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Validated Stability-Indicating HPLC Method for the Separation of Pindolol Enantiomers and Its Related Substances

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ABSTRACT

A simple, rapid and stability-indicating reverse-phase liquid chromatographic (RP-LC) method was developed for the separation of pindolol (PDL) and its related substances in the presence of its degradation products generated from forced decomposition studies. The chromatographic separation was achieved on a C18 Inertsil ODS-3V column (250 mm × 4.6 mm i.d.), using a mobile phase of 20 mM sodium dihydrogen orthophosphate-acetonitrile containing orthophosphoric acid to maintain pH at 4.0. Gradient elution was used at a flow rate of 1.0 mL/min. The UV detector was operated at 205 nm and the column temperature was maintained at ambient. Separation of PDL enantiomers was achieved on normal phase liquid chromatography (NP-LC) using a chiral pack AD-H (250 mm × 4.6 mm i.d.) column, with mobile phase consisting of n-hexane : ethanol : diethylamine (860 : 140 mL : 0.05%, v/v). A flow rate of 0.9 mL/min and detection wavelength of 215 nm were used. The method was validated for specificity, linearity and range, precision, accuracy, solution stability, robustness, limit of detection (LOD) and limit of quantification (LOQ). Selectivity of the RP-LC method was validated by subjecting the stock solution of PDL to acidic, basic, photolysis oxidative and thermal degradation. The calibration curve was found to be linear in the concentration range of 0.01 - 100 µg/mL ($r^2 = 0.9998$) and 0.001 - 50 µg/mL ($r^2 = 0.9982$) of PDL for the RP-LC and NP-LC method, respectively. The peaks of degradation products did not interfere with that of pure PDL. The utility of the developed method was examined for chemical and chiral purity by analyzing the tablets containing PDL and also by determining the related substances of PDL. The results of analysis were supported by statistical parameters.

Key words: pindolol, related substance, chiral separation, validation, forced degradation

INTRODUCTION

Pindolol (PDL) (Figure 1A), a synthetic beta-adrenergic receptor blocking agent[(1)] with intrinsic sympathomimetic activity, is 1-(indol-4-yloxy)-3-(isopropylamino)-2-propanol. It is an effective agent for treating hypertension in pregnancy, a disease that complicates up to 5% of all pregnancies[(2-5)]. PDL is a non-selective beta-adrenergic antagonist (beta-blocker) which possesses intrinsic sympathomimetic activity (ISA) in therapeutic dosage ranges but does not possess quinidine-like membrane-stabilizing activity. PDL causes no changes in utero or in umbilicoplacental vascular impedance or blood flow, has no effect on fetal haemodynamics and does not affect fetal cardiac function[(3)]. PDL non-selectively blocks beta-1 adrenergic receptors mainly in the heart, inhibiting the effects of epinephrine and norepinephrine, resulting in a decrease in heart rate and blood pressure. PDL inhibits the production of renin, thereby inhibiting angiotensin II and aldosterone production and therefore inhibits vasoconstriction and water retention due to angiotensin II and aldosterone, respectively. It is marketed as a racemic mixture of the (−)-S and (+)-R enantiomers, because PDL is eliminated stereoselectively by the kidney[(6)]. It is moderately lipid-soluble[(7)] and about 40 - 60% is reported to be bound to plasma proteins[(8)].

A few LC methods for quantification of PDL in biosamples have been reported using chiral columns and derivative method for separation[(10,11)]. Determination of PDL in human serum or urine has also been achieved by LC/MS/MS[(12,13)]. The United States Pharmacopeia (USP) describes the HPLC method with the mobile phase consisting of 0.05 M sodium acetate (pH 5.0) buffer and acetonitrile (65 : 35, v/v) on a 150 × 4.6 mm column using UV detection at 219 nm for the assay of PDL. In this method, the low concentration of acetonitrile resulted

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in less resolution between PDL and impurities, while increased acetonitrile concentration also resulted poor resolution between PDL and impurities with longer retention times. Further, the USP method described a TLC method for the determination of impurities and for the assay of formulations containing PDL. This method limits the detection of any individual impurity to a maximum of 0.5%. The British Pharmacopoeia (BP) describes a TLC method to report any impurity that exists greater than 0.3%. The present study reports for the first time the development and validation of RP-LC method for the assay of PDL and its related substances (Figure 1B) in shorter run time and at higher detection levels. No method has been reported so far for the analysis of PDL and its related substances. Further, the drug substance was subjected to stress degradation conditions *viz.*, acidic, basic, oxidation, photolysis and thermal degradation in liquid and solid states to evaluate the PDL purity. The proposed method is observed to be specific as it can be used to determine the presence of its degradation products. In addition, the separation of enantiomers was effectively achieved. This observation was not described in the official methods.

MATERIALS AND METHODS

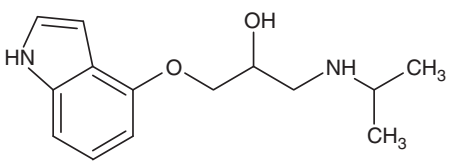
I. *Chemicals and Reagents*

The pure compound of PDL was obtained from Novartis Pharmaceuticals, India. HPLC-grade acetonitrile and n-hexane were purchased from Spectrochem Pvt. Ltd., India. Sodium dihydrogen orthophosphate, orthophosphoric acid, hydrochloric acid, sodium hydroxide, diethylamine and hydrogen peroxide were obtained from Merck (Darmstadt, Germany). HPLC-grade ethanol was obtained from Hayman, England. HPLC-grade water was obtained from a Milli-Q water purification system (Millipore, MA, USA) and used throughout the study.

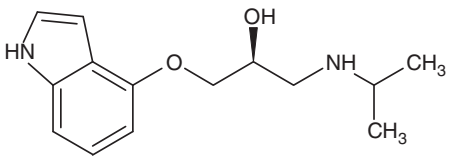
II. *Instrumentation*

For PDL and its related substances, a Waters 2695 separation module system consisting of an online degasser, quaternary pump, auto-sampler equipped with cooling system, thermostat column compartment and 2996 module photo-diode array (PDA) detector was used. An

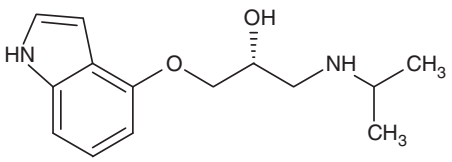
(A)



1-(1*H*-indol-4-yloxy)-3-(propan-2-ylamino) propan-2-ol

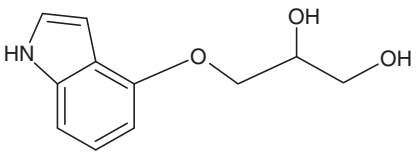


(2S)-1-(1*H*-indol-4-yloxy)-3-(propan-2-ylamino) propan-2-ol

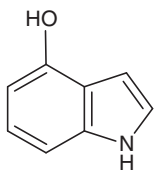


(2R)-1(1*H*-indol-4-yloxy)-3-(propan-2-ylamino) propan-2-ol

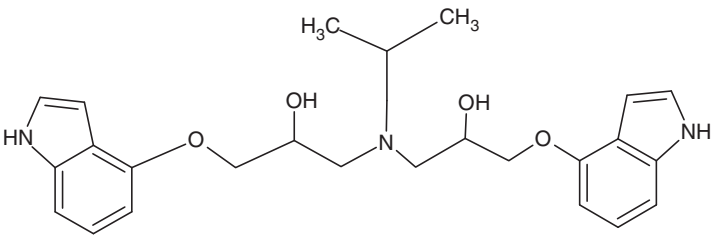
(B) Impurity-I: 3-(1*H*-indol-4-yloxy) propane-1,2-diol



Impurity-II: 1*H*-indol-4-ol



Impurity-III: 1, 1-[(1-Methylethyl)imino]bis[3-(1H-indol-4-yloxy)propan-2-ol]



Impurity-IV: 2, 3-bis {[(4-methylphenyl) carbonyl] oxy}butanedioic acid

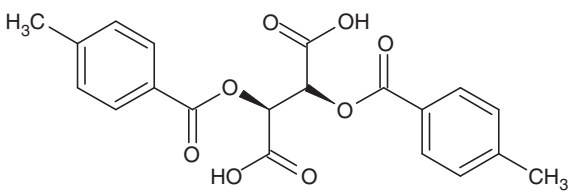


Figure 1. (A) Structures of pindolol (PDL). (B) Structures of related substances of PDL.

Inertsil C18 column (250 mm × 4.6 mm i.d., particle size of 5 μm, G L Science, Japan) was used in the study. The data were processed on the Empower software version 2.0. Mobile phase and sample/standard preparations were degassed using a sonicator (S. V. Scientific, India).

For separation of (R)- and (S)- enantiomers of PDL, a Waters 2695 separation module system consisting of a quaternary pump, auto-sampler equipped with cooling system, thermostat column compartment and 2487 module dual wavelength detector was used. A chiral pack AD-H column (250 mm × 4.6 mm i.d., particle size of 5 μm, Daicel, Japan) was used in the study.

III. *Chromatographic Conditions*

The mobile phase consisting of (A) 20 mM sodium dihydrogen orthophosphate and orthophosphoric acid in water of pH 4.0 and (B) acetonitrile was used, with a gradient programmed pump to deliver the buffer and solvent to the column at a flow rate of 1.0 mL/min. The solvent and buffer delivery pump was programmed to gradient system and it started with initially 30% of acetonitrile and 70% of phosphate buffer and maintained up to 4 min, then the acetonitrile and buffer ratio was increased to 70% and 30% respectively in 10 min and maintained at the same ratio for 5 min. The column was re-equilibrated for 5 min. The mobile phase was filtered through a 0.45-μm filter (Millipore, Bedford, USA). Water and acetonitrile in the ratio of 70 : 30 v/v was used as diluent for sample and standard preparations and the same was injected as blank. The UV detector was operated at 205 nm and peak purity was determined over 200 - 400 nm using a custom-made PDL spectrum match library. The column temperature was maintained at ambient.

The separation of enantiomers was achieved on a chiral pack AD-H (250 mm × 4.6 mm i.d.) column, using a mobile phase consisting of n-hexane-ethanol-diethylamine (860 mL : 140 mL : 0.05%, v/v) with an isocratic pump to deliver the solvent to the column at a flow rate of 0.9 mL/min. An n-hexane and ethanol mixture in the ratio 50 : 50 v/v was used as diluent for sample and standard preparation and the same was injected as blank. The UV detector was operated at 215 nm and the column temperature was maintained at ambient.

IV. *Procedures*

(I) *Preparation of Stock and Standard Solutions of PDL and Related Substances*

A stock solution of PDL (0.2 mg/mL) and impurity solution (0.1 mg/mL) was prepared by dissolving an appropriate amount of substance in diluent (water : acetonitrile ; 700 mL : 300 mL, v/v). For NP-LC analysis, (R)-enantiomer, (S)-enantiomer and racemate stock solutions (0.1 mg/mL) were prepared in diluent (n-hexane : ethanol; 450 mL : 550 mL, v/v).

I. *Analysis of Tablets*

Twenty tablets of PDL were finely powdered. An amount equivalent to 20 mg of the drug was weighed accurately and transferred into a 100-mL beaker. Using a mechanical stirrer, the powder was completely disintegrated in mobile phase. The solution was filtered and the filtrate was made up to 100 mL with the mobile phase. It was further diluted, as required for analysis.

II. *Linearity and Range*

The test solutions were prepared from the stock solution of PDL so as to contain the drug in the range of 0.01 - 100 μg/mL. The solutions were injected in triplicate into the HPLC column, using a constant injection volume of 10 μL and the chromatograms were recorded. The peak area *versus* concentration data was treated by least-squares linear regression analysis. Chromatograms were recorded using the above test solutions for three consecutive days in the same concentration range. The % Relative Standard Deviation (RSD) values were calculated.

III. *Precision*

Intra-day precision and inter-day precision were evaluated by analyzing six replicates of three different concentrations (20, 60, 80 μg/mL) on the same day and on different days, respectively. The respective % Relative Standard Deviation (RSD) values were calculated.

IV. *Accuracy*

The accuracy of the assay method was evaluated in triplicate at three concentration levels (20, 60 and 80 μg/mL). It was also evaluated by fortifying a mixture of formulation sample with three known concentrations of the drug. The recovery of the added drug was determined.

V. *Specificity and Selectivity*

Specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak and also among all the other peaks[14]. The presence/absence of peaks due to excipients, impurities and degraded products was examined to study the interference from these substances in the assay of the drug[15].

VI. *LOD and LOQ*

Determination of the signal-to-noise ratio was performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected and quantified.

The values of LOD and LOQ were determined at a signal to noise ratio of 3 : 1 and 10 : 1, respectively by injecting a series of test solutions of known concentrations within linearity range[15]. Precision study was also carried out at the LOQ level by injecting six preparations.

VII. *Robustness*

To determine the robustness of the developed method, experimental conditions were deliberately altered to check the reproducibility and quantitative recovery of the drug. This was carried out by maintaining the flow rate of the mobile phase in the range of 0.8 - 1.2 mL/min, column temperature in the range of 22 - 30°C and the strength of the phosphate buffer was changed from 20 mM to 10 mM and the resolution of PDL and related substances was evaluated. Chromatograms were recorded and compared with those obtained under optimum chromatographic conditions. In case of chiral separations n-hexane, ethanol and diethylamine compositions were varied from 830 mL to 890 mL v/v, 170 mL to 110 mL v/v and 0.03% to 0.07%, respectively.

VIII. *Solution Stability and Mobile Phase Stability*

The solution stability of PDL in the assay method was carried out by leaving both the test solutions of the sample and reference standard in tightly capped amber-colored volumetric flasks separately, at room temperature, up to the study period of 72 h. The chromatograms of these solutions were recorded separately at an interval of 1 h up to 72 h and the peak responses were compared.

The mobile phase stability was carried out by assaying the freshly prepared bulk drug and formulation sample solutions against freshly prepared reference/standard solution at an interval of 8 h. The same mobile phase was used throughout the experiment. % Relative Standard Deviation (RSD) values were calculated for mobile phase and solution stability experiments. In the case of chiral separations, solution stability and mobile phase stability were studied for 24 h.

IX. *Oxidation of PDL*

For oxidation study, 5.0 mL of stock solution of PDL (50 µg/mL) was transferred to a 25-mL amber-colored volumetric flask and made up to volume with 3% hydrogen peroxide. The flask was placed at 80°C for 8 h, cooled to room temperature and the volume was readjusted with 3% hydrogen peroxide. Then the solution was filtered through a 0.45-µm syringe filter and 10 µL solution was injected into the liquid chromatographic system to detect peaks of degradation due to oxidation.

X. *Thermal Degradation of PDL*

This was carried out by transferring 5.0 mL of PDL (50 µg/mL) into a 25-mL volumetric flask and diluted to the mark with the mobile phase. The flask was closed and placed at 80°C for 8 h, cooled to room temperature and the volume of solution was readjusted with the diluent. The chromatogram was recorded.

XI. *Degradation of PDL by an Acid*

For this study, 5.0 mL of PDL (50 µg/mL) was transferred into a 25-mL volumetric flask and the volume was made up to the mark with 1 M hydrochloric acid. The flask was placed at 80°C for 8 h, cooled to room temperature and the volume of solution was readjusted to 20 mL with 1 M hydrochloric acid. The solution was adjusted to neutral pH using 1 M sodium hydroxide to avoid column damage at too acidic a condition, as it would lead to peak splitting or to unsymmetrical peak shape. The neutral solution was injected into the liquid chromatograph and the chromatogram was recorded.

XII. *Degradation of PDL by an Alkali*

This was conducted by transferring 5.0 mL of PDL (50 µg/mL) into a 25.0-mL volumetric flask and diluted to the mark with 1 M sodium hydroxide. The flask was kept at 80°C for 8 h, cooled to room temperature and the volume was readjusted to 20 mL with 1 M sodium hydroxide. The solution was neutralized with 0.1 M hydrochloric acid solution and the chromatogram was recorded.

XIII. *Photodegradation of PDL*

For photodegradation studies, 5.0 mL of PDL (50 µg/mL) was transferred into a 25.0-mL volumetric flask and diluted to the mark with diluent. The flask was exposed to UV light for 8 h continuously. The experiment was also repeated with solid drug sample (10 µg/mL in diluent). Both solutions were separately injected into the liquid chromatograph and the chromatograms were recorded.

RESULTS AND DISCUSSION

I. *Method Development and Optimization for PDL and its Related Substances*

Identification and control of impurities for drug substances is a critical task in pharmaceutical process development for quality and safety. The most commonly used analytical technique for impurity analysis in drug substances and a drug product is undoubtedly a chromatographic method, namely high performance liquid chromatography (HPLC). Impurity profiling is typically performed by HPLC and impurities are further tested for identification and confirmation by other techniques. Upon identification of the impurity, the impurity formation was monitored and controlled throughout the synthesis. It is demonstrated that

identification and monitoring of the unknown or known impurities enabled chemists to pinpoint the chemical step of impurity generation, aiding the effort to reduce or even eliminate the impurity in the drug substances.

PDL is a weak base with pK_a of 9.7. The selection of buffer assumes importance. Ammonium acetate buffer was found to be unsuitable due to poor resolution and the UV cut off at 215 nm. Gradient program was employed using phosphate buffer and acetonitrile with an initial ratio of 60 : 40. Due to less interaction of the compound with the stationary phase, PDL and impurities were eluted very early and the peaks were unsymmetrical in nature. Moreover, the peaks of degradants were not separated from that of the pure drug. Well-resolved and symmetric peaks were obtained with the mobile phase consisting of sodium dihydrogen orthophosphate buffer of pH 4.0 ± 0.1 and acetonitrile. In order to optimize the chromatographic conditions, the effect of composition of mobile phase, flow rate and the detection wavelength were investigated. Ideal chromatographic conditions were observed with the mobile phase consisting of 20 mM sodium dihydrogen orthophosphate-acetonitrile containing ortho-phosphoric acid adjusted to pH 4.0. The gradient program started initially with 30% of acetonitrile and 70% of phosphate buffer and maintained up to 4 min, and then the ratio of acetonitrile and buffer was changed to 70 : 30 in 10 min and maintained at the same ratio for 5 min. The column was re-equilibrated for 5 min with a flow rate of 1.0 mL/min and a wavelength of 205 nm was used throughout the run time. Typical chromatograms of PDL and related substances overlaid with blank are shown in Figure 2. The selection of wavelength to monitor the quality evaluation of drug substance and related impurities was done by overlaying the individual UV spectra of PDL and its impurities-I, II, III and IV (Figure 3). The wavelength of 205 nm was selected due to maximum absorbance for all the related impurities and drug substance. From the purity plot of PDL, lower purity angle value of PDL compared to that of the purity threshold (Table 1 and Figure 4) revealed that the PDL was free from interference from its impurities and its degradants. This

Table 1. System suitability parameters

Name	Retention time (min)	Purity angle	Purity threshold	USP tailing	Resolution	USP plate count
PDL	3.383	0.557	0.567	1.201	Not applicable	5159
Impurity-I	5.287	0.537	0.574	1.180	8.160	7556
Impurity-II	8.267	0.497	0.500	1.089	10.572	11093
Impurity-II	9.261	0.523	0.592	0.958	3.639	27996
Impurity-IV	10.089	0.622	0.630	1.211	3.287	22483

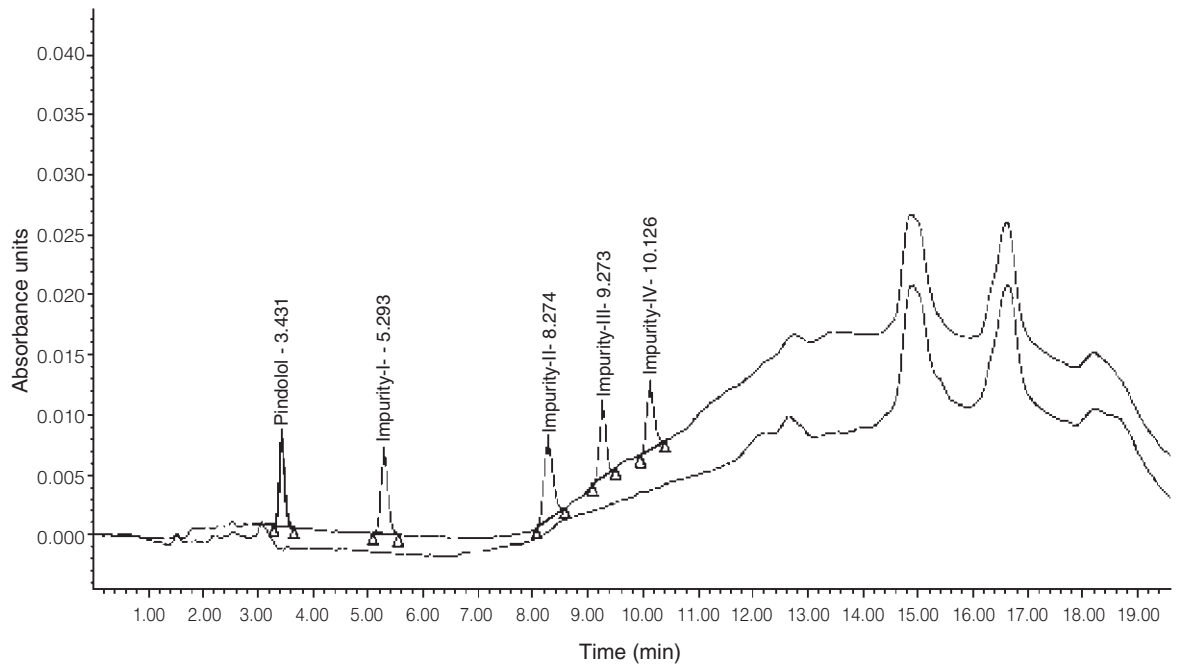


Figure 2. A typical chromatogram of PDL and its related substances overlaid with blank using an intial eluent of 70 : 30 (v/v, 20 mM sodium dihydrogen orthophosphate of pH 4.0 – acetonitrile) ramped to 30 : 70 within 10 min (UV absorption at 205 nm).

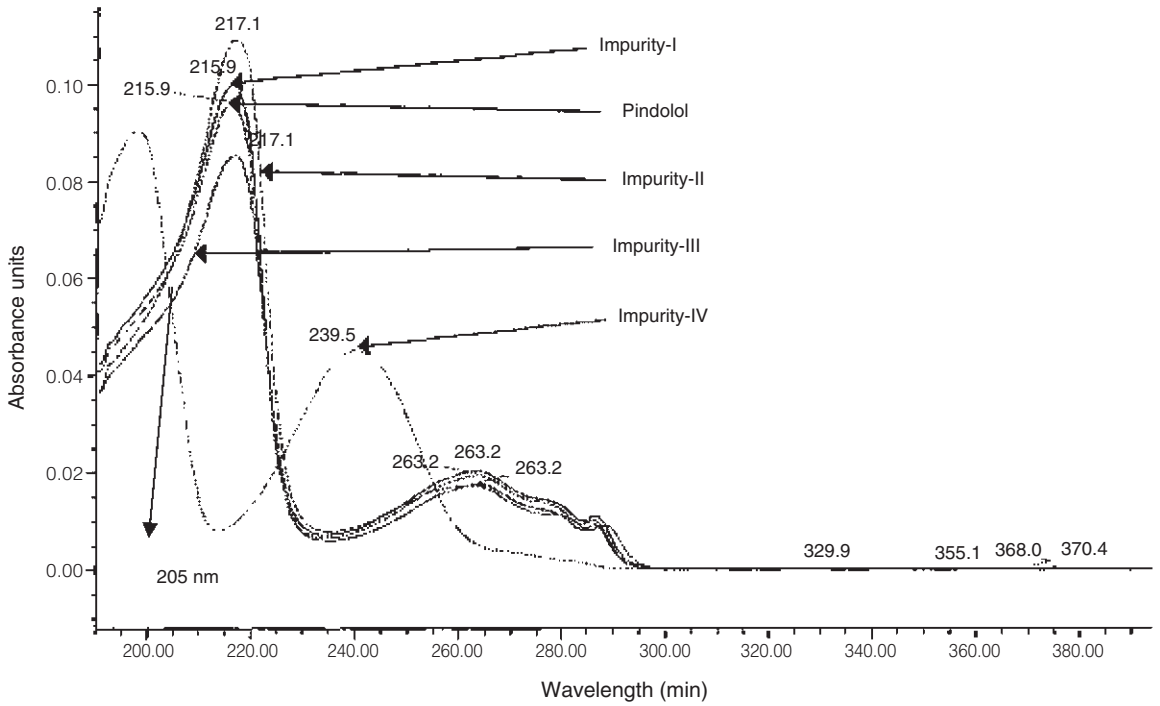


Figure 3. UV spectra of PDL and its related substances overlaid.

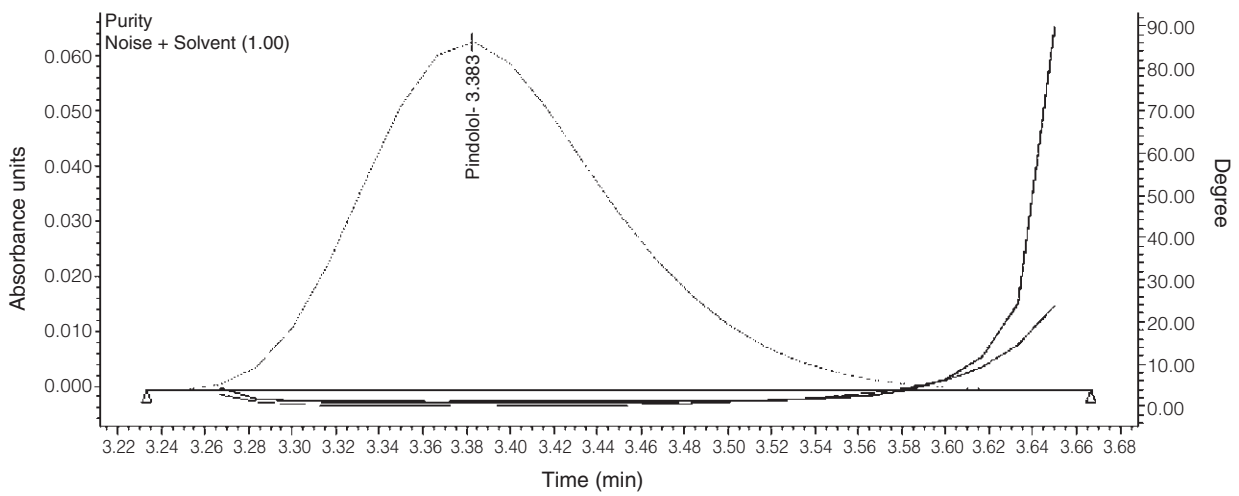


Figure 4. Purity plot of PDL.

was also evident by comparing the 3D plot of the PDL standard with that of the degraded product. The representative 3D diagrams for pure PDL and its related substances are shown in Figure 5.

II. *Method Development and Optimization for (R) and (S) Enantiomers of PDL*

The method development strategies adopted using chiral pack AD-H column involve different experiments based on the nature and structure of the compound. The design of mobile phase consists of a combination of alkane and polar alcohols based on normal or polar interactive modes. Attempts to separate (R) and (S) isomers of PDL using chiral columns in the reverse phase were not successful. The use of normal phase chromatography employing various chiral columns (chiral cel-OJ, chiral cel-OD) were also not successful. When using chromatographic conditions, such as flow rate of 0.9 mL/min and mobile phase of n-hexane and 2-propanol (800 mL : 200 mL, v/v) mixture on a chiral pack OD-H column with dimension 250 mm × 4.6 mm, 5 µm, it was observed that the (R) and (S) isomers of PDL were eluted as broad peaks with longer retention times. However, an improvement in peak shapes was observed when a chiral pack AD-H column with n-hexane and ethanol ratio (860 mL : 140 mL) mobile phase

and the same flow rate were used. However, there were still certain constraints like longer retention times and poor separation between the enantiomers. Due to the presence of amide functional group in PDL, 0.05% of diethylamine was introduced to the above mobile phase, i.e. n-hexane : ethanol (860 mL : 140 mL, v/v), which gave comparatively good peak shapes as well as good resolution. In the present optimized method, the typical retention times of (S)-enantiomer and (R) enantiomer of PDL were noticed to be 10.1 min and 13.1 min, respectively. Typical chromatograms of the PDL racemate are shown in Figure 6. The resolution between the two enatiomers was about 5.923. Diluent, n-hexane : ethanol

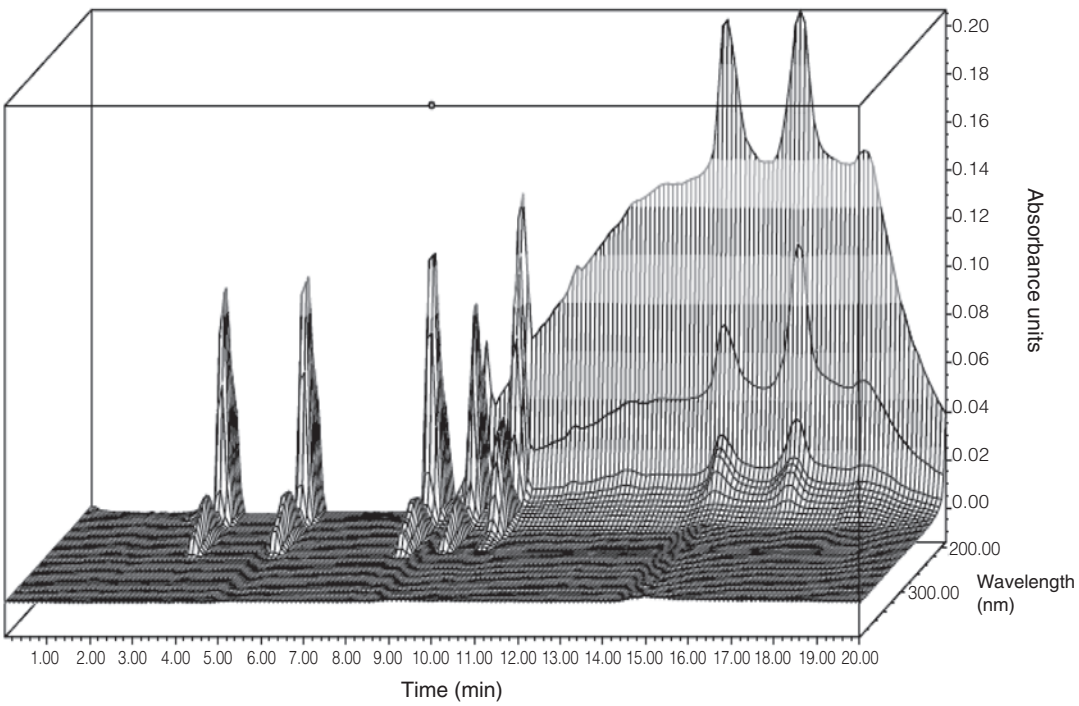


Figure 5. 3D plot of PDL standard and its related substances.

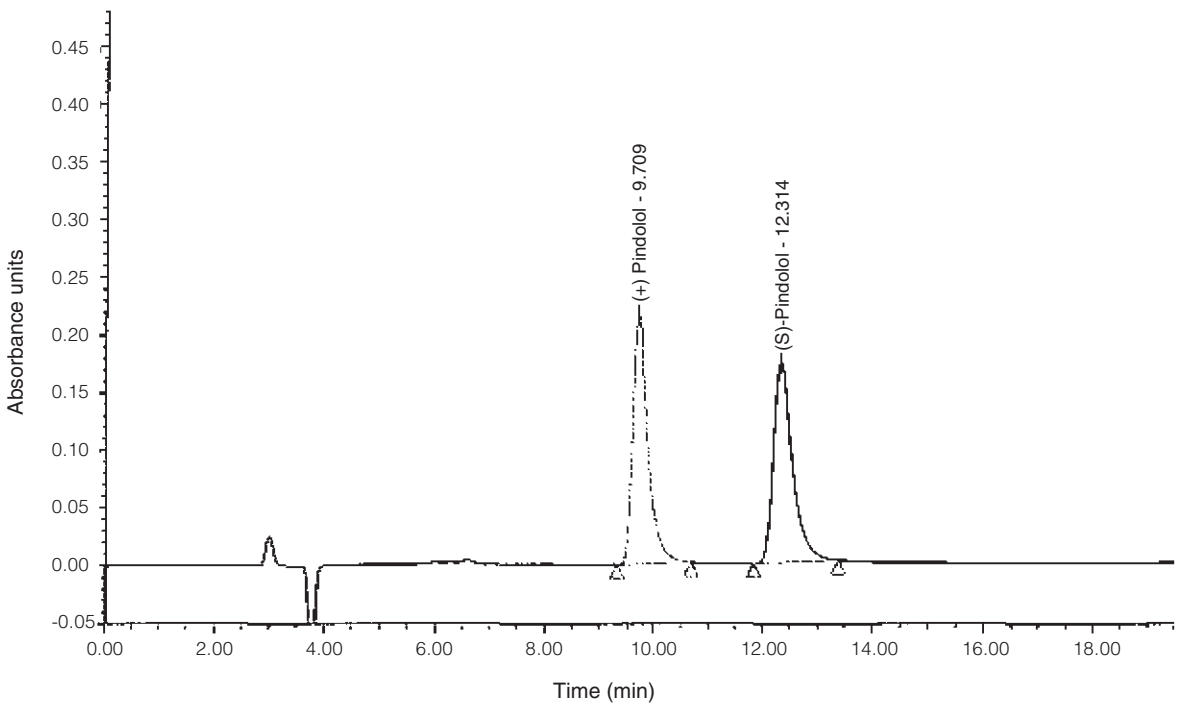


Figure 6. Typical chromatogram of PDL enantiomers (racemate) at a concentration of 8 µg/mL using an isocratic eluent of n-hexane : ethanol in 86 : 14 v/v ratio for 20 min (UV absorption at 215 nm).

(1 : 1) was used as blank and there was no interference of the blank and of excipients with (S) and (R) enantiomers of PDL. The elution was monitored at 215 nm.

III. *Precision*

The % RSD values for intra-day and inter-day precision study were found to be less than 0.5% and 0.8%, respectively, indicating that the method was sufficiently precise.

IV. *Accuracy*

The % recovery values of PDL in pharmaceutical formulation ranged from 99.8 to 100.1 (Table 2). High percentage recovery values revealed that the proposed method is accurate and could be adopted for routine quality control analysis.

V. *Limit of Detection (LOD) and Limit of Quantification (LOQ)*

ICH guidelines[15] were followed to calculate the values of LOD and LOQ for PDL and they were observed to be 0.0030 µg/mL and 0.01 µg/mL, respectively. The % RSD value was noticed to be less than 1.2 for related substances at the LOQ concentration level.

VI. *Linearity*

The calibration plot for the assay of PDL was linear over the investigated range of 0.01 - 100 µg/mL with a correlation coefficient value of 0.9998. In the case of racemate, (R) and (S) enantiomers of PDL, the calibration plot was noticed to be linear in the range of 0.001 - 50 µg/mL (r^2 = 0.9982) and 0.002 - 50 µg/mL (r^2 = 0.9989) respectively. For the related substances, the values are given in Table 3.

Table 2. Recovery results of PDL sample

Added (µg) (n = 3)	Recovered (µg)	% Recovery	% RSD
50.1	50.0	99.8	0.8
100.5	100.6	100.1	0.9
150.2	150.4	100.1	1.1

VII. *Robustness*

Under the deliberately varied chromatographic conditions (flow rate, column temperature and composition of organic solvent or buffer), the reproducibility of results was observed to be reasonably good. Hence, it can be concluded that the proposed method has good robustness for the analysis of PDL in bulk or tablets.

VIII. *Solution Stability and Mobile Phase Stability*

The % RSD of the assay of PDL during solution stability and mobile phase stability experiments were found to be less than 0.9%. This indicated that the sample solutions and mobile phases used during quantification of PDL and its related substances were stable for at least 72 h.

IX. *Results of Forced Degradation Studies*

Forced degradation is the process of subjecting the drug compound to extreme chemical and environmental conditions to determine product breakdown levels, assess preliminary degradation kinetics and identify degradants. The ICH guideline indicates that stress testing is designed to help determine the intrinsic stability of the molecule by establishing degradation pathways, in order to identify the likely degradation products and to validate the stability-indicating power of the analytical procedures used. The hydrolytic degradation of a newly proposed drug in acidic and alkaline conditions can be studied by keeping the solution of pure drug in HCl and NaOH medium respectively at room temperature over 12 h. If degradation is not observed for more than 5% even after increasing the strength of the acid or alkali, then the same solution can be refluxed for 8 h. Further, to examine the degradation by oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3 - 30%. If degradation is not observed for more than 5%, the same solution may be refluxed for 8 h.

Degradation was not observed in thet PDL sample when it was subjected to acid, alkali and oxidation at room temperature with 1 M hydrochloric acid, 1 M sodium hydroxide and 3% hydrogen peroxide, respectively. However, when the same solutions were kept at 80°C, degradation was observed under all the three conditions and chromatograms of these solutions were recorded. Oxidative degradation of PDL pure sample

Table 3. Related substances linearity and LOQ data (n = 6)

Name	R^2	LOQ (µg/mL)	Accuracy @ 50%	Accuracy @ 100%	Accuracy @ 150%
Impurity-I	0.9991	0.0206	101.1	99.1	98.4
Impurity-II	0.9994	0.0516	101.5	100.2	101.5
Impurity-III	0.9983	0.0509	99.5	99.7	99.5
Impurity-IV	0.9989	0.0510	98.7	100.9	99.3

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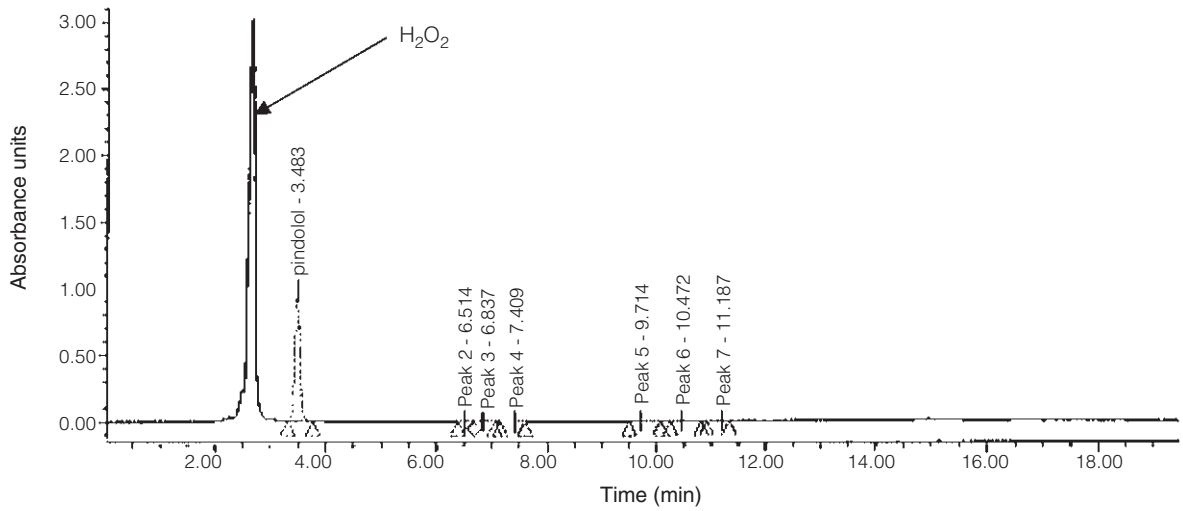

Figure 7. A typical chromatogram of PDL exposed to 3% hydrogen peroxide.

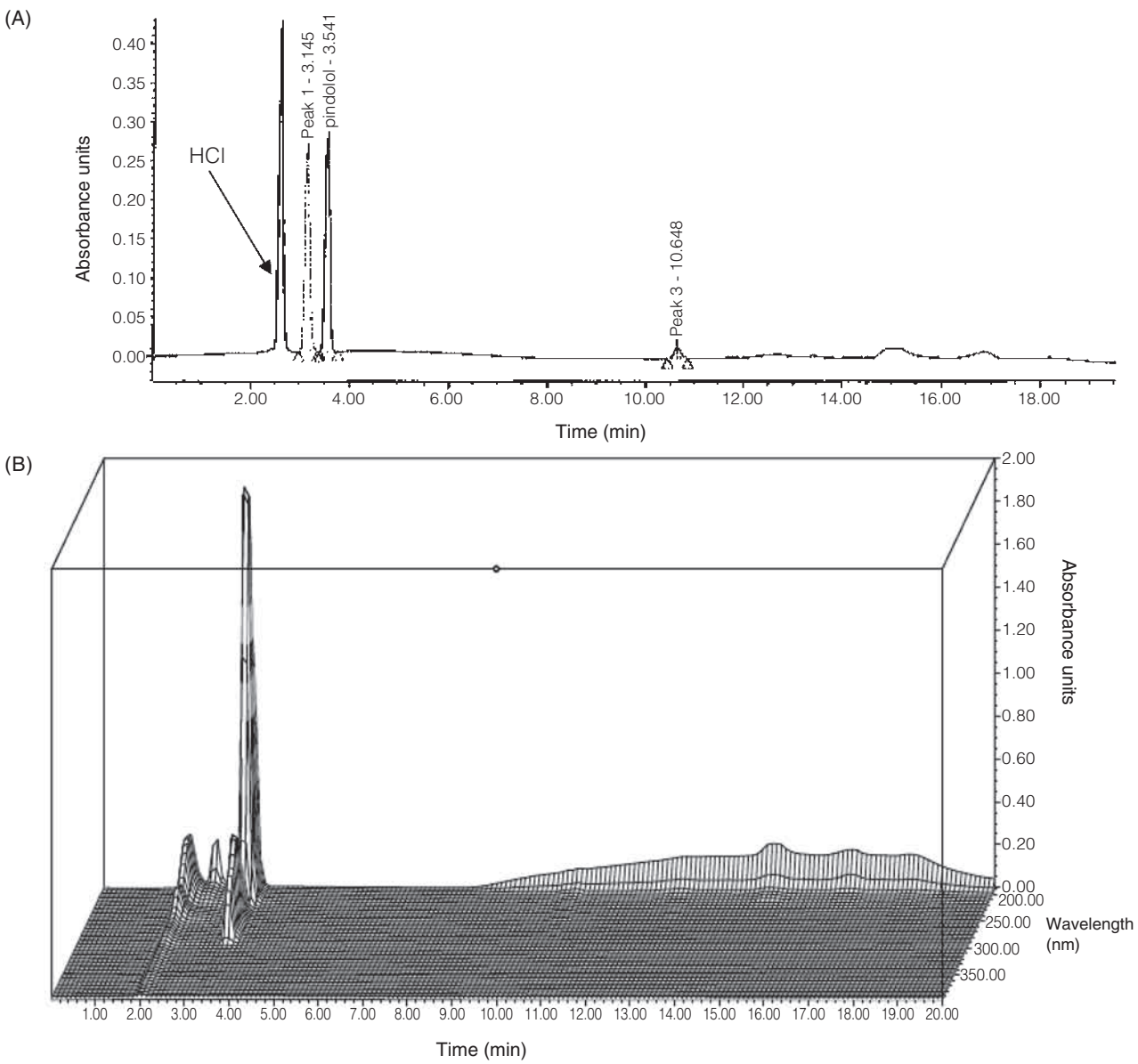

Figure 8. (A) A typical chromatogram of PDL exposed to 1 M hydrochloric acid.(B) 3D plot of PDL in acidic condition.

yielded six degradation products as shown in Figure 7. In acid hydrolysis of pure drug, two major degradation products were observed (Figure 8A). Moreover, the 3D plot of acid hydrolysis of PDL (Figure 8B) confirmed the two major degradation products. Two major degradation products were also noticed in alkali hydrolysis of PDL (Figure 9A) and this was evident from the 3D plot of alkali hydrolysis of PDL (Figure 9B). However,

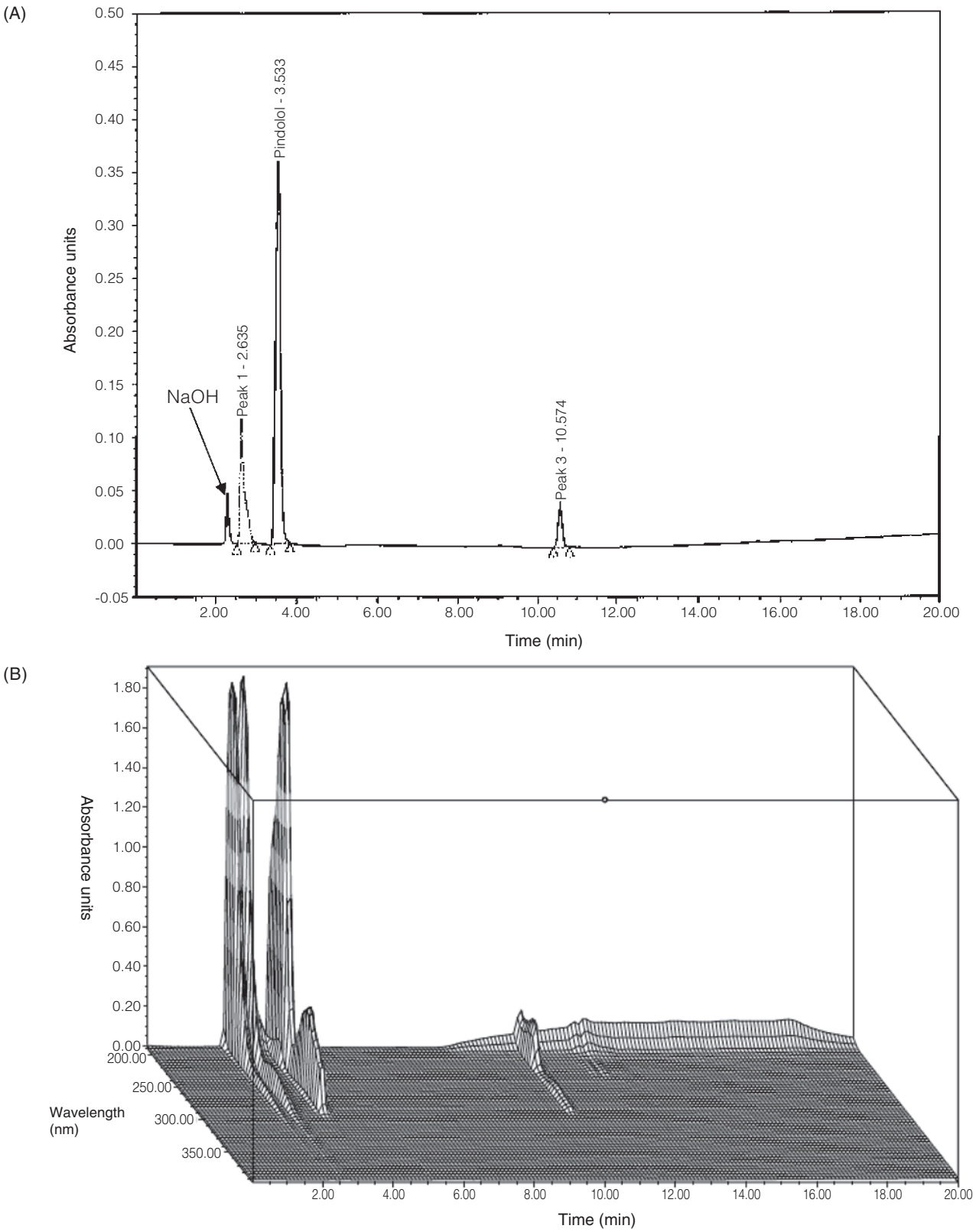

Figure 9. (A) A typical chromatogram of PDL exposed to 1 M sodium hydroxide. (B) 3D plot of PDL in alkaline condition.

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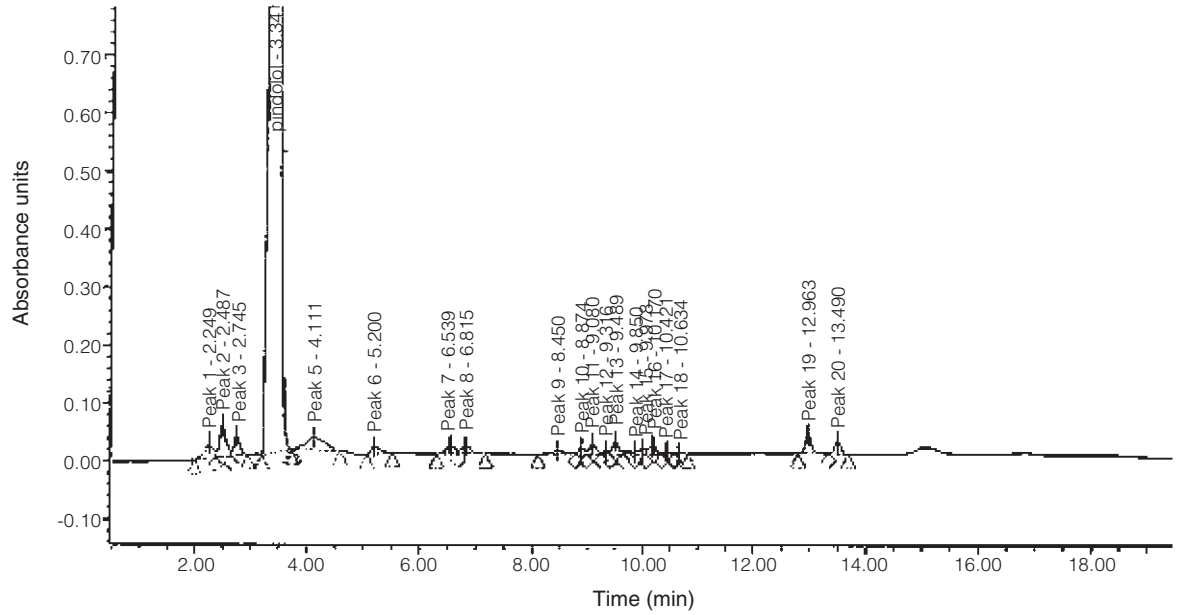

Figure 10. A typical chromatogram of PDL solution exposed to UV light (photolysis).

Table 4. Summary of forced degradation results

Stress condition	Time (h)	% Assay of active substance	% Mass balance (% assay + impurity)
Acid hydrolysis (1 N HCl) reflux at 80°C	8	81.2	18.8
Base hydrolysis (1 N NaOH) reflux at 80°C	8	49.3	50.7
Oxidation (3% H_2O_2) reflux at 80°C	8	90.1	9.9
Thermal (80°C)	12	99.4	0.6
Photolysis	24	92.5	7.6

Table 5. Analysis of PDL in pharmaceutical formulations

Formulation	Labeled (mg)	Found* (mg)	% RSD	% Recovery*
Visken®	5	4.95	0.96	99.0
	10	9.96	0.82	99.6
	15	15.06	0.74	100.4

*Average of nine determinations.

19 degradation products were found in the photolysis of pure PDL (Figure 10). Peak purity test results confirmed that the PDL peak was homogenous and pure in all the analyzed stress samples. The mass balance assay due to forced degradation is tabulated in Table 4. The clear solution of PDL changed to pale pink, yellow and light yellow under acidic, oxidation and photolytic conditions, but remained unchanged in alkaline condition. As the sample solutions obtained during stress studies were either too acidic or too basic, the solutions were neutralized before running the chromatograms. This was done in order to avoid column damage, peak splitting and unsymmetrical peak shapes.

X. *Analysis of Pharmaceutical Preparations*

The proposed method was successfully applied to the analysis of PDL in tablets and the results are shown in Table 5. The low values of % RSD indicated high precision of the method. High percentage recovery values indicated that the commonly employed excipients, colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose and pregelatinized starch did not interfere with the analysis of PDL in tablets.

CONCLUSIONS

The proposed RP-LC and NP-LC methods were observed to be precise, specific, accurate and stability indicating. PDL and its related substances and enantiomers can be determined in bulk powder and pharmaceutical formulation in the presence of its degradation

products. As a better alternative to the official methods, the currently described LC method separates PDL and its related substances within a short time span of 20 min. Moreover, the separation of enatiomers was achieved effectively while USP makes no mention about this. ICH guidelines were followed throughout the study for method validation and stress testing. In view of this, the proposed method could be adopted for quality control and routine analysis.

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