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Research Article

Development And Validation Of HPLC Method For The Determination Of Suvorexant In Pharmaceutical Dosage Forms

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Abstract

The present experiment was focused in developing an accurate and precise method for the estimation of Suvorexant in pharmaceutical Tablet dosage form. The mobile phase consisted of 65:35 % (v/v) of Methanol & 0.1% orthophosphoric acid solution operated on isocratic mode. The flow rate is 1.0 ml/min. Chromatographic determination of Suvorexant was performed on Agilent Zorbax 300°A Extend-C_{18} column (150 X 4.6 mm id, 5µm). The wavelength of detection is 248 nm. The injection volume is 20µL. The retention time of Suvorexant is 5.50 ± 0.10 minutes. The method was validated as per ICH guidelines and has been successfully used for the estimation in tablet dosage forms.

Keywords: Suvorexant; Insomnia; Neurokinin receptor; HPLC, MK-4305, Orexin Receptor

Introduction

The usage of orexin receptor antagonists for treatment of insomnia is gaining importance over the recent years. Almorexant was the first identified in 2007, [1] with equal affinity for OX_1R and OX_2R receptors. Suvorexant (MK-4305, ([(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan- 1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone)) **[Fig-1]** is similar in the mechanism of therapeutic action and has a diazepine based chemical structure. Suvorexant inhibits the wakefulness-promoting orexin neurons of the arousal system and promotes sleep [3–5]. The Food and Drug Administration has approved the usage of suvorexant in August 2014. Suvorexant must be administered atleast 30 minutes prior to sleep. At a recommended daily dose is 10 mg, the onset of action is within 1 hour with a peak plasma concentrations (250–300 ng/mL) occurring within 2–3 h [8]. In humans, the drug is extensively protein bound (99%) and has a good bioavailability of 82%. [2, 3, 7]. Fecal elimination (66%) is the major route of elimination for Suvorexant while the urinary elimination is approximately 23% [3]. Suvorexant is commercially available as (Belsomra®). The analysis of suvorexant by LC-MS/MS and GC-MS techniques in various biological fluids was reported earlier[6, 9]. We have developed the method and validated the method as per ICH Guidelines [9].

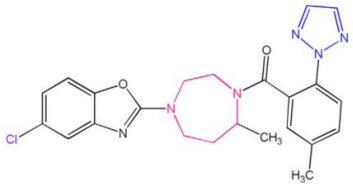


Fig-1: Structure of Suvorexant

2. EXPERIMENTAL

2.1. Reagents and chemicals

Orthophosphoric acid (GR Grade, SD Fine Chem Ltd), Methanol (HPLC grade, Merck ltd), Milli-Q water, Suvorexant (Reference standard purchased from Beijing Mesochem Technology Co. Ltd., China). All other chemicals are of the highest grade commercially available unless otherwise specified.

2.2. Apparatus and chromatographic conditions

A Shimadzu Class VP Binary pump LC-10ATvp equipped with a SIL-10ADvp Auto sampler and a CTO-10Avp Column Temperature Oven was used as the chromatographic system. Detection was facilitated by SPD-10Avp UV-Visible Detector connected to the SCL-10Avp System Controller. Data acquisition was done using LC Solutions software Version 1.23.

An Agilent Zorbax 300°A Extend-C_{18} column (150 X 4.6 mm id, 5µm) employing the use of mobile phase consisting of 65:35 % (v/v) of Methanol & 0.1% orthophosphoric acid solution at a flow rate is 1.0 ml/min by isocratic mode was found to be the most suitable for this estimation. The wavelength of detection is 248 nm. The injection volume is 20µL.

2.3. Preparation of standard solutions, Calibration Standards & Quality Control Samples

Calibration curve standards ranging between 2.16 – 10.80 µg/mL were prepared from a standard stock solution of Suvorexant (1 mg/mL in methanol) by using diluent solution (50:50 %v/v Methanol & Milli-Q water). Samples for Specificity were also prepared as required.

To evaluate the performance of the linear calibration curve, quality control samples representing 3 different levels (viz LQC, MQC & HQC) were prepared from a separate stock containing approximately the same concentration of the durg substance. The concentration of LQC, MQC and HQC are 2.70 µg/mL, 5.40 µg/mL and 8.10 µg/mL respectively

2.4. Assay

The assay of tablets containing Suvorexant (Belsomra®)is done using the procedure given in Indian Pharmacopoeia for tablets. Briefly, twenty tablets, each containing 10 mg of Suvorexant as labeled claim were weighed and finely powdered; a quantity of powder equivalent to 10.0 mg of Suvorexant was weighed and transferred to a 100 mL volumetric flask. To this 70 mL of methanol was initially added and vortexed thoroughly. The final volume is made up to volume with 0.1N HCl. The final solution was mixed well. This mixture is then carefully filtered using 0.45 µm membrane filter. The filtrate is then taken and suitably diluted and injected for analysis. The assay content was evaluated using the regression equation of linear calibration curve.

2.6 Method Validation

2.6.1 System Suitability

The system suitability was assessed by six replicate analysis of the drug at a concentration of 25.0µg/ml. The acceptance criterion is ± 2 % for the per cent coefficient of the variation for the peak area and retention times for the drug.

2.6.2 Detection and Quantitation Limits (Sensitivity)

Limits of detection (LOD) and quantification (LOQ) (**Fig-3**) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, and a precision (%CV) not more than 2%.

2.6.3 Linearity (Calibration Curve)

The calibration curve was constructed with eight concentrations ranging from 2.16 to 10.80 µg/mL. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (**Fig- 4**).

2.6.4 Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days).

2.6.5 Specificity

Specificity of the method was determined by comparing the Blank sample with that of the sample containing Suvorexant. (**Fig-5**). A less than 2% interference of the peak area at the retention time of the drug in the blank sample is taken as acceptance criteria for the analyte. Sample Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

2.6.6 Stability

The stability of the drug is determined by placing the MQC samples for the short term stability by keeping at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hrs was studied and established.

3.0 RESULTS AND DISCUSSION

3.1 Method Development and Validation

A 10 µg/mL solution of suvorexant is initially prepared and scanned for the wavelength maxima using a double beam UV Spectrophotometer. Suvorexant exhibited a λ_{max} of 248 nm **(Fig-2)** and accordingly the HPLC detection was also carried out at the same wavelength. The method was optimized with a view to develop a stability indicating assay method. Suvorexant is insoluble in water and has a pKa value of 2.19±0.02. The drug is freely soluble in methanol. Therefore we evaluated the chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 have been tried with different buffer salts such ammonium Formate, ortho phosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran. However less tailing and high theoretical plates are obtained with Agilent Zorbax 300^{o}A Extend-C18 column 150 X 4.6 cm 5µm. Mobile phase composition consisted of (65: 35v/v) of Methanol and 0.1% orthophosphoric acid in water on isocratic mode. The flow rate of the method is 1.0 ml/min. Calibration standards were prepared in diluents solution containing 50:50 % v/v of methanol and Milli-Q water. The column temperature is maintained at 35 ^{o}C. At the reported flow rate, peak shape was satisfactory. Hence 1.0 ml/min was optimized flow rate decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis.

3.2 Method Validation

3.2.1 System Suitability

The % RSD of the peak area and the retention time for both drug and internal standard are within the acceptable range (**Table-1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 9600 ± 25 and the USP tailing factor was 1.29 ± 0.1.

Fig-2: Scan Spectrum of Suvorexant

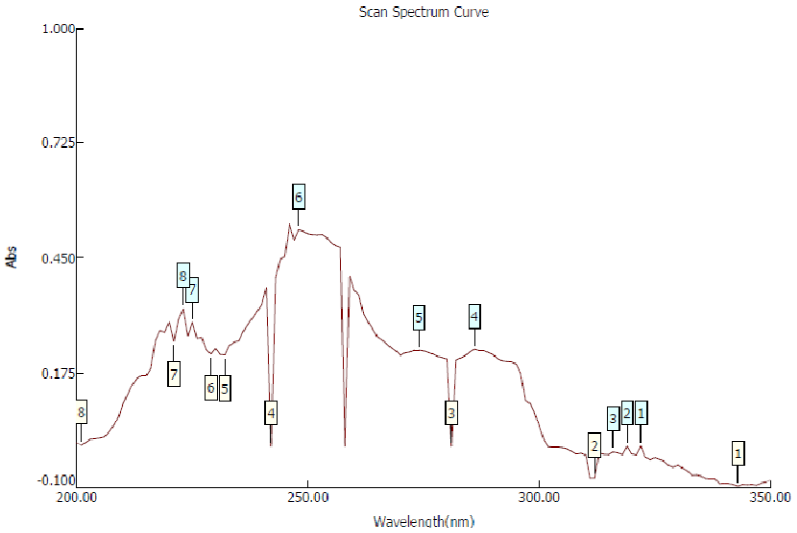


Table-1. System Suitability test for Suvorexant

S.No	Retention time (min)	Peak Area	Theoretical plates	Tailing factor
1	5.49	647882	4596	1.27
2	5.53	638619	4431	1.29
3	5.56	642434	4701	1.27
4	5.62	633349	4472	1.28
5	5.62	633562	4700	1.26
6	5.6	629665	4687	1.26
Mean	**5.57**	**637585.2**	**4597.8**	**1.272**
Std.Dev	**0.05**	**6744.85**	**120.58**	**0.01**
% RSD	**0.95**	**1.06**	**2.62**	**0.92**

Table-2. Sensitivity of Suvorexant by HPLC

LOD (0.1 µg/mL)				
S.No	**Retention time (min)**	**Peak area**	**Theoretical Plates**	**Tailing factor**
1	5.73	14147	5464	1.22
2	5.72	14152	5364	1.31
3	5.72	14174	5506	1.24
Mean	**5.72**	**14157.67**	**5444.67**	**1.26**
Std.Dev	**0.01**	**14.36**	**72.95**	**0.05**
% RSD	**0.10**	**0.10**	**1.34**	**3.76**
LOQ (0.2 µg/mL)				
S.No	**Retention time (min)**	**Peak area**	**Theoretical Plates**	**Tailing factor**
1	5.68	26490	5519	1.26
2	5.72	26846	5429	1.27
3	5.71	27118	5311	1.28
Mean	**5.70**	**26818.00**	**5419.67**	**1.27**
Std.Dev	**0.02**	**314.93**	**104.31**	**0.01**
% RSD	**0.36**	**1.17**	**1.92**	**0.79**

3.2.2 Determination and Quantification Limits (Sensitivity)

Fig-3 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table-2)**.

3.3.3 Linearity

The linearity was demonstrated in triplicate. The results of the best fit line (y = mx + c) for the triplicate analysis is given in **Table-3**. The accuracy of the calibration standards was evaluated from the back calculated concentrations **(Table-4)**. All the standards were found to be within the range of 95 – 105 %.

3.3.4 Accuracy and Precision

Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given the (**Table-5)**. The intra-day (day-1) and inter-day accuracy ranged from 98.00 to 102.00 %. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

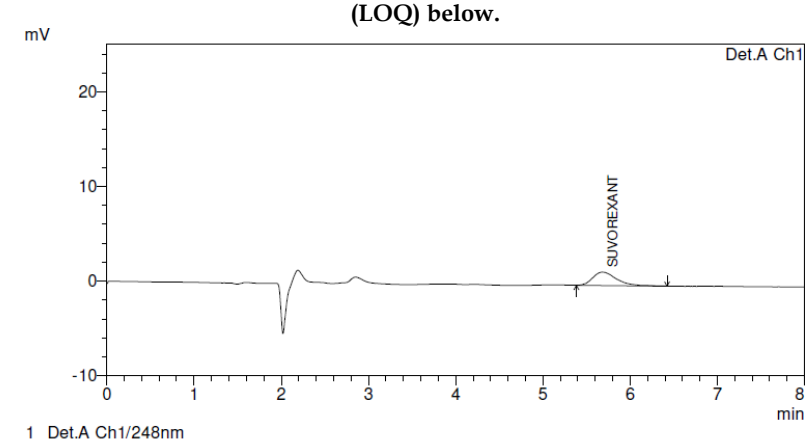
3.3.5 Specificity

Specificity was determined by comparison of the Blank chromatogram with that of the Standard chromatogram (**Fig-5**)

3.3.6 Room Temperature Stability

Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 105.61 %. The results are tabulated in **Table-6**.

Fig-3: Chromatograms shown below indicate limit of Detection (LOD) above and Limit of Quantitation (LOQ) below.



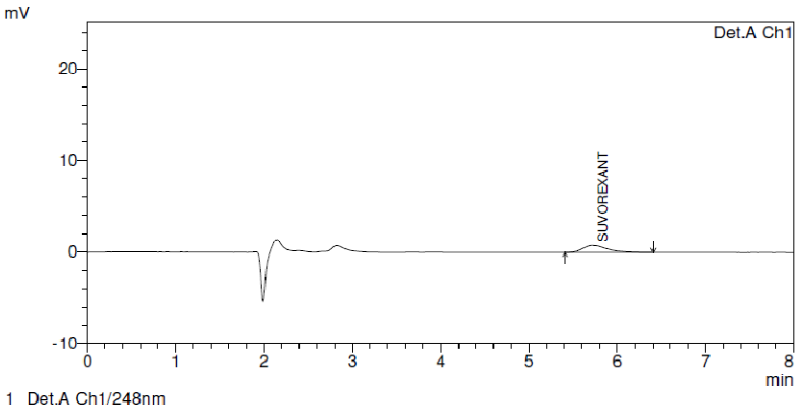


Fig-4: Linear calibration curve of Suvorexant.

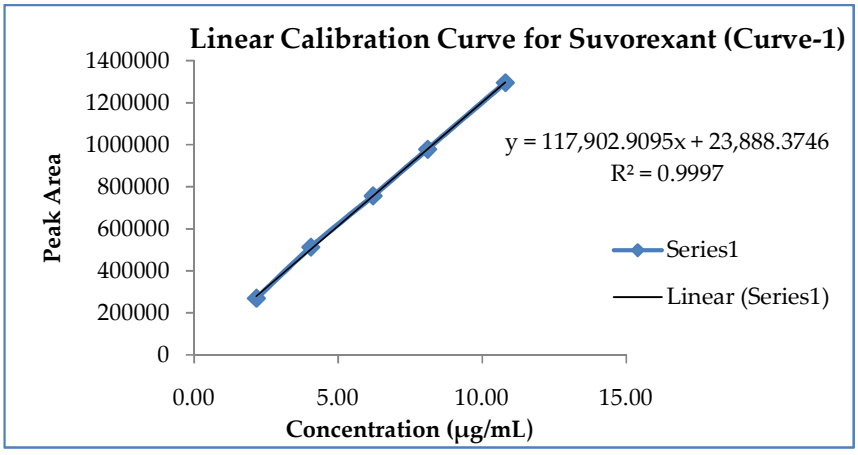
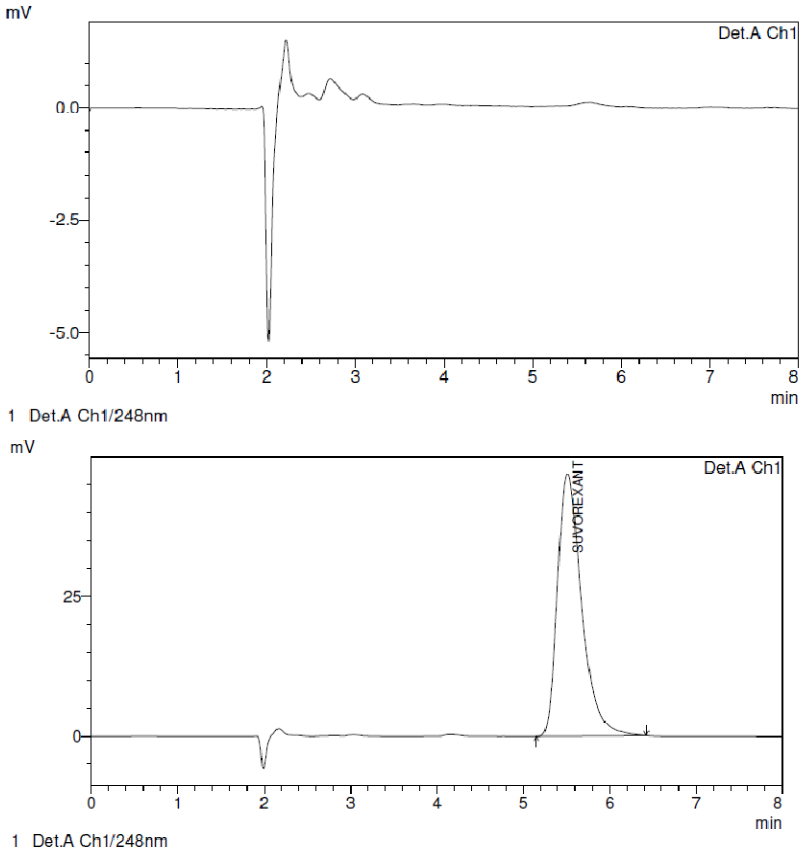


Fig-5: Comparison of Blank Chromatogram (below) to that of sample containing Suvorexant (above) standard



3.3.7 Robustness study

Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (1.0 ± 0.1 ml/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time, theoretical plates, and tailing factor, were studied**.** There was no significant variation due to the variation of mobile phase composition or flow rate variation. The results are tabulated in **Table-7.**

3.3.8 Ruggedness

The influence of analyst variation and column variation on the analysis was studied. The experiment was performed by a different analyst and using a different column. There method demonstrated good ruggedness. The results are tabulated in **Table-8.**

3.4 Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of Suvorexant. Also the method is validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Suvorexant tablets of 10 mg strength were evaluated. The amount of Suvorexant in tablet 1 is 99.05 ± 0.16. None of the tablets ingredients interfered with the analyte peak. The spectrum of Suvorexant is extracted from the tablets matched with that of standard Suvorexant showing the purity of Suvorexant in the tablets.

Table-3. Results of best-fit line (n=3)

Curve	Slope	Intercept	r^2
1	117902.9	23888	0.9997
2	118746.1	21660	0.9996
3	117845.1	24463	0.9999
Mean	**118164.7**	**23337**	**0.9997**

Table-4. Linearity and Range for Suvorexant demonstrating Mean accuracy, carryover effect and specificity of the method (Curve-1).

ID	Concentration (µg/mL)	Retention time (min)	Peak area	Theoritical plates	Tailing factor	Calculated concentration (µg/mL)	% Accuracy
Blank	0.00	no peak	NA	NA	NA	NA	NA
cc-1	2.16	5.56	269671	4753	1.27	2.08	96.51
cc-2	4.05	5.58	512712	4753	1.25	4.15	102.37
cc-3	6.21	5.55	756578	5001	1.24	6.21	100.07
cc-4	8.10	5.51	978179	4984	1.27	8.09	99.92
cc-5	10.80	5.5	1295021	4941	1.25	10.78	99.83
Carry over Blank	0.00	no peak	NA	NA	NA	NA	NA

- *NA - Not applicable*

Table-5. Results of inter and intra-day accuracy & precision for Suvorexant by HPLC

	Nominal Concentration in µg/mL (% Accuracy)		
	2.70	5.40	8.10
DAY 1			
MEAN (n=6) S.DSD	2.73 (101.11 %)	5.48 (101.48 %)	8.21 (101.36 %)
SD	0.01	0.04	0.05
% CV	0.37	0.73	0.61
DAY 2			
MEAN (n=6)	2.69 (99.36 %)	5.39 (99.81 %)	8.05 (99.38 %)
SD	0.02	0.08	0.07
% CV	0.74	1.48	0.87
DAY 3			
MEAN (n=6)	2.73 (101.11 %)	5.48 (101.48 %)	8.18 (100.99 %)
SD	0.01	0.09	0.07
% CV	0.37	1.64	0.86

Table-6: Room Temperature Stability of Suvorexant (n = 6).

RT Stability				
Sample ID	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
Fresh Sample	5.74	666449	5481	1.27
Fresh Sample	5.78	667344	5546	1.28
Fresh Sample	5.74	667610	5472	1.27
Fresh Sample	5.54	665337	5253	1.26
Fresh Sample	5.51	621912	5109	1.25
Fresh Sample	5.55	660750	5147	1.28
Mean	**5.55**	**660750.03**	**5147.00**	**1.28**
Std.Dev	**0.12**	**17969.23**	**188.55**	**0.01**
% RSD	**2.20**	**2.72**	**3.66**	**0.91**
Sample ID	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
Stability Sample	5.74	664883	5481	1.27
Stability Sample	5.78	666162	5546	1.28
Stability Sample	5.74	667303	5472	1.27
Stability Sample	5.54	660820	5253	1.26
Stability Sample	5.51	667344	5109	1.25
Stability Sample	5.55	655379	5147	1.28
Mean	**5.55**	**655379.19**	**5147.00**	**1.28**
Std.Dev	**0.12**	**4716.71**	**188.55**	**0.01**
% RSD	**2.20**	**0.72**	**3.66**	**0.91**
% Stability			**99.19**	

Table-7: Robustness of Suvorexant (n = 3).

Flow rate Variation-0.9 ml/min				
S.No	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
1	6.42	700405	6051	1.29
2	6.29	691083	5752	1.26
3	6.27	673141	5562	1.29
Mean	**6.33**	**688209.67**	**5788.33**	**1.28**
Std.Dev	**0.08**	**13857.25**	**246.52**	**0.02**
% RSD	**1.29**	**2.01**	**4.26**	**1.35**
Flow rate Variation- 1.1 ml/min				
S.No	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
1	5.06	561259	4763	1.27
2	5.16	558518	5183	1.29
3	5.21	559515	5163	1.29
Mean	**5.14**	**559764.00**	**5036.33**	**1.28**
Std.Dev	**0.08**	**1387.36**	**236.92**	**0.01**
% RSD	**1.48**	**0.25**	**4.70**	**0.90**
Mobile phase Variation- 60:40::Methanol: 0.1% Orthophosphoric acid in water				
S.No	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
1	9.64	562398	6750	1.27
2	9.62	621989	6765	1.26
3	9.58	618281	6919	1.25
Mean	**9.61**	**600889.33**	**6811.33**	**1.26**
Std.Dev	**0.03**	**33385.99**	**93.54**	**0.01**
% RSD	**0.32**	**5.56**	**1.37**	**0.79**
Mobile phase Variation- 70:30 ::Methanol: 0.1% Orthophosphoric acid in water				
S.No	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
1	4.38	589006	4835	1.29
2	4.4	586793	5306	1.29
3	4.79	618812	5126	1.27
Mean	**4.52**	**598203.67**	**5089.00**	**1.28**
Std.Dev	**0.23**	**17881.61**	**237.67**	**0.01**
% RSD	**5.11**	**2.99**	**4.67**	**0.90**

Table-8: Ruggedness of Suvorexant (n = 3).

Analytical Variation				
ID	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
Analyst 1	5.73	665451	5480	1.27
Analyst 1	5.74	665871	5450	1.28
Analyst 1	5.73	661700	5383	1.25
Mean	**5.73**	**664340.67**	**5437.67**	**1.27**
Std.Dev	**0.01**	**2296.51**	**49.66**	**0.02**
% RSD	**0.10**	**0.35**	**0.91**	**1.21**
ID	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
Analyst 2	5.54	661332	5253	1.26
Analyst 2	5.51	667720	5109	1.25
Analyst 2	5.55	666211	5147	1.28
Mean	**5.53**	**665087.67**	**5169.67**	**1.26**
Std.Dev	**0.02**	**3338.87**	**74.63**	**0.02**
% RSD	**0.38**	**0.50**	**1.44**	**1.21**
Column variation				
S.No	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
Column 1	5.74	664224	5481	1.27
Column 1	5.78	660746	5546	1.28
Column 1	5.74	660971	5472	1.27
Mean	**5.75**	**661980.33**	**5499.67**	**1.27**
Std.Dev	**0.02**	**1946.33**	**40.38**	**0.01**
% RSD	**0.40**	**0.29**	**0.73**	**0.45**
S.No	**Retention Time**	**Peak area**	**Theoretical Plates**	**Tailing factor**
Column 2	5.54	661332	5253	1.26
Column 2	5.51	667720	5109	1.25
Column 2	5.55	666211	5147	1.28
Mean	**5.53**	**665087.67**	**5169.67**	**1.26**
Std.Dev	**0.02**	**3338.87**	**74.63**	**0.02**
% RSD	**0.38**	**0.50**	**1.44**	**1.21**

Conclusions

The method gave accurate and precise results in the concentration range of 2.16 to 10.80 µg/mL. The mobile phase composition consists of (65:35 v/v) of Methanol and 0.1% orthophosphoric acid in water on isocratic mode at the flow rate of 1.0 ml/min. The retention times of the drug are 5.50 minutes. The column is Agilent Zorbax 300ºA Extend-C18 column 150 X 4.6 cm 5µm. A rapid sensitive and specific method for the determination of Suvorexant in the pharmaceutical formulations has been developed. The proposed RP-HPLC method for the estimation of Suvorexant in dosage form is accurate, precise, linear, rugged, robust, and rapid. The sample solution [prepared from product] is diluted in a mixture of methanol and 0.1N HCl. This step extends the application of the method to dissolution studies which are a part of *in vitro* formulation development. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies. The method is validated as per ICH Guidelines.

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