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Development and validation of stability indicating high performance thin layer chromatography assay method for estimation of duloxetine hydrochloride in bulk drug and pharmaceutical dosage form

Lakshmana Prabu S[1], Suriyaprakash T.N.K.[2] and Thiyagarajan S[1]

[1]Dept. of Pharm. Technology, Anna University of Technology, Tiruchirappalli – 620 024

[2]Dept. of Pharmaceutics, Periyar College of Pharmaceutical Sciences, Tiruchirappalli-620 021.

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ABSTRACT

A new simple, rapid, reproducible and stability indicating high performance thin layer chromatographic method for the analysis of duloxetine hydrochloride in bulk drugs and from pharmaceutical formulation was developed and validated. Chromatographic separation was achieved on aluminium precoated silica gel 60 GF 254 HPTLC plates with chloroform : methanol (80:20%v/v) as mobile phase gives a compact spot at R_f value of 0.42 ± 0.1 and enables excellent separation from its degradation products. Densitometric analysis of duloxetine hydrochloride was carried out in the absorbance mode at 216.0 nm. The correlation coefficient (r^2) was found to be 0.9990 ± 0.0007 with respect to peak area in the concentration range of 400-2000 ng band^{-1}. The method was validated for accuracy, precision and recovery. The limit of detection and limit of quantitation were found to be 100 and 350 ng band^{-1} respectively. Duloxetine hydrochloride was subjected to acid, alkali hydrolysis and oxidative degradation. The drug degradations were took under acidic, basic and oxidation conditions. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of duloxetine hydrochloride. The proposed HPTLC method can be applied for identification and estimation of duloxetine hydrochloride in bulk drug and marketed oral solid dosage form.

Introduction

Duloxetine hydrochloride, (+)-(S)-N-methyl-gamma- (1-naphthyloxy)-2-thiophenepropylamine hydrochloride1, although the exact mechanisms of the antidepressant and central pain inhibitory action of duloxetine in humans are unknown, the antidepressant and pain inhibitory actions are believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS. Preclinical studies have shown that duloxetine is a potent inhibitor of neuronal serotonin and nor-epinephrine reuptake and a less potent inhibitor of dopamine reuptake2.

A survey of literature revealed that the following analytical methods were reported for determination of duloxetine hydrochloride and its metabolites by spectrofluorimetric method3 and chromatographic methods4-20 and tandem mass spectrometry21-22. This developed method is easy, cheap, robust and reproducible when compared to already reported methods.

Nowadays, HPTLC has become a routine analytical technique because it is a simple micro analytical separation technique, economical, low consumption of solvent, reduces the time and cost of analysis and facilitates repeated detection (scanning) of the chromatograms with same or different parameters. The uniform particle size of precoated HPTLC plates enables the achievement of greater resolution and easily reproducible separation. In the present investigation an attempt was made to develop a simple and economical HPTLC method with great precision, accuracy and sensitivity for the analysis of duloxetine hydrochloride in bulk and its dosage form. The reagents utilized in the proposed method are cheaper, readily available and the procedure doesn't involve any critical mobile phase preparation or sample preparation.

Materials and Methods

Materials

Duloxetine hydrochloride (99.93% w/w) was procured as a gift sample from Zydus Cadila, Ahmedabad, India. Chloroform and methanol were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India. Precoated silica gel aluminium HPTLC plates 60 GF 254 with 200 µm thickness; E. Merck, Darmstadt, Germany were used.

Preparation of stock solution from the bulk drug

Duloxetine hydrochloride (50 mg, accurately weighed) was dissolved in 50 ml methanol. Different volume of stock solution 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml was taken and volume was made up to 10 ml by methanol to get working standard containing 40, 60, 80, 100, 120, 140, 160, 180 and 200 µgmL-1 solution respectively.

Sample Preparation

To determine the concentration of duloxetine hydrochloride in capsules (label claim: 20mg per capsule), the contents of 20 capsules were weighed, their mean weight determined and contents were finely powdered. The powder equivalent to 100 mg of duloxetine hydrochloride was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 100 ml. The resulting solution was filtered. The

Tele:

E-mail addresses: slaxmanvel@gmail.com

solution was further diluted to give the desired concentration (100 µgmL-1). From the above solution 10 µl (1000 ng band-1) was applied on HPTLC plate followed by development and analyzed. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

HPTLC Instrumentation

The chromatographic estimation was performed on precoated silica gel aluminium plates 60GF 254 (20 × 10 cm with 200 µm thickness; E. Merck, Darmstadt, Germany) using a Camag Linomat IV sample applicator (Camag, Muttenz, Switzerland) and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom and 15 mm from the left margin of the plate. A constant application rate of 100nls-1 was employed using nitrogen aspirator and space between two bands was 8.0 mm. The slit dimension was kept 5 mm × 0.45 micro and 5mm/s scanning speed was employed. Plates were developed using a mobile phase consisting of chloroform: methanol (80:20%v/v). Linear ascending development was carried out in 20.0× 10.0 cm twin trough glass chamber (Camag, Muttenz, Switzerland) equilibrated with mobile phase, the length of chromatogram run was 7 cm. Subsequent to the development, HPTLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 216 nm using deuterium lamp as radiation source.

Method validation

Calibration curve of duloxetine hydrochloride

A stock solution of duloxetine hydrochloride (1000µg/ml) was prepared in methanol. Different volumes of stock solution, viz., 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml were taken and the final volume was made up to 10 ml by methanol, to get 40, 60, 80, 100, 120, 140, 160, 180 and 200 µgmL-1 solution respectively. From each solution 10 µl were spotted in HPTLC plate to obtain concentrations of 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 ng band-1 of duloxetine hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicate applications of 1000 ng band-1 of duloxetine hydrochloride. The intra and inter-day variation for the determination of duloxetine hydrochloride were carried out by using standard substance and pharmaceutical product at three different concentration levels of 800, 1000 and 1200 ng band-1.

Robustness

Small deliberate changes in the chromatographic conditions, for example volume of mobile phase (± 1 ml), mobile phase composition (± 5 %) and wavelength (± 5 nm) were introduced and the effects on the results were examined. Robustness of the method was done at 1000 ng band-1.

Sensitivity

The sensitivity of the method was determined with respect to Limit of detection (LOD) and Limit of quantitation (LOQ). The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

Specificity

The specificity of the method was determined by analyzing standard drug, pharmaceutical product and placebo and compared the Rf of the standard with that of the sample to determine whether the pharmaceutical product and placebo led to interference.

Accuracy

The preanalyzed samples were spiked with known concentration of standard duloxetine hydrochloride and the mixtures were reanalyzed by the proposed method. At each level six determinations were performed to check the recovery of the drug at different levels from the formulation.

Forced degradation

Acid and base induced degradation

To 100 mg of duloxetine hydrochloride which was weighed and transferred into a clean 100 ml volumetric flask, 50 ml of methanol was added and dissolved. Volume was made up to the mark with methanol (Stock solution). Degradation experiments were performed by taking 10 ml of the above solution in each of the two different 50 ml round bottomed flasks. To the first flask, 10 ml 0.1 N HCl was added for acidic degradation. To the second flask, 10 ml of 0.1 N NaOH was added for basic degradation. Contents of the flasks were refluxed for 3 h in a water bath maintained at 60°C. Samples were allowed to cool to room temperature, neutralized and then suitably diluted with methanol to get 100 µgmL-1. The resultant solutions were applied on HPTLC plate in triplicate (10 µl each, i.e., 1000 ng band-1), the chromatograms were run and analyzed.

Hydrogen peroxide induced degradation

Hydrogen peroxide induced degradation experiment was performed by taking 10 ml of the stock solution in a 50 ml round bottomed flasks. To this 10 ml of 3.0% H2O2 was added for oxidative degradation. Flask was refluxed for 3 h in a water bath maintained at 60°C, samples were allowed to cool to room temperature and then suitably diluted with methanol to get 100 µg/ml. The resultant solutions were applied on HPTLC plate in triplicate (10 µl each, i.e., 1000 ng band-1), the chromatogram was run and analyzed.

Results and Discussion

There were no literature reports of HPTLC method of analysis for duloxetine hydrochloride for routine analysis and for stability studies, the selection of the mobile phase was carried out on the basis of polarity, i.e. choice of a solvent system that would give dense and compact band with appropriate Rf value for duloxetine hydrochloride. The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity, the mobile phase consisting chloroform: methanol (80:20%v/v) gave a sharp and well defined peak at Rf value of 0.42 ± 0.1, the chromatograph is shown in Figure 1.

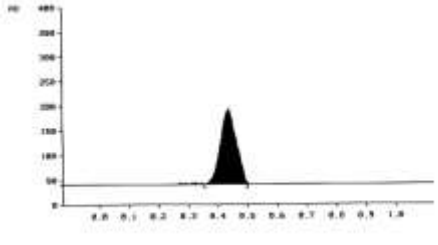

Figure 1. Typical HPTLC chromatogram of duloxetine hydrochloride (R_f = 0.42).

Method validation

Calibration

The calibration curve of amount of analyte against peak area was linear over the concentrations range 400-2000 ng band^{-1} (y= 2.045x-9.398) with the correlation coefficient of 0.9990

±0.0007. The correlation coefficient results showed that the developed method is having excellent linearity.

Precision

Repeatability study was carried out at three different concentration levels of 800, 1000 and 1200 ng band^{-1}. The repeatability of sample application and measurement of peak area were expressed in the terms of % RSD and results are depicted in Table 1. The results revealed that the developed method is having good repeatability.

Robustness

Small deliberate changes in the chromatographic condition did not affect the method and the results are shown in Table 2. The low values of % RSD results revealed that the robustness of the method.

Sensitivity

Under the experimental conditions employed, the lowest amount of drug which could be detected was found to be 100 ng band^{-1} (3*0.0007/2.045)and the lowest amount of drug which could be quantified was found to be 350 ng band^{-1} (10*0.0007/2.045), this indicates the adequate sensitivity of the method.

Specificity

The specificity of the method was confirmed by comparing the R_f value of standard with that of duloxetine hydrochloride in the marketed formulation. Absence of interfering peaks from the excipients commonly present in the tablets and therefore be interred that degradation of duloxetine hydrochloride had not occurred in the formulation that were analyzed by this method. Hence the developed method is specific and selective.

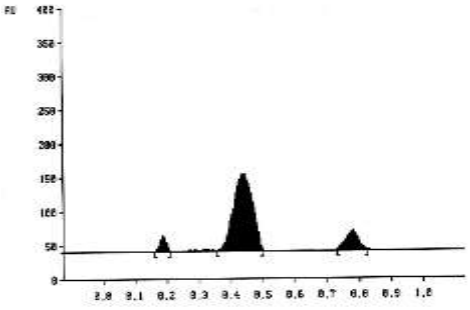

Figure 2. HPTLC chromatogram of acid degraded duloxetine hydrochloride

Accuracy

Accuracy and recovery studies were carried out and mean recovery was found to be between 99.37 ± 0.13% w/w. Analysis of duloxetine hydrochloride in marketed formulation showed a single spot of R_f 0.42 and the duloxetine hydrochloride content was found to be 99.24%, RSD 0.54%. Accuracy and recovery study results revealed that the developed method can be used for determination of duloxetine hydrochloride in pharmaceutical formulation. The low % RSD value for analysis of duloxetine hydrochloride in marketed formulation indicated the suitability of this method for routine analysis of duloxetine hydrochloride in pharmaceutical dosage forms.

Stability Indicating Property

Stability indicating property of the developed method was performed by degradation studies. Duloxetine hydrochloride undergoes acidic, basic and oxidative degradation and the chromatographs are shown in Figure 3-5. The degraded products peaks were well resolved from the drug and the results revealed that the stability indicating property of the developed method.

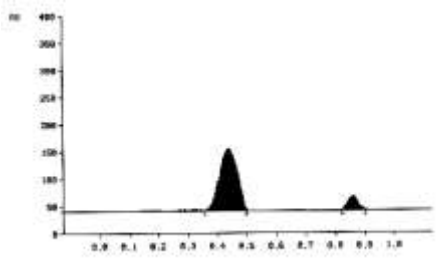

Figure 3. HPTLC chromatogram of base degraded duloxetine hydrochloride

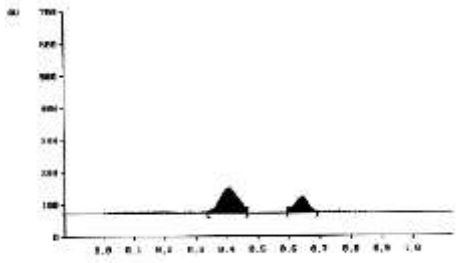

Figure 4. HPTLC chromatogram of hydrogen peroxide degraded duloxetine hydrochloride

Conclusion

The developed HPTLC method combined with densitometry was found suitable for determination of duloxetine hydrochloride in bulk drug and marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of duloxetine hydrochloride. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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Table 1. Intra and inter-day precision of HPTLC method[a]

Actual conc. (ng band $^{-1}$)	Intra-day precision			Inter-day precision		
	Mean area	S.D.	% R.S.D.	Mean area	S.D.	% R.S.D.
800	1604.67	20.40	1.27	1575.72	20.32	1.29
1000	1903.33	28.55	1.50	1895.94	23.54	1.24
1200	2404.83	35.86	1.49	2416.72	29.48	1.22

[a] n=6

Table 2. Robustness of the method[a]

Parameters	S.D. of peak area	% R.S.D.
Mobile Phase composition	27.43	1.42
Amount of mobile phase	22.53	1.21
Wavelength	20.65	1.09

[a] n=6

Table 3. Accuracy

Excess drug added (ng band^{-1})	Amount recovered (ng band^{-1})	Recovery (%)
800	796.17	99.52
1000	993.33	99.33
1200	1191.67	99.26

[a]n= 6
Mean recovery (± S.D) = 99.37 ± 0.13