Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

HPTLC METHOD FOR ESTIMATION OF DOMPERIDONE IN BULK AND MARKETED FORMULATION

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ABSTRACT

High performance thin layer chromatography (HPTLC) offers many advantages over HPLC. It reduces the cost of analysis as compare to HPLC. The mobile phase consumption per sample is extremely low in HPTLC, hence reducing the acquisition and disposal cost. Considering the cost and suitability of analysis for estimation of Domperidone (DMP) in bulk and its marketed formulation, HPTLC method was developed and validated. The Camag HPTLC system, employed with software win CATS (ver.1.4.1.8) was used for the proposed analytical work. Planar chromatographic development was carried out with the help of Silica Gel 60 F_{254} precoated TLC plates. Sample application was facilitated by Linomat 5 applicator. After sample application plates were subjected for ascending development in twin trough chamber of 10×10 dimension, using 10mlof solvent system. The mobile phase was ethyl acetate: methanol (6:4v/v). The detection of spots was carried out densitometrically using a UV detector at 286 nm in absorbance mode. The R_f value for DMP was found to be 0.57-0.64. The HPTLC method was found to be linear across the range 100-700ng/spot. The LOD and LOQ values were 37.72 and 114.30 ng/spot respectively. Performance characteristics of HPTLC method for estimation of DMP in bulk and its marketed dosage form were statistically validated as per the recommendations of ICH guidelines of analytical method validation.

KEYWORDS

HPTLC, Domperidone, Marketed dosage form and Analytical method validation.

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INTRODUCTION

HPTLC is a well-known and versatile separation method which is type of planar chromatography involves principle of adsorption. DMP chemically known as 5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1H-bezo[d]imidazol-1-yl)propyl]piperidin-4-yl)-1benzo [d]imidazol-2(3H)-one (Figure No.1) is synthetic benzimidazole compound which act as dopamine antagonist and antiemetic. It is poorly water soluble and has low absorbability after oral administration, and undergoes extensive first pass metabolism leading to poor bioavailability of 15%[1-4]. DMP is a

dopamine-receptor blocking agent[4-6]. DMP works primarily by blocking dopamine receptors found in an area of the brain known as the chemo receptor trigger zone, located just outside the blood brain barrier, which regulates nausea and vomiting[5,7,8]. It increases gastrointestinal peristalsis and motility that prevent reflux esophagitis and it is used to prevent nausea and vomiting[1,5,7,9,10].

It also used as prokinetic agent for treatment of gastrointestinal peristalsis which helps to increase transfer of food through the stomach[11-13].DMP increases the movements or contractions of the stomach and bowel. It is also used to treat nausea and vomiting. The use of DMP are in Hypokalaemia, Hypomagnesaemia, Congestive heart failure, and adverse effects Angioneurotic, Oedema, Allergic reaction, Dry mouth, Headache, Insomnia, Dizziness, Diarrhoea, Regurgitation, Appetite disorder, Nausea[3, 10-12].

It helps to increase lactation by release of prolactin in breast milk production. It does not produce dopamine antagonist effects in the CNS, probably because it fails to cross the blood brain barrier[14,15].

Review of literature survey reveals that UV spectro-photometric[16,17] and high pressure liquid chromatographic (HPLC)[3,4,9,12,17], LC-MS[15]methods have been developed for quantitative estimation of DMP. Not a single HPTLC method is reported for the estimation of DMP.

The objective of present work was to develop simple, rapid, accurate, specific and economic HPTLC method for the estimation of DMP in bulk and marketed formulation. HPTLC method was developed and validated as per the recommendations of ICH guidelines of analytical method validation.

MATERIALS AND METHODS

Materials and marketed formulation

DMP and drug tablets were procured as generous gift sample for the purpose of academic research from local pharmaceutical company. Merck HPTLC aluminium plates precoated with silica gel 60 F_{254} were procured from local scientific and chemical supplier.

Reagents

Chemicals of (A.R. and HPLC grade) were purchased from S.D. Fine Chemicals, Mumbai, Maharashtra, India.

Instrumentation

Details of HPTLC instrument are given in Table No.1.

EXPERIMENTAL

Analytical Method Development

Preparation of Standard Stock and Working Solution

Standard stock solution of DMP was prepared by dissolving 10 mg of drug in 10 ml methanol to obtain concentration 1000μg/ml (1000 ppm).

Selection of chromatographic layer

Silica Gel 60 F_{254}precoated TLC plates were selected as chromatographic layer.

Layer Prewashing-Precoated TLC plates were prewashed with methanol to remove adsorbed material, impurities which include water vapours and other volatile substances from the atmosphere when they get exposed in the lab environment.

Layer preconditioning - Prewashed plates were placed in oven at 100°C for 5 minutes prior to the sample application.

Preparation of Sample solution for estimation from marketed tablet formulation

Five tablets, each containing 10 mg of DMP were weighed and finely powdered. A quantity of powder equivalent to 10 mg of DMP was transferred to a 100 ml volumetric flask, dissolved in methanol and made the volume up to 100 ml with methanol. It was sonicated for 30 minutes in ultrasonication bath for complete dissolution of drug. The solution was double filtered, first through0.45 μm whtaman filter paper and after that through 0.45 μm syringe filter in order to get cleared solution. Further, it was diluted with methanol to get the concentration of 50 μg/ ml.

Selection of Detection Wavelength

10 μg/ml (10 ppm) solution of DMP was applied on HPTLC plate (suitable dimension), scanned densitometrically over the range of 200- 700 nm using camag HPTLC scanner 5.

Optimization of Chromatographic Conditions

Many preliminary trials were carried out for selection and optimization of,

- Mobile phase composition
- Chamber saturation time

ANALYTICAL METHOD VALIDATION

The developed HPTLC method was validated as per recommendations given by ICH guidelines Q2(R1) for validation of analytical procedures: text and methodology for following parameters[18].

RESULTS AND DISCUSSION

Selection of wavelength

UV absorption spectrum for 10 ppm solution of DMP (Figure No.2) was generated using camag HPTLC scanner 5 and win CATS (ver.1.4.1.8) software, 286 nm wavelength was selected as a detection wavelength for chromatographic determination of DMP since at 286 nm wavelength drug was showing maximum absorbance.

Optimization of chromatographic conditions

Many preliminary trials were carried out for selection of mobile phase; some are tabulated in Table No.3.

ANALYTICAL METHOD VALIDATION

Linearity

Linear relationship was observed by plotting peak area against sample concentration. DMP showed linear response in the concentration range of 100-700 ng/spot (Figure No.4). The linear regression data of calibration plot for DMP given in Table No.5.

Precision

Intra-day precision was performed at three different concentration levels within the same day low, mid and high i.e. 200 ng/spot, 400 ng/spot, 600 ng/spot respectively at three different times (session 1, 2, 3). Inter-day precision was carried out at same concentration levels on three consecutive days, using same homogeneous sample. The % RSD values for both intra-day and inter-day precision were found within acceptable limit as shown in Table No.6 and 7 respectively.

Accuracy

Accuracy of the method is reported as present recovery of known added amount of analyte in the sample. The accuracy of the method was established by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of DMP. Results obtained are given in Table No.9.

Specifity

When the densitogram of standard DMP was over layed with the densitogram of sample (tablet extract) it was observed that the densitogram of DMP was exactly matching with the densitogram of tablet extract as shown in (Figure No.5). Therefore the method is specific.

Robustness

To determine robustness of analytical HPTLC method changes observed in the mobile phase composition and saturation time. Effect of this change on both the R_f values and peak areas were evaluated by calculating the relative standard deviations (% RSD). The results obtained are tabulated in Table No.10.

Table No.1: Instrument and specifications of HPTLC

S.No	Instruments	Specification
1	Make and model	Camag, Switzerland
2	Scanner	TLC scanner 5
3	Sampling mode	Manual with Linomat applicator
4	Syringe	Hamilton (100 µl)
5	Detection	Ultraviolet (UV) detector
6	Software	WinCATS (ver.1.4.1.8)

Table No.2: Analytical method validation parameters and procedure followed

<table>
<tr><th>S.No</th><th>Parameters</th><th colspan="2">Method/ Procedure followed</th></tr>
<tr><td>1</td><td>Linearity</td><td colspan="2">As per ICH, for the establishment of linearity, a minimum of 5 concentrations are recommended.
A linear relationship was evaluated across the range of 100-700 ng/spot for DMP.
Linearity is reported by the value of the correlation coefficient, y-intercept, and slope of the regression line.
It was obtained by plotting peak area v/s concentration of standard and finding regression coefficient (R^2).</td></tr>
<tr><td>2</td><td>Precision</td><td colspan="2">Precision was carried out at two levels, as follows</td></tr>
<tr><td rowspan="3"></td><td rowspan="3"></td><td>Repeatability</td><td>Intermediate Precision</td></tr>
<tr><td>Repeatability was assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations/ 3 replicates each)</td><td>Intermediate Precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure.
Intraday and Interday precision studies were performed by taking 9 determinations of 3 concentrations/3 replicates each, at 3 times in a same day and on 3 different days, respectively.</td></tr>
<tr><td colspan="2">Precision is reported as standard deviation and relative standard deviation (coefficient of variation) for each type of precision investigated.</td></tr>
<tr><td>3</td><td>Detection of Limit and Limit of Quantification</td><td colspan="2">LOD and LOQ are determined based on the standard deviation of the response and the slope.
<table><tr><th>LOD</th><th>LOQ</th></tr><tr><td>$LOD = 3.3\times \frac{\sigma}{S}$</td><td>$LOQ = 10 \times \frac{\sigma}{S}$</td></tr></table>σ = Standard deviation of response estimated based on the calibration curve.
S = Slope of the calibration curve.</td></tr>
<tr><td>4</td><td>Accuracy</td><td colspan="2">Accuracy was established across the specified range of analytical procedure by adding known added quantities of analyte to the dosage form.
As per ICH, Accuracy should be assessed using a minimum of 9 determinations over a minimum of three concentration levels covering the specified range i.e. 3 concentrations levels in triplicate. (e.g. 3 concentrations/ 3 replicate each).
Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% of by adding known amount of standard solution of DMP.
Recovery studies were also performed and analysed comparing with standard on tablets containing DMP.</td></tr>
<tr><td>5</td><td>Specificity</td><td colspan="2">As per ICH, Specificity should be carried out to ensure identity of an analyte.
The specificity of the method was determined by comparing the R_f value and densitogram of standard DMP with marketed formulation (Tablet extract).</td></tr>
<tr><td>6</td><td>Robustness</td><td colspan="2">The robustness of an analytical procedure is a measure of its ability to remain unchanged by small, but considered variations in method parameters and provides an indication of its deliberate during normal usage.
For checking the robustness of the developed analytical method following parameters were purposely changed,
1. Small variations in a mobile phase
2. Changes in saturation time
The % RSD of peak area is calculated for each parameter.</td></tr>
</table>

Table No.3: Trials for selection of mobile phase composition

S.No	Mobile Phase Components	Composition (V/V/V/V)
1	N-Butanol: water	6:1
2	Ethyl Acetate:Methanol: Benzene:ACN	3:2:1:4
3	Acetone: Toluene :Methanol	4:4:2
4	Ethyl Acetate: ACN	7:3
5	Ethyl Acetate: Methanol: Carbon Tetrachloride	5:5:1
6	Ethyl Acetate: Methanol	6:4

Table No.4: Optimized Chromatographic Condition

1	Mobile phase composition	Ethyl acetate: methanol (6:4 v/v)
2	Sample application volume	10 μl
3	Detection wavelength	286 nm
4	Saturation time	25 min

Table No.5: Linear regression data of calibration plot for DMP

S.No	Parameter	HPTLC
1	Range	100- 700 ng/spot
2	r^2	0.993
3	y- intercept	295.7
4	Slope	11.82

Table No.6: Intra-day precision studies

S.No	Intra-day precision studies		DMP			Inference
1	Concentration levels		Low	Mid	High	Acceptable % RSD, hence precise
2	Concentration (ng/spot)		200	400	600	
3	Peak Area	Session 1	2300.6	4458.1	6271.1	
		Session 2	2344.4	4595.3	6113.5	
		Session 3	2381.3	4495.7	6102.3	
4	Average peak area		2342.1	4516.3	6162.3	
5	Standard deviation		40.3991	70.8963	94.3898	
6	% RSD		1.7249	1.5697	1.5317	

Table No.7: Inter-day precision studies

S.No	Inter-day precision studies		DMP			Inference
1	Concentration levels		**Low**	**Mid**	**High**	Acceptable % RSD, hence precise
2	Concentration (ng/spot)		200	400	600	
3	Peak Area	Session 1	2315.4	4333.8	6478.8	
		Session 2	2384.0	4478.2	6561.6	
		Session 3	2351.4	4413.3	6379.6	
4	Average peak area		2350.2	4408.4	6473.2	
5	Standard deviation		34.3140	72.3229	91.2774	
6	% RSD		1.4600	1.6405	1.4100	

Table No.8: LOD and LOQ of DMP

S.No	Parameters	Readings obtained
1	LOD	37.72 ng/spot
2	LOQ	114.30 ng/spot

Table No.9: Accuracy- Recovery studies

S.No	Drug	Level of percentage recovery (%)	Amount present in extract (ng/spot)	Amount added (ng/spot)	Total amount (ng/spot)	% recovery	Average % recovery	% RSD	Inferences
1	DMP	80	500	400	900	98.02	99.7	1.1537	Acceptable recovery hence accurate
		100	500	500	1000	101.78		1.5684	
		120	500	600	1100	99.3		0.7191	

Table No.10: Robustness results

S.No	Parameters	Parameter Changed	% RSD
1	Mobile phase composition (v/v)	Ethyl Acetate: Methanol (7:3)	0.3482
		Ethyl Acetate: Methanol (5:4)	2.5381
2	Saturation time (minutes)	+2	0.5487
		-2	1.3546

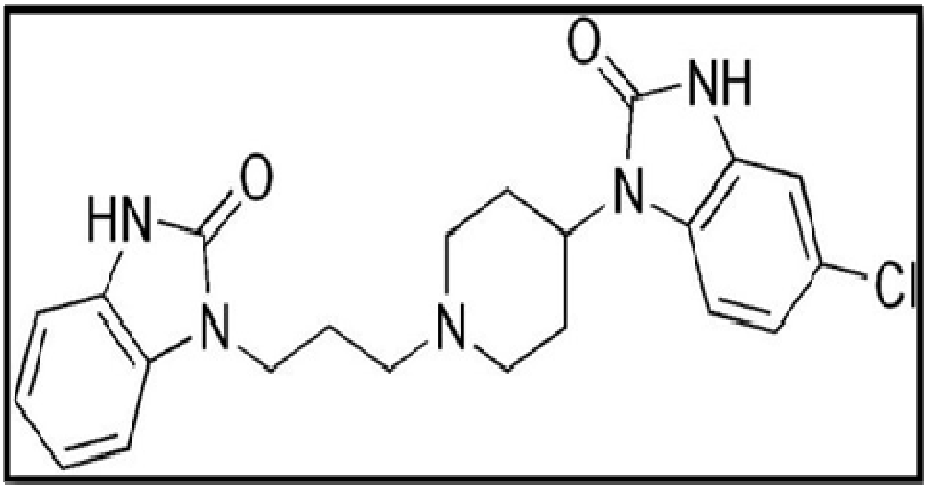

Figure No.1: Chemical Structure of Domperidone

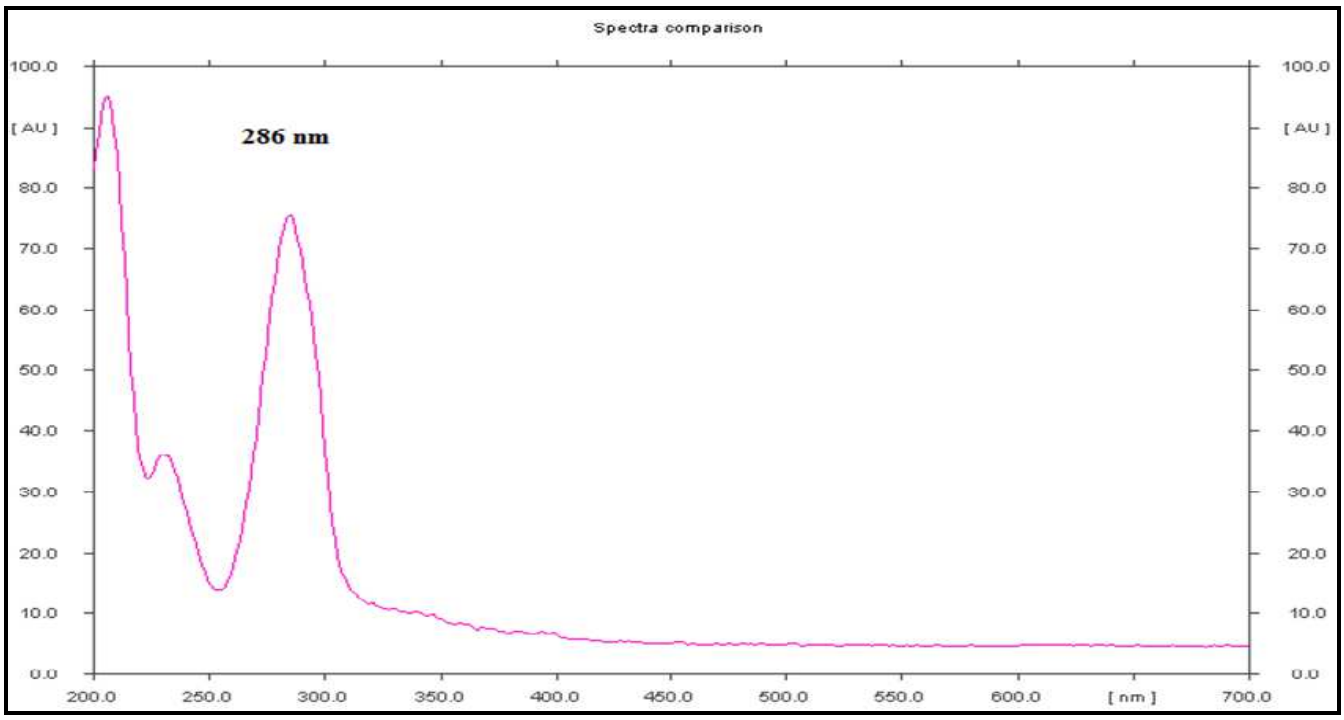

Figure No.2: HPTLC Spectrum of DMP

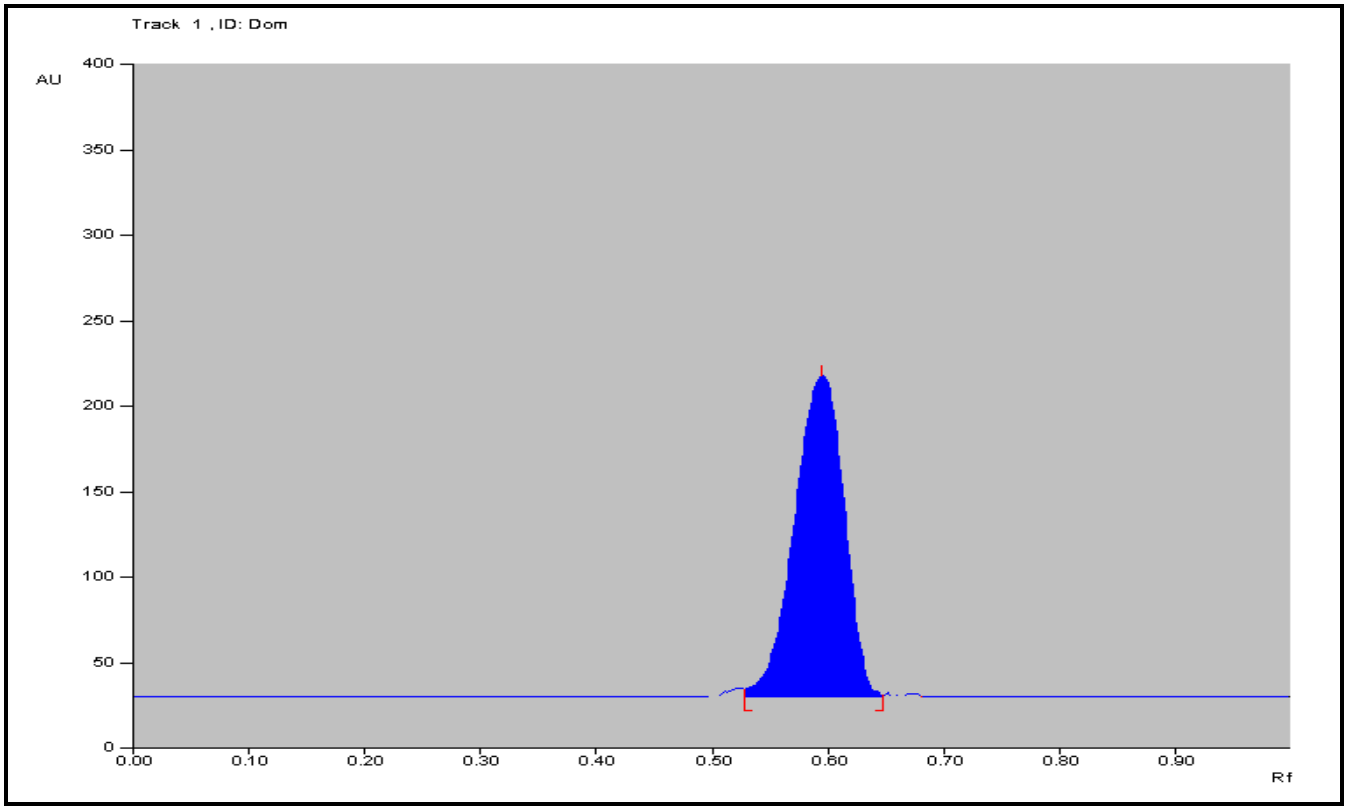

Figure No.3: Densitogram of DMP

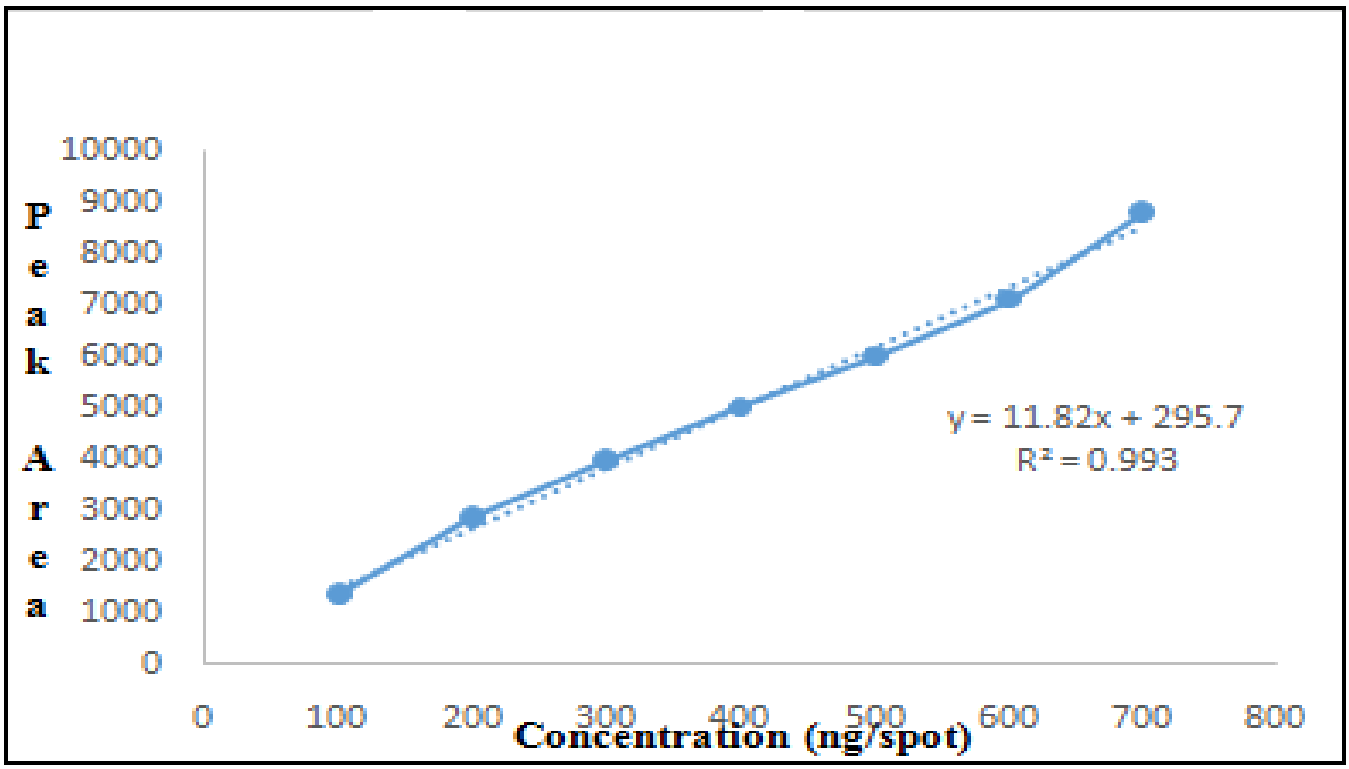

Figure No.4: Calibration Curve of DMP

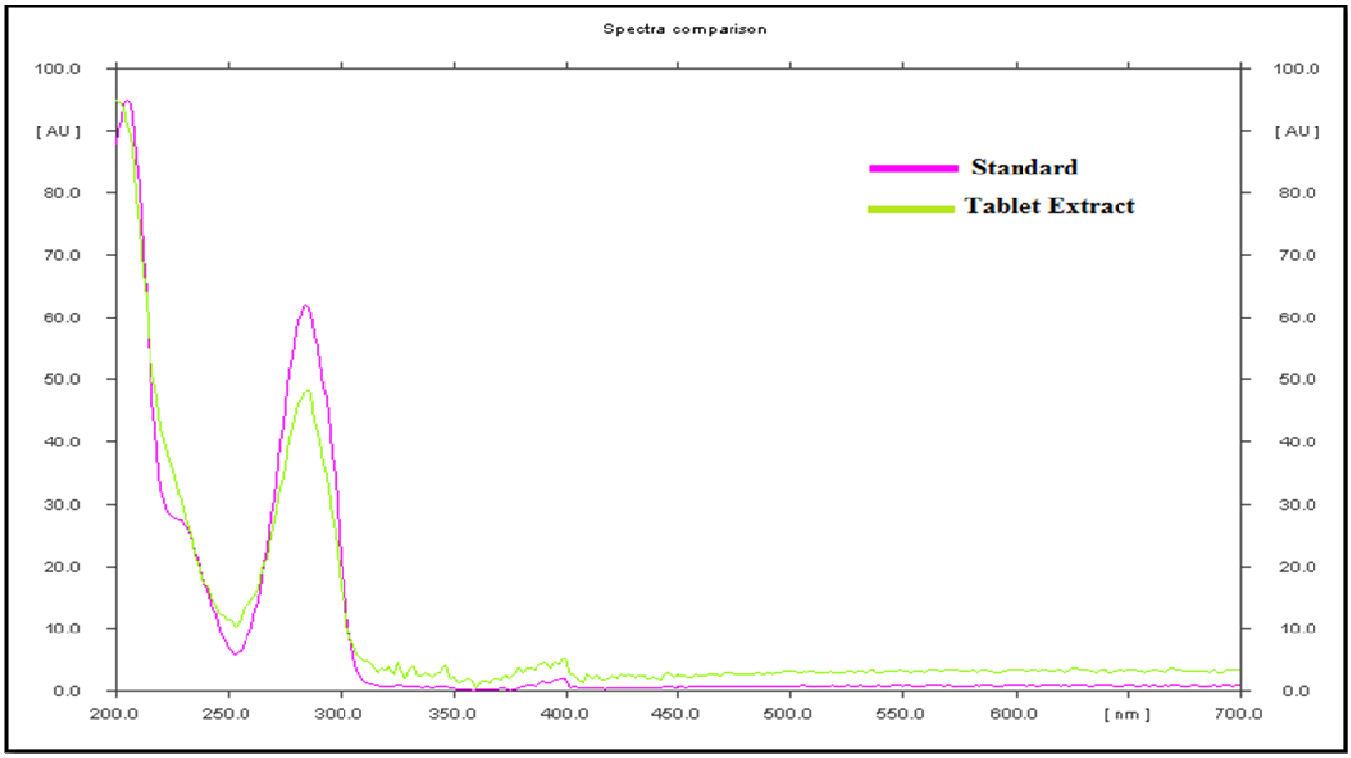

Figure No.5: Spectra of Tablet Extract and Standard of DMP

CONCLUSION

The developed HPTLC method has been statistically validated following the recommendations of ICH guidelines and it is found to be specific, accurate, precise and robust. Validation studies indicated that the proposed method is suitable for the estimation of DMP in bulk as well as from marketed dosage formulation without any interference of formulation excipients. The method can be conveniently adopted for routine analysis of the formulations containing DMP.

ACKNOWLEDGEMENT

This work has carried out in collaboration between all authors. We would specially like to acknowledge support given by precise chemipharma, Navi Mumbai. We are thankful to the Bharatividyapeeth's college of pharmacy, for providing the best analytical facilities and extended support for completion of research projects.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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