BIOANLYSIS OF ILOPERIDONE IN HUMAN PLASMA BY USING LC-MS/MS WITH ELECTRO SPRAY IONISATION TECHNIQUE AS INTERFACE

Amgad A. Awad El-Gied[1], Yuvaraj Y[2]., #J.S.K. Nagarajan[2], Dr. Murali Munisamy[3]

[1]Omdurman Islamic University, Sudan.

[2]Department of Pharmaceutical Analysis, JSS College of Pharmacy, RockLands, (Constituent College of JSS Academy of Higher Education and Research, Mysuru) Ootacamund, The Nilgiris, Tamil Nadu, India.

[3]Associate Professor, Associate Professor, Department of Translational Medicine, Member – IHEC, AIIMS, Bhopal

Abstract:

Iloperidone is atypical antipsychotic drug and its IUPAC name is 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1- piperidinyl]propoxy]-3methoxyphenyl]ethanone. The literature reveals that few methods established for the quantification of Iloperidone in biological fluids. We have developed and validated a simple, sensitive and rapid method for the quantification of Iloperidone in spiked human plasma by using LC-MS/MS. The seperation conditions for iloperidone by using Hibar® Octadecasilanecolumn (15cm x 4.6mm; 5µm) as stationary phase. Mobile phase composed of cyanomethane and 10mM ammonium formate (pH adjusted to 4.0 using formic acid) in the ratio of 85:15(v/v) at a flow rate of 0.5 mL/min. Injection volume was 10µL, ambient lab condition and the run time was 5.00 minutes. The quantification of Iloperidone and IS were achieved in positive ion mode in MRM (Multiple Reaction Monitoring) with retention times approximately 2.05 and 3.55 mins respectively. The precursor to product ion transitions and collision energies for Iloperidone and IS were m/z: 427.20→261.00 and 361.20→342.95 respectively. The overall precision of the matrix factor determined at lowest concentration was found to be 98.42% and highest concentration was found to be 99.54%. The analyte peaks were free from interferences in plasma samples. The method was validated as per USFDA guidelines which shows a high degree of precision and accuracy. Limit of Detection of Iloperidone and Limit of Quantification was found to be 0.15 ng/mL and 0.5ng/mL respectively.

Keywords: *Iloperidone, Bioanalysis, LCMSMS-ESI, Human plasma, Bioanalytical validation*

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1.Introduction

Iloperidone is atypical antipsychotic drug and its IUPAC name is 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1- piperidinyl]propoxy]-3methoxyphenyl]ethanone [1-4] The chemical structure of iloperidone is shown in Fig.1

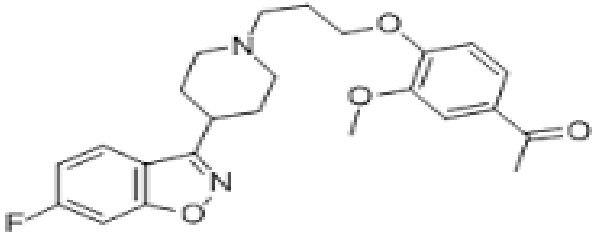

Fig.1

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The literature reveals that few methods established for the quantification of Iloperidone in biological fluids. TejasDadhaniya et al.,[5] had developed LC-MS/MS method for determination of iloperidone in Rabbit Plasma. Pranav S Shrivastav et al.,[6] had developed Stable-isotope dilution LC-MS/MS assay for determination of Iloperidone and its two major metabolites, P88 and P95 in Human Plasma. WwiyongLia et al.,[7] had developed simultaneous determination of Iloperidone and its two active metabolites in Human Plasma by LC-MS/MS.

The motto of the current study is to establish a simple, selective method for the quantification of Iloperidone in human plasma which employs Protein Precipitation for sample preparation using Liquid Chromatograph with triple Quadrapole MS/MS with electro spray ionization (ESI) as interface.

2. Experimental

2.1 Materials and reagents

Iloperidone and Prednisolone were procured from Indian Pharmacopoeia, New Delhi. Cyanomethane (Ranchem, Mumbai,), Ammonium Formate (S.D Fine Chemicals,). Milli-Q water was obtained by using a Milli-Q purification system.

2.2 LC-MS/MS Instrument and conditions

Separation of iloperidone was attained by using a LC system coupled with tandem quadrupole mass spectrometry (shimadzu 8030, Tokyo Japan) equipped with electrospray ionization (ESI) interface, LC-20AD pump, SPD-M20 PDA detector, CTO-20AC column oven, CBM-20 alite controller and SIL-20AC auto sampler. Data acquisition was performed using LC Solutions. Mass parameters optimized were as follows: DL temperature 250^{0}C, Temperature 200 ^{0}C, Detector voltage 1.3kv, Nitrogen gas was used as Nebulizer gas (1.5L/min) and drying gas (15L/min). Argon gas was used for collision induced dissociation (230kPa) experiments.

2.3 Seperation conditions

Hibar® Octadecasilanecolumn (15cm x 4.6mm; 5µm) was chosen as stationary phase. Mobile phase composed of cyanomethane and 10mM ammonium formate (pH adjusted to 4.0 using formic acid) in the ratio of 85:15(v/v) at a flow rate of 0.5 mL/min. Injection volume was 10µL, ambient lab condition and the run time was 5.00 minutes. The quantification of Iloperidone and IS were achieved in positive ion mode in MRM (Multiple Reaction Monitoring) with retention times approximately 2.05 and 3.55 mins respectively. The precursor to product ion transitions and collision energies for Iloperidone and IS were m/z: 427.20→261.00 and 361.20→342.95 respectively.

2.4 Preparation of calibration standard and quality control samples

Constituted 1mg/ml iloperidone, by weighing 100mg of iloperidone and solubilized in cyanomethane and made upto 100ml with cyanomethane. Nine calibration standards of Iloperidone were prepared using blank plasma spiked at different concentrations in the range of 0.5-5.0 ng/mL. Similarly prednisolen is also constituted by taking 100mg and dissolved and made the volume with cyanomethane and then further diluted and spiked to the blank plasma to maintain a concentration of 100ng/mL in each sample. The three quality control (QC) samples were similarly prepared at concentration of 1.0 ng/mL (LQC), 2.5 ng/mL (MQC) and 4.0 ng/mL (HQC).

2.5 Sample Preparation

Constituted 0.1 ml of Quality control sample into 1.0 ml centrifuge tube and 0.1ml of 100 ng/ml of internal standard solution, 0.5 ml of blank plasma and 0.3ml fo precipitating agent were added. Vortexed the resulting solution for 30 sec and centrifuged at 3500 rpm for 10

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min. Separated the supernatant layer and preserved for the analysis.

2.6 Method Validation

Method validation was performed based on the USFDA bioanalytical method validation guideline [8]. The current method was validated for as per the USFDA guidelines and all the values are within the guideline criteria.

3. Results and Discussion

3.1 Method Development and Optimization of Chromatographic Conditions

Liquid chromatography-tandem mass spectroscopy (LC-MS/MS) had led to major breakthrough in bio analytical method development because of its inherent speed, sensitivity and selectivity. The aim of this work was to develop and validate a simple, sensitive and rapid method for the quantification of Iloperidone in spiked human plasma. Mass spectrometer was operated in positive ion mode. The MRM transitions were selected as m/z 427.20→261.00 and 361.20→342.95 for Iloperidone and internal standard (Fig.2 & 3). In the optimization of chromatographic conditions use of mixture of organic solvents acetonitrile and methanol over single solvent in mobile phase had shown good response and symmetric peaks. Various buffers such as ammonium formate, ammonium acetate and formic acid were used with altered flow rates from 0.3 mL/Min to 0.7mL/min. Different mobile phase ratios like 90:10; 95:05; 85:15 were also tried. Different types of columns such as C_{18} and C_8 were used for the chromatographic separation. Finally, mobile phase composition of acetonitrile and and 10mM ammonium formate (pH adjusted to 4.0 using formic acid) in the ratio of 85:15(v/v) at a constant flow rate of 0.5 mL/min on a Hibar® C_{18} column(15cm x 4.6mm, 5µm) achieved good resolution with high sensitivity of Iloperidone and IS. The retention time of Iloperidone and IS were 3.55 and 2.05 mins respectively (Fig.4 & 5).

Fig.2: TYPICAL STANDARD MS -SPECTRUM OF ILOPERIDONE

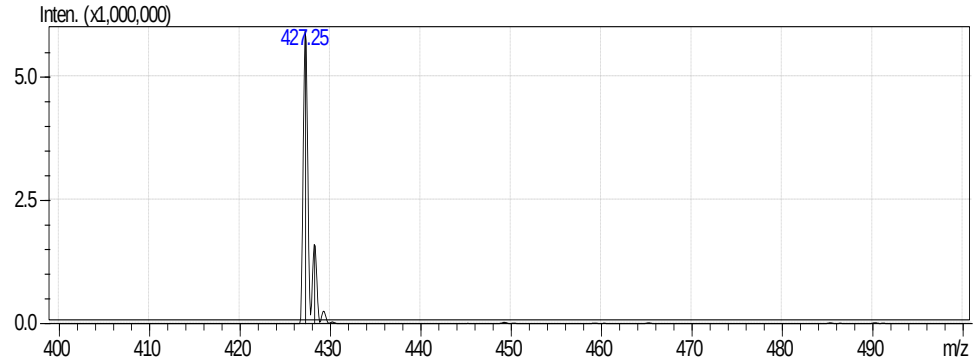

Fig.3: TYPICAL STANDARD MS -SPECTRUM OF PREDNISOLONE (IS)

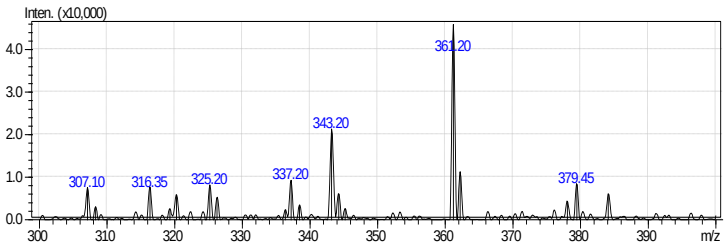

Fig.4: TYPICAL STANDARD CHROMATOGRAM OF ILOPERIDONE

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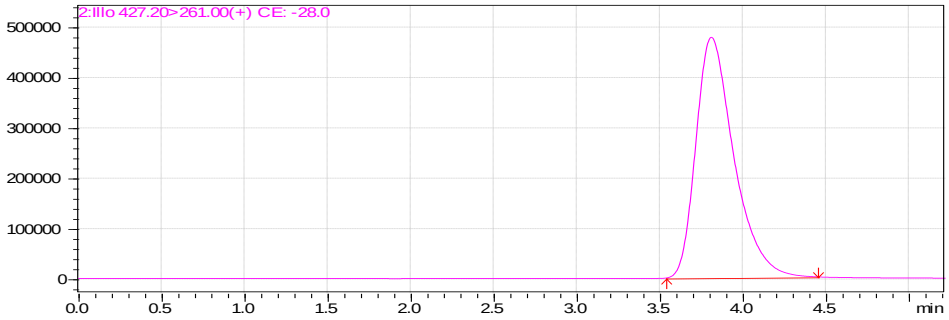
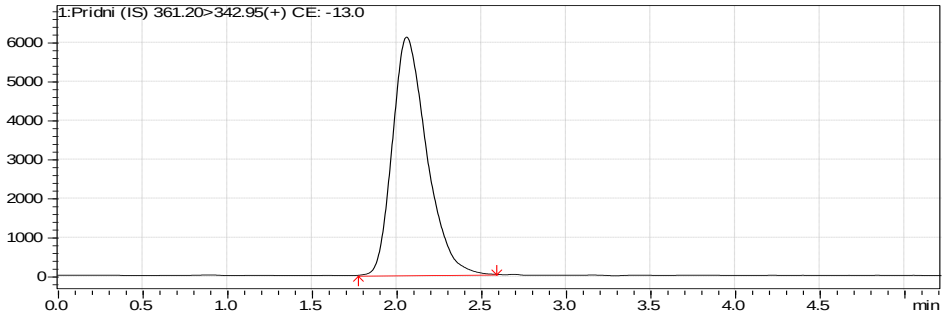


Fig 5: TYPICAL STANDARD CHROMATOGRAM OF PREDNISOLONE (IS)



3.2 Method Validation

3.2.1 Selectivity

No endogenous interferences were observed at the retention times of each analyte and the chromatograms are shown in the Fig. (6- 8).

Fig.6: TYPICAL STANDARD CHROMATOGRAM OF ILOPERIDONE IN PLASMA

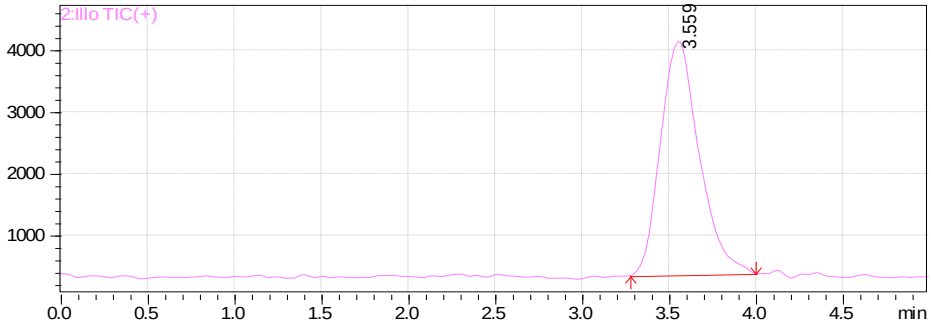


Fig.7: TYPICAL STANDARD CHROMATOGRAM OF PREDNISOLONE (IS) IN PLASMA

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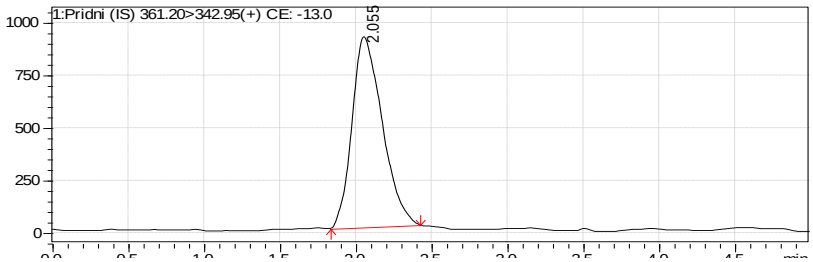
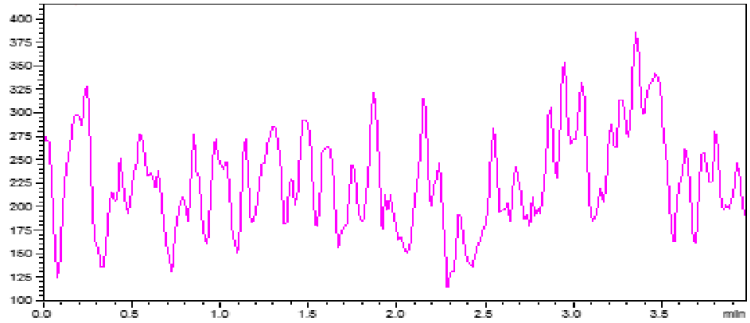


Fig.8: TYPICAL CHROMATOGRAM OF BLANK PLASMA



3.2.2 Linearity

The linearity for analyte was found to be 0.5-5.0 ng/ml. The r^2 value was found to be 0.998 and it is linear curve (Table.1)

Table 1. Linearity of Iloperidone

S.No.	Iloperidone Conc. (ng/ml)	IS Conc. (ng/ml)	Response Factor
1	0.5	100	0.553
2	1.0	100	1.688
3	1.5	100	3.097
4	2.0	100	4.385
5	2.5	100	5.395
6	3.0	100	6.597
7	3.5	100	7.824
8	4.0	100	9.012
9	5.0	100	11.836

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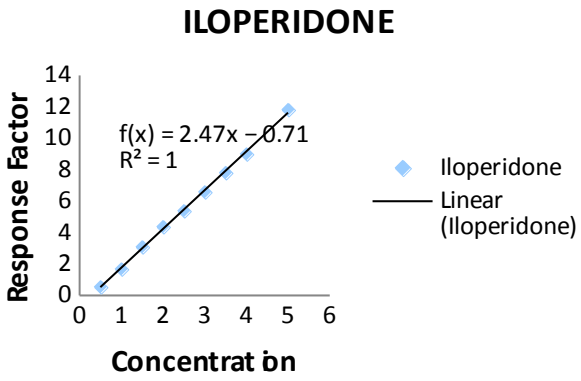
Fig.9: Linearity of Iloperidone



Linearity of Iloperidone

3.2.3 Matrix effect

Matrix effect was determined by the ration of peak area of post spiked concentration to the peak area of neat concentrations. The overall precision of the matrix factor determined at lowest concentration was found to be 98.42% and highest concentration was found to be 99.54%.

3.2.4 Accuracy and Precision

Accuracy, intra-day and inter-day precision were summarized in Table 2 & 3. All the values were in acceptance limit for all the analytes.

Table 2. Intra-day precision and accuracy data

Nominal concentration(ng/ml)	Measured concentration		
	Mean (ng/ml)	CV (%)	Recovery (%)
1.0	0.95	0.21	98.42
2.5	2.48	0.07	100.12
4.0	3.96	0.26	99.54

Table 3. Inter day precision and accuracy data

Nominal concentration(ng/ml)	Measured concentration		
	Mean (ng/ml)	CV (%)	Recovery (%)
1.0	0.92	0.83	92.00
2.5	2.45	0.81	98.00
4.0	3.93	0.14	98.25

3.2.5 Limit of Detection and Limit of Quantification

Limit of Detection of Iloperidone was found to be 0.15 ng/mL and Limit of Quantification was found to be 0.5ng/mL.

3.2.6 Stability studies

Stability studies in matrix were performed for freeze-thaw (three cycles), short term stability (25°C, 6h) and long term stability (-70ºC, 3weeks). No degradation of analytes were observed during the storage

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conditions. The stability studies were summarized in Table.4 which were found to be have acceptance limits for all analytes.

Table 4 Stability studies

Freeze and Thaw Stability			
Quantity of Iloperidone (ng/ml)	Obtained Conc.(ng/ml)	% CV	% Recovery
4.0	3.857	0.11	96.42
2.5	2.460	0.08	98.40
1.0	0.902	0.66	90.20
Short Term Stability (at Ambient Temperature)			
Quantity of Iloperidone (ng/ml)	Obtained Conc.(ng/ml)	% CV	% Recovery
4.0	3.854	0.11	96.36
2.5	2.458	0.25	98.33
1.0	0.900	0.43	90.05
Long Term Stability (-70ºC)			
Quantity of Iloperidone (ng/ml)	Obtained Conc.(ng/ml)	% CV	% Recovery
4.0	3.859	0.05	96.49
2.5	2.460	0.17	98.43
1.0	0.902	0.08	90.23

4. Conclusion

A rapid, selective and sensitive LC-MS/MS method was successfully developed for the quantification of Iloperidone in human plasma with a chromatographic run time found to be 5.00 min. The analyte peaks were free from interferences in plasma samples. The method was validated as per USFDA guidelines which shows a high degree of precision and accuracy. Limit of Detection of Iloperidone and Limit of Quantification was found to be 0.15 ng/mL and 0.5ng/mL respectively. The developed method can be used in clinical pharmacokinetics as well as in BA/BE studies.

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