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HPTLC METHOD DEVELOPMENT AND VALIDATION OF CINNARIZINE IN BULK AND MARKETED FORMULATION

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Key words:

HPTLC, cinnarizine, marketed dosage form, ICH, analytical method validation

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ABSTRACT: High performance thin layer chromatography (HPTLC) offers many advantages over HPLC. It reduces the cost of analysis as compared 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 cinnarizine 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 proposed analytical work. Planar chromatographic development was carried out with the help of Silica Gel 60 F254precoated TLC plates. Sample application was facilitated by Linomat 5 applicator. After sample application plates were subjected for ascending development in twin trough chamber of 10x10 cm dimension, using 10 ml of solvent system. The optimized mobile phase was composed of toluene: ethanol (7.5:2.5 v/v). In post development, the plates were air dried and then scanned densitometrically using a UV detector at 254 nm in absorbance mode. In HPTLC densitogram well defined peak was obtained for cinnarizine with starting position at 0.69 R_f, max position at 0.72 R_f and end position at 0.75 Rf. The optimal R_f value for cinnarizine was found to be 0.72. Performance characteristics of HPTLC method for estimation of cinnarizine in bulk and its marketed dosage form were statistically validated as per recommendations of ICH guidelines of analytical method validation. The HPTLC method was found to be linear across the range 50-400 ng/band. The LOD and LOQ values were found to be 0.05162 and 0.1564 ng/band respectively. The method was found to be accurate, precise, robust and economical for the analysis of cinnarizine from bulk and its formulation.

INTRODUCTION: HPTLC is a well-known and versatile separation method which is type of planar chromatography, involves principle of adsorption. It is a flexible enough to analyse a wide variety of samples. It is useful in many ways as it is simple to handle and requires short analysis time to analyse the simple or complex samples.

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Nowadays, HPTLC serves as a preferred analytical tool for quantitative analysis of drug substances in bulk, from their formulations, from biological matrix, analysis of herbal extracts and standardization of herbal drugs.[1]

Cinnarizine (CIN), chemically designated as 1-(diphenylmethyl)-4 - (3 - phenyl - 2 - propenyl) piperazine (**Fig.1**), widely used for prophylaxis and treatment of vertigo associated with meniere'sdisease.[2] CIN exerts its anti-vertigo effects primarily on the peripheral vestibular system through inhibition of calcium influx. [3] CIN acts by interfering with the signal transmission between vestibular apparatus of the inner ear and the vomiting centre of the hypothalamus.

Literature survey revealed that spectrophotometric, [4, 5] potentiometric [3] and reversed phase high pressure liquid chromatographic.[2, 6, 7] (RP-HPLC) methods have been developed for quantitative estimation of CIN. One HPTLC method has been reported for the simultaneous estimation of CIN and Domperidone maleate by Argekar *et al*. In referred scientific literature no HPTLC method has been found for the estimation of CIN as single chemical entitiy.[8]

The objective of research work was to develop accurate, precise, specific and economic analytical method for the estimation of CIN in bulk and marketed formulation. Considering the predefined objective of the research work, cost and suitability of analysis for estimation of CIN in bulk and its marketed formulation, HPTLC method was developed and then validated as per the recommendations of ICH guidelines of analytical method validation.

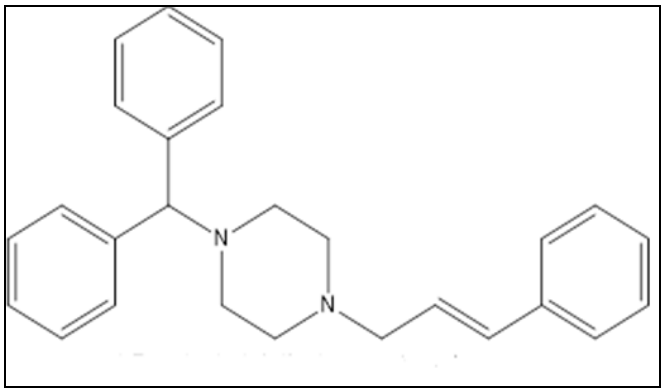

FIG.1: STRUCTURE OF CINNARIZINE

MATERIALS AND METHODS:

Materials and marketed formulation:

Cinnarizine was procured as generous gift sample for the purpose of academic research from Hikal Ltd., Bangalore. Commercial tablets containing Cinnarizine (25 mg) were used for the study. Merck HPTL Caluminium plates precoated with silica gel 60 F254were 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 1**.

TABLE 1: HPTLC INSTRUMENT AND SPECIFICATIONS

Sr.No.	Instruments	Specification
1.	Make and model	Camag, Switzerland
2.	Sample applicator	CamagLinomat V
3.	Densitometric scanner	Camag TLC scanner 3
4.	Sampling mode	Manual with Linomat applicator
5.	Syringe	Hamilton (100 µl)
6.	Detection	Ultraviolet (UV) detector
7.	Software	winCATS (ver.1.4.1.8)

Experimental:

Experimental work is presented in two sections, namely analytical method development (AMD) and analytical method validation (AMV).

Analytical Method Development (AMD):

Method development procedures:

HPTLC method was developed for estimation of CIN. The details of experimental work are presented in **Table 2**.

TABLE 2: EXPERIMENTAL PROCEDURES FOLLOWED FOR HPTLC METHOD DEVELOPMENT

Sr. No.	System/Method/ Step	Procedure followed
1.	Preparation of standard solution	Standard stock solution of CIN was prepared by dissolving 10 mg of drug in 10 ml methanol to obtain concentration 1000µg/ml (1000 ppm).
2.	Selection of stationary phase	Silica Gel 60 F_{254}precoated TLC plates were selected as chromatographic layer.
3.	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.
4.	Layer preconditioning	Prewashed plates were placed in oven at 100°C for 5 minutes prior to the sample application.
5.	Preparation of sample solution for estimation from marketed tablet formulation	Ten tablets, each containing 25mg of CIN, were weighed and finely powdered, A quantity of powder equivalent to 25 mg of CIN was transferred to a 50 ml volumetric flask, dissolved in methanol and made the volume up to 50 ml with methanol. It was sonicated for 30 minutes in ultra-sonication bath for complete dissolution of drug. The solution was double filtered, first through0.45μmwhatman filter paper and after that through 0.45μm syringe filter in order to get clear solution. Further, it was diluted

		with methanol to get the concentration of 250μg/ ml.
6.	Selection of detection wavelength	10µg/ml (10 ppm) solution of CIN was applied on HPTLC plate (suitable dimension), scanned densitometrically over the range of 200- 700 nm using Camag HPTLC scanner 3.
7.	Optimisation of chromatographic conditions	Many preliminary trials were carried out for selection and optimisation of, 1. Mobile phase composition 2. Chamber saturation time

RESULTS AND DISCUSSION:

Standard solution of CIN, sample solution of marketed tablet formulation were prepared as per aforementioned procedures. Selected stationary phase were prewashed and preconditioned before application of sample.

UV absorption spectrum for 10 ppm solution of CIN (**Fig.2)** was generated using Camag HPTLC scanner 3 and win CATS (ver.1.4.1.8) software, 254 nm wavelength was selected as a detection wavelength for chromatographic determination of CIN because at 254 nm wavelength CIN was showing maximum absorbance.

Selection of wavelength:

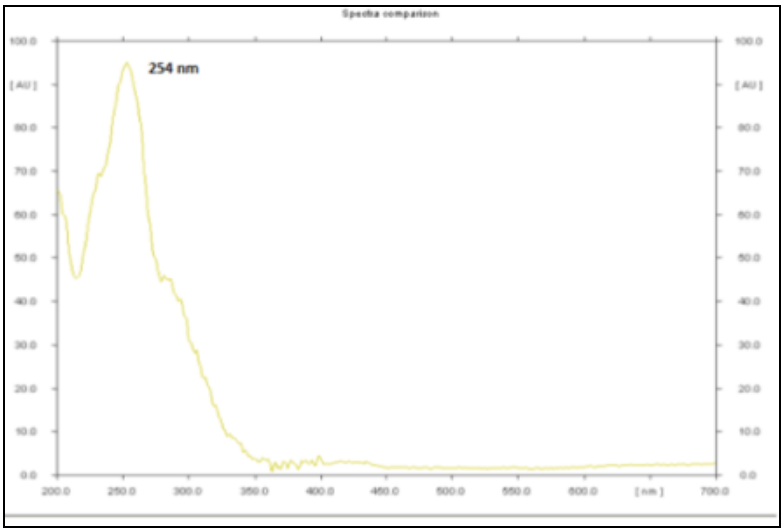

FIG.2: HPTLC SPECTRA OF CIN

Optimisation of chromatographic conditions:

Based on literature survey, polarity and solubility [9, 10] of CIN many preliminary trials were carried out for selection of mobile phase composition, some are tabulated in **Table 3**.

TABLE 3: TRIALS FOR SELECTION OF MOBILE PHASE COMPOSITION

Sr. No.	Mobile Phase Components	Composition (V/V/V)	Inference
1.	Methanol: Dichoromethane: Formic acid	1: 9: 0.05	Toluene: ethanol (7.5:2.5 v/v) was selected as optimised mobile phase composition
2.	Methanol: Toluene: Ethylacetate: Glacial acetic acid	2: 9: 0.5: 0.5	
3.	Toluene: Ethyl acetate: Methanol	7 : 0.5 : 2.5	
4.	Toluene: Methanol	6.5 : 3.5	
5.	Toluene: Methanol	7 : 3	
6.	Toluene: Ethanol	7 : 3	
7.	Toluene: Ethanol	7.5 : 2.5	

Toluene: ethanol (7.5:2.5 v/v) was selected as optimised mobile phase composition such as sample application volume, band width, chamber saturation time, relative humidity, temperature, separation technique, migration time etc. were also optimised by performing lab studies. All optimized chromatographic conditions are tabulated in **Table 4**.

TABLE 4: OPTIMISED CHROMATOGRAPHIC CONDITIONS

Sr. No.	Parameters	Optimised conditions
1.	Stationary phase	Silica Gel 60 F_{254}precoatedHPTLC plates, 10x10 cm (200 µm) – Merck
2.	Mobile phase composition	Toluene: Ethanol (7.5:2.5 V/V)
3.	Sample application	
	a. Application volume	10 µl
	b. Band width	6 mm
	c. Distance between the tracks	10 mm
4.	Saturation time	20min
5.	Relative humidity (%)	55±5
6.	Temperature (^{0}C)	25±2
7.	Separation technique	Ascending
8.	Quantity of Mobile phase	10 ml
9.	Migration distance	90 mm
10.	Migration time	15 min
11.	Densitometric evaluation -Detection wavelength	254nm

Densitogram obtained using these optimised chromatographic conditions for CIN is shown in **Fig.3**, R_f value for CIN was found to be 0.72.

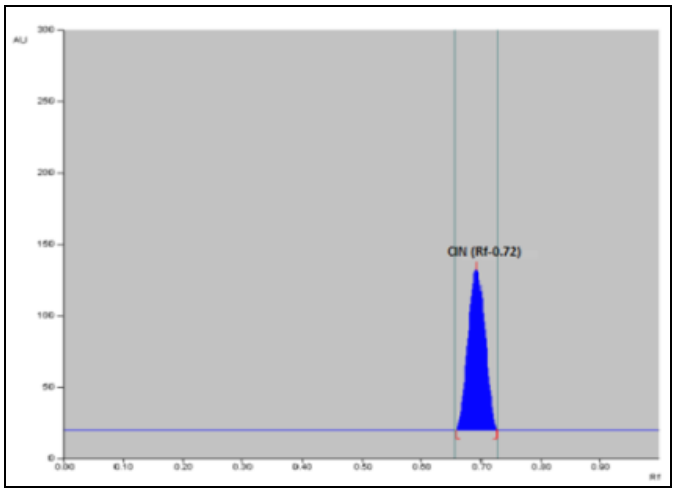

FIG. 3: DENSITOGRAM OF CIN

Analytical Method Validation (AMV):

Experimental procedures:

The developed HPTLC method was validated as per recommendations given by "ICH guidelines Q2(R1) for validation of analytical procedures: text and methodology".[11] Refer **Table 5** for parameters and procedure followed for AMV.

TABLE 5: ANALYTICAL METHOD VALIDATION: PARAMETERS AND PROCEDURES FOLLOWED. [12, 13, 14, 15]

Sr.No.	Parameters	Procedure Followed	
1.	Linearity	As per ICH guidelines, for determination of linearity, a minimum of 5 concentrations are suggested. By plotting peak area against concentration of standard and finding regression coefficient (R^2).	
2.	Specificity	As per ICH, specificity should be carried out to make sure the identity of an analyte. The specificity of the method was determined by comparing the R_f value and densitogram of standard CIN with sample (tablet extract).	
3.	Precision	Precision was carried out at two levels, as follows	
		Repeatability	**Intermediate Precision**
		Repeatability was estimated by using minimum of 9 determinations covering the described range for the procedure (e.g., 3 concentrations/ 3 replicates each)	Intermediate Precision was established to study the consequences of random events i.e. days, on the precision of the analytical procedure. Intraday and interdayprecision studies were conducted by taking 9 determinations of 3 concentrations/3 replicates each, at 3 different times

		in a same day and on 3 different days, respectively. Precision is reported as standard deviation and relative standard deviation (coefficient of variation) for each type of precision investigated.
4.	Limit of Detection (LOD) and Limit of Quantification (LOQ)	The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of the response and the slope of calibration graph. The quantitation was done with the help of equations. For LOD = 3.3 σ/ s,LOQ = 10 σ/ s, where σ = Standard Deviation of replication, s = Slope of calibration curve.
5.	Accuracy	Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample. Accuracy should be evaluated using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations/3 replicates each of the total analytical procedure). In the existing work percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by putting known amount of standard solution of CIN. These samples were then analysed and the results obtained were compared with expected results.
6.	Robustness	The robustness of an analytical procedure is a measure of its potential to remain unaffected by small, but intentional variations in method parameters and provides an mark of its reliability during normal usage. For inspecting the robustness of the developed analytical method following parameters were purposely changed, 1. Composition of Mobile Phase 2. Chamber saturation time

RESULTS AND DISCUSSION:

Linearity

Linear relationship was observed by plotting peak area against sample concentration. The calibration graph indicated that CIN produced a linear response across the range of 50-400ng/band (**Fig.4**). The linear regression data of calibration plot for CIN is given in **Table 6**.

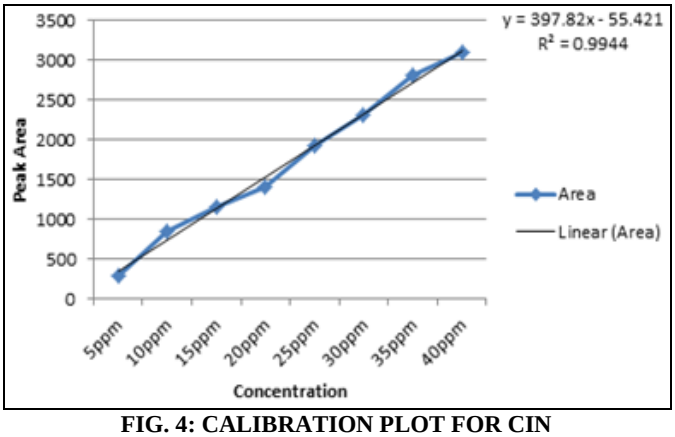

FIG. 4: CALIBRATION PLOT FOR CIN

TABLE 6: LINEAR REGRESSION DATA OF CALIBRATION PLOT

Sr.No.	Parameter	Results
1.	Range	50-400 ng/band
2.	R^2	0.9944
3.	y- intercept	55.421
4.	Slope	397.82

Specificity:

When the densitogram of standard CIN was over layed with the densitogram of sample (tablet extract) it was observed that the densitogram of CIN was exactly matching with the densitogram of tablet extract as shown in **Fig. 5**. Therefore the method is specific.

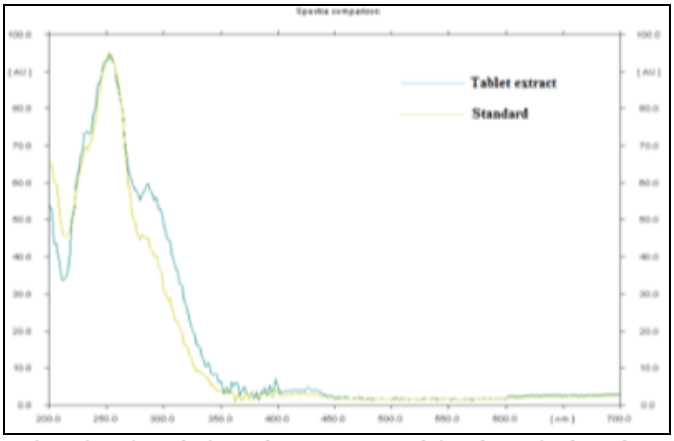

FIG.5: SPECTRA OF TABLET EXTRACT AND STANDARD OF CIN

Precision:

Intra-day precision:

It was performed at three different concentration levels low (100 ng/band), mid (250ng/band) and high (400 ng/band) respectively within the same day at three different times (session 1, 2, 3).

Inter-day precision:

It 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 7** and **8** respectively.

TABLE 7: INTRA-DAY PRECISION STUDIES

		Cinnarizine			Inference
Concentration levels		**Low**	**Mid**	**High**	
Concentration(ng/band)		100	250	400	
Peak area	Session 1	421.6	1327.9	2964.5	
	Session 2	431.6	1290.7	2944.6	Acceptable % RSD, hence Precise
	Session 3	425.5	1318.3	2950.7	
Average Peak area		426.2	1312.3	2953.3	
Standard Deviation		5.04	19.31	10.19	
% RSD		1.18	1.47	0.35	

TABLE 8: INTER-DAY PRECISION STUDIES

		Cinnarizine			Inference
Concentration levels		**Low**	**Mid**	**High**	
Concentration(ng/band)		100	250	400	
Peak area	Session 1	935	1168.7	2947.2	
	Session 2	947.4	1160.6	2956.6	Acceptable % RSD, hence Precise
	Session 3	928.2	1158.9	2978.6	
Average Peak area		936.9	1162.1	2960.8	
Standard Deviation		9.735	5.236	16.115	
% RSD		1.04	0.45	0.54	

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Values of LOD and LOQ calculated using slope of calibration plot for CIN is tabulated in **Table 9.**

TABLE 9: LOD AND LOQ

Sr. No.	Parameters	Readings obtained
1.	LOD	0.05162 ng/band
2.	LOQ	0.1564 ng/band

Accuracy:

Accuracy of the method is reported as percent 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 CIN. Results obtained are given in **Table 10**.

TABLE 10: ACCURACY- RECOVERY STUDIES

Drug	Level of percentage recovery %	Amount present in extract (*ng/band*)	Amount of standard added (*ng/band*)	Total amount (ng/band)	% Recovery	Average % Recovery	%RSD	Inference
Cinnarizine	80	250	200	450	99.13	100.38	0.41	Acceptable
	100	250	250	500	101.7		0.30	recovery,
	120	250	300	550	100.3		0.21	hence accurate

Robustness:

To determine robustness of analytical HPTLC method deliberate changes were made in the mobile phase composition and chamber saturation time. Effect of these changes 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 11**.

TABLE 11: ROBUSTNESS RESULTS

Sr. No.	Robustness parameters	Parameters changed	%RSD of area
1.	Mobile phase composition(V/V)	Toluene: Ethanol (7.7 : 2.3)	1.17
		Toluene : Ethanol (7.3: 2.7)	0.95
2.	Chamber saturation time (minutes)	+2	0.90
		-2	1.10

CONCLUSION: The developed HPLC method was found to be fast, simple, sensitive and economic. The method was validated and found to be specific, linear, accurate, precise and robust. Hence the HPTLC method can be conveniently adopted for routine analysis of the formulations containing CIN.

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