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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPY METHOD FOR ESTIMATION OF PALIPERIDONE HCL

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ABSTRACT
Paliperidone is an atypical antipsychotic drug .paliperidone is a selective dopamine type 2(D2) and serotonin type 2 (SHT2A) receptor antagonisms, developed for the treatment of schizophrenia. To develop and validate simple, accurate, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of paliperidone HCl in a pharmaceutical formulation. The developed UV spectrophotometric method for the quantitative estimation of paliperidone HCl is based on measurement of absorption maximum wavelength 236 nm using paliperidone HCl as a solvent. The stock solution of paliperidone HCl was prepared, and subsequent suitable dilution was prepared in distilled water to obtained standard curve. The standard solution of paliperidone HCl shows absorption maxima at 236.nm.

KEYWORDS: Paliperidone, UV spectroscopy, method development.

INTRODUCTION
Paliperidone 3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-9-hydroxy-2-Methyl-4H-pyrido [1,2-a]pyrimidin-4-one Hydrochloride (fig.1) is an atypical antipsychotic. It is the drug of choice for treatment of schizophrenia. The drugs therapeutic activity in schizophrenia is believed to be mediated through a combination of central dopamine type 2 (D2) and serotonin type 2 (5HT2A) receptor antagonism. It is a second generation antipsychotic and as an adjunct to treat depression. It is the primary active metabolite of risperidone, an older antipsychotic. It is a white or almost white powder is soluble in water. As per literature, various analytical methods have been developed for analysis of paliperidone. The present work focuses to develop a simple accurate, rapid, precise and reproducible spectrometric method for quantitative determination. In the present study a method was developed for determination of paliperidone and validation of the method as per ICH guidelines.[4]

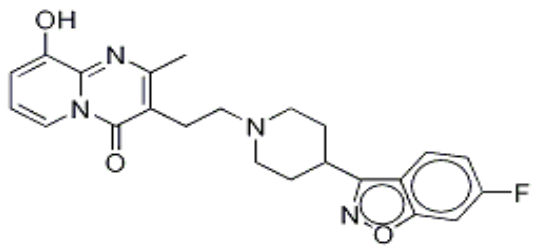

Fig 1: Structure of Paliperidone.

MATERIALS AND METHOD
Instrument
The analytical method was developed using Shimadzu UV 1700 spectrophotometer. The instrument consisted of matched quartz cells with 1 cm path length. Other instruments used were Ultrasonicater (Toshcon) and weighing balance (A&D Company Ltd.).

Material
Chemical reagents & Solvents
Paliperidone HCl was gifted from Wockhard Ltd. Aurangabad. Monobasic Potassium Phosphate(KH_2PO_4), Sodium Hydroxide(NaOH), Water, etc. all were of analytical grade.

Media
The buffer solution pH 6.8 was prepared using distilled water form purification unit.

METHOD DEVELOPMENT
Preparation of standard solution
Standard drug solution of paliperidone HCl was prepared by dissolving 100mg in 10 ml pH 6.8 phosphate buffer. The concentration of prepared stock I solution was 1000 mcg/ml. 1 ml of stock I was withdrawn and diluted to 10ml with pH 6.8 phosphate buffer (stock II, 100 mcg/ml).

Selection of wavelength for analysis of Paliperidone
1 ml of stock II was transferred to 10 ml volumetric flask

and diluted to 10 ml to give a concentrartion of 10 mcg/ml and this was used for initial spectral scan in the UV range of 400-200 nm to detect maximum wavelength and further dilutions can be prepared from the stock solution.

Preparation of serial dilutions
The serial dilutions were prepared from standard stock II solution to give respective concentrations of 8, 16, 32 and 40 mcg/ml.

METHOD VALIDATION[1]
The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines.

Linearity and range
The ability of an analyticl procedure to obtain test results which directly proportional to the concentration of an analyte present in the sample is called linearity of the method. Range of an analytical procedure is the interval between upper and lower concentration of an analyte in the sample for which it has been demonstrated the method has suitable level of precision, accuracyand linearity. Triplicate analysis (n=3) was done to determine the results. A graph is plotted for concentration (mcg/ml) vs absorbance. The correlation coefficient and linear regression equation can be obtained from the graph using the UV probe software.

Specificity
Specificity is the ability to assess the analyte unequivocally in the presence of components which may be expected to be present. Typically these might include impurities, degradant, matrix, etc. For this study specificity was done by using an excipient Lactose. The three different concentrations at three levels 80%, 100%, 120% respectively of standard solution (24µg/ml) spiked on Lactose sample. At each level of the amount, the triplicate study was performed to check the effect of Lactose.

Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value. The accuracy of the method was studied by recovery experiments, the recovery was performed at three levels 80, 100 and 120% of Paliperidone HCl standard concentration. The recovery samples were prepared for each recovery level. The final concentration of Paliperidone HCl was determined at each level of the amount; three determinations were performed and percentage recovery calculated as mean±standard deviation using calibration curve.

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 homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analysed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analysed, for three consecutive days and the absorbances were recorded. All study was performed in triplicates. The result was indicated by calculating % RSD.

Limit of detection (LOD)
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value. The limit of detection (LOD) was determined by preparing solutions of different concentrations from 2-14µg/ml.
$LOD = 3.3\ \sigma/S$

Where,
σ=Standard deviation
S= Slope

Limit of quantification(LOQ)
Limit of quantification is the amount above which can be productively quantified. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.
$LOQ=10\sigma/S$

Where,
σ=Standard deviation
S= Slope

Ruggedness (by two different analyst)
The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days.

To establish ruggedness of the proposed method, the solutions of 8 µg/ml of standard solution was prepared and analysed with the change in the different analyst.

Robustness
The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage analysed by a change in wavelength. The wavelength was selected max ± 2 i.e. 234 and 238 nm respectively for standard solution.

RESULTS AND DISCUSSIONS

Selection of wavelength

The spectrum of Paliperidone HCl in pH 6.8 buffer showed maximum absorbance at 236.nm which complies with reported λmax. Hence this wavelength was selected as λmax of Paliperidone in pH 6.8 buffer for further use.

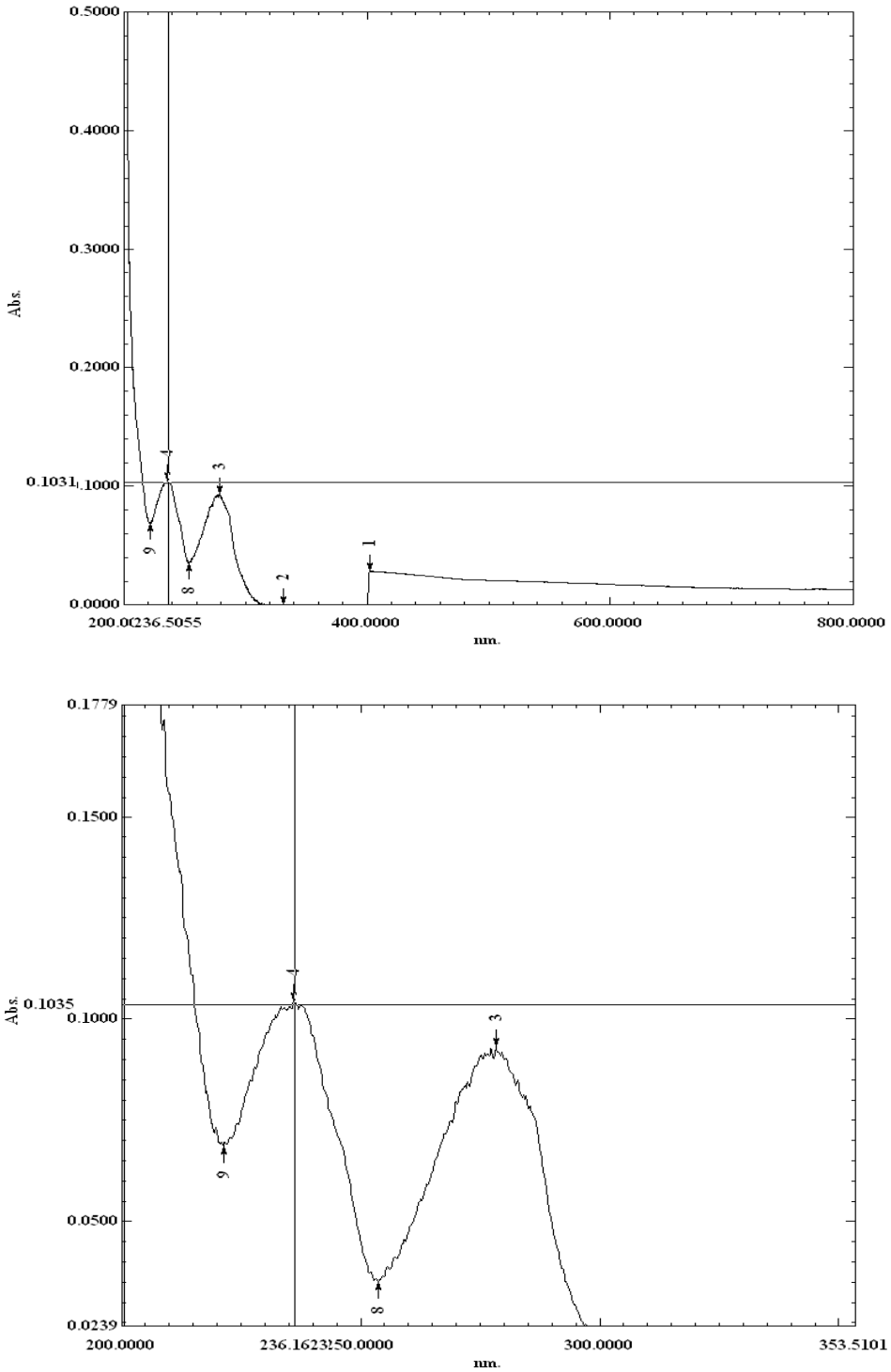

Fig 2: Lambda max spectrum.

Linearity and range

The linearity response of the drug was verified at 8-40 mcg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus concentration data. The data obtained was subjected to linear regression analysis. The equation of calibration curve for Paliperidone HCl obtained is y = 0.0128x + 0.004. The linearity range of curve was found to be 8-40 mcg/ml concentrations. The correlation coefficient (r^2) of

determination was 0.998. The linearity is shown in table.1 and fig.3.

Table 1: Calibration curve for Paliperidone HCl.

Sr. no.	Concentration (mcg/ml)	Absorbance
1	8	0.11
2	16	0.20
3	24	0.31
4	32	0.42
5	40	0.51

Calibration Curve of Paliperidone HCl

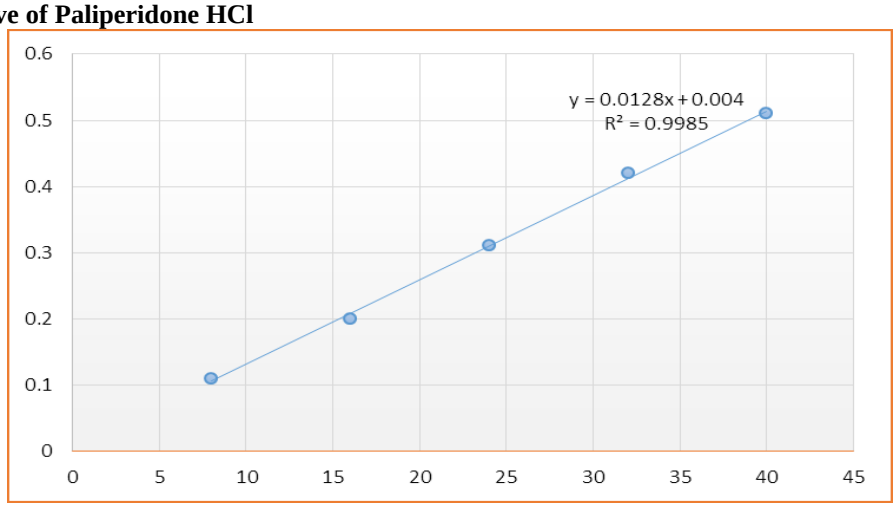

Fig 3: Calibration of Paliperidone HCl.

Table 2: Specificity study of Paliperidone HCL.

Level of addition	Standard API (µg/ml)	Lactose (µg/ml)	Total concentration (µg/ml)	Absorbance	Drug recovered (µg/ml)	% Recovery	Mean % Recovery
	24	19.2	43.2	0.311	24.00	100.00	100.00
80%	24	19.2	43.2	0.311	24.00	100.00	
	24	19.2	43.2	0.312	24.001	100.0338	
	24	24	48	0.321	24.00	100.00	99.9887
100%	24	24	48	0.322	24.0033	100.0676	
	24	24	48	0.322	23.9949	99.89858	
	24	28.8	52.8	0.313	24.0016	100.0338	100.00
120%	24	28.8	52.8	0.323	23.9966	99.93238	
	24	28.8	52.8	0.322	24.0016	100.0338	

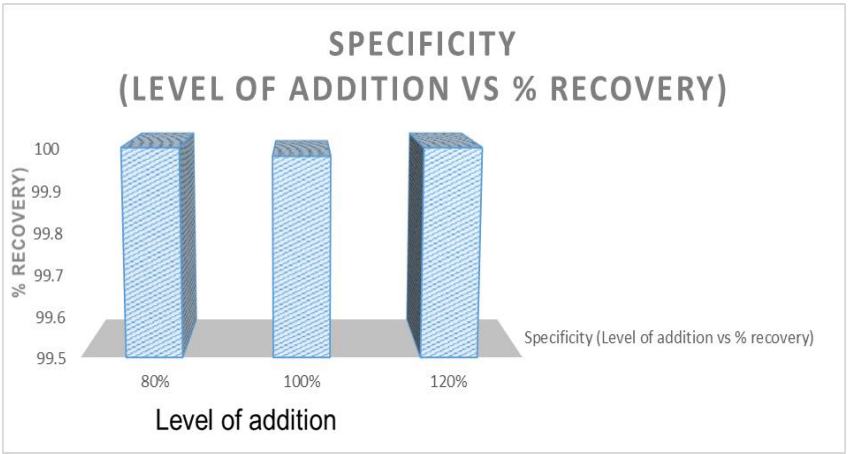

Fig. 4: % Recovery graph in specificity test.

Accuracy (Recovery Test)

Accuracy was studied by recovery experiments. The recovery was performed at three levels 80, 100 and 120% of Paliperidone HCl standard concentration. The recovery samples were prepared as per above mentioned procedure. The samples were prepared for each recovery level. The solutions were then analysed and the percentage recovery were calculated from calibration curve. The recovery value for Paliperidone HCl was 99.84 ± 0.332.

Table 3: Recovery of Paliperidone HCl.

Level of addition	Standard API (mcg/ml)	Formulation stock added (mcg/ml)	Total conc. (mcg/ml)	absorbance	Drug recovery (mcg/ml)	% recovery
				1.177	43.18	99.96
80%	24	19.2	43.2	1.172	43.00	99.53
				1.175	43.11	99.79
				1.307	48.00	100.0
100%	24	24	48	1.302	47.81	99.61
				1.305	47.92	99.84
				1.428	52.48	99.96
120%	24	28.8	52.8	1.425	52.37	99.75
				1.422	52.25	99.54

Table 4: Statistical validation of recovery studies.

Level of addition	% recovery (mean ± SD)	% RSD	SE	Conclusion
80%	99.96 ± 0.00252	0.2142	0.00145	Pass
100%	99.82 ± 0.00252	0.1928	0.00145	Pass
120%	99.75 ± 0.00252	0.2105	0.00173	Pass

Results obtained were found to be within range of pharmacopoeias standard for Paliperidone HCl. Limit for % accuracy is NMT 5% RSD.

Precision

The precision of proposed method was determined by Intra-day and Inter-day precision, and it was expressed in terms of percent relative standard deviation (%RSD). The reproducibility (inter-day precision) of the method and repeatability (intra-day) was evaluated in the same laboratory. For Inter-day and Intra-day %RSD were found in the range of 0.5745 and 0.5932 respectively as shown in table 4.

Table 5: Precision determination.

Sample name	Inter-day precision	Intra-day precision
1	99.49	99.47
2	99.03	99.80
3	98.40	99.21
Mean ± SD	98.97 ± 0.549	99.49 ± 0.291
%RSD	0.5745	0.5932

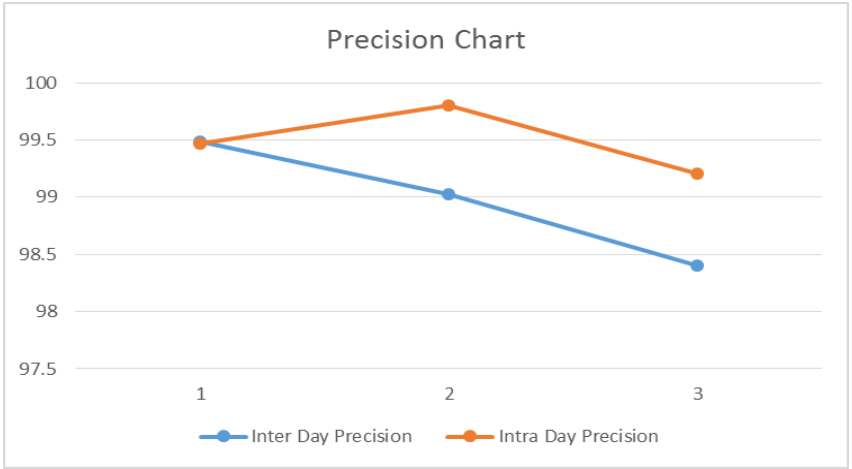

Fig. 5 Precision Chart.

Limit of detection (LOD)

LOD was determined by using the formula in equation.1 and was found to be 0.4277 mcg/ml. From this value it could be conclude that the concentration of Paliperidone HCl as less as 0.4277 mcg/ml can be successfully detected.

Limit of quantification (LOQ)

LOQ was also determined using equation.2 and was found to be 1.2962 mcg/ml. from this value it could be concluded that concentration of Paliperidone HCl above 1.2962 mcg/ml can be productively quantified.

Table 6: Ruggedness data for Paliperidone HCL.

Observation	Analyst 1	Analyst 2
Absorbance	0.1106	0.1100
	0.1102	0.1093
	0.1101	0.1099
Mean*	0.1103	0.1096
SD	0.0005	0.0005
%RSD	0.4533	0.4562

*(n=3).

Table 7: Robustness data for Paliperidone HCL.

Wavelength (nm)	Absorbance	Mean absorbance±SD	%RSD
234	0.1041	0.1041±0.0001	0.0969
234	0.1041		
234	0.1042		
236	0.1044	0.1043±0.00011	0.1054
236	0.1044		
236	0.1041		
238	0.1042	0.1042±0.0001	0.0989
238	0.1043		
238	0.1042		

Robustness

Robustness of this method was determined by analysing the standard paliperidone hcl solution of 8 µg/ml at a different wavelength (i.e. λ max±2). Absorbance was measured. The standard deviation and percent relative standard deviation was calculated. Results of robustness study indicate that the selected factor remained unaffected by small variation with RSD 0.0969-0.0989 confirms the robustness of the method.

Table 6: Summary of Validation parameters.

Parameter	Standard value	Observed value
Linearity equation		y = 0.0128x + 0.004
Correlation coefficient (r^2)	>0.997	0.999
Accuracy (%recovery)		99.84%
Precision	RSD<2.0	0.5932% (intraday)
	RSD<4.0	0.5745% (interday)
lmax		236.nm
Range		8-40 mcg/ml
Limit of detection (LOD)		0.4277 mcg/ml
Limit of quantitation (LOQ)		1.296 mcg/ml
Robustness		0.0969-0.0989
Ruggedness		0.4533-0.4562

CONCLUSION

The simple, rapid, precise and economical spectrophotometric method has been developed for the quantitative estimation of Paliperidone HCl. The method is validated as per the ICH and USP guidelines and it is found that the developed method is precise. Hence, this method can be successfully and suitably used for routine quality control analysis of Paliperidone HCl in pharmaceutical dosage form.

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