RP-UPLC Method Development and Validation for the Quantitative Determination of Lurasidone Hydrochloride

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Abstract

A simple, rapid, specific, linear, stability indicating reverse phase ultra-performance liquid chromatographic (UPLC) method was developed and validated as per the ICH guideline for quantitative determination of Lurasidone HCl, an antipsychotic drug. This method was performed by using Acquity CSH, Phenyl-Hexyl C18, (100 mm x 2.1 mm) column with 1.7 µm stationary phase particle size. 0.1 % Perchloric acid in water is used as mobile phase A and 0.1 % per-chloric acid with mixture of 550 ml acetonitrile and 450 of purified water as mobile phase B. An injection volume 1.0 µL, constant flow rate of 0.6 mL/min, 60°C column oven temperature and 230 nm detection wavelength were used. The sampling rate was 5 points per second. In this method Lurasidone peak is well separated from its known, unknown and degradant impurities. The degradation of Lurasidone under various stress condition was studied. Linearity was performed as between 50% to 150 % of standard concentration range. Solution stability performed on day basis (1 day, 2 days and 3 days).Robustness study was performed changing ± 10 % flow rate, Temperature Changed± 5°C. The new RP-UPLC method is precise, accurate as well as rapid and qualifies all the criteria of linearity, stability, and robustness.

KEYWORDS: Lurasidone Hydrochloride, Degradation study, RP-UPLC, Method validation and development, Quantitative Analysis.

INTRODUCTION:

Lurasidone Hydrochloride [1,2,3] is a potent atypical antipsychotic drug in active pharmaceutical ingredient used as an antipsychotic drug. The chemical name of Lurasidone Hydrochloride is (3aR,4S,7R,7aS)-{(1R,2R)-2-[1,2-Benzisothiazol-3-yl)piperazin-1-ylmethyl] cyclohexylmethyl}hehydro-4,7-methano-2H-isoindole-1,3-dion,hydrochloride. (See **Table 1**).

Lurasidone is approved drug for treatment of schizophrenia and depressive episodes associated with bipolar I disorder. Lurasidone useful for treating the cognitive and memory deficits seen in schizophrenia. Lurasidone has activity at several serotonin receptors that involve in learning and memory and unlike most other antipsychotics lacks any anti-cholinergic effect which are known to impair cognitive process and memory.

The mechanism of activity of Lurasidone HCl, as with other drugs having efficiency in schizophrenia, is unknown. It has been suggested that the efficiency of Lurasidone HCl in schizophrenia is mediated through a combination of central dopamine Type-2 and section Type 2(5HT2A) receptor antagonism .And it give Antipsychotic Activity. Lurasidone may be useful for treating cognitive and memory deficits seen in schizophrenia for several reasons different many other antipsychotics, Lurasidone does not block the muscarinic acetylcholine receptors, an action well known to impair learning and memory. Lurasidone has prominent activity at 5-HT1A, 5HT2A, 5HT7 and α2Cadrenergic receptors, all of which have been implicated in enhancement of cognitive function if modulated properly. Due to its low liability for extra pyramidal symptoms Lurasidone is unlikely to require co-administration of anticholinergics, which impair cognition in their own right. In animal studies lurasidone was found more active other than antipsychotics examined in receiving dizocilpine include learning and memory impairment, including risperidone, olanzapine, clozapine, aripiprazole and haloperidol. Side effect are Leucopenia, Neutropenia, Tachycardia, Blurred vision, Decreased appetite, Rhabdomyolysis, Dysuria, Amenorrhea, Dysmenorrhea Side effects are generally similar to other antipsychotics.

OBJECTIVE

Literature survey reveals that determination of Lurasidone HCl in biological fluids and rat plasma, methods such as LC/MS/MS [4,5],UV spectrophotometric [6,7,8], Pharmaco-Kinetic and Pharmaco-Dynamic [9,10] and HPLC [11].Infect no isocratic Reversed phase high performance liquid chromatography method has been found to estimate the Lurasidone HCl in tablet dosage form hitherto.

However the details stability indicating method with separation of known and degradant impurities separation are not reported. Thus herewith we are presenting the UPLC method for the assay of Lurasidone HCl.

The evolution of packing materials and its size led to the development of UPLC technique. According to van Deemter equation, decreased particle size increases the efficiency of separations, on other hand efficiency diminishes with the increased flow rates and/or linear velocities. At a particle size less than 2.5 µm, there is a significant gain in efficiency and the efficiency does not diminish at increased flow rates or linear velocities. By using smaller particle, speed and peak capacity can be extended to new, termed (UPLC).

Ultra performance liquid chromatography (UPLC) system take advantages of technological pace in particle chemistry performance, system optimization, detector design and data processing. When taken together, these achievements have created as improvement in chromatographic performance. UPLC remains the practically and principle of HPLC and along with that increase the overall interrelated attributes of speed, sensitivity and resolution. Speed allows a great number of analysis to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. Faster analysis and hence called as ultra-performance liquid chromatography, achieves both higher sample analysis throughput and better assay sensitivity. Analysis of operation cost and sample throughput UPLC cost advantages over HPLC.

Hence, Stability indicating reverse phase ultra-performance liquid chromatographic (UPLC) method for quantitative determination of Lurasidone HCl with separation of known and degradants impurities from analyte, method was developed and validated. Thus herewith we are presenting the UPLC method for the assay of Lurasidone HCl.

Table1: Chemical structure and name of Lurasidone HCl as well as its related impurities

Name	Chemical Structure and Name	Category
Lurasidone HCl	(3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2-benzisothiazole-3-yl)piperazine-1-yl methyl] cyclohexylmethyl} hexahydro-4,7-methano-2H-isoindole-1,3-dione,hydrochloride.	Active Pharmaceutical Ingredient

Name	Chemical Structure and Name	Category	Name	Chemical Structure and Name	Category
Impurity-A	3-(1-piperazinyl)-1,2-benzisothiazole (OR)3-(1-piperazin-1-yl)-1,2-benzothiazole.	Process Impurity Raw Material	Impurity-C	N-[4-(2-3aR,4S,7R,7aS)-4,7-methano-1*H*-isoindole-1,3-(2*H*)-dione.	Process Impurity Raw Material
Impurity-B	Trans (R, R)-3a, 7a-octahydro isoindolium -2-spiro-1-[4`-(1,2-benzo isothiazole-3-yl)] piperazine methane sulfonate	Process Impurity LUR-II Intermediat e stage	Impurity-D	3aR,4S,7R,7aS)-2-{(1,2)-2-[4-(1,2-benzisothiazole-3-yl) piperazine-1-yl methyl] cyclohexyl methyl} hexa hydro-4,7-methano-2H-isoindole-1,3-dione.	Process Impurity Raw Material

MATERIALS AND METHODS:

Lurasidone HCl standard, related impurities and test samples were received from Emcure Pharmaceuticals Limited, Analytical Research Centre, Hinjawadi, Pune. Acetonitrile, Glacial acetic, Perchloric acid used as highly pure and analytical grade. Purified water used as Millipore. Analytical balance and micro balance used as Mettler Toledo. Waters Acquity H-Class quaternary UPLC with PDA Detector and Empower-2 software used for data acquisition and calculations. All instruments are calibrated during method development and validation.

The standard and sample solutions were prepared by dissolving 50mg of Lurasidone HCl in 100 ml volumetric flask using binary solvent Acetonitrile: Water in 1: 4 proportions. Pipette the 25.0 ml above solution into 50 ml volumetric flask, and make up volume using same solvent.

Method Development:

The recent pharmaceutical analysis, UPLC used for the separation and quantification of various drugs. The principle of UPLC remains same as HPLC, however with increased speed, sensitivity and resolution. Speed allows a great number of analyses to be performed in shorter time thereby increasing sample throughput and lab productivity. The evolution of column packing materials primarily triggered the development of the UPLC. An increased efficiency of separations is observed with the decrease in particle size, while it diminishes on increased flow rates or linear velocities. Moreover, for a particle size smaller than 2.5µm significant gain in efficiency. Thus the UPLC technique is utilized for assay of Lurasidone HCl.

Lurasidone and its related impurities are polar molecules, therefore the method for the assay is developed using reversed phase chromatography. The stationary phase in reversed phase chromatography is non-polar like C4, C8, C18, while the mobile phase is polar such as water, acetonitrile, methanol and/or buffer solution. Thus during UPLC method development along with stationary and mobile phase other parameters such as column temperature, diluents, wavelength, and pH are also plays a crucial role. Stationary phase for UPLC method screened from particular Acquity CSH phenyl hexyl and Acquity BEH columns both with the C18 having 2.1 mm internal diameter and 1.7µm particle size. These columns with 50 mm and 100 mm length are used. When the BEH, C18 (2.1X 100 mm) 1.7µm is used in the method, broad of peak shape observed, and appropriate system suitability parameters such as tailing factor, theoretical plates are not met. Therefore change the column make, Acquity CSH phenyl hexyl, C18 (2.1X 100 mm) 1.7µm used for further development. When the Acquity CSH phenyl hexyl, C18 (2.1X 100 mm) 1.7µm is used in the method, the better separation of impurities, good peak shape, and appropriate system suitability parameters such as tailing factor, theoretical plates are met. The volatile buffer such as perchloric is selected for the mobile phase preparation. Thus 0.1% perchloric acid in water used as mobile phase A as well as mixture of 55:45 ratio of purified water and acetonitrile by adding 0.1% of perchloric acid in it, as mobile phase B. The appropriate linear program, flow rate, column oven temp is selected by performing different trial runs of standard preparation. Method development chromatographic conditions for Lurasidone HCI are listed in Table 2.

Table 2: Chromatographic conditions for the RP-UPLC method for assay of Lurasidone HCl

Component	Specification
Instrument	UPLC Acquity, H Class
Detector	UV, Photo diode array
Column	Acquity CSH Phenyl-Hexyl (2.1 x 100 mm) 1.7µm
Wavelength	230 nm
Flow rate	0.6 ml/min
Injection volume	1.0 µl.
Column oven temp.	60°C
Sampling rate	5 points/s
Run time	10 min

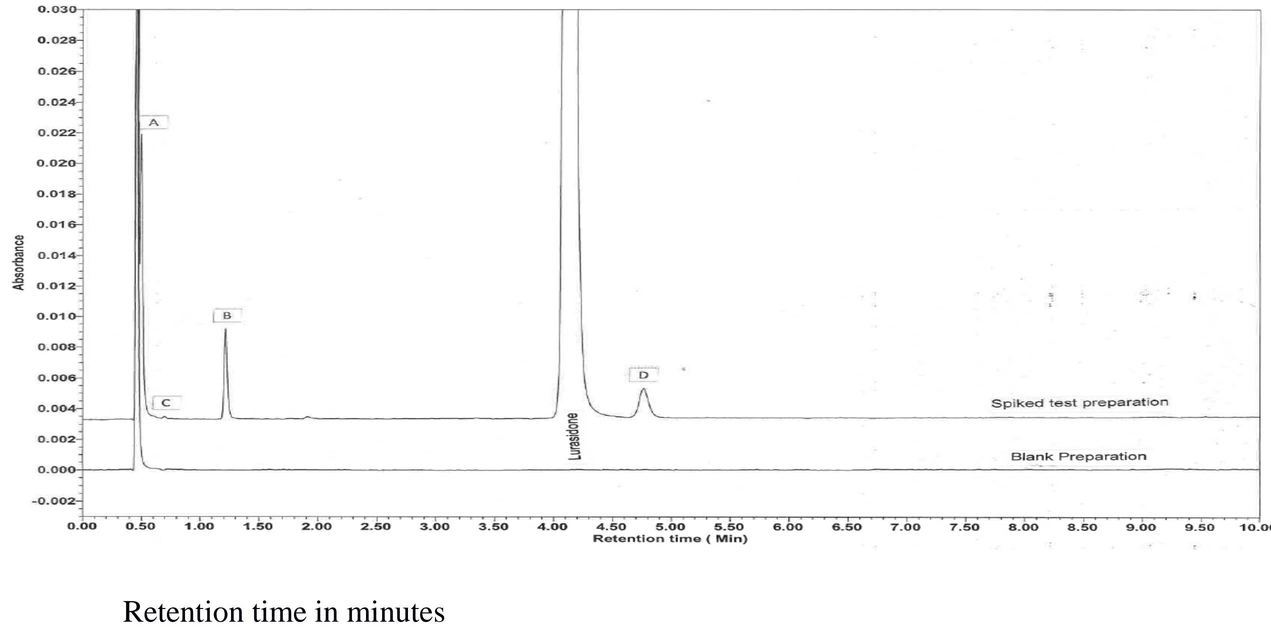
Furthermore the linear program have been used to perform the UPLC analysis, the composition of mobile phase A and B in 48:52 ratio have been used up to 10 min.

RESULTS AND DISCUSSION:

According to ICH guideline[12-18] different parameters has been tested and they are as fallows.

Specificity:

Specificity of method measures the analyte response in presence of related impurities. The assay method should present discrimination of the analyte and such impurities. Selectivity data is given in Table 3 and the related chromatograms are shown fig. 1. This is done by demonstrating that the assay results are unaffected due to the presence of spiked impurities with 1% concentration
(2.5 ppm) compared to the pure substance (250 ppm). Blank run do not shows any interfering peak with Lurasidone peak. The Lurasidone peak is well resolved from known impurities (fig. 1).Peak purity angle for Lurasidone peak and the peaks of its related impurities are below threshold in individual solutions of related impurities and unspiked as well as spiked tests. Peak purity angle of peak due to Lurasidone is less than the peak purity threshold in unspiked and spiked test preparation, which stated that peak purity passes. This indicates that these peaks are pure (**Table 3**). The % relative standard deviation (%RSD) in five replicates of Lurasidone is 0.07. Tailing factor and theoretical plates in prepared standard are 1.33 and 9184 respectively.



Retention time in minutes				
Impurity-A	Impurity-C	Impurity-B	Lurasidone	Impurity-D
0.499 min	0.697 min	1.214 min	4.118 min	4.763 min

Figure 1: Typical chromatogram for selectivity in the Lurasidone HCl assay over the spiked Impurities

Table 3:Selectivity data (spiked test preparation) for Lurasidone HCl.

	Unspiked test preparations			**Spiked test preparation**		
Eluent	**Retention time (min)**	**Peak purity angle**	**Peak purity threshold**	**Retention time (min)**	**Peak purity angle**	**Peak purity threshold**
Lurasidone	4.115	0.135	0.852	4.118	0.031	0.241

Solution Stability:

The stability of test prepared solution was performed at the room temperature on the day basis up to 3 days. The % assay of Lurasidone HCl was calculated for the study period of test preparation. % absolute difference between the individual results value and its Cumulative mean is not more than 2.0. % absolute difference values of assay are 0.53, 0.51, 0.60 and 0.62 for initial days 1, 2, and 3 respectively. It is within acceptance criteria (of % absolute difference less than 2.00) up to 3 days. This indicates that the test preparations are stable up to 3 days, when stored at room temperature.

Linearity and range:

Linearity of method is the measure of the proximity of the straight line obtained from a calibration plot of response versus concentration. It is obtained by performing the experiments using different concentrations of the Lurasidone HCl. Thus the linearity of present UPLC method is determined by using test solution with concentrations in 80–120 % of analyte as per ICH guidelines. The linearity data for 80,

90, 100, 110, and 120 % concentrations of standard preparation is given in Table 4. The peak area verses concentration data was treated by least squares linear regression analysis. The correlation coefficient obtained was 0.99997. The %Y intercept of calibration curve is 0.20 which is much below the allowed value 2.00. This is shows that less variation in different linearity concentration levels. All observed value and graph plotted for visual inspection (**fig. 2**) indicates that this method is linear for given range.

Table 4: Linearity data for the Lurasidone HCl assay from 80 – 120 % concentration range

Levels	Concentration (in ppm)	Peak area			
		Replicate-1	Replicate-2	Replicate-3	Average
1	124.64	398668	398092	398361	398374
2	199.42	633103	634141	635199	634148
3	249.27	793770	794740	798856	795789
4	299.13	958776	960264	959688	959576
5	373.91	1194280	1193725	1193370	1193792
	Correlation coefficient				0.99997
	Intercept				-1586.3626
	% Y Intercept				0.20

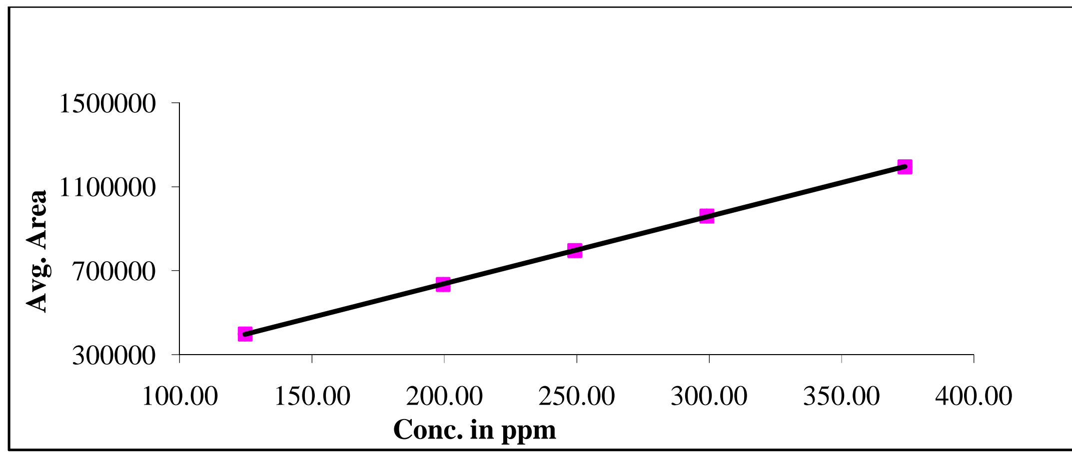


Figure 2: Linearity graph for the Lurasidone HCl assay in concentration range of 80 – 120 %.

Precision:

Precision provides an indication of random errors and can be subdivided into repeatability and intermediate precision. These precision studies are performed when the entire analytical method procedure is finalized. System precision is studies by carrying out five replicates of Lurasidone HCl standard. Comparative data of % assay of method precision and intermediate precision are shown in Table 5. The % RSD of five replicate Lurasidone HCl standards is 0.12. The method precision of assay has been performed by injecting six individual test sample preparation. The % assay and % RSD of test sample obtained are calculated. The intermediate precision is also

evaluated using different instruments and different columns by different analysts on different days in different laboratory. The % RSD for assay of method precision and intermediate precision study is 0.24 and 0.26 respectively. The overall % RSD of twelve test preparations (six each for method precision and intermediate precision) is 0.36.

Table 5: Comparative % assay data of method precision and intermediate precision for the Lurasidone HCl

Replicates	% Assay	
	Method Precision	**Intermediate precision**
1	100.69	100.10
2	100.69	100.08
3	100.11	99.75
4	100.48	100.09
5	100.74	99.60
6	100.69	100.30
Average	100.57	99.99
% RSD	**0.24**	**0.26**
Overall % RSD	**0.36**	

Robustness:

The robustness of analytical method is determined by purposely altering experimental conditions such as flow rate and column oven temperature. The flow rate was changed by ±10%. Actual flow rate of 0.60 ml/min is altered as 0.54 ml/min and 0.66 ml/min. The column oven temperature is changed with ± 5°C from 60°C in original method. In all above cases the retention times are varied by ± 0.2 min compared to actual retention times. System suitability parameter such as tailing factor is 1.28 to 1.32 and theoretical plates are 7786 to 10211. The % RSD for robustness studies are from 0.07 to 0.10. In all deliberate varied chromatographic conditions (flow rate and column oven temperature), significant change are not observed for the system suitability criteria like tailing factor, theoretical plates and % RSD. The values of these criteria are well within acceptance limit.

Forced degradation Studies:

Forced degradation[19-20] study gives a measure of specificity and it helps in selection of stability-indicating analytical procedures. The % degradation was determined by comparing chromatogram obtained under stress condition with untreated sample. The degradation studies include thermal, photolytic, humidity, aqueous, acidic, basic and oxidative stress conditions. The degradation data under these conditions are shown in Table 6.

In thermal degradation test sample was heated to 105°C for 12 h. Humidity degradation carried out with 75% relative humidity at 40°C for 24 h, while in the photolytic degradation the test samples were exposed to near UV light of 200 $W{\cdot}h{\cdot}m^{-2}$ intensity till the energy of 1.2 x 10^6lux.h. The physical appearance of the test solutions remained unchanged in heat, humidity and photolytic stress condition. All above test are performed using the present analytical method for the concentration of 250 ppm of

Lurasidone HCl. The peak area of the Lurasidone HCl remained constant without any degradant peak, which indicates that this molecule is stable for heat, humidity and photolytic stress.

The chromatograms of the acid, alkali and oxidation degradations are depicted in the **Fig. 3.** All known as well as degradants impurities are well separated from the Lurasidone peak and the peak purity criteria are also passed. Alkali degradation is performed by exposing test
sample to 7 ml of 1N Methanolic NaOH kept at room temperature for 18h. The sample is then neutralized with HCI and the analyzed on UPLC. The net 13.18 % degradation of Lurasidone is observed. Oxidation degradation has been performed by adding 7.0 ml of 50 % H_2O_2 to the test solution kept at room temperature only for 1.0 h 1h. The Lurasidone is degraded by 27.41 %.

In acid degradation study test sample exposed to 5.0 ml of 1N Methanolic HCI kept at room temperature for 15 h. The solution is then neutralized with NaOH. No any degradation is observed in acid condition Lurasidone HCl is quite stable in acidic condition. In aqueous degradation study test sample exposed to 5.0 ml of water at room temperature for 24 h.

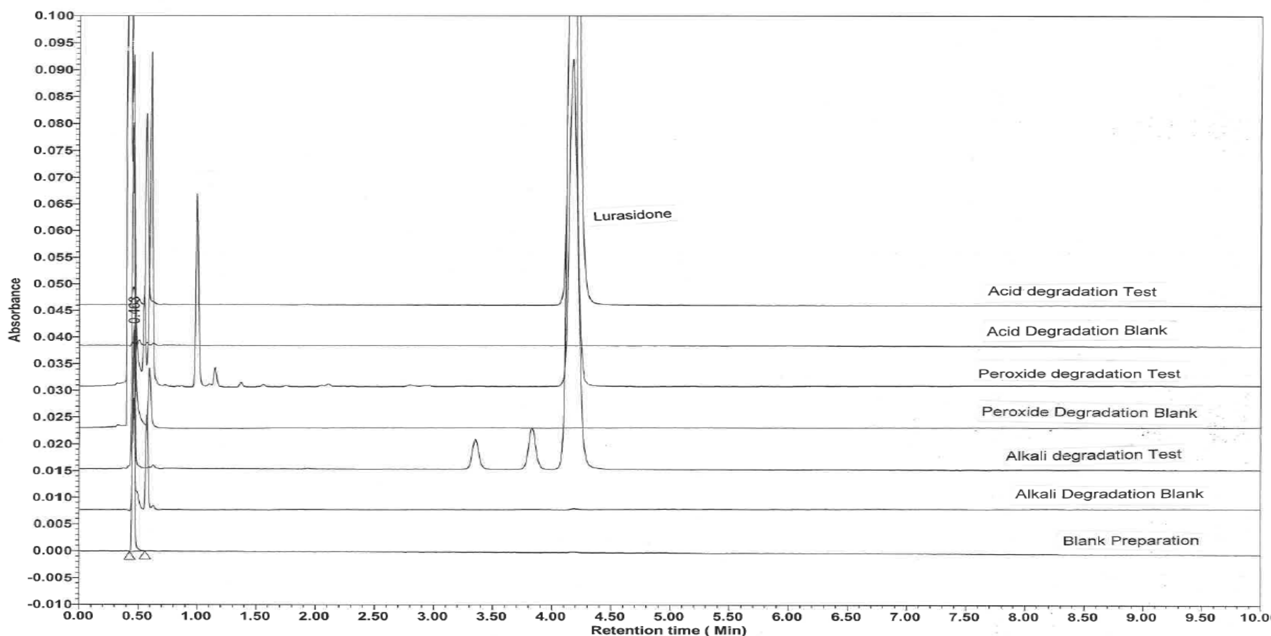


Figure 3:
Chromatogram for the acid, alkali, and oxidation degradation studies of Lurasidone HCl

Table 6: The stress conditions with % assay and % degradation of Lurasidone HCl

Stress (FD) Condition	Exposure period	% Degradation	Purity Angle	Purity Threshold	Peak Purity
Untreated		-	0.133	0.812	Pass
Heat	105°C for 12 Hrs	-	0.123	0.869	Pass
Humidity	75% RH and 40°C	-	0.143	0.816	Pass
Aqueous	5.0 mL kept for 24 hours at room temperature	-	0.139	0.794	Pass
Photolytic	1.2 million-lux hours and near UV at 200 watt hrs/m^2	-	0.128	0.821	Pass
Acid	5.0 mL 1N Methanolic HCl, kept at RT for 15.0hrs.	-	0.128	1.321	Pass
Alkali	7.0 mL of 1N Methanolic NaOH, kept at RT for 18.0 hrs.	13.18	0.037	0.246	Pass
Peroxide	7.0 mL 50% H2O2, kept at RT for 1.0 hrs.	27.41	0.033	0.239	Pass

CONCLUSIONS:

A new, accurate, linear, selective and stability indicating UPLC method for the quantitative analysis of antipsychotic drug Lurasidone HCl in active pharmaceutical ingredients is developed and successfully validated as per the acceptance criteria of pharmacopeia and ICH guidelines for validation of analytical methods.
The Lurasidone peak is well resolved from known and degradant impurities. The method is linear with correlation coefficient being 0.99997 and Y intercept of 0.20. The method and intermediate precision are evaluated and % RSD for assays are 0.24 and 0.26, respectively, with overall % RSD of 0.36. Robustness studies do not shows significant change for the system suitability criteria like tailing factor, theoretical plates and % RSD. The values of these criteria are well within acceptance limit.

The acid, heat, humidity and photolytic stress condition have not shown any change in the physical appearance of sample and the peak area of the Lurasidone HCl, indicating its stability for these stress conditions. In case of alkali and oxidation degradations all known as well as degradants impurities are well separated from the Lurasidone HCl peak and the peak purity criteria are also passed. The method was completely validated showing satisfactory data for all the tested method parameters.

In summary, the present stability indicating method is specific, linear, accurate, precise, selective, robust, as well as stable and can be used for the routine analysis in quality control.

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