DEVELOPMENT OF LC-MS/MS METHOD FOR DETERMINATION OF ILOPERIDONE IN RABBIT PLASMA: APPLICATION TO A PHARMACOKINETIC STUDY

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Received: 01 Feb 2015 Revised and Accepted: 28 Feb 2015

ABSTRACT

Objectives: Estimation of drug in plasma during in vivo studies requires a highly sensitive and precise method. Thus, the objective of the current study is to develop a LC-MS/MS method for quantitation of iloperidone (ILO) in rabbit plasma, which was further used for in vivo study as a part of development of long acting depot formulation.

Methods: The quantitation of the drug was determined by positive ion mode with multiple reaction monitoring (MRM). Plasma extraction was carried out using Celerity Deluxe cartridge showing more than 95% recovery. The chromatographic conditions for quantitation of ILO were use of Kromasil C 18 (50*4.6 mm, 5µm) column using, 0.01M Ammonium formate Buffer (pH 5.5): Acetonitrile (10:90) as mobile phase. Olanzapine was used as an internal standard. The developed method was applied for pharmacokinetics study of ILO in rabbits after intramuscular administration of 6.24 mg/kg dose.

Results: The method was linear over concentration range of 0.5-100 ng/ml with correlation coefficient more than 0.999. The lower limit of quantitation was 0.5 ng/ml and recovery was found more than 95% for ILO and olanzapine. Extraction and estimation of ILO were performed using the developed method. Steady release of ILO from depot formulation was observed up to 14 days in rabbits.

Conclusion: The developed LC-MS/MS method was precise and having detectability for lower drug concentration in rabbit plasma and it was successfully applied to study in vivo drug release in rabbits.

Keywords: Iloperidone, LC-MS/MS, Rabbit plasma, Pharmacokinetic, Long acting formulation.

INTRODUCTION

Iloperidone (ILO) is a commercially available second generation antipsychotic agent, approved for acute treatment of schizophrenia in adults [1]. Currently, ILO is official in Indian pharmacopoeia and under priority new monographs list in U. S. Pharmacopoeia [2, 3]. It is a piperidinyl-benzisoxazole derivative with antagonistic activity at serotonergic 5-HT₂A, dopaminergic D₂/D₃, and adrenergic receptors [4]. It is chemically known as 1-[4-[[3-[[4-(6-fluoro-1, 2-benzisoxazol-3-yl)-1-piperidinyl] propoxy]-3-methoxy-α-methylbenzenemethanol in human plasma [9]. Mengmeng Jia et al. also developed liquid chromatography-tandem mass spectrometry for pharmacokinetic study of ILO in human plasma along with its two active metabolites P88 and P95 [10]. A HPLC method was also reported by Leenata Mandpe et al. for determination of ILO and idebenone in human plasma [11]. Overall impression of literature search is that, all reported methods are based on pharmacokinetic study in human matrices. For the development of any long acting formulation preliminary animal study is required.

Therefore, the aim of this study was to develop a specific, fast and accurate method for an assay of ILO from rabbit plasma. In current research, LC-MS/MS method has been developed for drug release study from formulation. In this method, purification of sample was achieved by solid phase extraction (SPE) cartridges. The developed method has been successfully used to support the pharmacokinetic release study of ILO from intramuscular injection in rabbit model. It is expected that the results of these studies will provide useful information for development of long acting formulation.

Fig. 1: Chemical structure of ILO (A) and OLA (B)
Experimental

Chemicals and reagents

ILO (purity 99.8%) was procured from Jiochem Ltd (Jinan, China). Olanzapine (OLA) (purity 99.4%) used as an internal standard (IS) was a generous gift by Aum Laboratories (Ahmadabad, India). SPE cartridge, Celerity Deluxe DVB-LP 30 mg/ml and heparin sodium were purchased from Orochem India Pvt. Ltd. (Mumbai, India) and Biological E Ltd. (Himachal Pradesh, India) respectively. HPLC grade acetonitrile and analytical grade ammonium formate were purchased from Merck (Mumbai, India).

Standard solution preparation

A stock solution of ILO (0.5 ng/ml) was prepared in water: acetonitrile (50:50) as dihent. From the stock solution, working standard solution of 2.5 µg/ml concentration was prepared. A series of working calibration standards were prepared from working standard solution in order to have the final ILO concentration of 0.5, 1, 5, 10, 20 and 100 ng/ml in 200 µl rabbit plasma. The IS stock solution was prepared, having a concentration of 0.2 mg/ml in dihent. From this, aliquot was diluted to get 2000 ng/ml working IS solution. Finally, all prepared working standards were spiked with 10 µl working IS solution and vortexed for 30 sec before extraction procedure.

Sample preparation

For current development, different types of SPE cartridges were tried. Out of them, Celerity Deluxe DVB-LP cartridge was selected. Initial conditioning was carried out with 1 ml acetonitrile followed by 1 ml water. Amount of plasma sample loaded was 0.2 ml, followed by 1 ml water as washing solvent and finally elution was carried with 0.5 ml acetonitrile. Collected eluent was evaporated under nitrogen air and reconstituted in 100 µl mobile phase. All the reconstituted samples were analyzed by the developed method.

Chromatographic conditions

The liquid chromatography was performed using Jasco system having a binary gradient pump and auto injector. Detection was performed on AB Sciex (USA) manufactured API 2000 MS/MS system. The chromatographic separation was achieved using Kromasil C18 (50*4.6 mm, 5 µm). After the constructive analysis in order to develop a fast LC method, recommended chromatographic parameters were isocratic mode at a flow rate of 0.4 ml/min. Acetonitrile: buffer (90:10) was used as mobile phase where buffer was 0.01M ammonium format pH 5.5 in mill Q water. The pH of mobile phase was adjusted to 5.5 using 5 % formic acid or ammonia. Finally, mobile phase was filtered with 0.45 µm membrane filter and sonicated to remove dissolved gases. The column oven was maintained at room temperature throughout the study. The eluted peaks of ILO and OLA were detected by a mass spectrometer in MRM mode. The detection parameters set for ILO and OLA were peaks of ILO and OLA were detected by a mass spectrometer in MRM mode. The detection parameters set for ILO and OLA were

Optimization of sample preparation

Due to the complex nature of plasma, sample pre-treatment is often needed to remove proteins and other potential interferences prior to analysis. For this, protein precipitation (PPT) SPE and liquid-liquid extraction (LLE) methodologies are widely used [11]. Preliminary trials were carried out by the easiest way of extraction that is protein precipitation using acetonitrile. In the initial trial, 1500 µl acetonitrile was added to 500 µl rabbit plasma, vortexed for 2 min and centrifugated at 10000 RPM at a −10 °C for 10 min. Supernatant was collected and injected into LC system. The consistency and recovery of the method were not appropriate, so PPT method was not used further. Secondary trials were carried out using SPE process. As mentioned in sample preparation, different type of SPE cartridges like celerity deluxe DVB-LP, sagacity DVB HL, strata-XA 33µ polymeric strong anion and strata-X were used for extraction of analytes. But much better extraction was achieved using celerity deluxe DVB-LP cartridge which was further used in the study for sample preparation of ILO and OLA from rabbit plasma.

Method development and optimization

The primary goal of any quantitative analysis is good resolution of all sample components with accurate measurement of lowest concentration with fast analysis. HPLC method development for most of the pharmaceutical samples is carried out with reverse phase mode. Octylsilane and octadecylsilane columns are most widely used. Octylsilane columns have less retention for non polar substances than octadecylsilane and sometimes non polar analyte might be eluted in void volumes [12]. So far, faster elution with appropriate retention time, Kromasil C8 (octadecylsilane) column was used in the study. As the method is going to develop on LC-MS/Volatile buffers such as ammonium formate and ammonium acetate were tried in mobile phase. The sensitivity for ILO was hampered while using acetate buffer and much better while using ammonium formate, which might be due to ion pairing of drug in the MS/MS system. The composition of the mobile phase was optimized on the basis of retention time of an analyst. Different ratios of ammonium format buffer and acetonitrile were used running from 95:5 to 80:20, out of which the composition 90:10 was found optimum because as decreasing buffer concentration in mobile phase the response time reduces due to non polar nature of ILO and OLA. Finally, to achieve better sensitivity, shorter chromatographic analysis time and symmetrical peak shapes, mobile phase composed of acetonitrile: ammonium format buffer (pH 5.5) having a ration of 90:10 was optimized and peak of ILO and OLA were obtained at retention time of 2.5 and 3.2 min respectively as shown in fig. (2).

Validation of the method

The developed method was validated under lighting of bioanalytical validation guidelines for parameters such as specificity, linearity, lower limit of quantification (LLOQ), accuracy, and precision studies. Representative chromatograms of blank rabbit plasma, blank plasma spiked with OLA and blank plasma spiked with ILO are shown in fig. (2). As per LC-MS/MS chromatograms, there is no endogenous peak at retention time of ILO and OLA which indicates the method is specific for ILO and OLA. The linearity of the method is its ability to extract test results that are straight or by well defined mathematical conversion, compared to the application of analyte in a working range [13]. For this method, linearity was covered from 0.5 to 100 ng/ml. As per bioanalytical guideline IF5/N ratio is more than 10 then the represented concentration can be considered as LLOQ level. With this consideration linearity lowest level 0.5 ng/ml having S/N ratio more than 10 so it is consider as LLOQ for the method. The peak area ratio of ILO/OLA compared to the concentration of ILO was plotted linear regression method (1/x + x) weighting supplied to generate a calibration curve. The curve showed excellent linearity and good results of back calculated concentration over the selected range of 0.5-100 ng/ml and reported in table 1. A typical regression equation (y = 0.285x + 0.122) was founded with a
correlation coefficient (r) of 0.9996 as shown in fig. (3). The precision and accuracy of the method was calculated using linear regression equation and it should be less than 15% CV as per guideline (found less than 3% CV) for precision and accuracy was between 95.5 to 102%. These indicates the method is precised and accurate. Ones the method check specificity, accuracy, precise and linear over a range of 0.5-100 ng/ml then it was used for calculating unknown samples.

Fig. 2: Representative LC-MS/MS chromatograms of ILO and OLA in rabbit plasma (1) blank plasma at 427.0/190.1 (2) blank plasma at 313.1/256.2 (3) Standard spiking of ILO in plasma (4) Standard spiking of OLA in plasma. The retention times for ILO and OLA (IS) are approximately 2.5 and 3.2 min respectively.

Fig. 3: Linearity of standard preparation range from 0.5 to 100 ng/ml and least square regression equation

Table 1: Validation parameters results of ILO by proposed LC-MS/MS method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRM mode</td>
<td>427.0/190.1 (ILO)</td>
</tr>
<tr>
<td></td>
<td>313.1/256.2 (OLA)</td>
</tr>
<tr>
<td>Linearity range</td>
<td>0.5-100</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td></td>
</tr>
<tr>
<td>Coefficient of</td>
<td>0.9993</td>
</tr>
<tr>
<td>determination (r^2)</td>
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<tr>
<td>Correlation coefficient (r)</td>
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</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.285x + 0.122</td>
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<td>Limit of Detection</td>
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<tr>
<td>LOD (ng/ml)</td>
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<tr>
<td>Limit of quantitation LOQ (ng/ml)</td>
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<tr>
<td>Accuracy</td>
<td>95.5-102%</td>
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<td>Precision (% CV)</td>
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</tr>
</tbody>
</table>

Method application

The developed method was applied to study pharmacokinetic of ILO in rabbit after an intramuscular administration of ILO suspension. The plasma concentration (ng/ml) of ILO was calculated from the linearity regression equation and graph of concentration versus time (h) is shown in fig. (4). The pharmacokinetics parameters derived from plasma concentration profile are reported in table 2. The linear trapezoidal rule was applied for calculation of total area under the observed plasma concentration-time curve (AUC). The elimination rate constant (ke) calculated by the terminal log-linear decaying phase and it was found about 0.007 h^{-1}. Lower value of ke indicates very high mean half life (T1/2) of drug (96 h). From the drug release profile it is observed that, there is increased plasma concentration of...
ILO which is due to initial burst release and the $C_{\text{max}}$ of ILO was found to be 55.4 ng/ml achieved within 1.25 h ($T_{\text{max}}$) from the time of administration of intramuscular injection. Further, it was observed that, between 24 h to 96 h of administration of injection, ILO release is affected by sudden burst release, which leads to high fluctuations in plasma concentration. In the later stage, of study drug elimination was observed starting from 192 h to last point (336 h) of blood collection. The reason behind this fluctuation is administration of simple suspension of ILO without any excipients hence it can be considered as non-formulated ILO preparation.

**CONCLUSION**

In the present work rapid, sensitive and fast LC-MS/MS method has been developed and optimized. Very high extraction efficiency (more than 95%) with lowest detection limit (0.5 ng/ml) and simple chromatographic conditions make it applicable to study pharmacokinetic parameters of ILO in rabbit. Animal study shows that remarkable release of ILO after an intramuscular injection to rabbit. Release of ILO was observed up to 14 days, and in future if the issue of initial burst release is controlled using formulation techniques then the developed formulation can be used as long acting formulation. Thus, in vivo study result provides a very informative platform for the development of long acting dosage forms.

**ACKNOWLEDGEMENT**

We would like to thank B. V. Patel Pharmaceutical Education and Research Development (PERD) center Ahmadabad, India, for providing chromatographic instrumentation facility.

**CONFLICT OF INTERESTS**

Declared None

**REFERENCES**


