ejpmr, 2015,2[5], 529-540.

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LURASIDONE BY RP-HPLC AND HPTLC

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Article Received on 08/07/2015 **Article Revised on 01/08/2015** **Article Accepted on 26/08/2015**

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ABSTRACT

Lurasidone is an atypical antipsychotic of benzisothiazol class used in the treatment of schizophrenia. The drug is not official in any pharmacopoeia and hence validated analytical methods are needed for its determination in tablet dosage form. The HPTLC method was developed with a mobile phase of methanol: ethyl acetate: toluene in the ratio of 1:1:8 v/v/v with a Rf value of 0.68 at a wavelength of 230 nm. Linearity was found over the concentration range of 5-80 ng/spot with a correlation coefficient of 0.99421. LOD and LOQ was found to be 1ng/spot and 5ng/spot. Recovery studies at 50% and 100% levels were between 97.9 to 101.11%. The HPLC method was developed with a mobile phase system of ammonium acetate(pH 5.5): acetonitrile: methanol in the ratio of 35:30:35, v/v/v at a retention time of 7.39 minute and flow rate of 1ml/min. Linearity of lurasidone was found over the range of 0.1-4.5µg/ml. LOD and LOQ values were found to be 0.001 and 0.005µg/ml. Recovery studies at 50% and 100% levels were between 98 to 100.6%. Both the methods were validated as per the ICH guidelines. These methods can be employed for the routine analysis of lurasidone in tablet dosage form.

KEYWORDS: Chromatography, HPLC, HPTLC, Lurasidone.

INTRODUCTION

The U.S. Food and Drug Administration approved Latuda (lurasidone HCl) tablets for the treatment of adults with schizophrenia on Oct. 28, 2010. The most prominent symptoms

include hallucinations, delusions, disordered thinking and behavior, and suspiciousness. Latuda is included in the atypical antipsychotic class of drugs and manufactured by Sunovion Pharmaceuticals Inc. of Fort Lee, N.J.[1] Lurasidone is (3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2- benzisothiazol- 3-yl)piperazin 1-yl methyl]cyclohexylmethyl} hexahydro - 4,7-ethano2H isoindole-1,3-dione[Fig. 1] with a molecular weight of 492.67g/l. The drug was found to be not official in any pharmacopoeia. The reported methods include UV method, HPTLC and HPLC techniques for its estimation in formulations and stability indicating methods[2-7] .So our current aim is to develop an economic and validated HPTLC and HPLC method which can be used for its routine analysis in tablet dosage form.

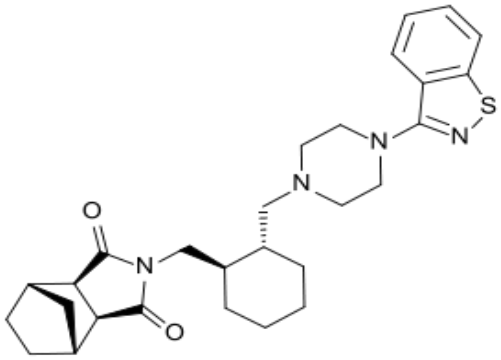

Fig. 1: Structure of Lurasidone

EXPERIMENTAL SECTION

Materials and Reagents

The HPLC grade solvents such as Water, methanol, ammonium acetate, acetic acid and acetonitrile were used, LR grade solvents such as toluene, ethyl acetate from Sigma-aldrich Chemicals Pvt. Ltd. were used. The other chemicals used for the buffer preparation are Potassium dihydrogen phosphate supplied by Qualigens Fine Chemicals Ltd. India.

Instrumentation and chromatographic conditions

Jasco V-600 UV/ Vis- spectrophotometer was used to record the spectrum. The HPTLC pre-coated silica gel $60F_{254}$ on aluminium sheets procured from Merck, of thickness 250µm[20cm x 10cm] was used as stationary phase. Sample application was done by using 100µl syringe and Camag Linomat V applicator. Linear ascending development was carried out in 20cm X 10cm twin trough glass chamber. The densitometric scanning was performed by using Camag TLC scanner III supported by Win cats soft ware. Evaluation of chromatogram was done by using peak areas.

For HPLC method Shimadzu Prominence UFLC equipped with Lichro CART C_{18}column (250mm×4.0mm, particle size 5µm**)**, LC-20 AD pump, SPD-M20A diode array detector, DGU-20A_3 degasser, SK-20 AC auto sampler and CTO- 10 ASVP column oven were used. Evaluation of chromatogram was done by using peak areas.

DEVELOPMENT AND VALIDATION OF HPTLC METHOD

Fixation of experimental conditions

Based on the solubility of the drug, methanol was selected as the solvent for the sample and ultra violet spectrum of lurasidone was recorded. The λmax of lurasidone was found to be 230 nm and was selected for the study [fig.2].

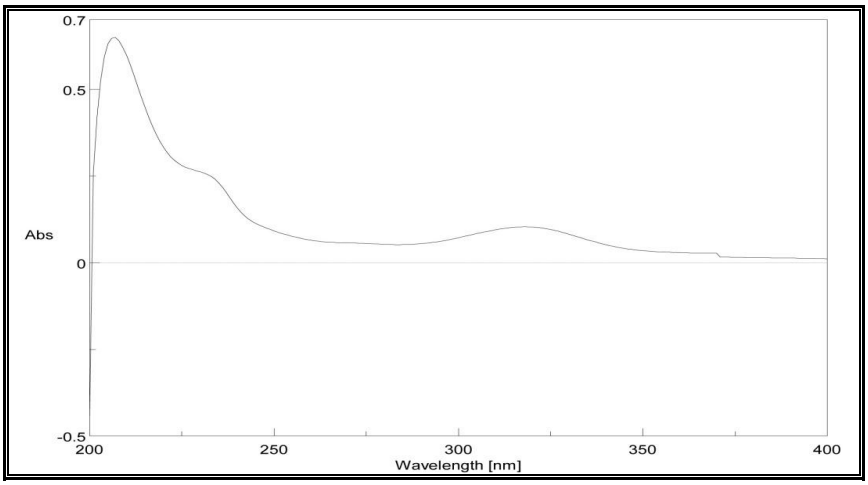

Fig. 2: UV spectrum of Lurasidone

Based on the trial and error with various solvents for the mobile phase and the saturation time, the optimum ratio of 1:1:8v/v/v of methanol:ethylacetate:toluene and the chamber saturation time of 30 minutes was fixed for good compact spots.

Method validation

Linearity

A stock solution of 10 µg/ml was prepared with methanol. Aliquots of lurasidone were applied on the plate to give a concentration of 5-80 ng/spot(Fig.3). The plate was developed, scanned and peak areas were noted.

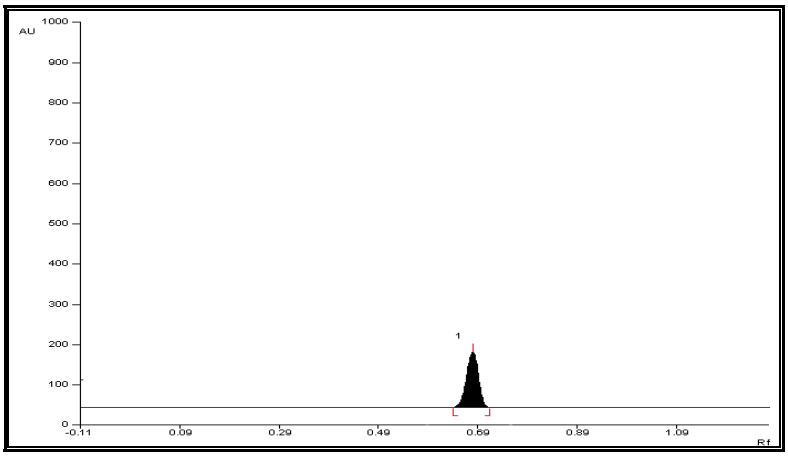

Fig. 3: Representative chromatogram of 5 ng/spot lurasidone solution

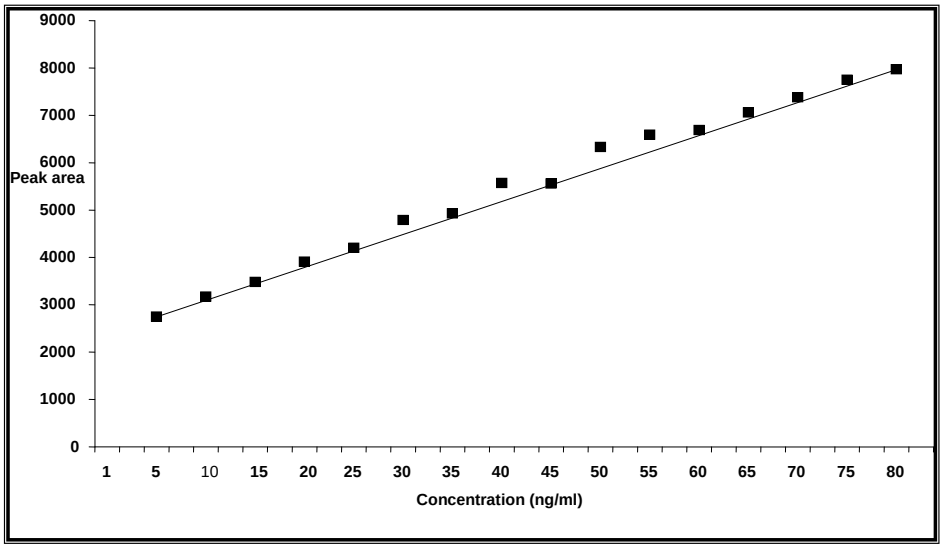

Fig. 4: Calibration graph of lurasidone

Accuracy

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out out at 50% and 100% levels. It was done by mixing known quantities of the standard drug with the analyzed sample formulation and the contents were reanalyzed by proposed method. The percentage recovery and its % RSD were calculated.

Precision

Intraday precision was studied by carrying out the analysis of the standard drug at two different concentrations in the linearity range of the drug for three times on the same day and % RSD was calculated. Inter day precision was studied by carrying out the analysis of the standard drug at two different concentration in the linearity range of the drug for three days

over a period of one week . Repeatability of sample application was studied by spotting 6 µl of drug solution six times on pre-coated TLC plate followed by development of plate.

Repeatability of measurement was determined by spotting 30ng/spot of drug solution on a precoated TLC plate and developed the plate and then scanned six times without changing the position of the plate. The %RSD was calculated for individual studies and values are tabulated below.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by applying decreasing amount of the drug in triplicate on the plate. The lowest concentration at which the peak is detected is called 'limit of detection' which was found out to be 1 ng/spot, (fig. 5). The lowest concentration at which the peak is quantified is called 'limit of quantification' which was found out to be 5 ng/spot (fig.6).

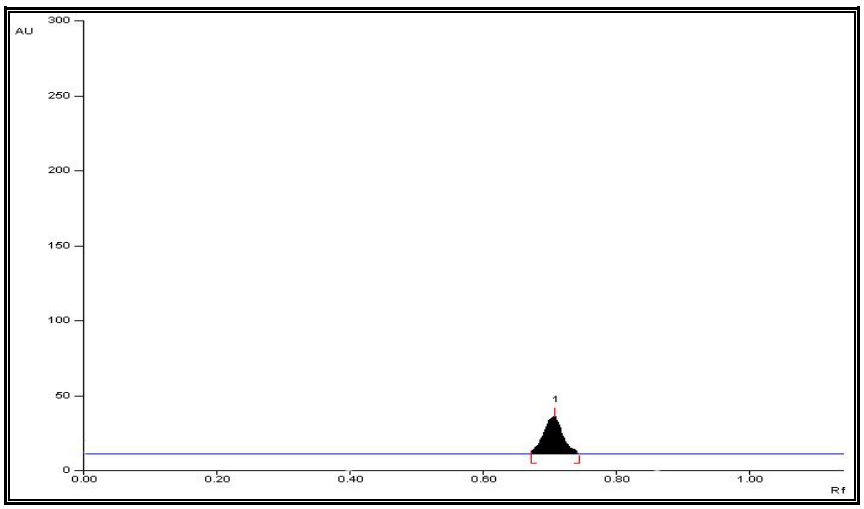

Fig. 5 : LOD of Lurasidone (1 ng/spot)

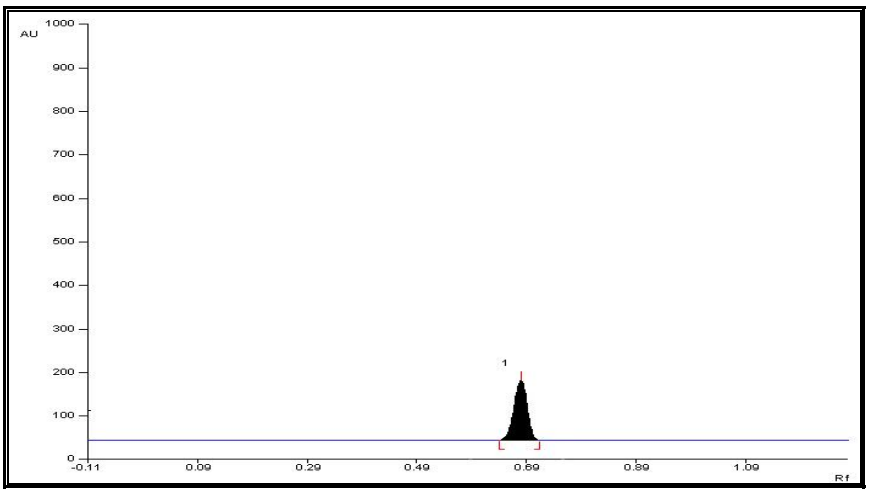

Fig.6: LOQ of Lurasidone (5 ng/spot)

Robustness

The robustness of the method was studied by varying the ratio of mobile phase and chamber saturation time and no appreciable changes in the peak area and peak symmetry were found.

Stability studies

Stability of lurasidone on the plate was studied at different intervals and the peak area were recorded. The developed plate was stable for three hours after which there was appreciable change in the peak area.

Analysis of formulation

Three tablets, each containing 20mg of lurasidone were taken for the studies and the average weight was determined. Quantity equivalent to 20 mg lurasidone was weighed and transferred to a 10 ml volumetric flask, extracted and made up to volume with methanol.Further dilutions were done to give a concentration of 10µg/ml solution.The 30 ng/spot solution was spotted, developed and detected for the peak areas.

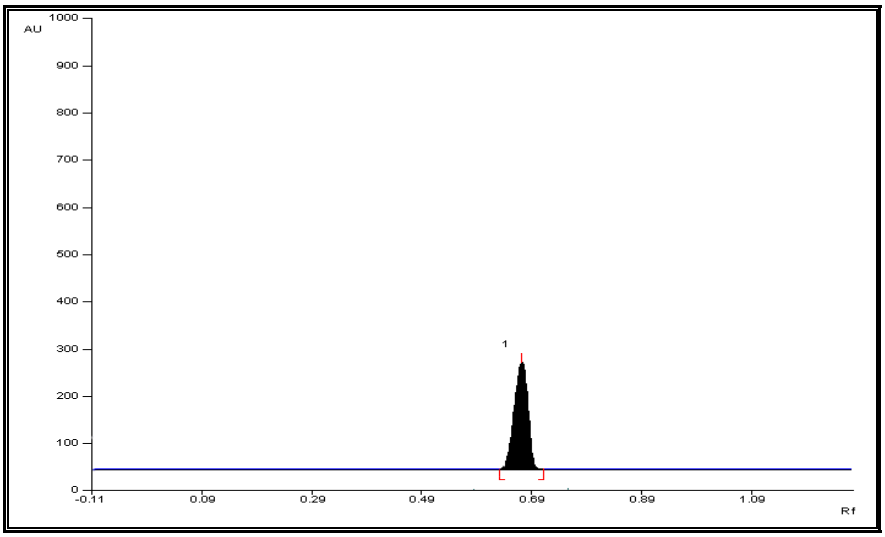

Fig.7: Chromatogram for the formulation solution.

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD

Optimized chromatographic conditions

Solvent selectivity, solvent strength (percentage of organic solvent in the mobile phase), strength of buffer, etc. were optimized to get the chromatographic conditions that gave the best separation with the ratio of mobile phase system consisting of ammonium acetate(pH 5.5)**:** acetonitrile: methanol (35:30:35) and flow rate of 1 ml/min. at 230 nm.

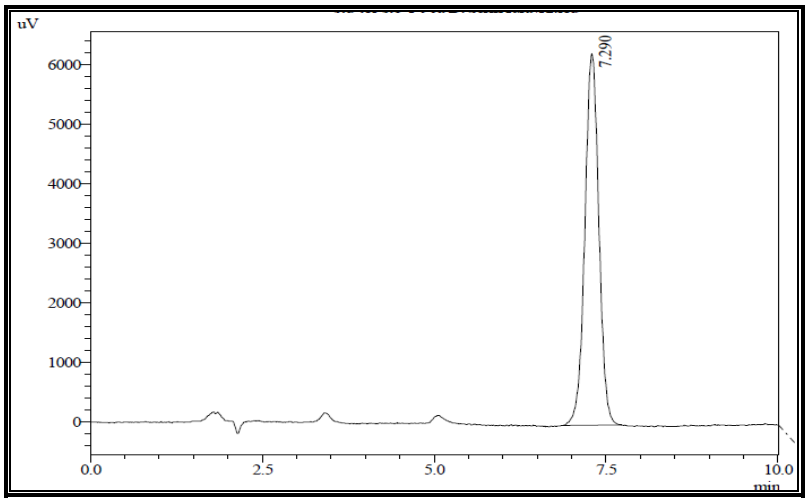

Fig 8: Representative chromatogram of Lurasidone

Linearity

From the stock solution of lurasidone (100µg/ml), standard solutions in the concentration range of 0.1 to 4.5µg/ml were prepared in methanol. These solutions were injected into HPLC system and chromatograms were recorded. The calibration graph is shown in figure 9.

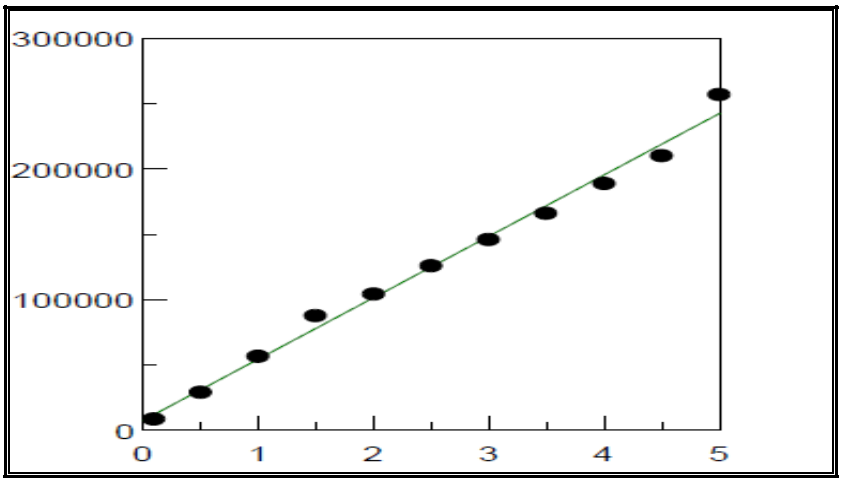

Fig.9: Calibration graph of lurasidone by HPLC.

Accuracy

Recovery studies of the drugs were carried out at 50% and 100% levels. It was done by mixing known quantity of standard drug with the analysed sample formulation and the contents were reanalysed by the proposed method. The percentage recovery and its %RSD were calculated.

Precision

Intraday precision was found out by carrying out analysis of standard drug solutions at one concentration in the linearity range for three times on the same day and % RSD was

calculated. Inter day precision was studied by carrying out the analysis of the standard drugs at one concentration in the linearity range of drugs for three days over a period of one week and % RSD was calculated. The standard solution (3 µg/ml) was injected six times and the response for each injection was recorded and %RSD was calculated.

LOD and LOQ

LOD and LOQ were calculated in terms of signal to noise ratio. LOD is the lowest concentration of the analyte that can produce a response detectable above the noise level of the system, typically, three times the noise level.LOD and LOQ were found to be 0.001 µg/ml (Fig.9) and 0.005 µg/ml(Fig.10) respectively.

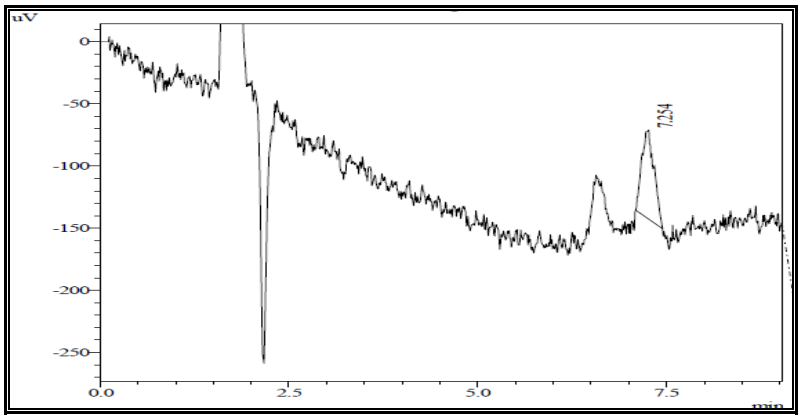

Fig 10: Limit of Detection - 0.001µg/ml

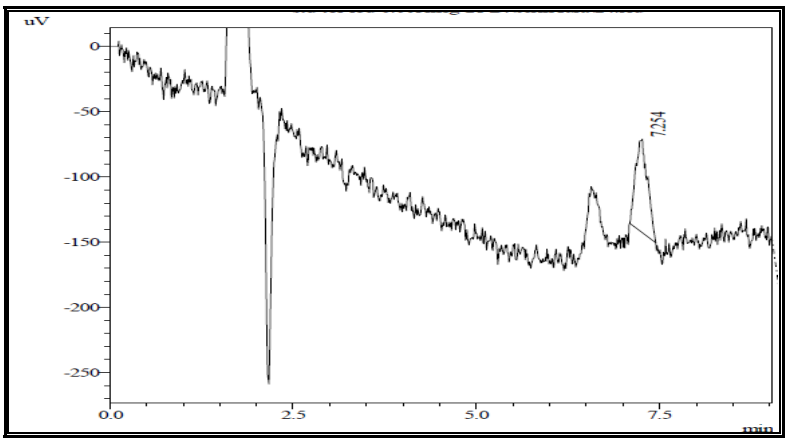

Fig 11: Limit of Quantification - 0.005µg/ml

Stability

Sample solution of Lurasidone was subjected to stability studies under room temperature. Stabilities were studied by looking for any change in retention time, resolution, peak shape, etc. when compared to chromatogram of freshly prepared solution. The solution stored under room temperature was stable upto 8hrs.

System suitability parameters

The system suitability parameters like tailing factor, plate count, asymmetric factor and resolution were calculated from the standard chromatogram.

Table I: System Suitability Studies

Tailing factor	Plate count	Assymetric factor	Resolution
1.2	3365.670	1.01	8.9

Robustness

In order to demonstrate the robustness of the method the ratio of methanol in mobile phase and the flow rate were varied and the chromatograms recorded. There was no appreciable change in the peak area and peak symmetry.

Analysis of formulation

Preparation of standard solution

Stock solution of Lurasidone 100 µg/ml was prepared using methanol. Suitable aliquots of drug solutions were transferred in 10 ml standard flask and diluted with methanol to get the concentrations ranging from 0.1-4.5 µg/ml.

Preparation of sample solution

Three tablets each containing 20 mg of Lurasidone were weighed and average weight of one tablet was calculated. Weight equivalent to 5 mg of Lurasidone was taken and transferred to a 50 ml standard flask and made up to volume with methanol (100µg/ml). From above solution, suitable aliquots of formulation solutions were prepared in methanol (Fig. 11).

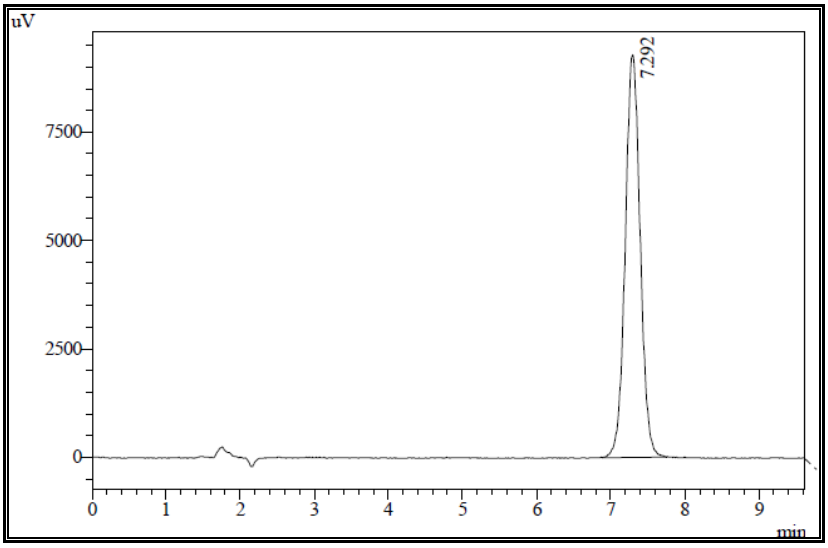

Fig.12: Chromatogram of lurasidone formulation.

RESULTS AND DISCUSSIONS

HPTLC method

For the determination of lurasidone by HPTLC method different mobile phase systems with Methanol:Acetonitrile, Methanol:acetonitrile:tetrahydrofuran, Methanol:Acetonitrile: Ethylether, Methanol:Acetonitrile:Dichloromethane, Methanol:Acetonitirle:Toluene, Methanol: Dichlorome -thane :Toluene, Methanol:Dichloromethane:Toluene:Acetic acid were tried. It was found that a system comprising of methanol: Ethyl acetate: toluene (1: 1: 8, v/v/v) was selected because this system gave good separation with symmetrical peak with an R_f value 0.68 at the selected wavelength of 230nm. The method was validated as per ICH guidelines. Calibration curve were plotted with peak areas of standard drug versus concentration. Linearity was found over the concentration range of 5-80ng/spot (r=0.99421). After the development, the plate was stable up to3 hours. Low relative standard deviation value shows that the developed method is precise. Limit of detection was found to be 1ng/spot and limit of quantification was found to be 5ng/spot. Recovery studies were carried out at 50%, 100% levels. Good recovery values show that the method is free from interferences. This method was successfully applied for the determination of lurasidone from tablet dosage form.

RP-HPLC Method

In RP-HPLC method, optimizations of different chromatographic parameters like selection of chromatographic method, detection wavelength, selection of mobile phase, ionic strength of mobile. phase, mobile phase ratio, flow rate etc. were done. A wavelength of 230nm was selected for the study. A mobile phase system consisting of ammonium acetate (pH 5.5): acetonitrile : methanol (35:30:35 v/v/v), was employed for the determination of lurasidone. With this system symmetrical peak and minimum tailing, retention time of 7.39minute was obtained at a flow rate of 1ml/min. The method was validated as per ICH guidelines.

Calibration curve was plotted using concentration (x) versus peak area (y). Linearity of lurasidone was found over the range of 0.1-4.5µg/ml, and correlation coefficient value was found to be 0.9985, showing good correlation between concentration and peak response. The LOD and LOQ values were found to be 0.001 and 0.005µg/ml respectively.

Precision of the developed method was studied under inter-day, intra-day and repeatability studies. A low relative standard deviation value shows that the developed method is precise.

Stability studies were carried for the standard solution and the solutions was found to be stable up to 8 hours under at room temperature.

Recovery studies were carried out at 50% and 100% levels. Good recovery values show that the method is free from interferences. System suitability parameters like number of theoretical plates (N), asymmetry factor (As), resolution (Rs) etc. were studied. The validated RP-HPLC method was applied to the determination of lurasidone from tablet dosage form.

Table II: Summary of the developed analytical methods

PARAMETER		HPTLC	HPLC
Linearity range		5-80ng/spot	0.1-4.5µg/ml
LOD		1ng/spot	0.001 µg/ml
LOQ		5ng/spot	0.005 µg/ml
% Recovery	50% level	97.9	98
	100% level	101.11	100.6
% label claim		98.26	98.13

ACKNOWLEDGEMENTS

The Authors are thankful to the SNR charitable trust for providing the laboratory facilities required for doing the work.

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