# DEVELOPMENT AND VALIDATION OF A FIRST-DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF AN ANTIPSYCHOTIC DRUG IN PHARMACEUTICAL FORMULATIONS AND FORCED DEGRADATION STUDIES**

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*A simple, cost-effective, and stability-indicating first-derivative spectrophotometric technique for quantifying Paliperidone in different pharmaceutical formulations is developed. In this method, the drug shows a maximum dA/dλ at 245 nm. The drug follows Beer–Lambert's law in the concentration range 2.5–70 μg/mL. Various degradation studies for the drug, such as acid hydrolysis, base hydrolysis, thermal, oxidative, and photolytic degradation are performed, and the results thereof are within the acceptable limit. The analytical method validation parameters like linearity, LOD, LOQ, precision, accuracy, etc. are conducted for the method as per the ICH Q2R(1) guideline, and the values are within the allowable range. Hence, for the determination of the Paliperidone quantity in pharmaceutical dosage forms, the developed process is a feasible one.*

***Keywords:*** *paliperidone, derivative spectroscopy, forced degradation.*

# РАЗРАБОТКА И ВАЛИДАЦИЯ СПЕКТРОФОТОМЕТРИЧЕСКОГО МЕТОДА НА ОСНОВЕ ПЕРВОЙ ПРОИЗВОДНОЙ ДЛЯ ОЦЕНКИ АНТИПСИХОТИЧЕСКОГО КОМПОНЕНТА В ФАРМАЦЕВТИЧЕСКИХ ПРЕПАРАТАХ И ИССЛЕДОВАНИЯХ ПРИНУДИТЕЛЬНОЙ ДЕГРАДАЦИИ

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*(Поступила 26 октября 2020)*

*Разработан спектрофотометрический метод на основе первой производной для количественного определения палиперидона в различных фармацевтических препаратах. В данном методе препарат показывает максимум dA/dλ при 245 нм, что соответствует закону Бера–Ламберта в диапазоне концентраций 2.5–70.0 мкг/мл. Исследованы кислотный гидролиз, щелочной гидролиз, термическое, окислительное и фотолитическое разложения лекарственного средства. Получены результаты в допустимых пределах. Параметры валидации аналитического метода (линейность, LOD, LOQ, прецизионность, точность и т. д.) определены в соответствии с рекомендациями ICH Q2R (1) и находятся в пределах допустимого диапазона.*

***Ключевые слова:*** *палиперидон, спектрофотометрия на основе производной, принудительная деградация.*

**Introduction.** Paliperidone (PD) (9-hydroxyrisperidone) is a major active risperidone metabolite [1]. The FDA approved it in 2006 for both acute and prolonged therapy of schizophrenia. Chemically, PD is (RS)-3-{2-[4-(6-fluoro-1,2-benzoxazole-3-yl)piperidin-1-yl]ethyl}-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-

**Full text is published in JAS V. 88, No. 6 (http://springer.com/journal/10812) and in electronic version of ZhPS V. 88, No. 6 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

pyrido[1,2-a] pyrimidin-4-one, and it is a derivative of benzisoxazole. PD acts as an antagonist of dopamine D(2) and serotonin 5HT(2A) [2]. It shows an antagonistic effect on adrenergic receptors like α(1), α(2), and histamine receptors H(1). It does not show any affinity for cholinergic receptors [3]. PD possesses one chiral carbon center used as a racemic mixture in the formulations. The racemate and enantiometer pharmacological profile matches for the *in vitro* binding assay and *in vitro* receptor occupancy studies [4–7].

A literature survey reveals that different authors quantified PD, present in different biological samples as well as in pharmaceutical dosage forms, by various analytical methods like spectrophotometry [8–12], HPLC [13–21], UPLC [22–24], HPTLC [25, 26], and LC-MS [27, 28]. Acetonitrile and methanol were used in a few reported spectroscopic methods [8–10] as diluents, but they are not cost-effective and cannot be used for dissolution studies. Korrapati U. et al. [11] developed two methods using isatin and chloranilic acid as a colorimetric reagent, which are complex and not cost-effective. Panda et al. [12] reported two spectrophotometric techniques by applying QbD, which is more complicated and requires trained personnel for its application. Therefore, there is a need to develop an easy and economical method for quantifying PD in dosage forms.

The authors used the first-derivative spectrophotometric method ($^1D$) in this study as it has many advantages over the conventional spectroscopic method ($^0D$). It removes irrelevant background absorption, and broad bands are suppressed and give rise to sharp bands compared to zero-order spectra. Therefore, the present research is to develop and validate a stability-indicating simple, accurate, sensitive, and precise $^1D$ method for PD as per the ICH guidelines.

**Methodology.** A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to a computer loaded with spectra manager software (UV Probe), with 1.0 cm matched quartz cells, was used for this study. The instrument influencing parameters are: 200–400 nm is the wavelength range; the scan speed is fast; the sampling interval is 1.0 nm; the derivative mode is $^1D$ (first-order derivative, $dA/d\lambda$); the bandwidth ($\Delta\lambda$) is 10.0 nm; the spectral slit width is 1 nm; the scaling factor is 1. For the weight measurement we used a high-precision electronic balance (Wensar, New Delhi).

The Roland Institute of Pharmaceutical Sciences supplied the working standard of PD (purity >98.5%) and further identified it by obtaining its DSC thermogram, ultraviolet (UV) spectra, and infrared (IR) spectra. Hydrochloric acid was purchased from Merck Ltd., Mumbai, India, and used to prepare 0.1 N HCl. Tablets of PD were prepared by direct compression due to the unavailability of the drug's pharmaceutical formulations in the local market.

A stock solution of PD (1000 μg/mL) was devised by dissolving 100 mg of the drug in 0.1 N HCl and diluted to 100 mL using the same solvent. From this, 5 mL was pipetted out to a 50 mL volumetric flask, and the volume was made up to the mark using 0.1N HCl to prepare a working standard solution having a concentration of 100 μg/mL. A series of dilutions was then made to prepare the concentrations ranging from 2.5 to 70 μg/mL and scanned in the range 400−200 nm against the solvent blank. The obtained zero-order spectra were derivatized to obtain first-order derivative spectra, and the response ($dA/d\lambda$) was measured at 245 nm. A linearity curve was plotted for the concentrations (2.5−70 μg/mL) versus the response at 245 nm.

For the preparation of PD tablet, all the components were first sieved through sieve No. 40, and then mixed following the geometric dilution to ensure the uniform mixing of the drug with selected excipients (Table 1). The prepared mixture of the drug and excipients was compressed into tablets using a Minipress-II (Karnavati, India) having a flat and round punch of a 6 mm diameter. A tablet batch consisted of 200 prepared tablets, and various quality control tests were carried out.

TABLE 1. Drug and Excipients Composition for a Unit PD Tablet Dosage Form

| Ingredients | Quantity, mg |
|---|---|
| Paliperidone | 9 |
| Microcrystalline cellulose pH 102 | 55 |
| Polyvinyl pyrrolidone K 30 | 20 |
| Starch | 10 |
| Talc | 3 |
| Magnesium stearate | 3 |
| Total weight | 100 mg |

The quality control tests were conducted following the IP and USP guidelines [29].

For the estimation of PD in the prepared formulation, 20 tablets from the ready batch were taken, weighed, and finely powdered. The tablet powder equivalent to 100 mg of PD was transferred into a 100 mL volumetric flask. Initially 50 mL of 0.1 N HCl was added, sonicated for 15 min, diluted to volume with 0.1N HCl, and filtered through Whatman filter paper (No. 41). Suitable dilutions of the sample solution were prepared with 0.1 N HCl, and the amount of PD was determined using the linearity curve. Both the percent labeled claim and the standard deviation (SD) were calculated.

The crushing strength of PD tablets was determined using a digital hardness tester. Each test used 10 tablets to assess the mean hardness using formula

$$\text{Mean hardness} = \frac{\text{total hardness of 10 tablets}}{10}.$$

The usual hardness value is 5–8 kg/cm$^2$ for standard compressed tablets with few exceptions such as effervescent tablets, dispersable tablet, chewable tablets, etc., and the value for sustained and controlled release tablets is more than 8–12 kg/cm$^2$.The friability test of PD tablets was performed using a digital friabilator. Tablets having a weight equivalent to 6.5 g (as per IP) were placed in the friabilator, rotated for 4min at a speed of 25 rpm, and the weight again taken. Finally, the loss in weight was calculated using formula

$$\% \text{ Friability} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100.$$

The maximum percentage friability should be between 0.5–1.0 % for standard compressed tablets. As per USP, 20 tablets were selected randomly and accurately weighed on an electronic balance, and the percent of the weight variation was determined using formula

$$\text{Weight uniformity} = \frac{\text{weight average} - \text{weight final}}{\text{weight average}}.$$

The acceptable weight variation for tablets weighing 130 mg or less should be 10%.

For disintegration test, six tablets were placed in six tubes (one each), and the basket rack assembly was kept in a beaker (1 L) containing water at 37±2°C in such a way that the PD tablets remained 2.5 cm below the upper level and 2.5 cm above from the bottom of the beaker during upward and downward movement, respectively. The disintegration period was noted (as per IP, the disintegration time for uncoated tablets is 15 min).

A USP Tablet Dissolution Tester (Type-2), which operated at a rate of 50 rpm and containing 0.1 N HCl as the dissolution medium (900 mL in one beaker) at a temperature of 37±0.2°C, was used to carry out the dissolution study. By maintaining the sink condition, the samples were withdrawn at 5, 10, 15, and 30 min (5 mL each time), and filtered through Whatman filter paper. After the suitable dilution, the samples were analyzed for the drug content using the first derivative spectroscopic calibration curve. We repeated the experiment three times. Later, the drug dissolution profile was evaluated for the quantity of the released drug in the initial 15 min ($Q_{15}$).

Validation is a method of establishing documentary confirmation that offers a higher degree of assurance that a particular action will constantly generate the preferred outcome or a product that meets the specified requirements and quality characteristics [30]. The planned method was validated for different parameters as per the ICH Q2 (R1) guidelines. The validation parameters like linearity, specificity, accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), and ruggedness were established.

The linearity range was checked over the concentration ranging from 2.5 to 70 μg/mL at six levels, each solution being prepared in triplicate. With the help of linear least-squares regression analysis, the calibration curve for PD was obtained by plotting $dA/d\lambda$ ($y$) versus the standard theoretical concentrations ($x$). The linearity was established as the correlation coefficient ($r^2$); its value should be ≥0.9990.

LOD and LOQ were determined based on the response SD based on the calibration curve and the slope.

The intraday and interday precision was determined by examining the standard samples selected based on the smile curve (Conc. *vs*. % RSD) [31], within the linearity range, at three concentration levels (30, 40, and 50.0 μg/mL), each solution being prepared in triplicate. For the interday precision, we examined three duplicates of the standard samples on the same day and three replications of the standards on three different days. The outcomes reported in terms of % RSD and one-way ANOVA at the 5% level of significance were used to compare the intraday and interday data.

The accuracy of an analytical method measures the percentage of the analyte recovered by the assay procedure. The study was carried out by spiking three known amounts of the analyte (16, 20, and 24 μg/mL; at three different levels, 80, 100, and 120% of sample solution) into a predetermined sample solution

of 20 μg/mL and preparing each solution in triplicate. The added drug percentage recovery was anticipated by measuring the $dA/d\lambda$ and fitting these values to the standard curve regression equation.

Recording the absorption spectra for the sample solution within the linearity range and comparing them with the standard solution of the same strength, we established the method's specificity. This study proves that there is no interference of excipients.

Ruggedness was established by examining aliquots from the homogenous slots (10 μg/mL) in different laboratories by two analysts and using two instruments under identical operational and environmental conditions. The result was presented as % RSD, and the data was compared using a *t*-test at a 5% level of significance.

The forced degradation study was performed as per the ICH Q1A-Q1E guidelines to confirm the developed method's stability-indicating power and reveal the intrinsic stability character of the active substance [32–34]. According to the procedure, the drug substance was exposed to diverse stressful conditions (more harsh conditions than the accelerated conditions), resulting in significant product degradation. The percentage of degradation should lie between 5% and 30% compared with nondegraded API [35]. This limit is selected so that little degradation will occur, but it should not be enough to generate secondary products [36].

A forced degradation study was carried out by preparing the drug solution (10 μg/mL) and subjecting it to various conditions. By adding 1 mL of 0.1 M NaOH to the standard and keeping it for 30 min at 50°C, we carried out the base hydrolysis. Similarly, by adding 1 mL of 0.1 M HCl and keeping it for 30 min at 50°C, the acid hydrolysis was performed. The standard drug was placed in a Petri dish for the thermal degradation study and held for 30 min at 50°C in a hot air oven. For the photolytic degradation, the standard drug was taken in a Petri dish and placed in a UV chamber for 30 min. For the oxidative degradation study, 1 mL of 3% $H_2O_2$ was added to the standard and kept at 50°C for 30 min in a water bath, neutralized by 3% sodium metabisulfite.

**Result and discussion.** The purity of PD was evaluated from the UV absorption spectrum (Fig. 2a) and the DSC thermogram (Fig. 1), which showed a sharp endotherm at 190.7°C in a nitrogen atmosphere at 100 mL/min, and an infrared spectrum ([C=O] stretching: 1888 $cm^{-1}$; [C-F] stretching: 1164 $cm^{-1}$; [-OH]: 3290 $cm^{-1}$ ( broad); [C=N, C-O] (Med): 1392, 1415 $cm^{-1}$ and [C-N, C=N](Med): 1217, 1732 $cm^{-1}$) (Fig. 3). These studies indicate that the drug was pure.

The PD solubility in different solvents like distilled water, 0.1 N NaOH, methanol, 0.1 N HCl, ethanol, and phosphate buffer (pH 6.8) was determined using the spectroscopic technique. In 0.1 N HCl, the drug showed a clear solution with the maximum solubility and good linearity range and was thus selected for this study.

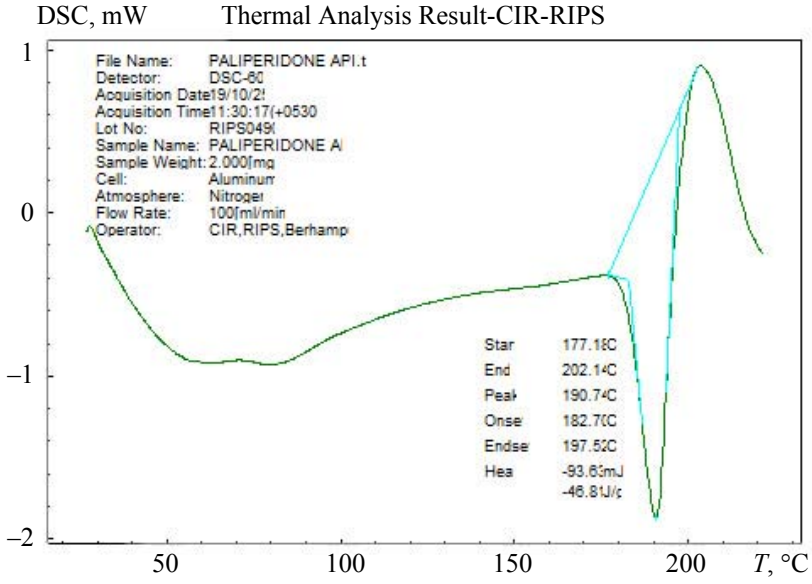


Fig. 1. DSC thermogram of Paliperidone.

In zero order spectra, the $\lambda_{max}$ was found at 235.0 and 271.0 nm. Still, the $^1D$ method was performed to remove irrelevant background absorption, suppress broad bands, and increase the sensitivity and specificity in the qualitative and quantitative analysis. The drug showed three peaks at 227, 245, and 286 nm in the $^1D$ spectrum (Fig. 2b). The study was conducted at 245 nm since, at this wavelength, Beer-Lambert's law shows a good linearity range, peaks are not deformed, and the maximum wavelength of the spectra and the zero-crossing point remain constant. It was noticed from the first derivative standard plot (Fig. 4) that there was a proportionate increase in the response concerning the concentration. It followed Beer-Lambert's law in the concentration range 2.5–70 µg/mL. The correlation coefficient value of 0.999 suggests that the developed spectrophotometric method had good linearity over the investigated range.

The calculated LOD and LOQ values were 0.316 and 0.958 µg/mL, respectively. This low value of LOD and LOQ indicates the sensitivity of the developed method.

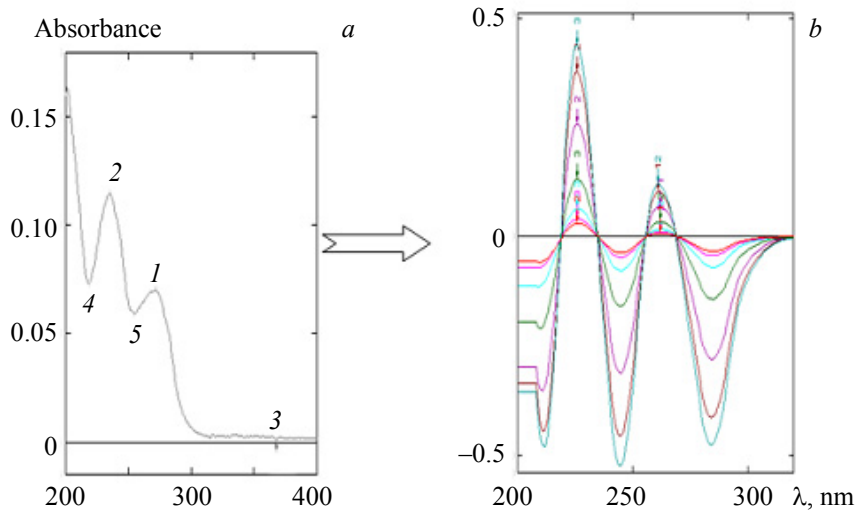


Fig. 2. UV absorption spectra of Paliperidone: a) $^0D$ spectra; b) $^1D$ spectra.

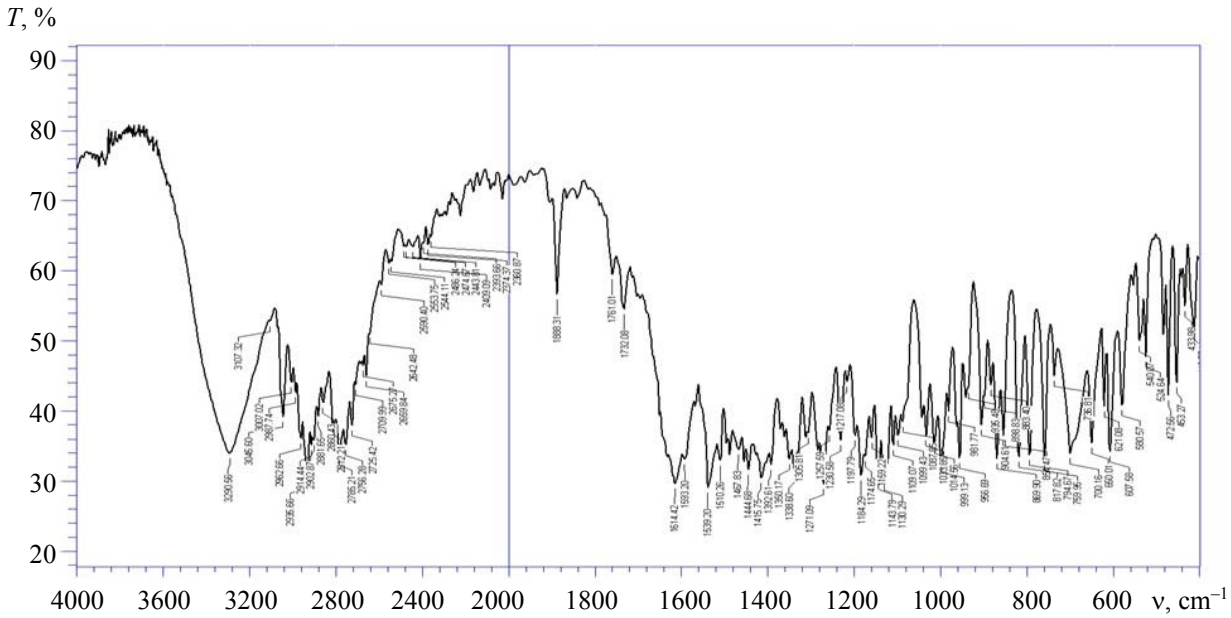


Fig. 3. FTIR spectra of Paliperidone.

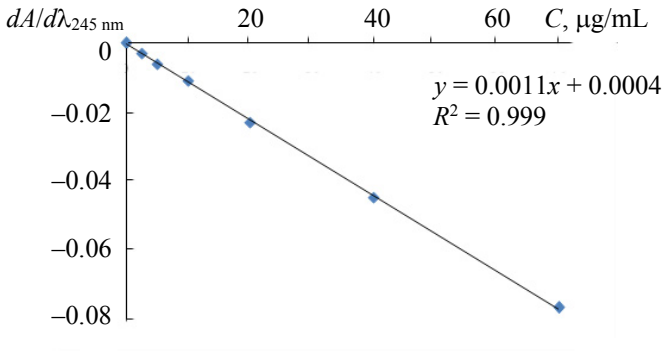


Fig. 4. Standard curve of PD by the $^1D$ spectroscopic technique.

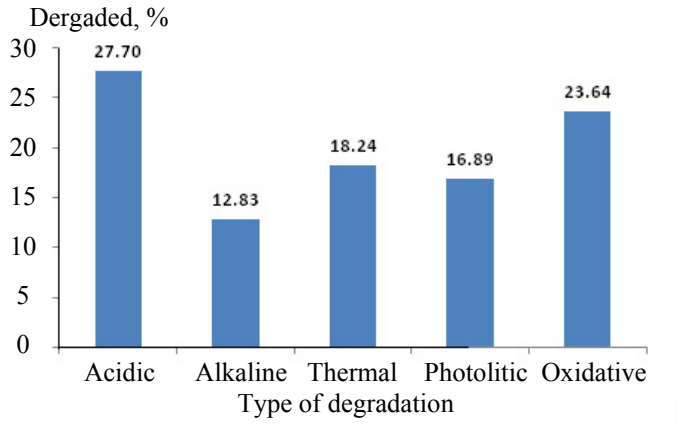


Fig. 5. Stress studies of PD by the $^1D$ spectroscopic technique.

The intraday and interday precision values were satisfactory with %RSD less than 2, which indicates that the developed method was reproducible. Further one-way ANOVA was applied to compare the intra and interday data, and the obtained *p*-value was 0.999, which was more than 0.05. The $F_{calc}$ (0.0002) value was less than the $F_{tab}$ value (5.143), indicating no difference in the results obtained on different days. Also, we calculated the confidence level at 5 and 1% levels. The values were less than 1.90 and 2.58 for 5 and 1%, respectively. The optical features of PD were calculated for the developed method and shown in Table 2.

TABLE 2. Different Validation Parameters of PD

| Parameters | Results |
|---|---|
| Beer–Lambert's range, µg/mL | 2.5–70.0 |
| Wavelength (nm) of measurement | 245.0 |
| Slope | 0.0011 |
| Intercept | 0.0004 |
| Correlation coefficient | 0.999 |
| Limit of detection, µg/mL | 0.316 |
| Limit of quantitation, µg/mL | 0.958 |
| Precision (RSD, %) | |
| Intraday (*n*=3) | 0.310–0.559 |
| Interday (*n*=3) | 0.310–0.874 |
| Accuracy (% recovery) | 98.395–102.759 |
| Ruggedness (RSD, %) | |
| Analyst 1/Instrument 1 | 0.900 |
| Analyst 2/Instrument 2 | 0.892 |

The prepared tablet quality control parameters were within the permissible limit as per the IP and USP guidelines and presented in Table 3. These tablets were analyzed to determine the drug content with the developed method, and it was found out that the content was close to the labeled claim by the authors (Table 4). The sample absorption spectrum overlapped with the standard, and both spectra were overlapped, which showed a lack of interference from the excipients. This proved that the method was specific.

TABLE 3. Quality Control Parameters for the PD Tablet

| Quality control test parameters | | Results* |
|---|---|---|
| Weight variation, % | | 99 ± 2.7 |
| Drug content, % | | 98.94 ± 1.06 |
| Hardness, kg/cm$^2$ | | 4.8 ± 0.6 |
| Friability, % | | 0.2 ± 0.005 |
| Disintegration time, min | | 7 ± 0.3 |
| Cumulative % drug release at | 15 min | 27.7 ± 2.6 |
| | 30 min | 56.5 ± 2.8 |
| | 60 min | 97.4 ± 2.3 |

*Mean ± SD, $n$ = 6.

TABLE 4. Assay PD Results in the Pharmaceutical Dosage Form (Tablet 100 mg) Using the Method

| Label claim, mg/tab | % Label claimed ±SD, $n$ = 5 | %RSD |
|---|---|---|
| 100 | 98.936 ± 1.060 | 1.072 |

The results of the accuracy study, presented in Table 2, were determined following the ICH guidelines. The percent recovery indicates that the developed method was accurate and there was no interference from the excipients.

The results obtained by two analysts confirmed the spectrophotometric method's ruggedness (Table 2) since the obtained %RSD value was less than 2. Moreover, to compare the data obtained by two analysts, a $t$-test was used. The $p$-value obtained was 0.288, which was more than 0.05, and the $t_{calc}$ value was 1.22, which was less than the $t_{tab}$ value (2.776), which indicates that there was no difference in the results obtained by the two analysts.

Forced degradation studies examined the stability-indicating capability of the developed method. The standard, subjected to various degradation conditions, was indicated by the decrease in the drug's expected concentration and the increase in the level of degradation products. The results from this test are presented in Fig. 5. The values are within the limit, and the drug is more susceptible to an acidic environment than to an alkaline medium. This study indicated that the drug was stable in various stress conditions.

**Conclusions.** A simple, sensitive, precise, accurate, and rugged $D^1$ method was developed and validated for PD. Industries can use this method for the regular analysis of PD in pharmaceutical dosage forms. The planned spectrophotometric method will not replace the currently well-known techniques accessible for drug analysis; still, it can serve as a choice where sophisticated analytical instruments like HPLC are not accessible for routine analysis.

**Acknowledgments.** The authors are thankful to the Roland Institute of Pharmaceutical Sciences, Berhampur-10, Odisha, India, for providing the research facilities.

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