of the response obtained from extracted plasma samples (by normal QC sample preparation) and the response obtained from post extracts of blank plasma samples spiked with the analyte. The extraction efficiency of the analytical method was assessed using a specific batch, containing the following samples:

- A calibration curve prepared by spiking the working standard solution in to post extracted blank plasma samples and diluted in methanol in order to quantify the QC samples;
- Quality control (QC) samples of each QC levels (Low, Medium & High), obtained by the normal extraction process. (QC Normal EX)
- Samples of each of the concentrations mentioned above, obtained by spiking the working standard solutions of analytes in post extracted blank plasma samples. (QC PEX)
- Samples of each of the concentrations mentioned above, obtained by diluting the working standard solutions by methanol. (QC MeOH)
Recovery was evaluated by calculating the mean of the response of each concentration and dividing the extracted sample mean by the spiked in post extracted blank plasma sample mean of the corresponding concentration.

\[
\frac{(QC \ Normal \ EX)}{(QC \ PEX)}
\]

This comparison was done in order to eliminate matrix effects, giving a true recovery. The recovery was also evaluated in different types (normal, hemolized and lipemic) of human plasma at a medium concentration value (for example QC medium).

The matrix effect was evaluated by comparing the peak areas obtained from analytes spiked in post-extraction blank plasma sample, with those for the respected compound dissolved at the same concentrations in methanol [22-23].

\[
\frac{(QC \ PEX)}{(QC \ MeOH)}
\]

The blank plasma used in this study was obtained as three different batches. Three different concentrations (each QC levels) of each analytes and 200 pg mL\(^{-1}\) of internal standard were evaluated by analysis of three different batches at each concentration. If peak-area ratios from the plasma extracts were ±15% those from methanolic solutions, a matrix effect was implied.

### 3.5.5 Stability

Characterization of the stability of analytes in biological samples collected during clinical studies together with that of critical assay reagents, including analyte stock solutions, is recognized as an important component of bioanalytical method [24]. Stability of the analytes and IS in human plasma were tested for short term, freeze-thaw and long term...
stability. All the stability studies were conducted at three concentration levels (low, medium and high QC levels) with three determinations for each. The samples were analyzed using freshly prepared calibration samples. Plasma samples of each QC levels were thawed and kept at room temperature for a period of time (8 h) exceeding that expected to be encountered during routine sample preparation. These samples were then analyzed for short-term temperature stability. Long-term temperature stability of analytes was studied for 30 days by analysing of QC samples at the three different levels. Freeze–thaw stability QC samples containing analytes were tested after three freeze cycles (-20°C) and thaw (room temp.). Post-preparative stability was determined by re-analysis of extracted QC samples kept under in the autosampler at 4°C for 24 h. The stability of the standard and internal standard working solutions was tested for 6 h at room temperature. The stability of working solutions was expressed in the same way as for percentage recovery.
4. RESULTS AND DISCUSSION

4.1 Sample Preparation

The mean extraction recovery of pramipexole was found to be below 70% when the other organic solvents (e.g. ether, ethyl acetate) were used as extracting solvents in our pilot studies, which did not assist to enhance the sensitivity of the analytical method. However better results were obtained by using methyl tert-butyl ether as extracting solvent.

4.2 Chromatography and Mass spectrometry

Typical chromatograms are shown in Figure 2. The retention times of pramipexole and IS were approximately 4.51 and 4.93 min, respectively. Caffeine was selected as the internal standard for its similarity in the retention and extraction recovery. In order to enhance the sensitivity of method, SIM mode was selected at the dominant and characteristic ions for pramipexole which were at m/z 211,212 and 152 and, for IS at m/z 194 and 165. The total run time was only 6 min, which was much shorter than those reported in the literature. After careful comparison of many temperatures, we finally adopted the temperatures of 150 °C at the injection port and 200°C at the oven for optimal monitoring of the analyte, and the solvent cutoff time of 4.0 min to minimize any early eluting plasma interferences, in addition, it yielded suitable retention time and peak shape for pramipexole and IS, which offered relatively short analytical runtimes.
Figure 2: (a) GC MS chromatogram and full scan mass spectra of (b) pramipexole and (c) caffeine (IS) .
4.3 Method validation

4.3.1 Specificity

Specificity with respect to plasma components was determined by analyzing hyperlipemic, and haemolysed blank samples from six different bathes of plasma samples collected under controlled conditions. No interference was observed at the retention times of pramipexole and IS. The samples were analyzed using the proposed extraction procedure and chromatographic conditions in order to compare them with an aqueous solution of the analyte at a concentration near to the limit of quantification. Representative chromatograms of blank plasma, blank plasma spiked with pramipexole at the limit of quantitation (20 pg mL\(^{-1}\)) and three QC sample of pramipexole were shown in Figure- 3a and 4a, and no interference was observed at the retention time of the analyte at 4.51 min and IS at 4.93 min due to endogenous substances in blank human plasma.
Figure 3: GC MS (TIC) chromatogram of QC samples of pramipexole: (a) LLOQ QC cone. 20 pg mL\(^{-1}\). (b) Low QC cone. 100 pg mL\(^{-1}\). (c) Medium QC cone. 250 pg mL\(^{-1}\). (d) High QC cone. 500 pg mL\(^{-1}\).
4.3.2 Linearity

The calibration curves (Figure 5) showed good linearity within the range 20–1000 pg mL$^{-1}$. Representative linear equation of calibration curve for the analyte was $Y = (0.0112 \pm 0.71)X + (0.058 \pm 2.18)$ with a correlation coefficient of 0.9988, where $Y$ was the peak area ratio of the analyte to the IS and the $X$ was the concentration of the analyte.
4.3.3 Precision and accuracy

The precision and accuracy of the method were evaluated by analyses of each QC level samples. The data of intra- and inter-day precision and accuracy from QC samples are summarized in Tables 1. The intra and inter day precision (%CV RSD, n=5) at LLOQ-QC was 2.61% and 9.50% respectively with an accuracy (relative error R.E.) of ±2.36 and ±7.80. The precision and accuracy of the present method confirm to the criteria for the analysis of biological samples according to the guidance of FDA where the R.S.D. determined at each concentration level is required not exceeding 15% (20% for LLOQ) and R.E. within ±15% (±20% for LLOQ) of the actual value [20]. The results were within acceptable limits showing satisfactory accuracy and precision.
Table 1  Intra and Inter day precision data of QC samples for pramipexole.

<table>
<thead>
<tr>
<th>Precision type</th>
<th>Nominal concentration (pg mL⁻¹)</th>
<th>Precision Mean ± SD</th>
<th>RSD (%)</th>
<th>Accuracy Mean relative (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Intra-day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td>20.459 ± 0.53</td>
<td>2.61</td>
<td>2.36</td>
<td></td>
</tr>
<tr>
<td>100.00</td>
<td>98.635 ± 3.86</td>
<td>3.91</td>
<td>3.63</td>
<td></td>
</tr>
<tr>
<td>250.00</td>
<td>247.325 ± 12.98</td>
<td>5.25</td>
<td>4.16</td>
<td></td>
</tr>
<tr>
<td>500.00</td>
<td>494.683 ± 24.55</td>
<td>4.96</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td>19.568 ± 1.86</td>
<td>9.50</td>
<td>7.80</td>
<td></td>
</tr>
<tr>
<td>100.00</td>
<td>102.882 ± 3.22</td>
<td>3.13</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>250.00</td>
<td>246.269 ± 11.48</td>
<td>4.66</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>500.00</td>
<td>500.717 ± 28.34</td>
<td>5.66</td>
<td>4.57</td>
<td></td>
</tr>
</tbody>
</table>
3.3.4 Recoveries and Matrix effect

The recovery in terms of extraction efficiency was determined by analyzing the QC samples. The recoveries were determined at four concentrations (LOQ, low, medium, and high QC) by comparing peak areas obtained from plasma samples with those obtained by unextracted (spiked in post extracted blank plasma) sample at the same concentration and conditions and the results are shown in Table-2. The recovery of IS was also tested using the same condition, and the mean recovery of IS was found to be 83.31± 4.07%. At the concentrations of 20, 100, 250, and 500 pg mL⁻¹ the recoveries of pramipexole were 86.6±4.2%, 87.8±5.7%, 82.4±7.1%, and 85.4±6.6% respectively.

The comparison of the response obtained from extracted plasma samples (by normal QC sample preparation) and the response obtained from post extracts of blank plasma samples spiked with the analyte to calculate the recovery were shown in Table 2.

Table-2 also shows results obtained during study of matrix effect. Matrix effects were absent as shown by the fact that concentrations of analytes as a percentage of nominal concentrations for low, medium and high QC samples were, respectively, 90.59±1.54%, 93.92±2.21 % and 92.99±2.13 % for pramipexole, and the percent nominal concentration of I.S. was 92.10±1.20 %.
Table 2  Recovery data of the extraction procedure of QC samples for pramipexole and IS in human plasma

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Conc. type QC level</th>
<th>Conc. found in methanolic sample pg ml(^{-1})</th>
<th>Post extracted spiked sample pg ml(^{-1})</th>
<th>Extracted sample pg ml(^{-1})</th>
<th>% Recovery</th>
<th>SD</th>
<th>%RSD</th>
<th>% Recovery in Matrix effect</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pramipexole *</td>
<td>Low</td>
<td>103.389</td>
<td>93.652</td>
<td>84.174</td>
<td>89.87</td>
<td>2.05</td>
<td>2.28</td>
<td>90.59</td>
<td>1.54</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>264.825</td>
<td>248.820</td>
<td>231.346</td>
<td>92.92</td>
<td>1.63</td>
<td>1.76</td>
<td>93.92</td>
<td>2.21</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>523.989</td>
<td>487.451</td>
<td>449.862</td>
<td>92.24</td>
<td>1.61</td>
<td>1.75</td>
<td>92.99</td>
<td>2.13</td>
<td>2.29</td>
</tr>
<tr>
<td>Caffeine (IS)**</td>
<td>200 pg ml(^{-1})</td>
<td>206.048</td>
<td>189.802</td>
<td>173.858</td>
<td>91.58</td>
<td>0.91</td>
<td>0.99</td>
<td>92.10</td>
<td>1.20</td>
<td>1.30</td>
</tr>
</tbody>
</table>

*recovery and matrix effect of Pramipexole calculated by area ratio of analytes/IS and for IS** only by area of IS.
4.3.5 Stability

No significant decrease of the analyte concentration was observed when kept at room temperature for 12 h during short-term temperature stability which indicated reliable stability behavior under the experimental conditions of the analytical runs. The stability data of the analytes in plasma over three freeze-thaw cycles indicated that the analytes were stable in human plasma for three freeze-thaw cycles, when stored at -20°C and thawed to room temperature. The samples were stable for a period of 30 days at -20°C. The stability of the working solutions was tested at room temperature. Based on the results obtained, these working solutions were stable over 6 h. The results from all stability tests are presented in Table-3, which demonstrate a good stability of each analytes over all steps of the determination and no stability-related problems are expected during the routine analyses. The method is therefore proved to be applicable for routine analysis for the pharmacokinetic, bioavailability or bioequivalence studies samples.
Table 3. Stability of Pramipexole in human plasma under various storage conditions (n = 5)

<table>
<thead>
<tr>
<th>Storage condition</th>
<th>Nominal concentration (pg mL(^{-1}))</th>
<th>Calculated concentration (ng mL(^{-1}))</th>
<th>Mean ± SD</th>
<th>RSD (%)</th>
<th>Mean relative Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability at 37°C for 48 h</td>
<td>100</td>
<td>95.607 ± 6.87</td>
<td>7.18</td>
<td>6.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>252.393 ± 11.32</td>
<td>4.48</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>507.429 ± 20.31</td>
<td>4.00</td>
<td>3.52</td>
<td></td>
</tr>
<tr>
<td>The long-term stability after storage at -20°C for 30 days.</td>
<td>100</td>
<td>108.709 ± 9.06</td>
<td>8.34</td>
<td>9.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>261.401 ± 6.81</td>
<td>2.60</td>
<td>4.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>510.049 ± 22.16</td>
<td>4.34</td>
<td>2.74</td>
<td></td>
</tr>
<tr>
<td>Stability in autosampler at 4°C for 24 h</td>
<td>100</td>
<td>107.867 ± 3.90</td>
<td>3.61</td>
<td>7.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>255.411 ± 10.07</td>
<td>3.94</td>
<td>3.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>502.865 ± 24.56</td>
<td>4.88</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td>Stability followed by three freeze–thaw cycles</td>
<td>100</td>
<td>101.320 ± 4.04</td>
<td>3.99</td>
<td>3.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>253.902 ± 3.42</td>
<td>1.35</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>503.481 ± 15.24</td>
<td>3.03</td>
<td>2.46</td>
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</table>
5. CONCLUSION

A fast, sensitive, specific analytical method has been developed and validated for the
determination of pramipexole in blood plasma by using gas chromatography mass
spectrometry (SIM) which is superior to the other reported LC and CE methods. The
technique provides a rapid, reliable and reproducible method with low detection limit for the
determination and quantification of pramipexole in blood plasma. Significantly lower limit of
quantification of 20 pg ml\(^{-1}\) was achieved in plasma, compared with the previously published
methods. The assay involves relatively simple sample preparation, Acceptable precision and
accuracy is obtained within the standard curve range of 20-1000 pg ml\(^{-1}\). The steps of sample
preparation were uncomplicated using 0.1 mL plasma sample. This method demonstrated a
simple sample preparation procedure and relatively short analysis run time which allows to
high sample throughput of samples for determination of the pramipexole in plasma. The
method fulfills all requirements for bio analytical method and can be successfully applied to
the pharmacokinetic study of pramipexole.
6. REFERENCES


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