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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF DOLASETRON MESYLATE

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ABSTRACT

Objective: To develop and validate stability indicating HPTLC method for determination of Dolasetron mesylate.

Methods: The chromatographic separation was performed on aluminium plates precoated with silica gel 60 F_{254} using Methanol: Choloroform: Ethyl acetate (7:2:1 v/v/v) as mobile phase followed by densitometric scanning at 280 nm.

Results: The chromatographic condition gave a compact spot for Dolasetron mesylate at Rf value of 0.65±0.03. Stress testing was performed in accordance with international conference on harmonization (ICH) Q1A R2 guidelines. Method was validated as per ICH Q2 R1 guidelines. The calibration curve was found to be linear in the concentration range of 100-800 ng/band for Dolasetron mesylate. The limit of detection and quantification was found to2.24 ng/band and 6.79 ng/band, respectively.

Conclusion: A new sensitive, simple, and stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for determination of Dolastron mesylate. The proposed method can be used for routine determination of Dolasetron mesylate stability.

Keywords: Dolasetron mesylate, HPTLC, Stability indicating method.

INTRODUCTION

Dolasetron mesylate (fig. 1), is chemically (2α,6α,8α,9aβ)-octahydro-3-oxo-2,6-methano-2*H*- quinolizin-8-yl-1*H*-indole-3-carboxylate mono methane sulfonate monohydrate, is an highly specific and selective serotonin subtype 3 (5-HT) receptor antagonist, used to treat nausea and vomiting in chemotherapy [1, 2].

Literature survey revealed that Dolasetron mesylate is official in the USP-NF [3] and few methods viz. HPLC [4-7], HPLC-EI-MS and GC-MS in human plasma [8,9] have been reported for the determination of Dolasetron mesylate either as single drug or simultaneous with its metabolite.

To the best of our knowledge, no stability indicating High Performance Thin Layer Chromatographic (HPTLC) method has been reported for Dolasetron mesylate. The present work involves stress degradation as per ICH Q1A (R2) and Q1B [10, 11] for developing new, simple, sensitive stability indicating HPTLC method; The method was validated as per the ICH guidelines Q2 R1 [12].

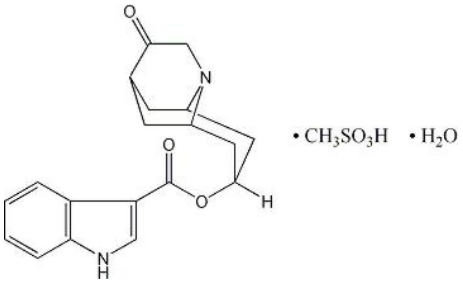


Fig. 1: Chemical structure of Dolasetron mesylate

MATERIALS AND METHODS

Chemicals and reagents

Dolasetron mesylate was provided as a gift sample by Emcure pharmaceutical Pvt. Ltd, Kurkumbh, Pune. Methanol, Chlorofrom, Ethyl acetate and all other chemicals used in this study were of AR grade purchased from Merck Pvt. Ltd. Mumbai, India.

Instruments

Pre-coated silica gel 60F_{254}aluminium plates (10 x 10 cm, 250 µm thickness; Merck, Germany), Linomat-5 sample applicator (Camag, Switzerland), twin trough chamber (10 x 10 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner 3 (Camag, Switzerland), winCATS version 1.4.3 software (Camag, Switzerland), Photostability chamber (Newtronics NEC103RSPI), Shimadzu balance (Model AX-200) were used in the study.

Experimental

Preparation of standard solutions

Standard stock solution of Dolasetron mesylate was prepared by dissolving 5 mg of the drug in 10 ml of methanol to get concentration of 500µg/ml. From the standard stock solution, working standard solution was prepared containing 50µg/ml of Dolasetron mesylate.

Selection of analytical wavelength

The standard solution Dolasetron mesylate in water was scanned over wavelength range 200 to 400 nm by using UV-Visible spectrophotometer. Wavelength 280 nm was selected for analysis were Dolasetron mesylate showed higher absorbance (fig. 2).

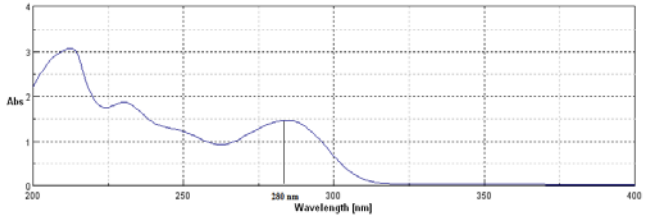


Fig. 2: UV spectrum of Dolasetron mesylate between 200-400 nm

Chromatographic conditions

TLC plates precoated with silica gel 60 F_{254}, were used as stationary phase. TLC plates were pre-washed with methanol and activated at

110 °C for 10 min prior to application. The standard solution of Dolasetron mesylate, was applied on the pre-coated TLC plate as a band with 6 mm width. The chromatographic development was performed by using methanol: chloroform: ethyl acetate (7:2:1 v/v/v) as mobile phase with 15 minutes chamber saturation time and migration distance of 80 mm. Densitometric scanning was performed at 280 nm. The standard densitogram of Dolasetron mesylate (400 ng/b and) are shown in fig. 3.

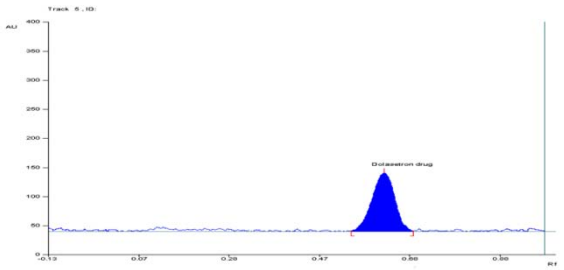


Fig. 3: Densitogram of standard Dolsetron mesylate (400 ng/b and)

Stress degradation studies of bulk drug

Stress degradation studies were carried under a condition of acid/base/neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared, the blank subjected to stress in the same manner for the drug solution, working standard solution of Dolasetron mesylate subjected to stress degradation. Dry heat and photolytic degradation were carried out in a solid state. The concentration of degrading reagent and time of exposure was optimized to get 10-30 % degradation.

Acid hydrolysis

Optimization trial

Initially, trials were conducted by exposing the sample solution to one ml 0.1N HCL for 8 hours and no degradation was observed.

Optimized trial

One ml working standard solution of Dolasetron mesylate (500µg/ml) was mixed with one ml of 0.1 N HCL (methanolic) and 8 ml of methanol. Solution was kept for overnight. 8 µl (400 ng/b and) of the resulting solution was spotted on TLC plate. Average 86.17% of Dolasetron mesylate was recovered with no peak of degradation.

Alkaline hydrolysis

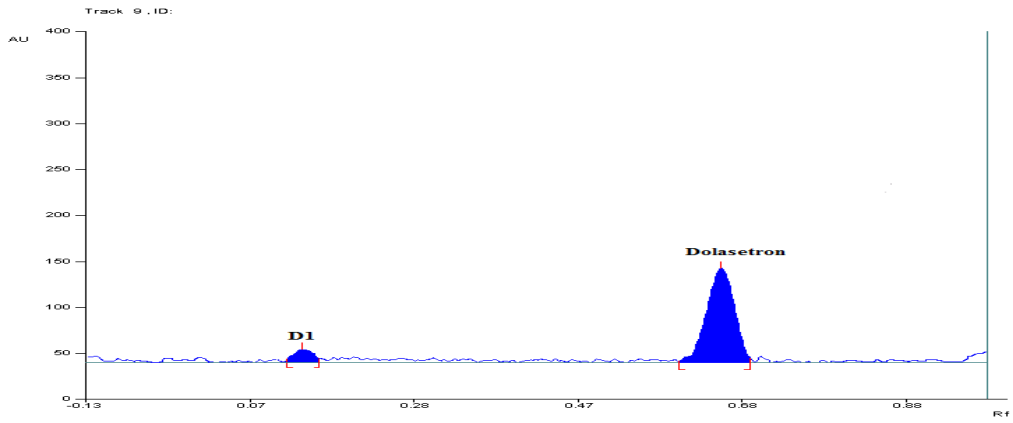
Optimization trial

Initially, trials were conducted using 0.1N NaOH by exposing the sample solution for 12 hrs and No degradation were observed.

Optimized trial

One ml working standard solution of Dolasetron mesylate (500 µg/ml) was mixed with one ml of 1 N NaOH (methanolic) and 8 ml of methanol. Solution was kept for 2 hr. 8 µl of the resulting solution was spotted on TLC plate, and densitogram was developed. Average 80.31% Dolasetron mesylate was recovered with one peak of degradation product (D1) at Rf value 0.14 (fig. 4, I & II).

I)



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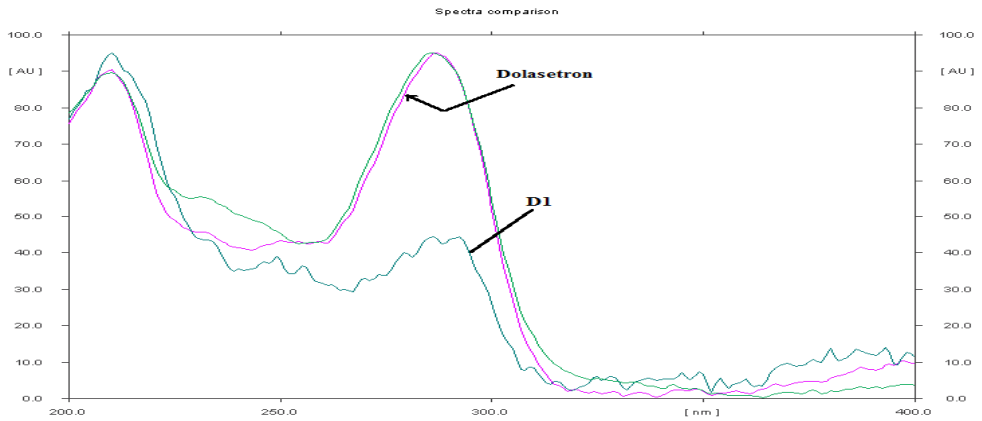


Fig. 4: I) Densitogram of alkali treated Dolasetron mesylate, II) UV overlay spectrum of Standard drug, alkali treated Dolasetron mesylate, Degradation product (D1)

Degradation under neutral condition

One ml working standard solution of Dolasetron mesylate (500 µg/ml) was mixed with one ml of distilled water and 8 ml of methanol. Solution was kept for 48 hrs. 8 µl of the resulting solution was spotted on TLC plate. Average 86.84% of Dolasetron mesylate was recovered with no peak of degradation in neutral condition.

Degradation under oxidative condition

Optimization trial

Initially, trials were conducted using 10% v/v Hydrogen Peroxide by keeping the sample solution for 2 hrs. It was observed that drug get degraded completely.

Optimized trial

One ml working standard solution of Dolasetron mesylate (500 µg/ml) was mixed with one ml 10% solution of H_2O_2 (methanolic) and 8 ml of methanol. Solution was kept for 30 minutes. 8 µl (400 ng/b and) of the resulting solution was spotted on TLC plate.

Average 87.74% Dolasetron mesylate was recovered with one peak of degradation Product (D2) at Rf 0.15 (fig. 5I). Oxidative degradation study was performed by increasing exposure time of Dolasetron mesylate with 10 % H_2O_2 at 30, 60 and 120 minutes. (fig. 5 II). Results are mentioned in table 1.

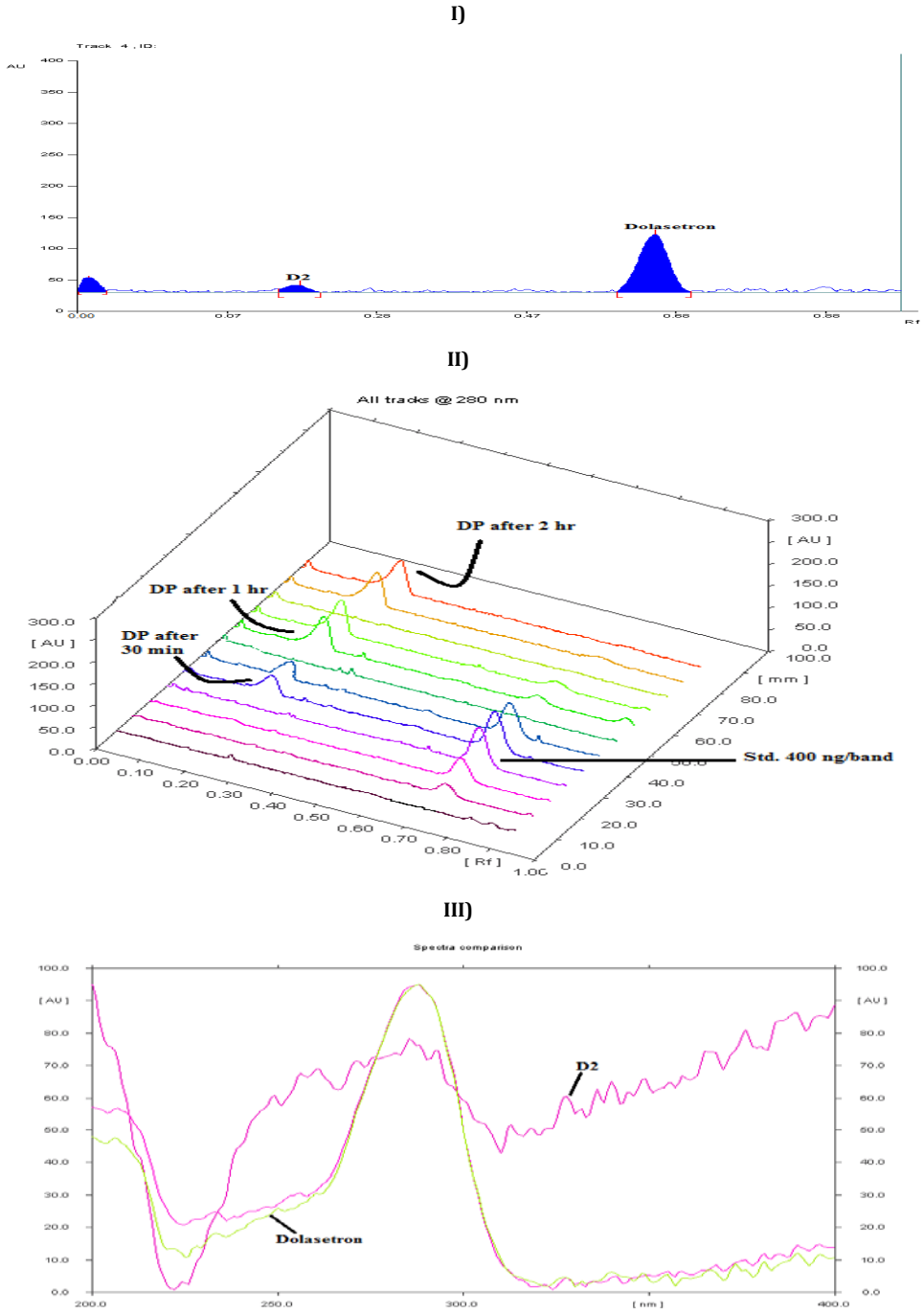


Fig. 5: I) Densitogram of peroxide treated Dolasetron mesylate, II) Oxidative degradation monitored at 30, 60 and 90 min. III) UV overlay spectrum of standard drug, Peroxide treated dolasetron mesylate and Degradation product (D2)

Table 1: Oxidative degradation kinetics of Dolasetron mesylate

S. No.	Exposure time to oxidative condition (min)	% Recovery	% Degradation
1	30	87.74	12.26
2	60	38.53	61.47
3	120	1.62	98.38 (Complete degradation)

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in an oven (80 ° C) for a period of 24 hours. Sample was withdrawn, dissolved in methanol and diluted to get 500µg/ml. One ml was further diluted to get 50 µg/ml solutions of which 8µl volume were spotted on TLC plate. Average 71.87 % Dolasetron mesylate was recovered with no peak of degradation was observed in dry heat.

Photo-degradation studies

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool white fluorescent light to achieve an illumination of 1200 Lux. Hr. Sample were weighed, dissolved and diluted to get 500 µg/ml.

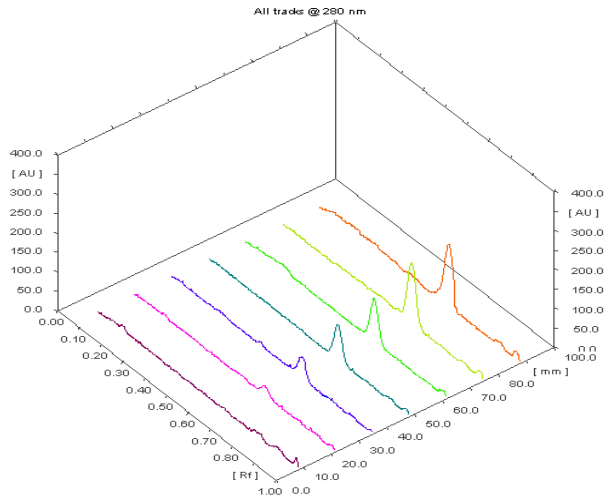
One ml was further diluted to get 50 µg/ml solutions of which 8 µl volumes was spotted on TLC plate. Average 85.48% and 68.69% of Dolasetron mesylate was recovered after exposure to UV and Fluorescent light respectively with no peak of degradation was observed in both.

Analytical validation parameters

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample (drug-excipient blend). The spot for the drug in a sample was confirmed by comparing the Rf and spectra of the spot with that of the standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at the peak end. The peak purity was determined on Win CATS software 5.

I)



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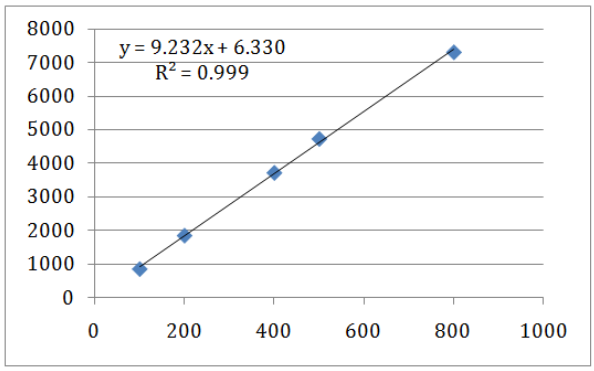

Fig. 6: Calibration curve of Dolasetron mesylate

Linearity and range

Appropriate volumes were spotted from standard stock solution to obtain 100, 200, 400, 500 and 800ng/band respectively. Each standard was analysed in five replicates, and peak area was noted. The relationship between peak area and concentration was established by the simple regression equation method. (fig. 6)

Accuracy

To check accuracy in the method, recovery studies were carried out by standard drug addition to drug-excipient blends at three different levels 80%, 100% and 120 %. Basic concentration of sample chosen was 200 ng/band of standard. The drug concentrations were calculated by using regression equation of Dolasetron mesylate (table 2).

Precision

The precision to the method was demonstrated by intra-day and inter-day studies. In the intra-day studies, 3 replicates of 3 standard solutions (200, 400 and 500 ng/band) were analyzed in a same day and percentage RSD was calculated (table 3). For the inter-day variation studies, 3 standard solutions (200, 400 and 500 ng/band) were analyzed on three consecutive days and percentage RSD was calculated (table 4).

LOD and LOQ

The LOD (limit of detection) and LOQ (limit if quantification) of Dolasetron mesylate were estimated from the standard deviation of the response and the slope to the calibration curve. LOD and LOQ were found to be 2.24 ng/band and 6.79 ng/band respectively.

Robustness

The robustness of the method was studied, during method development, by small but deliberate variations in chamber saturation time (13, 17 min), change in mobile phase composition, time from application to development (30, 120 min), and time from development to scanning (30, 120 min). One factor at a time was changed at a concentration level of 400ng/band to study the effect on peak area of drug (table 5).

Table 2: Determination of accuracy for Dolasetron mesylate

Level (%)	Concentration (ng/band)		Avg. area	% Recovery	% RSD
	Sample conc.	Amount added			
80%	200	160	3058.4	99.14	0.78
100%	200	200	3358	98.71	1.02
120%	200	240	3648.5	98.08	1.43

Table 3: Intra-day precision

S. No.	Concentration (ng/band)	Intraday mean area*	% recovery	SD	% RSD
1	200	1862.93	100.57	10.92	0.52
2	400	3751.33	101.43	14.83	0.39
3	500	4750.06	102.78	13.18	0.27

* Average of 3 determinations

Table 4: Inter-day precision

S. No.	Concentration (ng/band)	Inter day mean area*	% recovery	SD	% RSD
1	200	1899.33	102.54	11.12	0.58
2	400	3745.53	101.27	13.75	0.36
3	500	4730.76	102.30	51.38	1.08

* Average of 3 determinations

Table 5: Robustness study of Dolasetron mesylate

S. No.	Parameters	Robust condition	% RSD
1.	Chamber saturation time(15 min)±2 min.	13 min	0.71
		17 min	0.52
2.	Mobile phase compositionMethanol: Chloroform: Et. acetate: (7:2:1 v/v)±0.2 Methanol	Methanol: Chloroform: Et acetate (7.2: 2:1 v/v)	1.2
		Methanol: Chloroform: Et acetate (6.8: 2:1 v/v)	0.62
3.	Time from Application to development (immediate)	After 30 min.	1.25
		After 2 hrs	1.68
4.	Time from Development to Scanning(immediate)	After 30 min.	0.82
		After 2 hrs	1.32

RESULTS AND DISCUSSION

Optimization of mobile phase

Method development for Dolasetron mesylate was started with the development of densitogram using neat solvents and combinations of Methanol, Chloroform, Ethyl acetate, and Toluene in different ratios. Methanol: Chloroform: Ethyl acetate in the ratio of (7:2:1 v/v/v) was selected as the mobile phase for Dolasetron mesylate, which resulted in acceptable peak parameters. The Rf found to be 0.65±0.03 for Dolasetron mesylate.

Stress degradation

Drug was subjected to various forced degradation conditions. The conditions of stress were optimized with respect to the strength of the reagent and exposure period so as to achieve degradation in the range of 10 to 30%. During optimization of degradation conditions, if the higher percentage of degradation was observed, milder conditions were used with lesser duration of exposure. If percent degradation is high, there are chances of formation of secondary products.

This was carefully avoided. Although percent assay reduced under all conditions; the separate peak for degradation product was observed only under base and oxidative conditions. In order to make the product peaks easier to locate in other conditions, 10 times higher concentrations was spotted. But no product peak could be located. The degradation under oxidative stress is being investigated further. Summary of stress degradation results is given in table 6.

Table 6: Summary of stress degradation study of Dolasetron mesylate

S. No.	Stress degradation condition	% Recovery	% Degradation	R_f of degradation product
1	Acid (0.1 N HCl,kept for overnight)	86.17	13.83	-
2	Base (1N NaOH,kept for 90 mins)	80.31	19.69	0.14
3	Water(Kept for 48 hrs.)	86.84	13.16	-
4	H_2O_2 10%(Kept for 30 min.)	87.74	12.26	0.15
5	Heat dry(80 °C, 24 hrs.)	71.87	28.13	-
6.	Photo stability[UV, 200 watt hrs/square meterFlorescence, 1.2 million Lux. Hrs]	85.48 68.69	14.52 31.31	- -

Method validation

The method validation results were satisfactory as per ICH Q2R1 guidelines. The peak areas were found to be linear over the concentration range 100–800 ng/band with a correlation co-efficient of 0.999. Method specificity can be proved using "Peak Purity" parameter in WinCats software of HPTLC. There is a provision to compare the UV spectrum at the start, middle and end of any peak. The percent matching indicates peak purity. Peak purity results greater than 0.999 indicate that peak is homogeneous in all stress conditions tested. The unaffected assay of drug in the blend confirms the non-interference by any excipient. Inter and Intraday precision were less than 2%. Percent recovery in accuracy study was within the limit of 98 to 102%. The results of validation are summarized in table 7.

Thus the optimized HPTLC method for Dolasetron is based on use of simple ternary mixture of organic solvents. The method is found to be sensitive with limit of quantitation less than 10 ng/band. The drug was found to be prone to oxidation and alkali catlaysed hydrolysis. The oxidative degradation was found to be faster than hydrolytic. The degradation products observed under these conditions were well resolved from the drug peak.

The spectral purity of drug peak indicates specificity of the method. Dolasetron was found to be photolabile. The peak area was found to reduce upon exposure to ultraviolet and fluorescent light as per ICH Q1B guidelines. But the peak for the product of degradation was not noticed. The peak purity of drug peak is maintained even in photodegraded sample. Thus the developed method is stability indicating.

Table 7: Summary of validation parameters

S. No.	Validation parameter	Results
1.	Linearity	Y= 9.23X+6.330 R^2 = 0.999
2.	Range	100-800 ng/band
3.	Precision	(%RSD)
	A) Intraday precision	0.39
	B) Interday precision	0.67
4.	Accuracy	% Recovery
	80%	99.14
	100%	98.71
	120%	98.08
5.	LOD	2.24 ng/band
6.	LOQ	6.79 ng/band
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION

The developed method is stability indicating where well resolved peaks were observed for analyte and degradation product. The method is specific, accurate, precise, robust and can be used for routine quality control as well as assessing the stability of Dolasetron mesylate in bulk.

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CONFLICT OF INTERESTS

Declared None

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