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Research Article

DEVELOPMENT AND VALIDATION OF HPLC AND HPTLC METHODS FOR DETERMINATION OF CINNARIZINE AND DOMPERIDONE MALEATE IN COMBINATION

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Abstract: Rapid, accurate and sensitive methods for the identification and quantification of Cinnarizine (CINN) and Domperidone maleate (DOM) were developed by HPTLC-densitometry and HPLC at 270 nm. The first developed method was HPTLC, where mixture of Cinnarizine and Domperidone maleate were separated on silica gel TLC 60 F_{254} plates using absolute Benzene:Tolune:Methanol (5.5:3:1.5 v/v/v) as mobile phase and scanning of the separated bands at 270 nm. Linearity was observed in the concentration range of 0.5-1.0 µg/band for both the drugs. The second method was HPLC, where the mixture of Cinnarizine and Domperidone maleate separated on thermo scientific MOS-I hypersil C8 column (5µm p.s, 150 mm × 4.6 mm), column temperature 25°c, flow rate 1 ml/min. The mobile phase selected was Acetonitrile : Ammonium acetate (50 mM) (78:22 v/v) with detection at 270 nm. Linearity was observed in the concentration range of 40-280 µg/ml for Cinnarizine and 30-210 µg/ml for Domperidone maleate. Both methods were validated according to ICH guideline and value of linearity, precision, robustness, LOD, LOQ, selectivity, recovery were found to be in good accordance with the prescribed value.

Key words: HPTLC, HPLC, Cinnarizine, Domperidone maleate

INTRODUCTION

Nausea and Vomiting are complex mechanism and the symptoms are influenced by the origin of the emetic response which can occur when the sensory inputs about body position contradict what is expected. Vomiting can occur due to the stimulation of the vestibular apparatus in the inner ear, which has connections to the emetic centre in the brain stem.

Cinnarizine (CINN) is chemically, 1-benzhydryl-4-cinnamyl-piperazine.(fig.1). Antihistamine which is mainly used for the control of nausea and vomiting due to motion sickness. It acts by interfering with the signal transmission between vestibular apparatus of the inner ear and the vomiting centre of the hypothalamus. The disparity of signal processing between inner ear motion receptors and the visual senses is abolished, so that the confusion of brain whether the individual is moving or standing is reduced.[1,2]

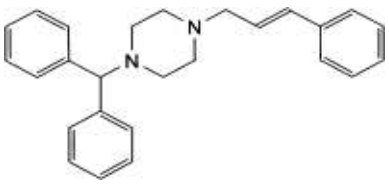

Fig.1 Structure of Cinnarizine

Domperidone maleate (DOM) is chemically, 5-Chloro-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl] piperidin-4-yl] -1,3-dihydro-2H-benzimidazol-2-onehydrogen (Z)-butenedioate.(fig.2). used in the treatment of nausea and vomiting.[3-6] Domperidone is a first choice antiemetic in some countries. Domperidone blocks the action of dopamine. It has strong affinities for the D_2 and D_3 dopamine receptors. Which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which, among others, regulates nausea and vomiting.

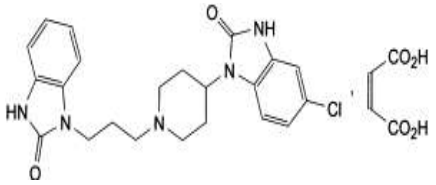

Fig.2 Structure of Domperidone maleate

Domperidone alone or in combination with other drugs is reported to be estimated that U.V. spectrophotometry[7], HPTLC[8], RP-HPLC[9,10] ,where as to our knowledge no HPTLC and HPLC method are reported for estimation of Cinnarizine and Domperidone maleate in combination therefore, present work was aimed to developed new, alternative, rapid, validated, sensitive and selective HPTLC and HPLC method for the qualitative and quantitative analysis.[11-16] The proposed method were optimized and validated as per ICH guidelines [17-19].

EXPERIMENTAL

Materials

Pure Cinnarizine and Domperidone maleate were kindly gifted by Micro Lab, Bangalore, India. Commercial tablets (VERTIDOM, Geno pharmaceutical Ltd, Goa) containing Cinnarizine (20 mg) and Domperidone maleate (15 mg) were used for the study.[20] Benzene, Toluene and methanol were used of analytical grade (Merck, Mumbai, India) and Acetonitrile used was of HPLC grade. Ammonium acetate and all the other chemicals used were also of analytical grade (Merck, India). Double distilled water used in all experiments was obtained from Milli-Q System (Millipore).

Instrumentation and Chromatographic conditions

For HPTLC

Aluminium plates precoated with silica gel 60 F_{254} plates (Merck, Mumbai, India) were used as stationary phase. Camag TLC scanner III (Densitometer) with WinCAT's software version 1.4.3.6336 was used for scanning and documentation. Camag Linomat V sample applicator with 100µl syringe was used for sample application. Camag hightec UV cabinet fitted with dual wavelength 254/366 nm, 8 volt UV lamp was used for inspection of HPTLC plates. Camag twin trough glass chamber with stainless steel lid, pre-saturated with mobile phase (benzene- toluene-methanol 5.5:3:1.5 v/v/v) was used for chromatographic development. The optimized chamber saturation time was 15 min at room temperature. Densitometric scanning was performed at 270 nm. Source of radiation was deuterium lamp emitting a continuous UV spectrum between 200-400 nm. Slit dimension used was 5 × 0.45 mm. The intensity of diffused light was used for determination of concentration of substance densitotogram. Peak areas were determined with linear regression.

For HPLC

HPLC system (Lachrom 2000 HPLC) equipped with Winchrom software consist of quaternary solvent delivery pump (L-7100 Double reciprocating pump), UV Detector L-7400 (190-666nm). All separation were carried out on thermo scientific MOS-I hypersil C8 column (5.0µm ps, 150mm × 4.6mm). Mobile phase was acetonitrile : ammonium acetate (50 Mm) (78:22 v/v) It was filtered and ultrasonically degassed before use. The flow rate was 1.0 ml/min, column temperature was maintained at 25^{o}C and injection volume was 2µl.

Preparation of standards solutions

For HPTLC

Standard stock solution of CINN and DOM were prepared by dissolving quantity of CINN (5 mg) and (5 mg) of DOM separately in methanol in separate 10 ml volumetric flask and final volume of both solutions were made up to mark with methanol to get stock solution of 500µg/ml.

For HPLC

Standard stock solution of CINN and DOM were prepared by dissolving quantity of (25 mg) of Cinnarizine and (25 mg) of Domperidone maleate separately in mobile phase in separate 25 ml volumetric flask, dissolved and diluted to the mark with mobile solution to get concentration (1000 µg/µl) of CINN and DOM. From these solutions concentration of 40-280 µg/ml for CINN and 30-210 µg/ml for DOM were made in 10 ml volumetric flask. The peak areas were plotted against the corresponding concentration to obtain calibration graph.

Analysis of marketed formulation.

To determine the content of CINN and DOM in marketed tablet (VERTIDOM label claim 20 mg CINN and 15 mg DOM per tablet), 20 tablet were weighed their mean weight was determined and finely powdered and powder equivalent to 20mg CINN and 15mg DOM was used for analysis.

For HPTLC

The tablet powder equivalent to 10 mg of CINN and 7.5 mg of DOM was transferred into 10 ml volumetric flask, 5 ml of methanol was added and sonicated for 30 min and were diluted up to 10 ml with methanol. The sample solution was then filtered using Whatman filter paper. 2µl sample was applied on plate, the spot concentration was 2 µg/band of CINN and 1.5 µg/band of DOM. The plates were developed in previously described chromatographic conditions. The peak areas of spot were measured at 270 nm and concentrations in the sample were determined using multilevel calibration.

For HPLC

The equivalent weight of 25 mg CINN and 18.75 mg DOM was taken and dissolved in mobile phase and sonicated for 30 min and then volume was made up to the mark with mobile phase. The sample solution was then filtered using Whatman filter paper and filtrate were approximately diluted to get approximate concentration of 120µg/ml CINN and 90µg/ml DOM and injected into HPLC for six times under the condition describe above. The peak areas were measured at 270 nm.

RESULT AND DISCUSSION

The optimization of procedures

For HPTLC different proportion of benzene, tolune, methanol were tried while selection of mobile phase. Ultimately benzene : tolune : methanol (5.5:3:1.5 %v/v/v) was finalized as mobile phase. The spot developed were dense,

compact and typical peak of CINN and DOM obtained was shown in fig.3. Peak was symmetrical in nature and no tailing was observed when plates were scanned at 270 nm.

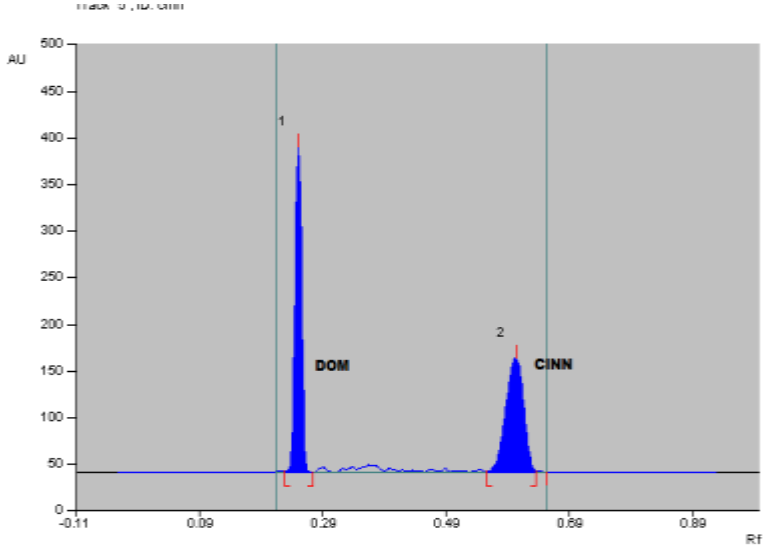

Fig.3 HPTLC-densitogram at 270nm

For HPLC Acetonitrile : ammonium acetate (50 Mm) (78:22 v/v) was selected as mobile phase, typical peak of CINN and DOM obtained was shown in fig.5. Peak was symmetrical in nature and no tailing was observed when plates were scanned at 270 nm.

HPTLC and HPLC method was validated for linearity, precision, robustness, LOD and LOQ, specificity and accuracy.

Linearity and range

For HPTLC, the calibration curve area versus concentration (ng/band) was found to be linear, the concentration range of 0.5–3.0 µg/band for Cinnarizine and 0.5–3.0 µg/band for Domperidone maleate were spotted on TLC plates. The linear regression data for the calibration curve for the two samples Cinnarizine and Domperidone maleate showed good linear relationship over the concentration with respect to peak area. (Table.1)

Regression equations were calculated as,
$Y = 587.4 + 3.17X$……$r^2 = 0.996$ for CINN
$Y = 1761 + 1701X$….. $r^2 = 0.996$ for DOM

For HPLC, The calibration curve were constructed in concentration range of 40–240 µg/ml for Cinnarizine and 30-180 µg/ml for Domperidone maleate respectively. Average peak-area values for CINN and DOM were directly correlated to the concentration. Beers low is obeyed over this concentration range.

Regression equations were calculated as,
$Y = 289103X + 1E+06$……$r^2 = 0.999$ for CINN
$Y = 99345X + 41836$…..... $r^2 = 0.999$ for DOM

LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) were determined by calculation of the signal to noise ratios (S/N) were 3 and 10, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background (N). Result depicted in (Table.1) for both methods.

Precision

The intra-day precision and inter-day precision of the method was evaluated by using linear regression data for the calibration curve analyzing Cinnarizine and Domperidone maleate. The repeatability of sample application and measurement of peak area were expressed in terms of %RSD was found to be less than 2%. HPTLC and HPLC method was determined by assaying tablets six times per day. The intra- and inter-day (n = 6) precisions are summarized in (Table.2).

Specificity

For HPTLC the specificity of the method was determined by analysis of drug standard and test samples. The identity of the Cinnarizine and Domperidone maleate spot from the samples was confirmed by comparison of its R_F CINN (0.64) and DOM (0.33) spectrum with those from a standard (Fig.3). For HPLC the identity of the Cinnarizine and Domperidone maleate peak for the samples was confirmed by comparison of its R_T CINN (8.0) and DOM (3.70) shown in (Fig.5).

Recovery

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100%, 120%.

Known amount of standard CINN and DOM were added to pre-analyzed samples and were subjected to the proposed method. Result of recovery study of both methods are shown in (Table.3).

Robustness

The robustness of the HPTLC method was determine by optimized chamber saturation time of 15 min (±2 min) and amount of mobile solution used 10 ml (±1 ml) were small change at three levels, to study the effect of R_F and peak area of the drug. Result depicted in (Table.4)

The robustness for HPLC, one factor at the time was changed at concentration level of 120 µg/ml for CINN and 90 µg/ml for DOM to estimate the effect on R_T and peak area of the drug. Optimized chromatographic parameter was flow rate 1 ml/min (± 0.2 ml/min) and % of acetonitrile solution used 78% (± 2%) were small change at three levels result depicted in (Table.4)

The method was found to be unaffected by small changes with %RSD for all the parameters less than 2% indicating that methods are robust.

Table.1: Assay parameters and method validation obtained by applying proposed HPTLC and HPLC method for determination of CINN and DOM in binary mixture.

Parameter	Compound	HPTLC method*	HPLC method*
Linearity range	**CINN**	0.5-3.0 µg/band	40-240 µg/ml
	DOM	0.5-3.0 µg/band	30-180 µg/ml
Slope	**CINN**	3.298	29464
	DOM	1794.64	99234
Intercept	**CINN**	430.88	1E+06
	DOM	1656.08	41836
LOD	**CINN**	0.1664	0.0485
	DOM	0.0797	0.020
LOQ	**CINN**	0.5044	1.1471
	DOM	0.2414	0.077
r^2	**CINN**	0.998	0.998
	DOM	0.995	0.998
Mean	**CINN**	100.63	99.99
	DOM	100.45	99.98
%R.S.D.	**CINN**	1.164	0.313
	DOM	0.680	0.179

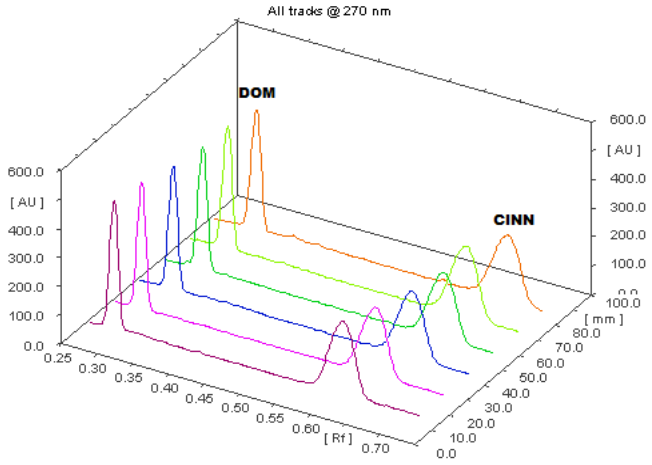

Fig.4 3D HPTLC-densitogram at 270nm.

Table.2: Intra-day and Inter-day Precision

Parameter	HPTLC				HPLC			
	% mean amount estimated*		%R.S.D.*		% mean amount estimated*		%R.S.D.*	
	CINN	DOM	CINN	DOM	CINN	DOM	CINN	DOM
Intraday	99.27	99.88	0.9169	0.9805	99.95	99.87	0.4866	0.4404
Interday	100.52	99.98	0.7451	0.7051	99.79	99.58	0.3503	0.5141

**Mean of six estimation.*

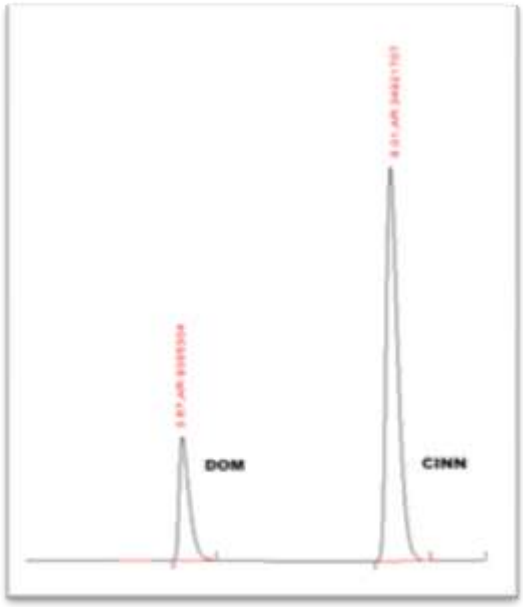

Fig.5 HPLC chromatogram of cinnarizine and domperidone maleate at 270nm.

Table.3
Application of standard addition technique for pharmaceutical preparation by HPTLC and HPLC method.

% Recovery	HPTLC				HPLC			
	% mean recovery		%R.S.D.*		% mean recovery		%R.S.D.*	
	CINN	DOM	CINN	DOM	CINN	DOM	CINN	DOM
80%	99.75	99.64	0.7384	0.6125	99.95	100.31	0.1229	0.3241
100%	100.14	100.09	0.4868	0.2224	99.86	99.86	0.4642	0.4209
120%	99.26	100.37	1.0584	1.0172	100.16	100.03	.02941	0.2940

**Mean of three estimation.*

Table.4
Robustness

HPTLC				HPLC			
Saturation time 15 min.	**Level**	**R_F***		**Flow rate ml/ min**	**Level**	**R_T***	
		CINN	**DOM**			**CINN**	**DOM**
13	-2	0.64	0.26	**0.8**	-0.2	8.21	3.88
15	0	0.65	0.27	**1**	0	8.04	3.68
17	+2	0.66	0.29	**1.2**	+0.2	7.39	3.35
Amt of mobile solution (ml)				**%ACN in mobile phase**			
9	-1	0.63	0.26	**77**	-1	8.01	3.59
10	0	0.64	0.26	**78**	0	8.04	3.68
11	+1	0.65	0.28	**79**	+1	8.08	3.51

**Mean of three estimation.*

CONCLUSION

Rapid, accurate and sensitive methods based on HPLC and HPTLC, has been developed for routine analysis of Cinnarizine and Domperidone maleate in fixed-dose combination.

The proposed HPTLC and HPLC methods are validated as per ICH guidelines. The accuracy of the proposed methods was determined by recovery study. The developed methods were found to be selective, accurate, and precise for the concurrent estimation of drug in perspective tablet dosage form.

The results obtained indicate that the introduced methods can be classified amongst highly selective and sensitive procedures. These merits suggest the use of the proposed method in routine and quality control analysis without interference of commonly encountered dosage form additives.

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