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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD OF PALIPERIDONE PALMITATE IN BULK AND ITS APPLICATION FOR DRUG LOADING AND ENTRAPMENT EFFICIENCY ANALYSIS OF LONG-ACTING INJECTABLE MICROSPHERES

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ABSTRACT

Objective: The objective of the current study was to develop simple, precise and cost-effective ultraviolet (UV) spectroscopic method for estimation of paliperidone palmitate (PP) as a bulk and in the polymeric depot dosage form.

Methods: UV spectrophotometer with quartz sample cells and spectral manager software was used for analysis samples. 20 % Methanol in Mili-Q-Water was used as a solvent for the sample and standard preparations. Method validation was carried out as per ICH Q2 (R1) guidelines. Stress degradation studies were carried out to check the stability-indicating performance of the developed method. The validated method was applied for the estimation of the PP content of long-acting injectable microspheres of PP.

Results: The wavelength of maximum absorption was found to be 283 nm. The proposed method was validated in the range of 5-30 µg/ml of PP. The mean recovery for 80%, 100% and 120% standard solution was found to be 99.03%, 99.21% and 99.35% respectively with less than 2% relative standard deviation (RSD). Intra-day and inter-day method precision ranged from 0.54 to 1.62% and 0.67 and 1.24% respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated to be 0.03 and 0.10 µg/ml respectively. The developed method was found to be robust and rugged. Stress degradation studies were carried out under acidic, alkaline, hydrolytic and oxidative and photo stress. The proposed method was capable to detect changes in assay due to stress conditions.

Conclusion: The developed method was successfully employed for routine analysis of drug loading in long-acting injectable microspheres of PP.

Keywords: UV spectroscopy, Method validation, Paliperidone palmitate, Stress degradation, Drug loading, Microspheres

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INTRODUCTION

Paliperidone palmitate (PP) is newer widely prescribed atypical antipsychotics for the treatment of schizophrenia [1-3]. PP belongs to the class of benzisoxazole derivatives. The chemical name is (9RS)-3-[2-[4-(6-Fluoro1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4Hpyrido [1,2-a] pyrimadin-9-yl hexadecanoate. Paliperidone palmitate is a prodrug and it gets hydrolyzed to paliperidone. It was postulated that the drug's therapeutic effect in schizophrenia may be due to a combination of central dopamine type 2 (D2) and serotonin Type 2 (5HT2A) receptor antagonism. PP is very slightly soluble in ethanol and methanol, practically insoluble in polyethylene glycol 400 and propylene glycol, and slightly soluble in ethyl acetate [4].

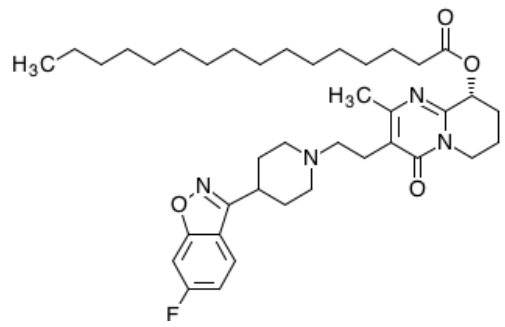

Fig. 1: Chemical structure of paliperidone palmitate

In the treatment of schizophrenia, the recommended dose of PP tablets in adults is 6 mg administered once daily and is recommended for long-term administration. PP was considered to be an ideal drug candidate to formulate in extended-release drug formulation via the parenteral route of administration. Poly(lactic-co-glycolic acid) (PLGA) is a well-known biodegradable and biocompatible polymer used to formulate drug in long acting injectable dosage form. PLGA based microsphere formulations of various drugs were successfully developed for the injectable application. Polymeric microspheres are capable to deliver the encapsulated drug for long term via parenteral administration [5-7].

Historically, UV spectroscopic methods were proved very beneficial for quantification purposes in pharmaceutical analytical research [8-11]. The literature search of available UV spectroscopy methods [12-14] showed that three UV methods were reported for estimation of PP in pharmaceutical drug products. Several high-performance liquid chromatography (HPLC) methods [15-18] have been reported for the determination of paliperidone-free base in bulk and tablet dosage forms. Very limited scientific investigations were found that demonstrate the development and validation of analytical methods for PP and in long-acting injectable microsphere formulation.

Based on the detailed review of methods for estimation PP, a new UV spectrophotometric method was developed for the determination of PP in bulk as well as to estimate drug loading and entrapment efficiency of long-acting injectable microspheres. In this research work, significant efforts were made to develop a newer, simple, easy, precise, sensitive, accurate and cost-effective UV spectroscopy method for analysis of PP in bulk and microspheres dosage form.

MATERIALS AND METHODS

Instruments and reagents

A double beam UV-visible spectrometer (UV-1700, Shimadzu, Japan) with the spectra manager software was used for the analysis with quartz cells having 10 mm path length for spectral measurement.

Weighing balance (Mettler Toledo, USA) with internal calibration mode was used for the accurate weighing purpose. PP was obtained as a gift sample from Glenmark life sciences, India. Methanol, sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Merck, Germany. All the chemicals of analytical grade were used in the proposed study.

Preparation of stock solution

Accurately weighed 10 mg of PP was transferred to a 100 ml volumetric flask, dissolved in 20 ml methanol by shaking manually for 10 min. The volume of solution was adjusted with the Mili-Q-Water up to the 100 ml mark to give the final strength i.e.100 µg/ml.

Selection of wavelength of maximum absorbance

Appropriate volume (3 ml) of the standard stock solution was transferred into a 10 ml volumetric flask, diluted to a mark with distilled water to give a concentration of 30 µg/ml. The resulting solution was scanned in the UV range (200 to 400 nm). The UV spectrum of PP was shown in fig. 2. In UV spectrum, PP showed maximum absorbance at a wavelength of 283 nm.

Preparation of calibration curve

Different aliquots of stock solution in the range of 0.5 to 3 ml were transferred into a series of 10 ml volumetric flasks, and the volume was made up to the mark with Mili-Q-water to get concentrations 5, 10, 15, 20, 25 and 30 µg/ml, respectively. The solutions were scanned on a UV spectrophotometer in the range of wavelength from 200 to 400 nm. The absorbance was recorded at a wavelength of 283 nm. The values of absorbance against the concentration of calibration standard were represented in table 1. The calibration plot was constructed as concentration vs. absorbance and represented in fig. 3.

Method validation

The proposed UV method for the estimation of paliperidone palmitate was validated in terms of parameters like linearity, range, precision, robustness, ruggedness, accuracy and sensitivity. The method was validated as per the guidelines of the International Conference on Harmonization (ICH) [19-21].

Linearity and range

Linearity of the proposed UV method was established using six different calibration standards viz. 5, 10, 15, 20, 25 and 30 µg/ml. Calibration curves in terms of absorbance vs. concentration were plotted. Linear least square regression analysis and R square value was considered for establishing the linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method. Calibration curve was shown in fig. 3. Overlay graph of concentration vs. absorbance was established and depicted in fig. 4.

Accuracy

The recovery studies were carried out to evaluate the accuracy of the proposed UV method. Three different solutions of PP were prepared in triplicate at a level of 80%, 100% and 120%. Accuracy of the method was determined based on percent recovery from prepared solutions. The results of the accuracy study were shown in table 2.

Precision

The precision of the assay method was examined in terms of repeatability by carrying out six independent assays of PP test arrangement and the % RSD of measurement (intra-day). The intermediate precision of the method was checked by performing same methodology on three consecutive days. The values of % RSD of intra-day and inter-day precision study were shown in table 3 and table 4 respectively. % RSD of less than 2 was considered to be acceptable.

Robustness

The robustness of the developed UV method was studied by varying composition co-solvent system. Methanol concentration in the composition of diluent was deliberately changed to 15 and 25% of bulk. PP in a concentration of 20 µg/ml was prepared utilizing above mentioned co-solvent system independently, (n=3) and sample were analyzed at λmax of 283 nm. The result was determined in terms of % RSD. % RSD of less than 2 was considered to be acceptable. The analysis results of the robustness study were represented in table 5.

Ruggedness

Ruggedness study was carried out by analyzing samples of PP utilizing two different analysts (n=3). The result was depicted in terms of % RSD. % RSD of less than 2 was considered to be acceptable. The analysis results of ruggedness study were represented in table 6.

Sensitivity

The sensitivity of measurements of PP by the use of the proposed method was estimated in terms of the limit of detection (LOD) and limit of quantitation (LOQ) [20, 21]. The sensitivity data of the study were shown in table 7. The LOD and LOQ were calculated using the following equations.

$$\text{LOD} = 3.3 \text{ X } \frac{\text{N}}{\text{B}}$$

$$\text{LOQ} = 10 \text{ X } \frac{\text{N}}{\text{B}}$$

Where, 'N' is the standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Stress degradation studies

Stress degradation studies with optimized stress conditions were carried out to study the stability-indicating nature of the developed UV spectroscopic method. For each stress condition, three samples were prepared. The stress degradation studies include acid, alkali, hydrolytic, oxidative and photodegradation studies [22-24]. The results of stress degradation studies were represented in table 8.

Acidic degradation

10 mg of PP was added in 20 ml volumetric flask, followed by a methanolic 1N hydrochloric acid and volume was made up to 20 ml mark. The solution was stored for 24 h time.

Alkali degradation

10 mg of PP was added in 20 ml volumetric flask, followed by methanolic 1N sodium hydroxide and volume was made up to 20 ml mark. The solution was stored for 24 h time.

Hydrolytic degradation

10 mg of PP was added in 20 ml volumetric flask, followed by methanolic Mili-Q-Water and volume was made up to 20 ml mark. The solution was heated at 80 °C for 6 h time.

Oxidative degradation

10 mg of PP was added in 20 ml volumetric flask, followed by 4 ml, 10% hydrogen peroxide solution and methanolic Mili-Q-Water and volume was made up to 20 ml mark. The solution was stored for 24 h time.

Photodegradation

The photolytic stability study of the drug was studied in solid-state. The PP was exposed to UV light providing illumination of not less than 200-watt h/m^2 followed by cool white fluorescence light of not less than 1.2 million Lux-hr. The drug concentration of each exposure was studied by using the proposed UV spectroscopic method [25].

Estimation of drug loading and entrapment efficiency of PP in long-acting injectable microspheres

Validated method was employed for the estimation of drug loading of PP in injectable depot microspheres formulation. In this study, long acting injectable microspheres formulation of PP was analyzed using pre-validated UV-method and results were reported in terms of average percent assay or percent drug loading. 100 mg of long acting depot injectable microspheres were added in 20 ml methanol and sonicated for 5 min. Volume of the solution was made up to 100

ml with Mili-Q-water and mixed by sonication for 2 min. 5 ml of the solution was withdrawn and added to 50 ml volumetric flask and diluted with Mili-Q-water. The absorbance of the solution was recorded at wavelength 283 nm and percent drug loading was calculated. The results of drug loading and entrapment efficiency were represented in table 9. Drug loading and entrapment efficiency in long acting injectable microspheres was calculated using following equations [26-28].

$$\text{Drug Loading}(\%) = \frac{\text{Weight of drug contained in microspheres}}{\text{Weight of microspheres}} \text{ X } 100$$

$$\text{Entrapment Efficiency}(\%) = \frac{\text{Practical drug content}}{\text{Therotical drug content}} \text{ X } 100$$

RESULTS AND DISCUSSION

Preliminary method development trials were executed to find out the solvent system for solublization of PP. Methanol was selected as solvent of choice for initial trials. Firstly, estimation of the wavelength of maximum absorbance was carried out by running absorption spectrum of 30 µg/ml solution of PP in 20% v/v methanol in Mili-Q-water solution. Generally, a solution showing absorbance value less than 1 is considered to be suitable for the determination of wavelength of maximum absorbance (λmax). Full scan was processed using UV software and the λmax was identified with the help of software. It was found to be 283 nm for PP. UV spectrum of PP in 20% methanolic water was shown fig. 2.

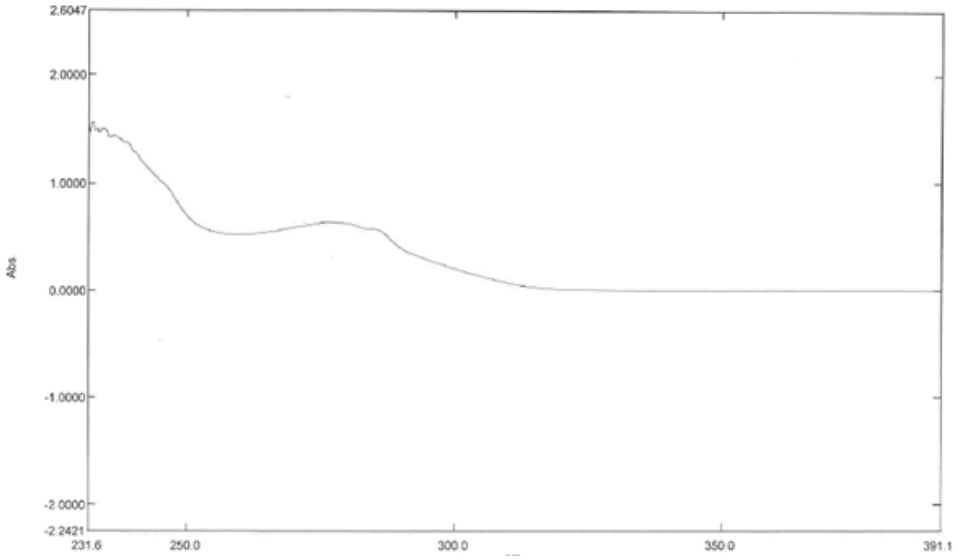

Fig. 2: UV spectrum of paliperidone palmitate in 20% methanolic water

Different aliquots of PP in the range 0.5 to 3 ml were transferred into a series of 10 ml volumetric flasks, and the volume was made up to the mark with Mili-Q-water to get concentrations 5, 10, 15, 20, 25 and 30 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. Overlay UV curves of different calibration standards were shown in fig. 4. The absorbance of calibration standards was recorded at wavelength 283 nm and reported as shown in table 1. The calibration plot was constructed as concentration vs. absorbance and represented in fig. 3. Calibration curve shown R square value of 0.996 as represented in fig. 3. R square value of more than 0.95 percent is acceptable considering 5% error. R square value closer to unity suggests the calibration curve is linear.

Table 1: Concentration vs. absorbance of paliperidone palmitate at 283 nm

Concentration (µg/ml)	Absorbance* (%)
5	0.1108±0.02
10	0.2204±0.06
15	0.3210±0.01
20	0.3990±0.04
25	0.5087±0.08
30	0.6355±0.11

*Data represents mean±SD (n=3)

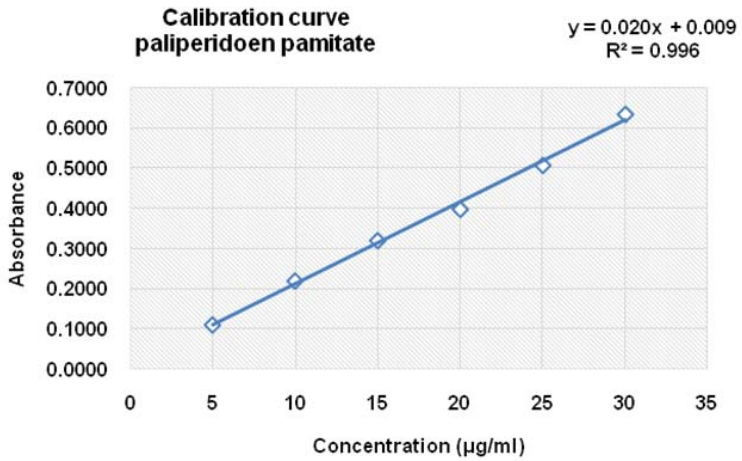

Fig. 3: Calibration curve of paliperidone palmitate

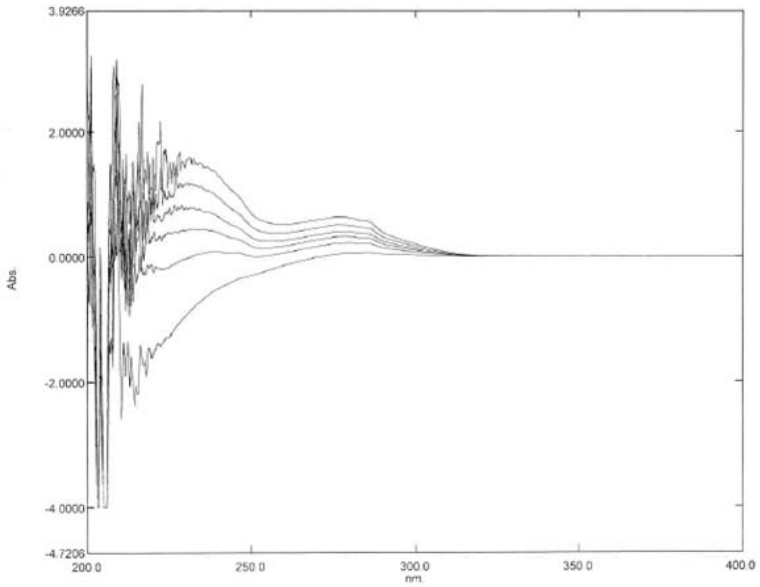

Fig. 4: Overlay UV curves of calibration standards for paliperidone palmitate

A six-point calibration curve of PP with a range from 5 to 30 µg/ml was developed. Concentrations and mean absorbance values are presented in table 1. The least-square regression analysis equation of calibration curve was y = 0.0204x+0.0093 with correlation coefficient 0.996 as shown in fig. 3. Based on the linearity study, it can be concluded that developed UV method was linear in the prepared concentration range of calibration solutions from 5 to 30 µg/ml. Accuracy is a measure of closeness of the estimated value to the actual amount of the analyte in solution. For PP, accuracy was established using recovery studies. The mean recovery of 80%, 100% and 120% standard solution was found to be 99.03, 99.21 and 99.35% respectively. % RSD was found to be 0.47%, 0.33% and 0.45% to 80%, 100% and 120% standard solution respectively. Results of the accuracy study were represented in table 2. The results of accuracy showed that % RSD of analysis was less than 1; it reveals that the developed UV method is highly accurate.

Table 2: Accuracy results

Concentration (%)	Original level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % recovery*	% RSD (n=3)
80	20	16	99.54	99.03	0.47
80	20	16	98.62		
80	20	16	98.94		
100	20	20	99.22	99.21	0.33
100	20	20	98.87		
100	20	20	99.53		
120	20	24	99.55	99.35	0.45
120	20	24	99.67		
120	20	24	99.08		

*Data represents mean±SD (n=3)

Precision is the closeness of a measurement to each other. It is expected that the analytical method should be precise. The precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 10, 15 and 20 µg/ml of PP solutions for three times in the same day and results are represented in table 3. Interday precision was determined by analyzing the 10, 15, and 20 µg/ml solutions of PP for three consecutive days and results represented in table 4. Overall, % RSD values of less than 2 in the case of both intra-day and inter-day precision showed that the developed UV method is precise.

Robustness of the analytical method was the study of the impact of small, deliberate changes in method parameters on method performance. It was desired that such small changes should not impact the performance of the analytical method. In this study, the robustness of the proposed UV method was established by modifying the composition of the co-solvent system in the range of 15 to 25 % of methanol in the Mili-Q-Water. The results of the robustness study were shown in table 5. % RSD values for the concentration of 20 µg/ml concentration with a small change in the composition of the solvent system were found to be less than 2% RSD. It showed that the proposed UV method is robust in nature.

Table 3: Intraday precision results

Conc. (µg/ml)	Morning			Afternoon			Evening		
	Mean absorbance*	% Assay	%RSD (n=3)	Mean absorbance*	% Assay	%RSD (n=3)	Mean absorbance*	% Assay	%RSD (n=3)
10	0.2107±0.01	98.73	0.62	0.2109±0.06	98.82	1.24	0.2115±0.04	99.12	0.95
15	0.3129±0.04	99.22	1.09	0.3133±0.02	99.35	0.98	0.3123±0.07	99.02	1.14
20	0.4104±0.07	98.31	1.43	0.4102±0.08	98.26	0.54	0.4118±0.08	98.65	1.62

*Data represents mean±SD (n=3)

Table 4: Interday precision results

Conc. (µg/ml)	Day 1			Day 2			Day 3		
	Mean absorbance*	% Assay	%RSD (n=3)	Mean absorbance*	% Assay	%RSD (n=3)	Mean absorbance*	% Assay	%RSD (n=3)
10	0.2104±0.09	98.58	0.85	0.2105±0.04	98.63	1.04	0.2108±0.09	98.77	1.07
15	0.3127±0.05	99.15	1.24	0.3137±0.08	99.48	0.67	0.3127±0.05	99.15	1.24
20	0.4110±0.06	98.46	1.07	0.4105±0.11	98.33	0.88	0.4119±0.06	98.68	0.79

*Data represents mean±SD (n=3)

Table 5: Robustness results

Concentration (µg/ml)	% Methanol	Absorbance	% RSD (n=3)
20	15	0.4104	0.61
		0.4124	
		0.4154	
	20	0.4069	0.59
		0.4114	
		0.4107	
	25	0.4112	0.43
		0.4125	
		0.4147	

Data represents mean±SD (n=3)

The ruggedness of the proposed method was determined for 20 µg/ml concentration of PP by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions. The results of the ruggedness study were shown in table 6. % RSD of UV absorbance testing under defined method conditions for analyst 1 and analyst 2 was found to be 1.33 and 1.27%. The data of robustness (table 6) of UV method showed the proposed method is robust.

Table 6: Ruggedness results

Concentration (µg/ml)	Analyst 1		Analyst 2	
	Absorbance	% RSD (n=3)	Absorbance	% RSD (n=3)
20	0.3995	1.33	0.3993	1.27
	0.3997		0.3989	
	0.3994		0.3994	

Data represent mean±SD (n=3)

Sensitivity of UV spectroscopic method can be estimated by calculating LOD and LOQ. LOQ is the lowermost concentration that can be estimated with acceptable accuracy and precision. LOD and LOQ of the proposed UV method were found to be 0.03 and 0.10 µg/ml, respectively as shown in table 7. The results of LOD and LOQ showed that the developed method is sensitive.

Table 7: UV method sensitivity data

Concentration (µg/ml)	Absorbance (%)		
	Sample 1	Sample 2	Sample 3
10	0.2206	0.2207	0.2203
Standard Deviation (n=3)	0.0002082		
LOD	0.03 µg/ml		
LOQ	0.10 µg/ml		

Stress degradation studies revealed that PP prone to degradation on acidic, alkaline and oxidative stress. PP was found stable under hydrolytic and photodegradation challenges. The developed method was not capable to quantify degradation products, but able to estimate percent degradation calculated from assay term. The developed method was found suitable to study the stress degradation of PP. Stress degradation parameters, stress conditions, conc. (% assay) and % degradation was represented in table 8.

Table 8: Stress degradation studies

S. No.	Parameter	Stress condition	Conc. (% assay)	% Degradation
1	Control	---	99.88	0.12
2	Acid	1N-HCl, 24 H	83.60	16.4
3	Alkali	1N-NaOH, 24 H	81.60	18.4
4	Hydrolytic/Thermal	80 °C, 6 H	99.86	0.14
5	Oxidative	10% H2O2, 24 H	91.58	8.42
6	Fluroscent	1.2 Million Lux. Hrs	99.86	0.14
7	UV	200 Watt Hrs/Square Meter	99.81	0.19

The results of the stress degradation study of PP were in accordance with the forced degradation study carried out by *Sawant SD* and *Barge VU,* which reported that PP undergone degradation in acidic and basic stress conditions; while PP was stable under hydrolytic, photo and oxidative conditions [29]. As well, *Mathad VT et al.* reported stress degradation profile of paliperidone showed degradation under acidic, basic and oxidative stress [30].

The developed UV spectroscopic method was successfully applied for the estimation of drug loading in long-acting injectable microspheres dosage form. Average practical drug loading (n=3) of PP in long-acting injectable microspheres dosage form was found to be 32.77±0.58%. The developed method was studied to estimate drug loading and entrapment efficiency in microspheres during stability study of developed microspheres. The analysis data of drug loading after exposure to different stability conditions were tabulated in table 9.

Entrapment efficiency of microspheres was found to be 98.32%. Drug loading and entrapment efficiency were studied on stability conditions like 40 °C/75%RH and 25 °C/60%RH for 3 mo as per ICH guidelines of stability testing [31]. Results of drug loading were found to be in the range from 32.70 to 32.77%. The results of entrapment efficiency were found to be between 98.12 and 98.32%. The data in table 9 showed that the developed method was capable to estimate drug loading and entrapment efficiency of prepared microspheres during initial analysis and stability analysis.

Table 9: Drug loading analysis of long acting injectable microspheres of PP

Stability condition/Interval	Drug loading±SD* (%)	Entrapment efficiency±SD* (%)
Initial	32.77±0.58	98.32±0.84
40 °C/75%RH-1 Mo	32.73±0.32	98.19±0.57
40 °C/75%RH-3 Mo	32.70±0.69	98.12±1.09
25 °C/60%RH-1 Mo	32.75±0.43	98.26±0.68
25 °C/60%RH-3 Mo	32.73±0.55	98.19±0.80

*SD: Standard Deviation, n=3

CONCLUSION

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of PP was developed and validated. The Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of drug loading of PP in injectable depot microspheres.

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Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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