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Development and Validation of Stability Indicating Analytical Method for Estimation of Cinnarizine and Dimenhydrinate in Tablet Dosage Form

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ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Cinnarizine and Dimenhydrinate in Tabletdosage form. Chromatography was carried on PHENOMENEX C18 (250 x 4.6) mm; 5µm) analytical column using mobile phase (0.2 % Formic Acid and 0.2 % tri ethyl amine (TEA) in water pHadjusted to 5.0 with formic acid and methanol (40:60 % v/v)) at a flow rate of 01.0 ml/min. The detection was carried out at 260 nm. The retention time of Cinnarizine and Dimenhydrinateis found to be 2.954 min and 5.128 min respectively. Correlation co-efficient for cinnarizine and Dimenhydrinate was found to be 0.999. Assay result of marketed formulation wasfound to be in 99.4 % and 98.8 % for Cinnarizine and Dimenhydrinate respectively . The proposed method was validated with respect to linearity, accuracy, precision androbustness. Recovery was found in the range of 99.4 %–101.5 %. Statistical Analysis proves that the developed methods weresuccessfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in QualityControl laboratories.

KEYWORDS Cinnarizine, Dimenhydrinate ,hplc ,analytical method development , validation

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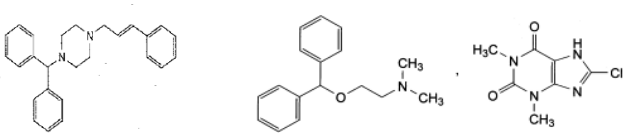
INTRODUCTION:

The IUPAC name of the cinnarizine is (*E*)-1-(Diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine withmolecular formula and molecular weight $C_{26}H_{28}N_2$ and 368.514 g/mol respectively.The molecular structure of the drug is given in Fig.1.a [1,2,3]

Figure 1 –Cinnarizine(a) and Dimenhydrinate (b)



(Dimenhydrinate -b)

Cinnarizine is an antihistamine and a calcium channel blocker classified as a selective antagonist of T-type voltage-operated calcium ion channels, because its binding blocks the channels and keeps them inert. In treatment of nausea and motion sickness it was previously hypothesized that Cinnarizine exerts its effects by inhibiting the calcium currents in voltage gated channels in type II vestibular hair cells within the inner ear

Cinnarizine is Official in Indian Pharmacopeia 2014, British Pharmacopeia 2015, and European pharmacopeia 2015

The IUPAC name of the Dimenhydrinate is 2-benzhydryloxy-N,N-dimethylethanamine;8-chloro-1,3-dimethyl-7H-purine-2,6-dionewithmolecular formula and molecular weight $C_{24}H_{28}ClN_5O_3$ and 469.96382 g/mol respectively.The molecular structure of the drug is given in Fig.1.b[4,5]

Dimenhydrinate is an antiemetics drug combination that contains diphenhydramine and theophylline. It is not effective in the treatment of nausea associated with cancer chemotherapy. Dimenhydrinate directly inhibits the stimulation of certain nerves in the brain and inner ear to suppress nausea, vomiting, dizziness, and vertigo. diphenhydramine and dimenhydinate both reduce vestibular neuronal excitation due to angular or linear acceleration motions.[6]However no stability indication method has been reported till date for theestimation of Cinnarizine and dimmenhydrinate using the RP-HPLC method[7]. The present paper describesthe analytical method development and validation of estimation of Cinnarizine and Dimenhydrinate inPharmaceutical dosage form using RP-HPLC . The proposed methodare optimized and validated as per ICH guidelines.[8-11]

Dimenhydrinate is official in British pharmacopeia 2015 and USP -30 NF-25[12]

Materials and methods

Materials

SHIMADAZU HPLC 2010 C HT . Cinnarizine and dimenhydrinate was obtained as a gift

sample from Montage laboratories pvt .ltd , Himatnagar , and Gujarat, India. The commercialfixed dose combination stugeron plus was procured from local market. All solvents (HPLCgrade) were obtained from Merck Chemicals and Rankem .

Methods

Working Standard preparation

Preparation of Dimenhydrinate + Cinnarizine solution: (100 µg/mL and 50 µg/mL

respectively)

About 10mg of Cinnarizine and 20mg of Dimenhydrinate weigh and tranferred into 200mL of

volumetric flask, 130mL of diluent added in to it sonicated to dissolve, cooled and then dilute up to 200mL of diluent.

Sample Preparation for marketed formulation:

Transferred 5 intact tablets in to 200mL of volumetric flask, added about 150mL of Diluent in to it,sonicated for 30 minutes with intermittent shaking, cooled to attain room temperature and made upto volume with Diluent. and filtered the solution with 0.45µ nylon filter. Further 5 ml of stock solution pipette out in 50mL of volumetric flask and made up the volume with Diluent.

Cinnarizine: 50 ppm

Dimenhydrinate: 100 ppm

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters:

Pumps: Mode of chromatography: Reversed Phase Chromatography
Mode of Elution: Isocratic
Flow Rate: 1.0 ml/min
Oven: Ambient Temperature
Detector: Type: UV detector
Wavelength: 260 nm
Column: Phenomenex C18 (250 x 4.6) mm, 5um
Sample Volume: 20 µl
Run time: 10 min
Mobile Phase: (0.2 % Formic Acid and 0.2 % tri ethyl amine (TEA) in water pH adjusted to 5.0 with formic acid and methanol (40:60 % v/v))
Diluent: Methanol
System Suitability Parameters:
Table 1-

Sr. No	Peak name	Retention time (min)	Area	Tailing factor	Theoretical plate	Resolution
1	Cinnarizine	2.954	6813762	1.2	8742	-
2	Dimenhydrinate	5.128	9837000	1.1	13257	6.8

Linearity and Range (n=3):

The linearity response was determined by analyzing 5independent levels of calibration curve in the range of25-75µg/ml for Cinnarizine and 5independent levels of calibration curve in the range of50-150 µg/ml for Dimenhydrinate .The plot of peak area against concentration was plotted.Correlation coefficient and regression line equationswere calculated. Linearity range was established throughconsideration of required practical range and accordingto each drug concentration present in thepharmaceutical product, to give accurate, precise andlinear results**.**

Precision

Repeatability

Repeatability was determined by analyzing standardsolution of Cinnarizine and Dimenhydrinate having the concentration 50µg/ml and 100µg/ml. Scanned these solutions six times in a day. Theresults were reported in terms of % RSD (relativestandard deviation).

Interday Precision

The inter-day precision of the proposed method wasdetermined by measuring the corresponding responseson 3 different days over a period of 1 week for 3 different

concentration of cinnarizine (25,50 and 75 µg/ml) and Dimenhydrinate (50, 100 and 150 µg/ml) and Theresults were reported in terms of % RSD.

Accuracy (% Recovery)

The accuracy of the method was determined bycalculating recovery of cinnarizine and Dimenhydrinate by the Standardaddition method. Each solutionwas injected in triplicate and the percentage recoverywas calculated by measuring the peak areas and fittingthese values into the regression equation of therespective calibration curves.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit ofquantification (LOQ) were calculated using the standarddeviation of y-intercept of calibration curve (σ) and average of slope (S) of the calibration curve.

LOD = 3.3 × σ /s ,
LOQ = 10 × σ /s

Robustness

The robustness was studied by analyzing the sample ofCinnarizine and Dimenhydrinate by deliberate variation in the methodparameters. The change in the response was noted.Robustness of the method was studied by changingdifferent experimental conditions like pH of Solution by ± 2 unit , Flow rate by ±0.2 ml/min, Mobile phaseby ± 2 %.

Result and discussion

VALIDATION PARAMETER

Linearity and Range

Linear correlation was obtained between peak area andconcentration of Cinnarizine in the range of 25-75µg/ml and for Dimenhydrinate 50-150 50µg/ml . The linearity of the calibration curves wasvalidated by the value of correlation coefficients of theregression (r). The overlay chromatogram is presented inFigure 2. The linearity data are presented in Table 2.Calibration curve is presented in Figure 3 and Figure 4 .

Figure 2: Chromatogram of Linearity Overlay

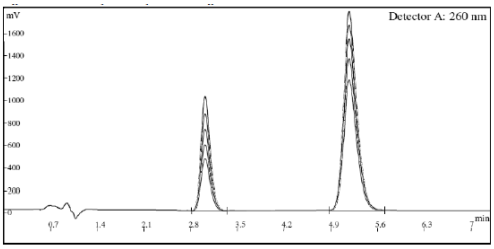
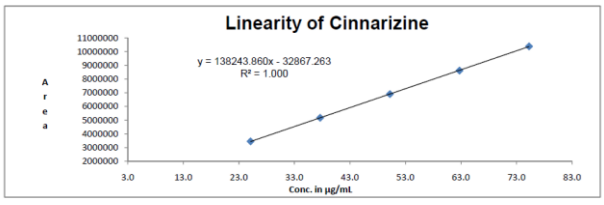


Figure 3: Linearity Curve of Cinnarizine and Dimenhydrinate



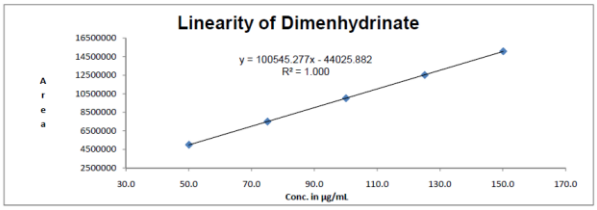


Table 2: Data indicating Linearity of cinnarizine and diemhydrinate

Cinnrazine		Dimenhydrinate	
Conc. (µg/ml)	**Area**	**Conc. (µg/ml)**	**Area**
25.1	3444524	50.1	4998621
37.6	5169245	75.1	7500480
50.2	6897270	100.1	10020618
62.7	8619032	125.2	12522030
75.3	10390977	150.2	15071087

Table 3.1and 3.2 Repeatability data from tabletFormulation

Repeatability (Method precision, n=6):

Table 3.1: Repeatability Study of Cinnarizine (n=6)

Sr. no	Area	Mean	% RSD
1	6799105		
2	6777871		
3	6823608	6785822.66	0.4
4	6750747		
5	6772204		
6	6791401		

Table 3.2: Repeatability Study of Dimenhydrinate (n=6)

Sr. no	Area	Mean	% RSD
1	9801078		
2	9854117		
3	9826108	9836833.16	0.6
4	9797524		
5	9941263		
6	9800909		

Repeatability

The data for repeatability for CINNARIZINE AND DIMMENHYDRINATE is shown in

Table-3. The % RSD for Repeatability data was found tobe 0.4% and 0.6% Respectively

Intraday precision

The data for intraday precision forcinnarizine and Dimenhydrinate is shownin Table-4. The % RSD for intraday precision for Cinnarizine was found tobe between 0.21 – 0.31% and for Dimenhydrinate was found in range of 0.27 – 0.72 %

Table 4.1 &4.2 : Intraday Precision data for Cinnarizine and Dimenhydrinate from

Tablet formulation

Table 4.1 : Intraday Precision Study of Cinnarizine (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	25.0	3427543±7902.0	0.23
2	50.0	6856913±14264	0.21
3	75.0	10330339±32056.4	0.31
Mean			0.35

Table. 4.2: Intraday Precision Study of Dimenhydrinate (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	50.0	4964547±13182.1	0.27
2	100.0	9952345±30486.2	0.31
3	150.0	14968775±107571.3	0.72
Mean			0.82

Interday precision

The data for interday precision for Cinnarizine and dimmenhydrinate is shown

in Table-5. The % RSD for intraday precision for Cinnarizine was found to

be between 0.69– 0.83% and for Dimenhydrinate 0.46 – 0.86 % .

Table 5.1 and 5.2 : Intraday Precision data for Cinnarizine and Dimenhydrinate from

tablet formulation

Interday Precision (Intermediate precision, n=3):

Table. 5.1: Interday Precision Study of Cinnarizine (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	25.0	3413401±23602.7	0.69
2	50.0	6828621±46730.7	0.68
3	75.0	10287770±85031.3	0.83
		Mean	0.73

Table. 5.2: Interday Precision Study of Dimenhydrinate (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	50.0	4893583±32915.6	0.67
2	100.0	9885975±45485.2	0.46
3	150.0	14868961±120354.6	0.81
Mean			0.64

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Cinnarizine and Dimenhydrinate was found
to be 99.4 %– 101.5 %. The results are shown in Table-6. Recovery greater than 98 % with low SD justifies the accuracy of the method.

Table 6.1: Recovery Data of Cinnarizine

Accuracy Level %	Set no.	Area	Amount added (mg)	Amount recovery(mg)	%Recovery	Mean	%RSD
80 %	1	6130643	80.00	79.57	99.5	100.4	0.8
	2	6183429	80.50	81.12	100.8		
	3	6156286	79.60	80.32	100.9		
100%	1	6886098	100.50	101.73	101.2	100.1	1.0
	2	6812357	100.00	99.57	99.6		
	3	6790364	99.50	98.92	99.4		
120%	1	7566185	120.50	121.68	101.0	100.2	0.8
	2	7542341	120.70	120.98	100.2		
	3	7472309	119.80	118.92	99.3		

Table: Recovery Data of Dimenhydrinate

Accuracy Level %	Set no.	Area	Amount added (mg)	Amount recovery(mg)	%Recovery	Mean	%RSD
80 %	1	8898535	160.00	159.02	99.4	100.3	0.8
	2	8923393	159.00	160.02	100.6		
	3	8995382	161.40	162.93	100.9		
100%	1	9884031	200.00	198.85	99.4	100.2	0.9
	2	9995446	201.00	203.35	101.2		
	3	9901395	199.60	199.55	100.0		
120%	1	10988581	240.00	243.50	101.5	100.5	1.0
	2	10919067	241.60	240.69	99.6		
	3	10926462	240.20	240.99	100.3		

Limit of detection and limit of quantification

The Limit of detection (LOD) were found to be 0.654 and 0.1305 for Cinnarizine and Dimenhydrinate respectively and Limit of quantitation

were found to be 1.9834 for Cinnarizine and 0.3957 for dimenhydrinate

Robustness

The method is found to be robust as the results were not

significantly affected by slight variation in composition of mobile phase, pH of mobile phase and flow rate of the mobile phase (Table-7).

Table 7.1 & 7.2 : Robustness data for Cinnarizine and Dimenhydrinate from tablet Formulation

Table 7.1: Change the ratio of mobile phase

Standard repetitions (n=6)	**38: 62**		**42 : 58**	
	Cinnarizine	Dimenhydrinate	Cinnarizine	Dimenhydrinate
Mean Area ± SD	6875185± 21948.6	9952499± 32879.0	6876954± 53524.5	9870500± 24279.0
% RSD	0.3	0.3	0.8	0.2

Table 7.2: Change the flow rate

Standard repetitions (n=6)	**0.8 ml/min**		**1.2 ml/min**	
	Cinnarizine	Dimenhydrinate	Cinnarizine	Dimenhydrinate
Mean Area ± SD	751420± 40995.9	10930565 ±39501	6219078± 34274.7	8959694± 38035.6
% RSD	0.4	0.2	0.6	0.4

Table 7.3: Change the pH

Standard repetitions (n=6)		**pH**	**pH 5.1**	
	Cinnarizine	Dimenhydrinate	Cinnarizine	Dimenhydrinate
Mean Area ± SD	6855236± 16216.6	9952499± 24279.0	6853645± 19193.3	9878899± 41036.8
% RSD	0.2	0.3	0.3	0.4

Applicability of the method

The proposed RP-HPLC method was successfully applied for determination of Cinnarizine and Dimenhydrinate in tablet dosage form.

The percentage was found to be satisfactory, which is comparable with the corresponding label claim amount (Table-8).

Table 8-Application of RP-HPLC method to Cinnarizine and Dimenhydrinate

Tablet 8 formulation

Sr no	**Formulation**	**Drug**	**% assay of Cinnarizine**	**% assay of Dimenhydrinate**
1	Stugeron plus	Cinnarizine(20mg) Dimenhydrinate (40mg)	99.4%	98.8%

CONCLUSION

In Estimation of cinnarizne and Dimenhydrinate in tablet dosage form,separation was achieved on Phenomenex C18 (250 x 4.6)mm ; 5µm at Ambient temperature by using a mobile phase(0.2 % Formic Acid and 0.2 % tri ethyl amine (TEA) in water pH adjusted to 5.0 with formic acid and methanol (40:60 % v/v)) at a flow rate of 1.0ml/min and UV detection was carriedout at 260 nm. Data suggests that peak purity index ofthe drug was found to be greater than 0.990, so there isno co-elution of any degradation products with mainpeaks and the results obtained were found within theacceptance criteria. Results of the validation for cinnarizine and Dimenhydrinate of the above method were linear in therange of 25-75 µg/ml and 50-150 µg/ml respectively . The % recovery was found to be 99.4 %– 101.5 %. The results of the precision studyindicate that the proposed method shown good

repeatability with a % RSD of 0.4 % for Cinnarizine and % RSD of 0.6 % for Dimenhydrinate . Similarly %RSD fromthe intraday precision data was found to be 0.23% -

0.31% for Cinnarizine and 0.27 % - 0.72% for Dimenhydrinate and %RSD from the Interday precision data werefound to be 0.68% - 0.83% and for Dimenhydrinate 0.46% - 0.81% . Absolute differencebetween mean assay values of method precision andintermediate precision was found to be less than 2.0 %.Robustness is performed by making changes in flow rate,Mobile phase composition and pH. The assayobtained after proposed changes compared with theassay obtained in normal conditions. According to theacceptance criteria difference in the assay should not bemore than 2%. The results obtained are well within theacceptance criteria. The % assay results of 99.4 % for Cinnarizine and 98.8 % for Dimenhydrinate indicates that the proposed method wassuccessfully utilized for the estimation Cinnarizine and Dimenhydrinate in Tablet dosage form. Hence, the method can be termedas robust. Since the results are well within the limit ofacceptance criteria for all validation parameters,therefore the method can be considered as validated andsuitable for intended use. So, the proposed stabilityindicating RP-HPLC assay method can be successfullyapplied for the estimation of Cinnarizine and Dimenhydrinate in tablet dosage form.

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