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Analytical method development and validation for the determination of lurasidone HCL in pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A simple, accurate, precise and sensitive RP-HPLC assay method have been validated for the estimation of Lurasidone HCl in bulk and marketed pharmaceutical formulation.Lurasidone HCl is separated using Symmetry C18 ODS (4.6mm×150mm) 5µm particle size column and Methanol: Phosphate Buffer (0.05M-pH-4.8) (34:66) used as a mobile phase at a flow rate of 1.0ml/ minand effluent was detected at 315 nm. Here resolution was good, theoretical plate count and symmetry was appropriate. The retention time of Lurasidone HCl was found to be 2.248 minutes. Linearity was observed over concentration range of 30-60ng ml-1. The Limit of detection and limit of quantification was found to be 1.2ng ml-1 and 3.7ngml-1 respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98% to 102%. The above method was afforded excellent percentage recovery was found to be within the limits i.e. 98-102% The % RSD values were less than 2%.The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of this method. The method was successfully applied for determination of drug in tablets, wherein no interference from tablet excipients was observed, indicating the specificity of the developed method.The proposed method was found to be simple, precise, accurate, rapid, economic and reproducible for the estimation of Lurasidone HCl in bulk and marketed pharmaceutical formulation.

Keywords: Lurasidone HCl, RP-HPLC, Accuracy, Precision, ICH Guidelines.

INTRODUCTION

Chemically lurasidone is [(3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl) piperazin-1-ylmethyl] cyclohexylmethyl} hexahydro-4,7-methano-2H-isoindole-1, 3-dione hydrochloride and it is an azapirone derivative. Lurasidone hydrochloride appears as a white to light yellow crystalline powder and is stable and its solubility in chloroform and acetonitrile. It is sparingly soluble in ethanol and it is slightly soluble in water and acetone. The improvement of memory impairment due to MK-801 induction was found to be higher in lurasidone compared to other anti- psychotics and hence proved to be a clinically useful drug for cognitive impairments in treatment of schizophrenia.[1]

Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the

presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.[2]

MATERIALS AND METHODS

Table 1: Drug details

S. No.	Drug name	Formulation	Manufacturer	Procurement
1	Lurasidone HCl	Luratrend 80mg (2.5mg)	Sun Pharmaceuticals Ltd	Sura labs

Water

Molecular Formula: H_2O
Molar Mass: 18.015
Refractive Index: 1.333
Double distilled water HPLC grade is used as the mobile phase for analytical and preparative separations. Water for HPLC is purified and tested to ensure that is has low UV, absorbance to provide most sensitive detection across all wave lengths.

Methanol

Molecular Formula: CH_3OH
Molar Mass: 32.04 g/mol
Pka: 15.5
Refractive Index: 1.32
Dipole moment: 1.69
Methanol is known as methyl alcohol. Methanol is used as HPLC mobile phase for analytical and preparative analysis. It shows high UV-transmittance and low evaporation residue and suitable for gradient and isocratic condition. Methanol mixes with water it forms adduct which has a viscosity even higher than that of water.

Acetonitrile

Molecular Formula: CH_3CN
Molar Mass: 41.05192 g/mol
Refractive Index: 1.34
Acetonitrile is basically a polar solvent which is miscible with water but has less sufficient dispersive properties to elute substances from a liquid chromatography column by dispersive interactions with solute. Acetonitrile used as HPLC mobile phase for analytical and preparative analysis. Is shows high UV transmittance and low evaporation residue and suitable for gradient and isocratic conditions.

METHOD DEVELOPMENT

Selection of initial conditions for method development

A. **Determination of solubility of drug**

Table 2: Solubility of Lurosidone HCl

Solvent	Lurosidone HCl
Water	Very Slightly Soluble
Acetonitrile	Soluble
Acetone	Soluble
Methanol	Freely Soluble
Ethanol	Soluble
DMSO	Soluble
Dimethyl Formamide	Soluble
Dichloromethane	Freely Soluble

B. **Selection of chromatographic methods**

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected.

C. **Optimization of Column**

The method was performed with various columns like Hypersil C_{18} column, X- bridge column and X-terra (4.6 ×150mm, 5µm particle size), Symmetry C18 ODS (4.6mm×150mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

D. **Mobile Phase Optimization**:

Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer (0.05M-pH-4.8) (34:66) v/v respectively.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Phosphate Buffer (0.05M-pH-4.8) (34:66)
Column : Symmetry C18 ODS (4.6mm×150mm) 5µm particle size
Flow rate : 1 ml/min
Wavelength : 315 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 6 minutes

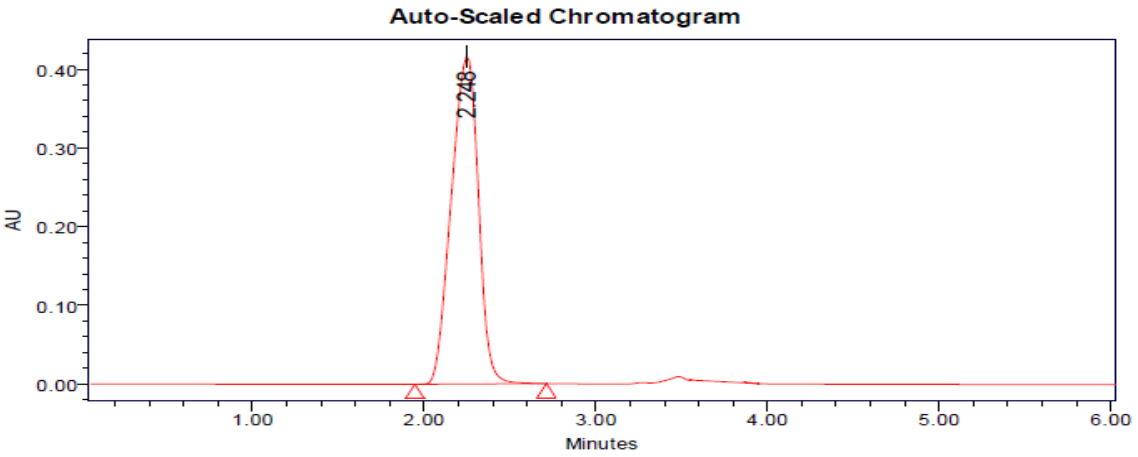

Fig 1: Optimized Chromatogram (Standard)

Table 3: Results of Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Lurosidone	2.248	1056524	63582	1.46	5876

In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So it is an optimized chromatogram.

Optimized Chromatogram (Sample)

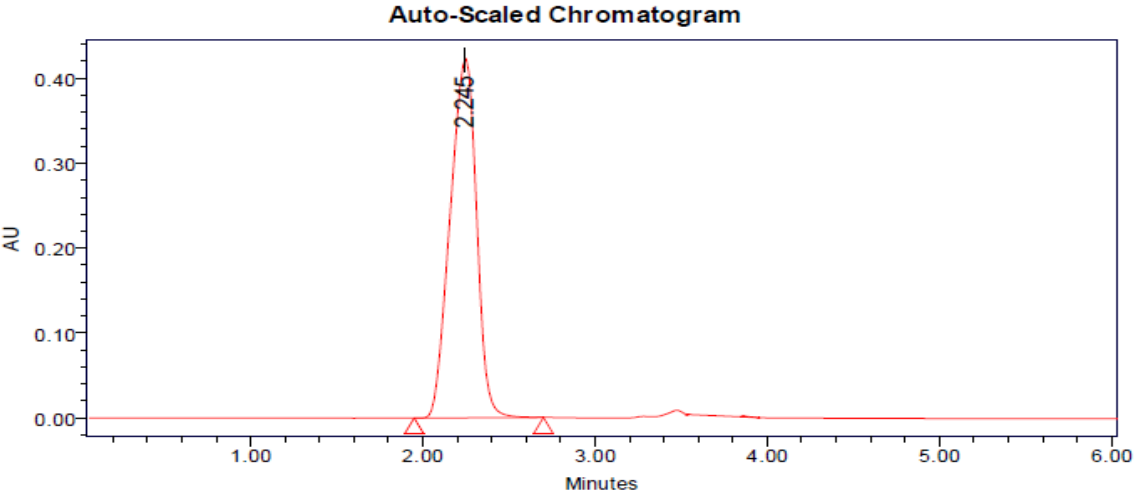

Fig 2: Optimized Chromatogram (Sample)

Table 4: Results of Optimized Chromatogram (Sample)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Lurosidone HCl	2.245	1068547	64587	1.48	5986

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

System Suitability

Table 5: Results of system suitability for Lurosidone HCl

S.No.			Area	Height		
1	Lurosidone HCl	2.260	1056859	63587	5846	1.46
2	Lurosidone HCl	2.262	1058745	63598	5874	1.47

3	Lurosidone HCl	2.262	1058474	63587	5869	1.46
4	Lurosidone HCl	2.255	1057586	63582	5874	1.46
5	Lurosidone HCl	2.263	1054874	63847	5846	1.47
Mean			1057308			
Std. Dev.			1551.432			
% RSD			0.146734			

%RSD of five different sample solutions should not more than 2.
The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.
Analytical method was tested for specificity to measure accurately quantitates Lurosidone HCl in drug product.

Assay (Standard)

Table 6: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lurosidone	2.241	1058745	63587	1.46	5879	1
2	Lurosidone	2.246	1056854	63589	1.47	5874	2
3	Lurosidone	2.245	1058462	63524	1.46	5869	3

Assay (Sample)

Table 7: Peak Results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lurosidone	2.248	1065874	64874	1.47	5986	1
2	Lurosidone	2.248	1066258	64258	1.48	5948	2
3	Lurosidone	2.247	1069854	64587	1.48	5964	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Lurosidone HCl in pharmaceutical dosage form was found to be 99.25%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Table 8: Chromatographic Data for Linearity Study

Concentration µg/ml	Average Peak Area
20	548745
30	806487
40	1056528
50	1285845
60	1538542

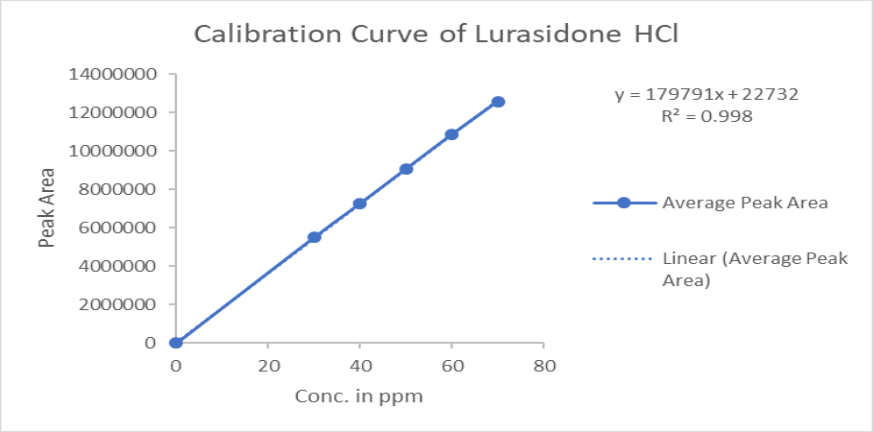

Fig 3: Calibration Curve of Lurosidone HCl

LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Letrozole is a straight line.
Y = mx + c
Slope (m) = 25499
Intercept (c) = 22732
Correlation Coefficient (r) = 0.99
The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Correlation Coefficient (r) is 0.99, and the intercept is 0.22732. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Table 9: Results of repeatability for Lurosidone HCl

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lurosidone HCl	2.255	1056524	63525	5869	1.46
2	Lurosidone HCl	2.258	1056485	63587	5874	1.47
3	Lurosidone HCl	2.252	1056985	63985	5896	1.47
4	Lurosidone HCl	2.253	1054874	63548	5846	1.46
5	Lurosidone HCl	2.258	1053652	63854	5863	1.46
Mean			1055704			
Std.dev			1398.475			
%RSD			0.132468			

%RSD for sample should be NMT 2.
The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 10: Results of Intermediate precision Analyst 1 for Lurosidone HCl

S.No.			Area	Height (µV)		
1	Lurosidone HCl	2.255	1065874	65245	6154	1.48
2	Lurosidone HCl	2.262	1068547	65241	6185	1.47
3	Lurosidone HCl	2.257	1069854	65231	6158	1.48
4	Lurosidone HCl	2.260	1065341	65784	6192	1.47
5	Lurosidone HCl	2.262	1065848	65842	6154	1.48
6	Lurosidone HCl	2.263	1065232	65894	6135	1.47
Mean			1066783			
Std. Dev.			1935.366			
% RSD			0.181421			

%RSD of Six different sample solutions should not more than 2.

Table 11: Results of Intermediate precision Analyst 2 for Lurosidone HCl

S.No.			Area	Height (µV)		
1	Lurosidone HCl	2.266	1075483	65842	5986	1.47
2	Lurosidone HCl	2.275	1078564	65285	5978	1.48
3	Lurosidone HCl	2.266	1078542	65365	5982	1.48
4	Lurosidone HCl	2.267	1078547	65985	5914	1.47
5	Lurosidone HCl	2.276	1078549	65421	5974	1.48
6	Lurosidone HCl	2.270	1076545	65487	5961	1.47
Mean			1077705			
Std. Dev.			1352.23			
% RSD			0.125473			

%RSD of Six different sample solutions should not more than 2.

ACCURACY

Table-12: The accuracy results for Lurosidone HCl

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	534704.3	20	20.078	100.390%	100.18%
100%	1043632	40	40.036	100.090%	
150%	1553443	60	60.030	100.050%	

The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION FOR LUROSIDONE HCL

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 1.2µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 3.7µg/ml

Robustness

Table 13: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	1056524	2.248	5876	1.46
Less Flow rate of 0.9 mL/min	1098698	2.879	5986	1.42
More Flow rate of 1.1 mL/min	1021454	1.915	5784	1.45
Less organic phase	1012431	1.916	5642	1.43
More organic phase	1005874	1.950	5465	1.44

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lurasidone HCl in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Lurasidone HCl is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. Methanol: Phosphate Buffer (0.05M-pH-4.8) (34:66) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Lurasidone HClin bulk drug and in Pharmaceutical dosage forms.

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