ORIGINAL ARTICLE

Establishment of inherent stability of pramipexole and development of validated stability indicating LC–UV and LC–MS method

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Abstract Pramipexole belongs to a class of nonergot dopamine agonist recently approved for the treatment of early and advanced Parkinson's disease. A validated specific stability indicating reversed-phase liquid chromatographic method has been developed for the quantitative determination of pramipexole in bulk as well as in pharmaceutical dosage forms in the presence of degradation products. Forced degradation studies were performed by exposition of drug to hydrolytic (acidic and basic), oxidative and photolytic stress conditions, as defined under ICH guideline Q1A (R2). Significant degradation was observed under hydrolytic, oxidative and photolytic conditions and the degradation products formed were identified by LC–MS.

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1. Introduction

Pramipexole is a new drug used in the therapy of Parkinson's disease. Chemically it is (S)-2-amino-4,5,6,7-tetrahydro-6-(propylamino)benzothiazole (Fig. 1). Pramipexole is a nonergot dopamine agonist with high relative in vitro specificity and full intrinsic activity at the D2 subfamily of dopamine receptors, binding with higher affinity to D3 than to D2 or D4

receptor subtypes [1–4]. Several methods like HPLC [5–8] and UV spectrophotometric [9] have been reported for the estimation of pramipexole. Gradient ultra fast liquid chromatographic analysis of pramipexole [10], chromatographic method for determination of dissociation constants of pramipexole and its impurities [11,12], stability studies and structural characterization of pramipexole [13] and quantification of pramipexole in human plasma by HPLC-MS/MS [14] method have been reported in literature.

To our knowledge, no article related to stability indicating method for pramipexole in the presence of degradation products and identification of degradation products by LC–MS method has been published. So it was felt necessary to carry out forced degradation studies according to the ICH requirements [15] and develop a selective and validated stability-indicating HPLC method. The aim of the present study was to identify degradation products and to elucidate

complete degradation pathway of the drug. The proposed method was validated according to ICH guidelines [16].

2. Experimental

2.1. Materials and chemicals

A pure drug sample of pramipexole (Batch no. PH0051109, Expiry date: Jan 2011) was obtained from Dr. Reddy's Laboratories Ltd. (Hyderabad, Andhra Pradesh, India) as a gift sample (purity 99.97%, w/w). Twenty tablets of pramipexole (Brand name: PRAMIPEX, label claim: 1 mg pramipexole per tablet, Batch no. PXPF0934, Expiry date: July 2012) were procured from a local Pharmacy. All analytical grade chemicals and reagents used were purchased from Merck Chemicals, Mumbai, Maharashtra, India.

2.2. Instrumentation

The LC method development, forced degradation studies and validation were done using Jasco Inc. (Easton, MD) Model PU 2080 Intelligent LC Pump with autosampler programmed at 20 µL injection volume with UV detector (Jasco Model UV
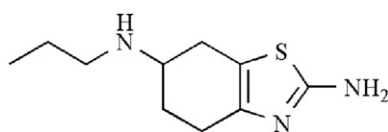

Fig. 1   Structure of pramipexole.
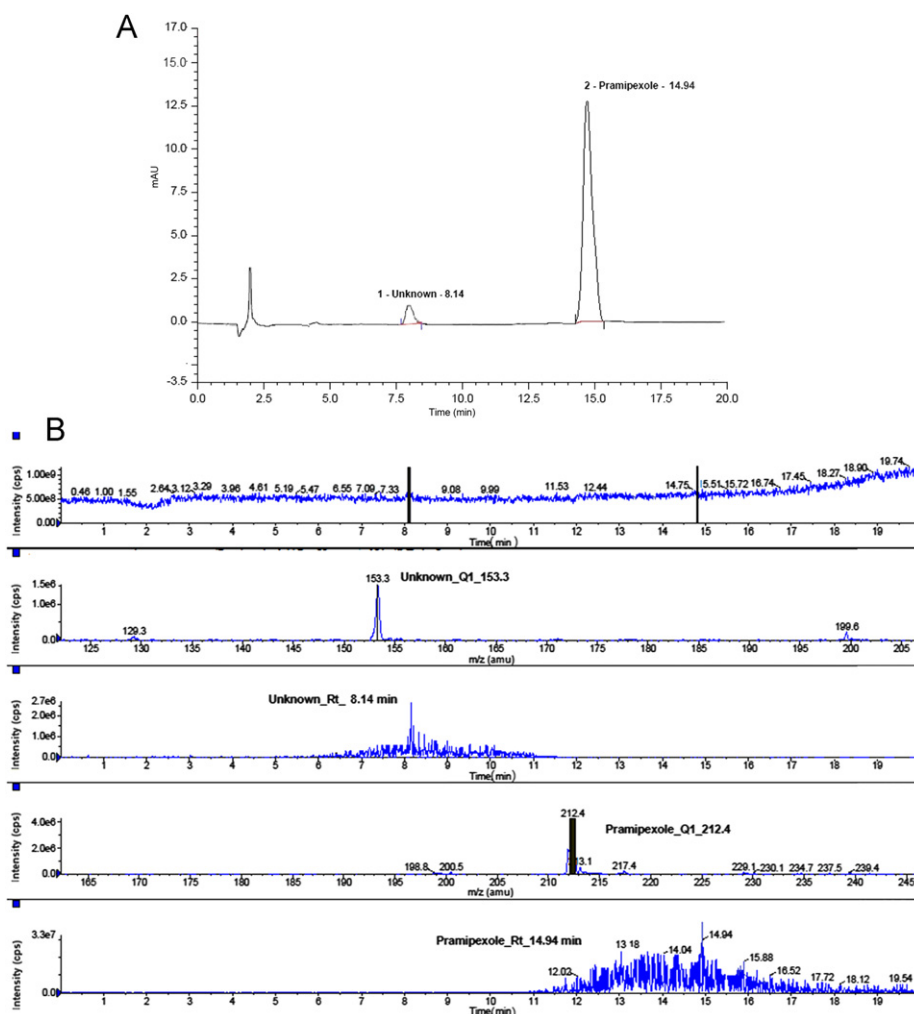

Fig. 2   (A) Chromatogram of acid treated (3 M HCL at 80 °C for 48 h) pramipexole sample. Peak 1: degraded, t_R: 8.14 min. Peak 2: pramipexole, t_R: 14.94 min. (B) Positive ESI-quadrupole (+Q1) mass spectrum of acid degradation sample.

2075) set at a wavelength of 263 nm. Integration of data was done using the Jasco Borwin Version 1.5, LC-Net II/ADC system. The column used was SymmetryShield column C_{18} (5 µm, 250 mm × 4.6 mm i.d.) from Waters, Milford, USA.

MS studies were performed on a 4000 Q-TRAP Linear Ion Trap Quadrupole Mass Spectrometer (Applied Biosystems Sciex, USA). Pramipexole and the degradation products mass spectra were taken with an electrospray interface (ESI) and the mass spectra were recorded in positive mode in mass range of 50–800 amu and analyzed in the triple quadrupole analyzer. Methanol was used for dilution of samples to prepare concentration of 50 µg/mL and injected into the inlet. The data were processed using an Analyst 1.4.2 software. The liquid chromatographic system attached to the mass spectrometer was Ultimate 3000 equipped with pump, autosampler and an Ultimate 3000 RS column compartment (Dionex, CA, USA) and data were integrated using a Chromeleon v 6.8 SR10 operating software.

The mobile phase used was a methanol: 0.1% triethylamine (70:30, v/v) and the column used was C_{18} (5 µm, 250 mm × 4.6 mm i.d.) from Waters, Milford, USA. The flow rate was set at 1 mL/min and the effluent from the column was introduced into the mass spectrometer through a flow splitter which splits volume of mobile phase and deliver minimum amount of mobile phase into mass spectrometer. The split ratio was 20:80 (In: Out).

2.3. Forced degradation studies

A stock solution of pramipexole (1000 µg/mL) was prepared in methanol and this solution was used for forced degradation to provide an indication of the stability-indicating property of the proposed method. The average peak area of pramipexole after application (50 µg/mL) of seven replicates was obtained in all degradation studies.
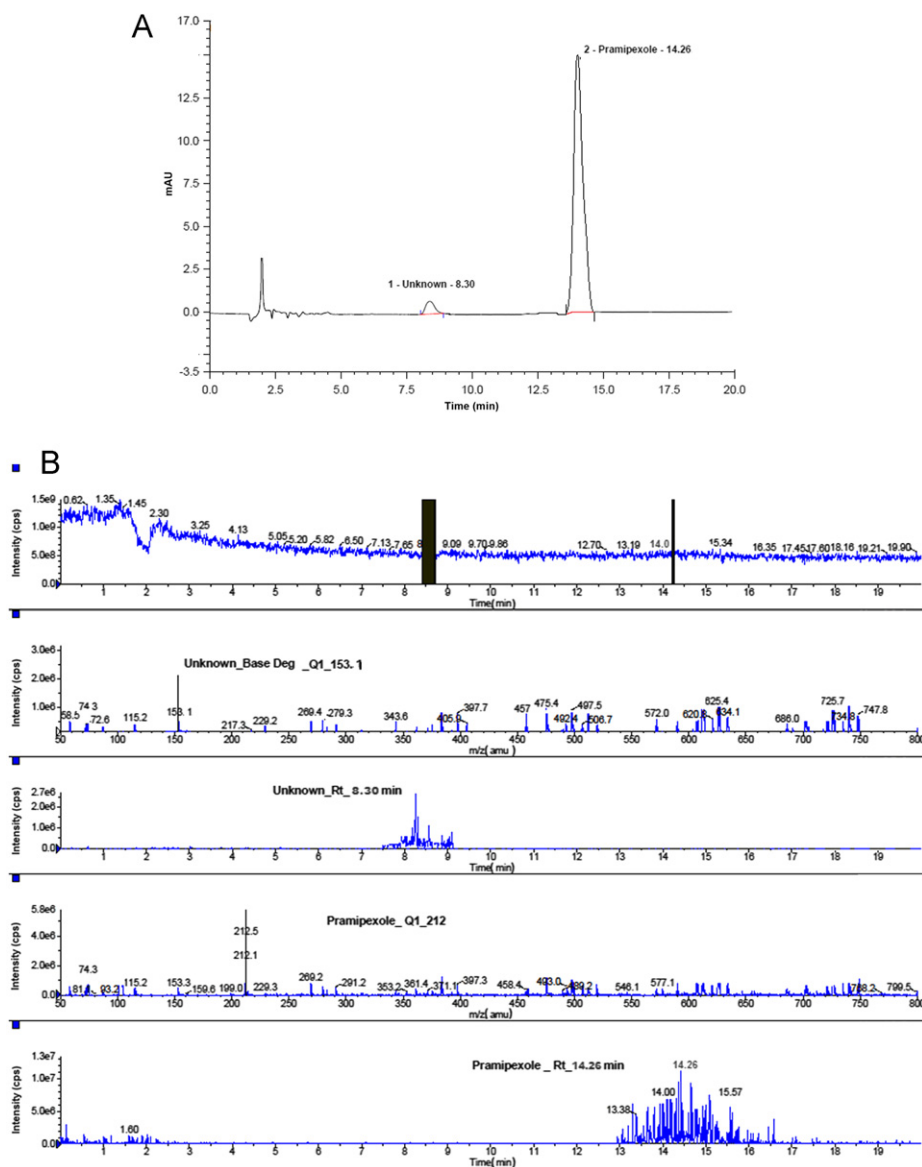

Fig. 3 (A) Chromatogram of base treated (2 M NaOH at 80 °C for 24 h) pramipexole sample. Peak 1: degraded, t_R: 8.30 min. Peak 2: pramipexole, t_R: 14.26 min. (B) Positive ESI-quadrupole (+Q1) mass spectrum of base degradation sample.

2.3.1. Acid and basic conditions

Acid decomposition studies were carried out by refluxing the solution of drug in 3 M hydrochloric acid at 80 °C for 48 h. The studies under alkaline condition were carried out in 2 M sodium hydroxide and the solution of drug was refluxed for 24 h at 80 °C. The resultant solutions were neutralized and diluted with methanol to obtain 50 μg/mL solutions and a volume of 20 μL was injected into the system.

2.3.2. Oxidation with hydrogen peroxide

To study hydrogen peroxide induced degradation, the drug solution was exposed to 6% hydrogen peroxide at room temperature for a period of 8 day and then heated in boiling water bath for 10 min to completely remove the excess of hydrogen peroxide. The resultant solutions were diluted to obtain 50 μg/mL solutions and a volume of 20 μL was injected into the system.

2.3.3. Photochemical degradation

The photochemical stability of the drug was studied by exposing the stock solution of drug (1000 μg/mL) to direct sunlight (60,000–70,000 lux) for a period of 8 day on a wooden plank and kept on terrace. A calibrated lux meter (Model ELM 201, Escorp, New Delhi, India) was used to measure intensity of sunlight. The solution was diluted with methanol to obtain a solution of 50 μg/mL and then a volume of 20 μL of the solution was injected into the system.

2.3.4. Dry heat and wet heat degradation

The solid form of pramipexole standard was placed in an oven at 50 °C for a period of 30 day to study dry heat degradation. For wet heat degradation, the standard drug was kept in a humidity chamber for 3 months at 50 °C, 75% relative humidity.

2.4. Optimization of stability-indicating HPLC method

The developed HPLC procedure was optimized to get the stability-indicating assay method. The pure drug along with its degraded products was injected and run in different solvent systems. Methanol and water in different ratios were tried. Elution of degradation product in dead volume was observed

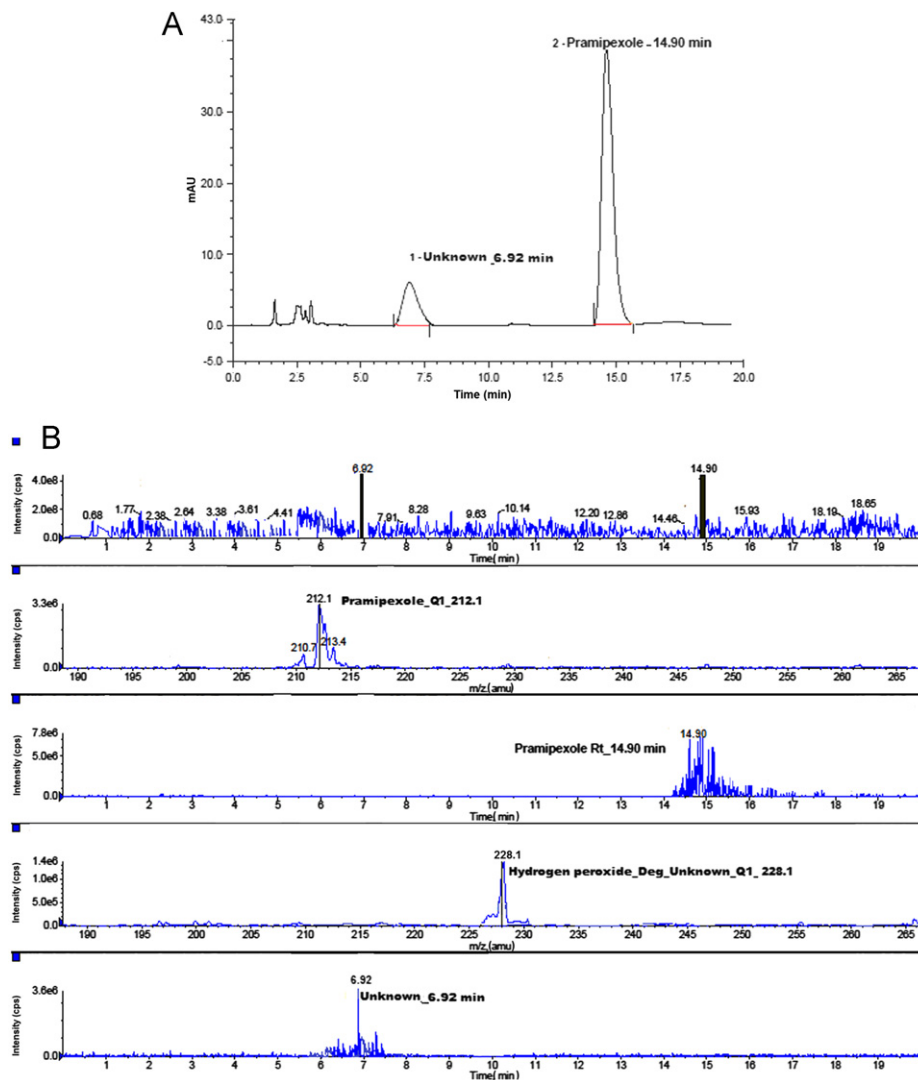**Fig. 4** (A) Chromatogram of H_2O_2-treated (6% H_2O_2 at room temperature for 8 day) sample. Peak 1: degraded, t_R: 6.92 min. Peak 2: pramipexole, t_R: 14.90 min. (B) Positive ESI-quadrupole (+Q1) mass spectrum of oxidative degradation sample.

when methanol concentration was increased in the mobile phase. Hence by decreasing the concentration of the methanol it was found that there was improvement in resolution. It can be seen that methanol: 0.1% triethylamine (70:30, v/v) as a mobile phase at a flow rate of 1 mL/min gave acceptable retention time (t_R), theoretical plates and good resolution of the drug and degradation products.

2.5. Validation of the method

Validation of the optimized LC method was done with respect to following parameters.

2.5.1. Linearity
Linearity of the method was studied by injecting seven concentrations of the drug prepared in the mobile phase in the range of 5–50 µg/mL in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.5.2. Precision
Precision was computed here by repeatability and intermediate precision. Repeatability studies were carried out by analysis of three different concentrations (5, 20, and 50 µg/mL) of the drug ($n=6$) on the same day. Intermediate precision of the method was checked by repeating studies on three different days.

2.5.3. Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ was determined on basis of signal-to-noise ratio. LOD and LOQ represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. The LOD and LOQ were experimentally verified by diluting known concentrations of standard solution of pramipexole and calculating the signal-to-noise ratio for pramipexole by injecting series of solutions until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.
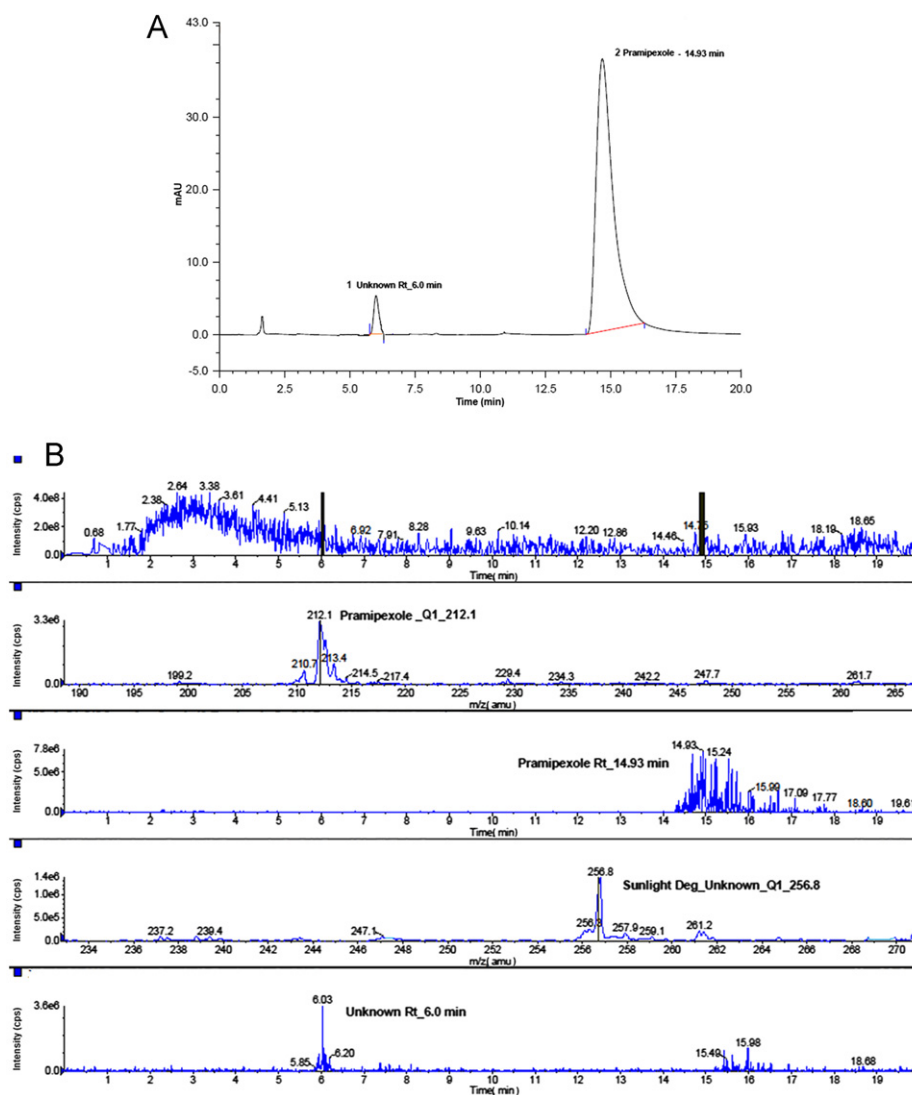

Fig. 5 (A) Chromatogram of sunlight treated (direct sunlight for 8 day) pramipexole sample. Peak 1: degraded, t_R: 6.0 min. Peak 2: pramipexole, t_R: 14.93 min. (B) Positive ESI-quadrupole (+Q1) mass spectrum of photodegradation sample.

2.5.4. Robustness of the method

To evaluate robustness of the HPLC method, few parameters like variation of flow rate and percentage of methanol in the mobile phase were deliberately varied. The resolution of drug in a mixture of stressed samples was studied by performing the analysis on different chromatographic systems. Robustness of the method was done at three different concentration levels of 5, 20, and 50 µg/mL for pramipexole. Also robustness was verified by studying the resolution of drug in a mixture of degraded samples on different chromatographic systems on different days.

2.5.5. Specificity

The specificity of the method was established through study of resolution factor of the drug peak from the nearest resolving peak. Overall selectivity was established through determination of purity for each degradation peak using PDA detector.

2.5.6. Analysis of marketed formulation

To determine the pramipexole content in conventional tablets (Brand name: PRAMIPEX; Sun Pharmaceuticals Industries Ltd. [Batch no. PXPF0934], label claim: 1 mg pramipexole per tablet, expiry date: July 2012), the twenty tablets were weighed, their mean weight was determined and finely powdered. A weight of the powder/triturate equivalent to 1 mg pramipexole was transferred to 10 mL volumetric flask containing 7 mL methanol, sonicated for 30 min and diluted to 10 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using a 0.45 µm Whatman filter paper (Millipore, Milford, MA). Filter saturation time was 15 min. The above stock solution was further diluted to get sample solution at three different concentrations of 5, 20 and 50 µg/mL, respectively. A 20 µL volume of each sample solution was injected into the LC system ($n=6$) under the conditions described above. The peak areas were measured at wavelength 263 nm and using multilevel calibration developed on the same LC system under the same conditions using linear regression equation, and the concentrations in the samples were determined.

2.5.7. Accuracy

Accuracy of the developed method was determined by applying the method to a pramipexole sample (tablets) to which a known amount of pramipexole standard powder equivalently 80%, 100%, and 120% of label claim was added (standard addition method). From the slope and Y-intercept of the calibration curve, the recoveries were calculated.
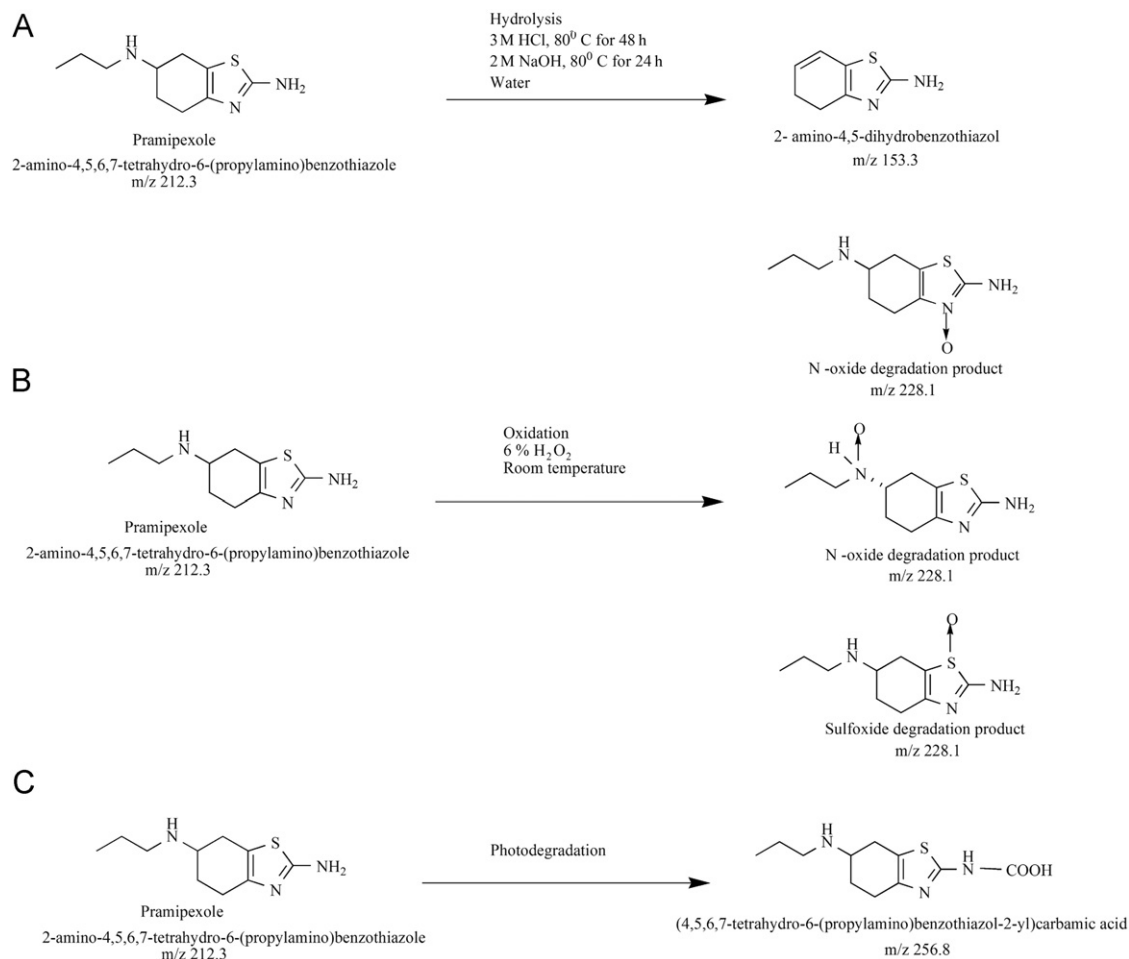

Fig. 6 Possible degradation pathway. (A) hydrolysis, (B) oxidation and (C) photolysis.

3. Results and discussion

3.1. Results of forced degradation studies

3.1.1. Hydrolysis
The rate of degradation in base was faster as compared to acid. The drug was highly labile to basic degradation. After reaction in 3 M hydrochloric acid at 80 °C for 48 h, one major degradation product was formed, as shown by the chromatogram in Fig. 2A. The LC–MS analysis of acid degraded sample was performed which showed that a peak at 8.14 min was observed with m/z value 153.3 (Fig. 2B).

Initially drug solution with 1 M sodium hydroxide was refluxed at 80 °C for 48 h but negligible degradation was observed, hence the strength of base was increased, 10–20% degradation was observed by refluxing drug solution with 2 M sodium hydroxide at 80 °C for 24 h forming degradation product at retention time 8.30 min in HPLC (Fig. 3A). The LC–MS analysis of base degraded sample showed a major degradation product at 8.30 min with m/z value 153.1 (Fig. 3B).

In both acid as well as base hydrolysis, the degradation products formed were the same, which was confirmed by the study of m/z values observed in mass spectra. The major degradation products showed m/z value 153 in both acid and base hydrolysis. From mass data, the major acid and base degradation product was confirmed as 2-amino-4,5-dihydro-benzothiazol (Fig. 6A).

3.1.2. Oxidation
More than 10% of degradation was observed on exposure to 6% hydrogen peroxide at room temperature for a period of 8 day, which indicated that drug was unstable against oxidative stress. The chromatogram (Fig. 4A) had a single degradation product peak at 6.92 min.

The LC–MS analysis of degraded sample (6% H_2O_2 at room temperature for 8 day) was performed; a peak at 6.92 min was observed with m/z value 228.1 (Fig. 4B). From mass data, it was depicted that oxidation can take place either on nitrogen or sulphur of benzothiazole ring or nitrogen of aminopropyl chain; hence the structures of three possible degradation products which have the same m/z value were proposed as 2-amino-6-(propylammonio)-4,5,6,7-tetrahydrobenzo[d] thiazole-3-oxide or 2-amino-N-propyl-4,5,6,7-tetrahydrobenzo[d] thiazole-6-aminium1-oxide or 4-amino-[1,2,6,7-tetrahydrobenzo[d]thiazole]propyl-ammonio-N-oxide, respectively (Fig. 6B).

3.1.3. Photochemical degradation
Pramipexole was found to be unstable to photochemical degradation as more than 10% degradation was seen after exposing drug to sunlight for 8 days (Fig. 5A).

The LC–MS analysis of photodegraded sample showed major degradation product at 6.0 min. When pramipexole is exposed to sunlight, carboxylic group is attached to amino group of thiazole ring which has m/z value 256.8 (Fig. 5B). Possible degradation product formed under photochemical degradation was confirmed as (4,5,6,7-tetrahydro-6-(propyla-mino)benzothiazol-2-yl)carbamic acid (Fig. 6C).

3.1.4. Dry and wet heat degradation
Pramipexole was found to be stable as negligible degradation was seen when subjected to dry and wet heat.

3.2. Results of method validation

The results of validation studies on the stability-indicating method developed for pramipexole involving methanol: 0.1% triethylamine (70:30, v/v) as mobile phase are summarised below.

3.2.1. Linearity
The drug response was linear (0.9998) in the concentration range of 5 µg/mL–50 µg/mL. The mean ($\pm$RSD) values of slope, intercept and correlation coefficient were 8705.3 ($\pm$1.20), 2201.4 ($\pm$1.75) and 0.9998 ($\pm$0.79), respectively.

3.2.2. Precision
The results of the repeatability and intermediate precision experiments are indexed in Table 1. It can be seen that RSD values for both repeatability and intermediate precision studies were <2% as recommended by ICH guideline, illustrating that developed method was found to be precise.

3.2.3. LOD and LOQ
The LOD and LOQ were found to be 0.5 µg/mL and 2 µg/mL, respectively.

3.2.4. Robustness of the method
Each selected factor was changed at three levels (−1, 0 and 1). One factor at a time was changed to determine the effect. Thus, replicate injections of mixed standard solution at three concentration levels ($n=6$) were carried out under small changes of two chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed (Table 2). The resolution of drug in the mixture of stressed sample was found to be similar when studies were performed on different chromatographic systems on different days, indicating that the method has sufficient ruggedness.

3.2.5. Specificity
In Figs. 2, 3, 4, 5A complete separation of pramipexole in the presence of its degradation products was observed illustrating

Table 1 Precision studies for pramipexole.

Concentration (µg/mL)	Intra-day precision ($n=6$)			Inter-day precision ($n=6$)		
	Measured conc. (µg/mL)	RSD (%)	Recovery (%)	Measured conc. (µg/mL)	RSD (%)	Recovery (%)
5	4.96	0.31	99.20	5.01	0.10	100.20
20	20.14	0.13	100.70	19.43	0.50	97.15
50	49.69	0.25	99.38	48.91	0.38	97.82

Table 2 Robustness testing for pramipexole[a] ($n=6$).

Factor[b]	Level	t_R[c] (min)	K[d]	T[e]
Flow rate (mL/min)				
0.9	−1	15.01	3.62	1.09
1.0	0	14.71	3.57	1.05
1.1	+1	14.50	3.54	1.20
Mean±SD		14.74±0.13	3.49±0.04	1.11±0.65
Percentage of methanol in the mobile phase (v/v)				
69	−1	14.64	3.40	1.31
70	0	14.70	3.89	1.22
71	+1	14.73	3.23	1.13
Mean±SD		14.69±0.11	3.50±0.10	1.22±0.08

[a]Average of three concentrations: 5, 20 and 50 μg/mL.
[b]Two factors were slightly changed at three levels (−1, 0, and +1).
[c]t_R=retention time.
[d]K=retention factor.
[e]T=tailing factor.

Table 3 Analysis of commercial formulation (PRAMIPEX, 1 mg) ($n=6$).

Commercial formulation PRAMIPEX (1 mg)	Drug found (mean±SD, mg)	Recovery (mean±SD, %)
1st lot	0.989±0.569	98.90±0.67
2nd lot	0.995±0.247	99.50±0.53

specificity of the HPLC method. The peaks obtained were sharp and had clear baseline separation. The resolution factor from nearest peak was >3 for pramipexole. The PDA detector scanned all the components present in the mixture in whole wavelength range from 200 to 400 nm and it indicated that there were no degradation peaks hiding under or unresolved from the analyte peak (pure drug), which also reflected the specificity of the method.

3.2.6. Analysis of marketed formulation
Commercially available pramipexole tablets from two different lots were analyzed using the proposed procedures and the results are indexed in Table 3.

3.2.7. Recovery studies
As can be seen in Table 4 good recoveries of the drug in the range from 97.75% to 100.45% were obtained at various added concentrations.

4. Conclusion

An analytical method for determination of pramipexole in the presence of degradation products was developed. Using various ICH recommended stress conditions, the behavior and intrinsic stability of pramipexole under various stress conditions was established and all degradation products were identified by the LC–MS method. The drug was well separated

Table 4 Accuracy (% recovery) of pramipexole in tablet formulation at three concentration levels ($n=6$).

Label claim (mg per tablet)[a]	Amount added (%)	Total amount (mg)	Amount recovered (mg)±RSD (%)	Recovery (%)
0.992	80	1.78	1.74±0.12	97.75
	100	1.98	1.97±0.11	99.49
	120	2.18	2.19±0.10	100.45

[a]Average of amount found in two different lots of pharmaceutical formulation.

from the degradation products demonstrating the stability indicating capability of the method. This study could be very useful for checking the quality of drug during stability studies.

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