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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF OPIPRAMOL HYDROCHLORIDE AS BULK DRUG AND IN TABLET DOSAGE FORM

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ABSTRACT

A new simple, accurate, precise and selective stability- indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for estimation of Opipramol Hydrochloride in tablet dosage form. The mobile phase selected was Toluene: Methanol (7: 3, v/v) with UV detection at 256 nm. The retention factor for Opipramol Hydrochloride was found to be 0.45 ± 0.006. The method was validated with respect to linearity, accuracy, precision and robustness as per ICH guidelines. The drugs were subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Results found to be linear in the concentration range of 100-600 ng band$^{-1.}$ The method has been successfully applied for the \analysis of drug in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 99.56 ± 1.48. The developed method can be used for checking the stability of Opipramol Hydrochloride in bulk drug and pharmaceutical dosage form.

KEYWORDS: Opipramol, HPTLC, Forced degradation, Validation.

INTRODUCTION

Opipramol Hydrochloride (OPH), chemically, 2-{4-[3-(5H-Dibenzo[b,f]azepin-5-yl)propyl]-1-piperazinyl}ethanol hydrochloride is a member of the tricyclic antidepressants and acts as a sigma receptor agonist and used in the treatment of generalized anxiety disorder and somatoform disorders.[1]

Literature survey reveals High Performance Liquid Chromatographic (HPLC) methods for determination of opipramol in human plasma.[2-4] Electroanalytical method for Determination of Opipramol in Pharmaceutical Preparations and Biological Fluids has been also reported.[5] Synthesis, characterization and pharmacological screening method[6] and thin layer chromatographic method[7] is also reported.

To best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of OPH in tablet dosage form. This paper describes simple, precise, accurate and selective HPTLC method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per International Conference on Harmonisation Guidelines.[8, 9]

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of OPH was kindly supplied by Sun Pharma (Mumbai, India). The pharmaceutical dosage form used in this study was Insidon tablets (Novartis Ltd., India) labeled to contain 50 mg of OPH was procured from the local market. Toluene, Methanol (HPLC grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on precoated silica gel aluminium plate 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: methanol (7: 3, v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 256 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Solution

Working standard solution of OPH was prepared by dissolving 10 mg of drug in 10 mL of methanol to get concentration of 1 mg mL^{-1} from which 0.5 mL was further diluted to 10 mL with methanol to get solution of 50 ng μL^{-1}.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 256 nm. So, 256 nm was selected as the wavelength for detection.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 50 mg of OPH was weighed and transferred to a 100 mL volumetric flask containing 50 mL of methanol and the content was sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 500 ng $band^{-1}$. One mililitre volume of above solution was diluted with methanol to obtain final concentration of 50 ng $band^{-1}$. Four µL volume of this solution was applied on TLC plate to obtain final sample concentration of 200 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 256 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid/base hydrolytic, oxidative, thermolytic, and photolytic stress conditions.

Acid treatment

1 mL working standard solution of OPH (50 ng μL^{-1}) was mixed with 1 mL of 0.1 N methanolic HCl and 8 mL of methanol. Solution was refluxed for 24 hrs. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Alkali treatment

1 mL working standard solution of OPH (50 ng μL^{-1}) was mixed with 1 ml of 1 N methanolic NaOH and 8 mL of methanol. The solution was refluxed for 4 hrs. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Neutral Hydrolysis

1 mL working standard solution of OPH (50 ng μL^{-1}) was mixed with 1 mL of water and 8 mL methanol. The solution was refluxed for 72 hrs. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Oxidative degradation

1 mL working standard solution of OPH (50 ng μL^{-1}) was mixed with 1 mL of 3 % solution of H_2O_2 and 8 mL of methanol. The solution was refluxed for 6 hrs. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Photo-degradation

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours square $meter^{-1}$ and subsequently to fluorescence light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved in methanol to get concentration of 50 ng μL^{-1}. 6µL of the resulting solution was applied to HPTLC.

Degradation under dry heat

Dry heat study was performed by keeping drug in oven at 60ºC for period of 24 hrs. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 50 ng μL^{-1}. 6 µL of the resulting solution was applied to HPTLC.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary objective in developing this stability indicating HPTLC method is to achieve the resolution of OPH and its degradation products. The chromatographic separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Toluene: Methanol (7: 3, v/v) as mobile phase and detection was carried out at 256 nm. The

retention factor for OPH was found to be 0.45 ± 0.006. Representative densitogram of standard solution of OPH is shown in Figure 1.

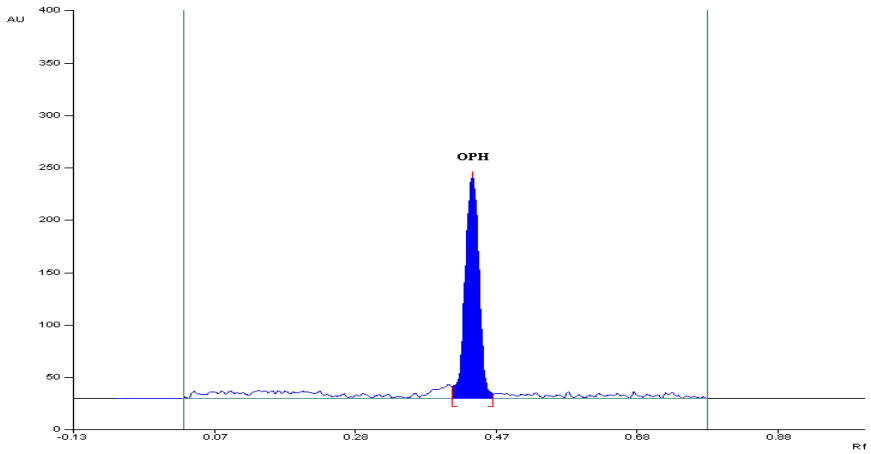


Fig. 1: Representative densitogram of standard solution of OPH (300 ng/band, R_F = 0.45 ± 0.006)

Result of forced degradation studies

Forced degradation study showed that the method is highly specific and there was no interference of degradation products observed at retention time of drug.

Acid treatment

The densitogram obtained from the acid-degraded sample of OPH contained peak of degradation at R_F 0.58. The representative densitogram obtained after acid treatment is shown in Figure 2.

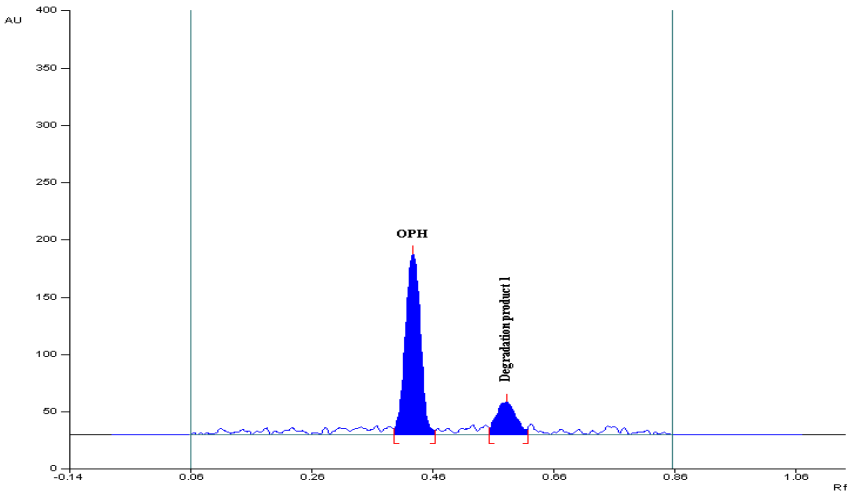


Fig. 2: Representative densitogram after acid treatment with degradation product at R_F = 0.58

Alkali treatment

22.34 % of degradation of OPH was observed in alkaline condition. The representative densitogram after alkali treatment is shown in Figure 3.

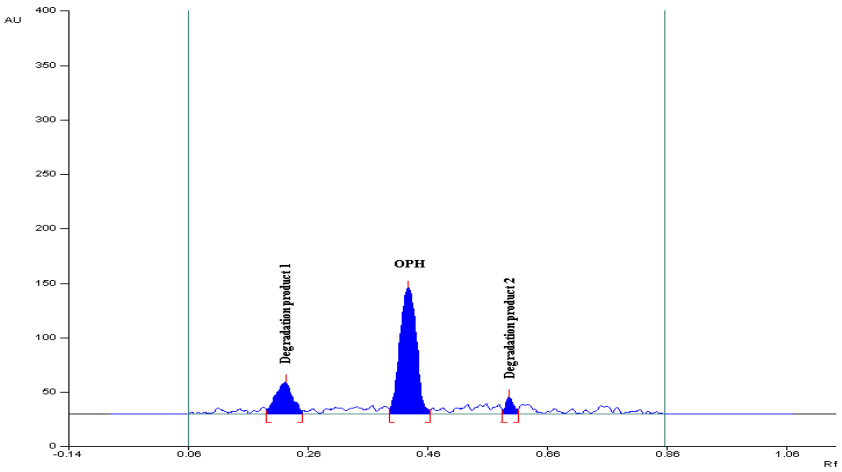


Fig. 3: Representative densitogram after alkali treatment with degradation products at R_F = 0.23 and 0.56

Oxidative degradation

19.33 % degradation was observed for OPH when treated with 3 % H_2O_2. The representative densitogram after oxidative degradation is shown in Figure 4.

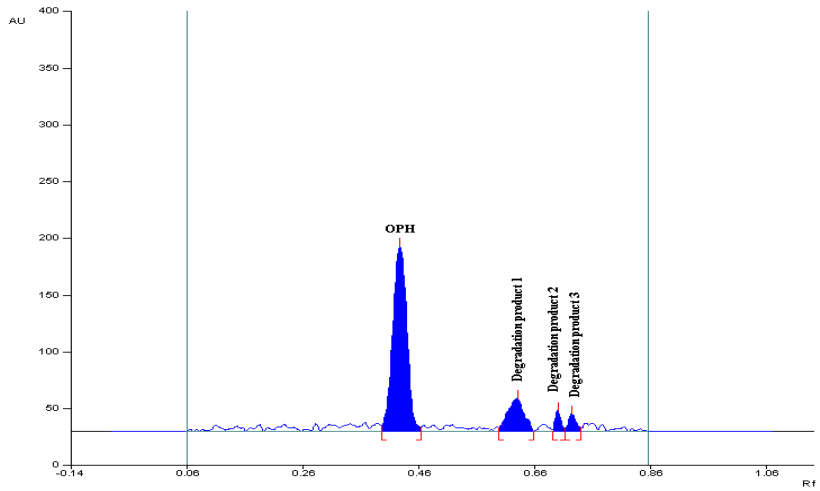


Fig. 4: Oxidative degradation densitogram with degradation products at R_F = 0.62, 0.70 and 0.73

Neutral Hydrolysis

24.32 % degradation was observed for OPH in neutral condition when refluxed for 72 hours. The representative densitogram after neutral degradation is shown in Figure 5.

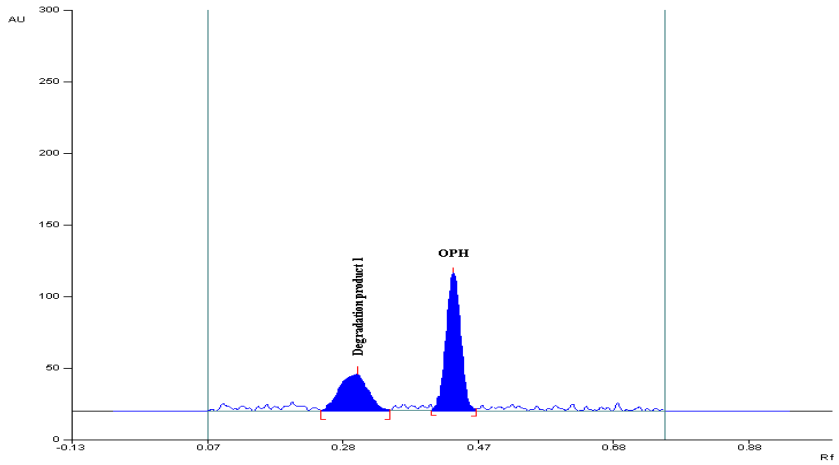
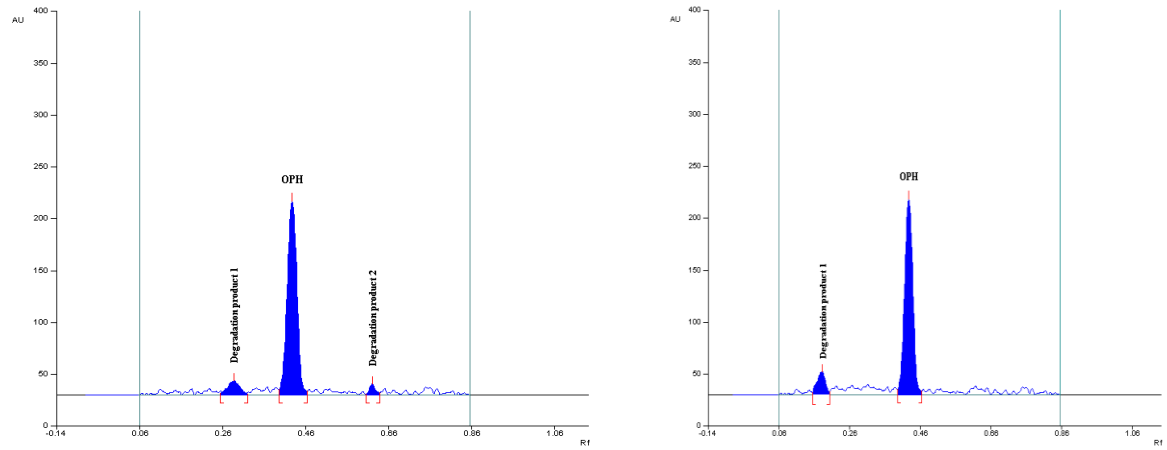


Fig. 5: Representative densitogram obtained after neutral degradation with degradation product at R_F = 0.32

Photodegradation Studies

OPH exhibited 12.37 % of degradation with degradation product at R_F 0.29 and 0.63 when exposed to ultraviolet light (200 Watt hours/Sequre meter) and 8.67 % of degradation with degradation product at R_F 0.18 when exposed to fluorescence light (1.2 million lux hours). The representative densitogram after photo degradation is shown in Figure 6.



(a) **(b)**

Fig. 6: Photo degradation densitogram obtained after exposure to (a) UV light (R_F = 0.29 and 0.63) (b) Fluorescence light (R_F = 0.18)

Dry Degradation Studies

15.40 % of degradation was observed for OPH when the powdered drug substance was exposed to dry heat at 60º C for 24 hrs. The representative densitogram obtained from sample subjected to dry heat is shown in Figure 7.

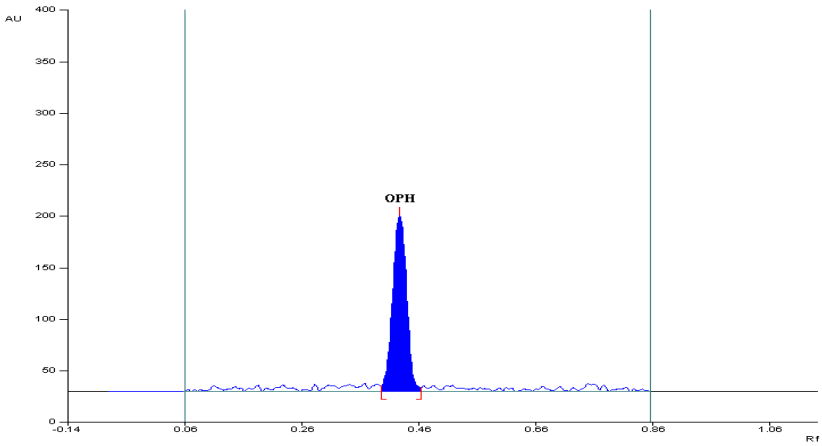


Fig. 7: Densitogram obtained from sample subjected to dry heat

Peak purity results greater than 990 indicate that OPH peaks are homogeneous in all stress conditions tested. The unaffected assay of OPH in the tablet confirms the stability indicating power of the method. The forced degradation studies data are summarized in Table 1.

Table 1: Data of forced degradation studies of OPH

Stress conditions/ duration	% Assay of active substance	Rf values of degraded products
Acidic / 0.1 N HCl/ Reflux for 24 hrs	83.33	0.58
Alkaline /1 N NaOH/ Reflux for 4 hours	77.66	0.23, 0.56
Oxidative /3 % H_2O_2 / Reflux for 6 hrs	80.66	0.62, 0.70, 0.73
Neutral/H_2O/ Reflux for 72 hrs	75.68	0.32
Photolysis UV/200 watt hours/square meter	87.63	0.29, 0.63
Fluorescence / 1.2 million lux hours	91.33	0.18
Dry heat/ 60ºC/ 24 hours	84.6	--

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.[8, 9]

Preparation of Calibration Curve

The standard stock solutions of OPH (50 ng μL^{-1}) were applied by overspotting on TLC plate in range of 2, 4, 6, 8, 10 and 12 µL. Straight-line calibration graphs were obtained in the concentration range 100-600 ng $band^{-1}$ with high correlation coefficient > 0.99

Precision

Set of three different concentrations in three replicates of standard solutions of OPH were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.66 to 1.31. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.82 to 1.38. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 10 ng $band^{-1}$ and 30 ng $band^{-1}$, respectively.

Recovery Studies

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 200 ng $band^{-1}$ from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are shown in Table 2.

Table 2: Recovery Studies of OPH

Drug	Amount taken (ng $band^{-1}$)	Amount added (ng $band^{-1}$)	Total amount found (ng $band^{-1}$)	% Recovery	% RSD
OPH	200	160	363.45	100.73	1.24
	200	200	400.32	100.08	1.00
	200	240	438.10	99.56	1.05

*Average of three determinations

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase saturation time, wavelength and the effect on the area of drug were noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results are given in Table 3.

Table 3: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)*	
1	Mobile phase saturation (± 10 %)	1.55	0.50
2	Wavelength	1.18	0.78

*Average of three determinations

CONCLUSION

The developed HPTLC method is precise, specific, accurate, reproducible, and stability-indicating, without interference from the excipients or from degradation products resulting from treatment with acid, alkali, oxidizing agent, or from UV irradiation and can be used for quantitative analysis of OPH in pharmaceutical dosage form. As the method is stability indicating one it may be extended to study the degradation kinetics of drug.

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