RESEARCH ARTICLE

Development of Stability Indicating Robust Chromatographic Method for the Simultaneous Estimation of Cinnarizine and Dimenhydrinate in Dosage Form

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ABSTRACT

Estimation of Cinnarizine (CNN) and Dimenhydrinate(DMH) was carried out using a stability-indicating method using ultra-performance liquid chromatographic (UPLC). Both the drugs have pKa values, 8.4 for Cinnarizine and 8.8 for Dimenhydrinate, resulting in a challenge for chromatographic method development with poor resolution. Design of experiment with full factorial 2^3 design facilitated optimization of various method parameters like Mobile phase pH, column temperature, and flow rate as these are critical factors for the best resolution between Cinnarizine and Dimenhydrinate. The separation was achieved with a simple gradient method using a 50 mm x 4.6 mm, 3.5 µm, column (Waters, X-bridge C-18) with 0.3 mL/min as flow rate, column temperature set at 40°C, and wavelength for analysis selected was 260 nm. The method fulfilled the validation criteria as per ICH guidelines. The method was linear within concentration range of 10 to 150 µg.mL^{-1} for Cinnarizine and 20 to 300 µg mL^{-1} for Dimenhydrinate. The chromatographic peak purity in the degradation study revealed no co-eluting peaks and standard Cinnarizine and Dimenhydrinate. The method can successfully quantify the drugs in the commercially available dosage form.

Keywords: Cinnarizine, Dimenhydrinate, Full factorial design, Quality by Design (QbD), Response surface methodology (RSM), Ultra-performance liquid chromatographic (UPLC).

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INTRODUCTION

Cinnarizine (CNN) chemically,1-(diphenylmethyl)-4-(3-phenylprop-2-en-1-yl) piperazine) (Figure 1A) is reported for antihistamine, sedative, and calcium-channel blocking activity.

Dimenhydrinate (DMH) is chemically 2-benzhydryloxyethyl(dimethyl)azanium;8-chloro-1,3-dimethyl-2-oxopurin-6-olate (Figure 1B) and is a combination salt of two drugs namely Diphenhydramine and 8-chlorotheophylline (a chlorinated derivative)[1]. It is prescribed as an antihistaminic with antimuscarinic and is associated with sedative effects.

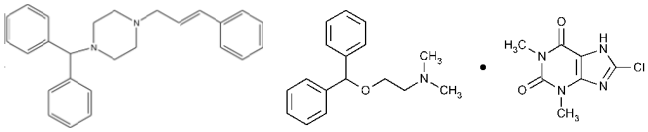

Figure 1: Chemical structure of Cinnarizine (A) and Dimenhydrinate(B)

In combination, both CNN and DMH are prescribed to treat emesis related to motion sickness and for treating nausea, vertigo, and other vestibular disturbances.[2,3] The drugs are official in IP,[4] BP.[5] USP.[6] Literature survey reveals different liquid chromatographic methods for the determination of CNN and DMH in combination in pharmaceutical dosage form[7-9] high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) methods[10,11] UV Spectroscopy.[12,13] Tawakkol SM *et al.* reported worked on chemometric methods to estimate Cinnarizine and Dimenhydrinate in mixture prepared in house along with pharmaceutical dosage form[14] and spectrophotometric methods for the analysis of combination in the presence of cinnarizine impurity[15] and biological samples.[16]

Adequate resolution and quantitative determination of both the drugs with pKa values 8.4 for CNN and 8.87 for DMH has been a major challenge during method development and validation. UPLC technique comprising of better speed, resolution and sensitivity was introduced in 2004 by Waters

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wherein column efficiency was improved using smaller particle size and minimizing the column length. With these features, UPLC had a better stand when compared with HPLC. Therefore, it was decided to develop an UPLC method and validate it using quality by design (QbD). Presently, QbD is an important tool to handle the analytical process during the method development itself, as Beike Deijaegher *et al.*[17]. Analytical method optimization reported by several scientists includes full factorial design and fractional factorial designs to optimize critical method parameters (CMPs), and further method optimization can be done with central composite design (CCD)[18] or Box–Behnken design (BBD).[19,20] The logical use of experimental design helps in understanding factor–response relationship, which can be applied to method validation. Among the various statistical design tools, the response surface methodology (RSM) approach is considered best to understand the optimized conditions suitable for method development. The responses obtained from the Design of Experiments (DoE) are interpreted statistically to understand and get the relationship between the responses and the independent variables. Best operating conditions can be determined as a part of the design space using these design models.[21,22]

It was also considered necessary to ascertain the stability of CNN and DMH by applying deliberate stress studies using ICH recommended test conditions and to develop a stability-indicating assay.[23-25] Hence stability-indicating UPLC method was developed, optimized (using DoE approach) and validated as per ICH guidelines for determination of CNN and DMH in the pharmaceutical dosage form.

MATERIAL AND METHOD

Chemicals and Reagents

The CNN and DMH samples were kindly gifted by S.S. Pharmachem, Thane, Maharashtra, India. Use of HPLC grade Acetonitrile as solvent and reagents of analytical grade are recommended. Orthophosphoric acid [OPA], hydrochloric acid (HCl), sodium hydroxide (NaOH), and 30% hydrogen peroxide (H_2O_2) were procured from Merck. Potassium dihydrogen Phosphate Anhydrous (KH_2PO_4) and Dipotassium hydrogen Phosphate (K_2HPO_4) for buffer preparation were sourced from Emparata.

Instrumentation

The UPLC acquity system from Waters Corporation had a binary solvent manager, a sample manager and a Dual wavelength UV detector, and PDA detector. Empower software was used to monitor output signals. Other instruments *viz*. Analytical Balance from Mettler Toledo, pH Meter of Lab India, Ultra Sonic Bath Sonicator of PCI analytics, Vacuum oven of Biotechnics India, Humidity Chamber, Photo-stability Chamber of Newtronic.

Preparation of Standard and Sample Stock Solution

Cinnarizine weighed accurately about 10 mg and 20 mg of drug Dimenhydrinate and transferred to 100 mL volumetric flask, about 50 mL of Acetonitrile was added and sonicated for two minutes phosphate buffer pH 6, volume made to 100 mL. This gave stock standard solution of 100 ppm of Cinnarizine and 200 ppm of Dimenhydrinate.

Preparation of Sample Solution

Vertizac® from Ajanta Pharma Limited was taken and twenty tablets were weighed, and grinded in a clean and dry mortar. Then the powder equivalent to 10 mg of CNN (20 mg of DMN) was transferred to volumetric flask (100 mL). Acetonitrile added (50 mL), on sonication for two minutes, powder was dissolved, volume made up to 100 mL with phosphate buffer pH 6. The solution was filtered using 0.45 µ (Nylon 66-syringe) filter. Discarded initial 2–3 mL filtrate and remaining used for analysis.

Chromatographic Conditions

The initial chromatographic runs were carried out on a trial and error basis wherein variation in stationary phase like C18 Column with different particle size of column like 1.7 micron (Aquity UPLC (2.1 × 50 mm), 1.9 micron (YMC Column 150 x 2 mm), 2.7 micron (Agilent 4.6 × 50 mm) and 3.5 micron (X bridge 4.6 × 50 mm) were tried. The mobile phase was composed of buffer solution pH 6 and acetonitrile. A 0.22 µm nylon membrane filter was used to filter mobile phase and degassed using sonication for 15 min. For the chromatographic run, detection wavelength was set at 260 nm, the flow rate of 0.3 mL/min with column temperature maintained at 40°C. The injection volume was 1 µL and run time of 10 minutes was set.

Method Validation

The method was validated as per ICH guidelines. All System suitability parameters were evaluated to get good resolution and repeatability of the proposed method. Deviation in retention time (Rt), peak area, theoretical plates (HETP) and tailing factor (t) were investigated.

To determine linearity, appropriate dilutions from stock solution were prepared from 10 to 150 $\mu g.mL^{-1}$ for CNN and 20 to 300 $\mu g.mL^{-1}$ for DMH and analyzed under optimized chromatographic conditions. The calibration curve was plotted as main peak area vs respective concentration, and the regression equation was derived.

Precision studies were carried out wherein six standard solution injections were injected per optimized chromatographic conditions. For method precision, appropriate sample dilutions of Vertizac® Tablets were injected as described under the methodology. To determine the method's ruggedness, different columns were used on different days to analyze sample solutions (n = 6) of the same lot (method precision) of Cinnarizine and Dimenhydrinate Tablets 20 mg/40 mg on a different UPLC (other than that used in method precision) as described under methodology.

Accuracy of the method was analyzed by injecting appropriate dilutions of a known amount of the standards solution of Dimenhydrinate and Cinnarizine at three different levels 50, 100, and 150% levels with tablet sample solution and the percentage recovery at each level in triplicate (total nine determinations for each) were determined. Robustness of the method was evaluated with critical parameters such as flow

rate (0.3 ± 0.1 mL/min., pH 6 ± 0.5) and column temperature (40°C ± 5°C).

Assay

Twenty tablets (Vertizac®) were weighed, ground in a dry glass mortar to make fine powder. Weighed approximately accurate, powder equivalent to 10 mg CNN (20 mg DMH) and transferred to 100 mL volumetric flask. Further suitable aliquot were prepared and sample solutions (n=6) of the same lot (as used in method precision) of CNN and DMN Tablets 20 mg/40 mg were made and analyzed using optimized chromatographic condition.

Stress Degradation Studies

ICH guidelines were followed to determine the stress degradation using a drug concentration 100 ppm for CNN and 200 ppm of DMH. Based on the literature of both CNN and DMH, conditions for degradation were selected and drugs were individually subjected to stress conditions.

Acid and alkali-induced Degradation

Standard drugs were weighed, accurately about 10 mg of CNN (20 mg of DMH) and each taken in separate 100 mL volumetric flask, added 50 mL of Acetonitrile in each flask, sonicated and 10 mL 1N hydrochloric acid (HCl) was added to Flask A while 10 mL 1N sodium hydroxide (NaOH) was added in Flask B. Flasks were maintained at room temperature for about four hours and then neutralized. The volume was made up of diluents (buffer pH 6). The solution was analyzed under optimized chromatographic conditions.

Hydrogen Peroxide Induced Degradation

Solutions were diluted same as described above except 10 mL of 3 % hydrogen peroxide (H_2O_2) was added instead of acid/alkali and diluted up to the volume with diluents (buffer pH 6). The sample was injected immediately after filtering it through 0.45 µ Nylon filter and analyzed under optimized chromatographic conditions.

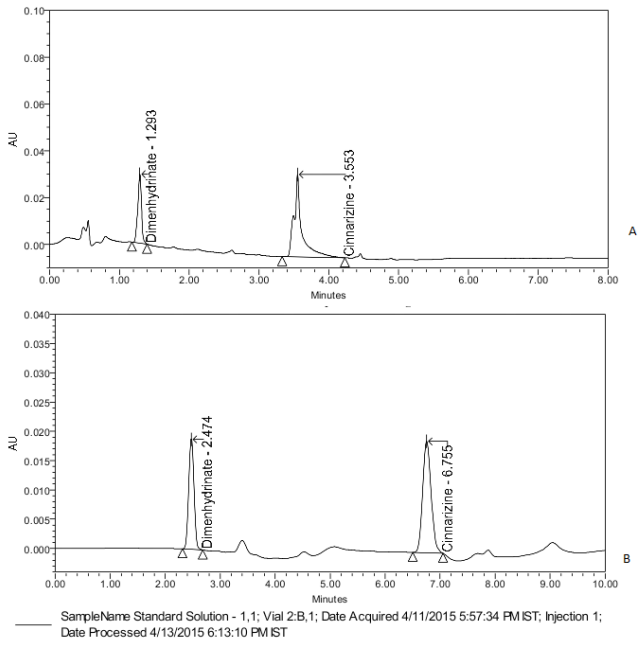


Figure 2: Stationary phase selection (A) Chromatogram of trial using column 1.7 / 1.9 µm (Aquity /YMC column); (B) Chromatogram of trial using column 3.5 µm (X-bridge) (optimum trial)

High Temperature/ Thermal (80°C) Induced Degradation.

For thermal degradation, the drug samples were placed in a covered Petri plate and exposed to 80°C for 72 Hours by keeping the container in a thermostatic oven. Further, the analysis was carried out as mentioned above.

Photo-degradation

The drug sample solution wrapped in Aluminum foil were exposed to 1.2 million lux hours of light and UV Energy of 200 watt-hours/Square meter for 72 hours. Further the analysis was carried out as mentioned above.

High Humidity (40°Cand 75%RH) Induced Degradation.

The sample was exposed at 40°C/75% RH Humidity condition for 72 hours and analyzed under optimized chromatographic conditions.

RESULTS AND DISCUSSION

Optimization of Chromatographic Method[26]

The chromatographic method is aimed to achieve good resolution between the drugs which were closely eluting due to their closer pKa values.

Selection of Stationary Phase

The stationary phases with different particle sizes were explored viz. stationary phase C18 with different particle size of column like 1.7 micron (Aquity UPLC 2.1 x 50 mm, 1.7 micron), 1.9 micron (YMC column 150 x 2 mm, 1.9 micron), 2.7 micron (Agilent 4.6 × 50 mm, 2.7 micron) and 3.5 micron (X bridge 4.6 × 50 mm, 3.5 micron) were tried. It was observed that the peak shapes were not proper with a particle size of 1.7 and 1.9 micron, as shown in chromatogram Figure. 2a. In contrast, peak shapes for both the drugs were good, and both the peaks were well resolved on 2.7- and 3.5-micron size columns. However, these two 3.5 micron size column was preferred (Figure 2b). Hence the column with C18 stationary phase having 3.5 micron particle size was selected.

Selection of Mobile Phase

The buffer solution like 0.1 % Triethylamine (TEA) and 0.1% Trifluoroacetic acid (TFA) were taken as mobile phase buffer (in A port) with Acetonitrile and Methanol as organic phase (in B port). The results obtained were unsatisfactory concerning peak separation and peak shapes with either TEA or TFA. As both the drugs are strong basic in nature with pKa values near 8, the pH of the mobile phase chosen was 6, which was two pH units away from the analyte pKa. Trials using buffer with pH 6, gave good peak separation and shape. Hence the mobile phase (pH 6) phosphate buffer was finalized. Acetonitrile was preferred rather than methanol as an organic solvent. Trials with variation in the proportion of buffer pH 6 and Acetonitrile were taken and based on the peak shapes the gradient program was optimized. Mobile phase was used as

diluents to get results as per the expectation in the proportion of 50:50 (Buffer pH 6: Acetonitrile).

Optimization of Column Temperature

Trials were performed using column temperature over a wide range of 30 to 50°C with variation of 5°C to improve the response, resolution, and peak shapes. Peak shapes with 40°C column temperature were satisfactory, and hence 40°C was selected as optimum column temperature, and robustness studies were performed at 40 ± 5°C, i.e., 35 and 45°C.

Flow Rate

Trials were performed using different flow rates ranging 0.1 mL^{-1} to 0.5 mL^{-1} being a UPLC method. Chromatograms obtained showed that with flow rate of 0.1 mL^{-1}, both the drugs were not eluted within 10 min run time. Also, the response with 0.2 mL^{-1} was not satisfactory. With the flow rates of 0.3 mL^{-1} to 0.5 mL^{-1}, both the peaks were eluting, however considering the peak shapes and resolution, 0.3 mL^{-1} was selected as optimum flow rate.

Selection of wavelength

The absorbance maxima reported forCinnarizine was 252 nm and Dimenhydrinate showed absorbance maxima at 277 nm. An isobestic point obtained at 260 nm wavelength gave satisfactory results and robustness studies were performed at 260 nm ± 5 nm.

Selection of injection volume

Since the standard and sample solution concentration was 100 ppm for Cinnarizine and 200 ppm for Dimenhydrinate, response with the injection volume selected 1-µL was satisfactory. Hence 1-µL injection volume was selected as optimum, considering the peak shape acceptance.

Table 1: Scouting of three parameters of UPLC

			Actual levels	
Factor	*Factor Name*	*Unit*	*Low*	*High*
A	Column Temperature	°C	30	50
B	Mobile phasepH	pH	4	8
C	Flow rate	mL/min	0.2	0.4

Method Optimization using QbD[26]

To identify critical robustness parameters and optimize the method, QbD approach was used to get a good resolution for Cinnarizine and Dimenhydrinate. With the simple method of analysis requiring variation in less than three factors, a good choice is CCD for robustness testing as it has the highest efficiency as far as the number of runs is taken. RSM was applied to explore performance of method using optimized factors and the impact on response produced by all variables used at a time to further get the experimental region around critical parameters for analysis.

Column temperature, pH of mobile phase, and Flow rate were considered critical method parameters for screening and evaluated at lower and higher limits Table 1. An experimental design of the above method conditions to be evaluated was developed using Design Expert® Software.

Eleven experiments, as listed in Table 2, were run using the conditions as described in Table 1. Minimum and maximum limit for column temperature (40 ± 10°C) were fixed, pH of mobile phase selected as 4 and 8, respectively. Flow rate (0.3 ± 0.1 mL/min) and resolution between the CNN and DMH were the response for these studies.

The advantage of this approach was to present design with three levels per factor without extreme vertices. The factors and ranges were selected based on chromatographic runs carried out initially.

The data generated was analyzed with statistical software (Design Expert Version 9.0.1, Stat-Ease Inc., and Minneapolis, MN, USA). The consequence of the applicable factors was calculated using Fisher's statistical test for analysis of variance (ANOVA) models that were assessed and run to relate the first-order interaction terms. When the probability of F-ratio is low, the model can be taken as a better statistical fit for the data (Table 3).

Based on outcome from DoE:

- Pareto Chart presented in Figure 3, useful to understand the interaction impact of the factors on the response. The Chart indicates that column temperature has more impact on resolution than flow rate and the combined effect of

Table 2: Resolution results of DOE trials/runs using Factorial design

	Factor 1	*Factor 2*	*Factor 3*	*Response*	
Run	*A:Temperature*	*B:pH*	*C:Flow rate*	*Resolution*	*Remarks*
1	50	8	0.4	16.42	Resolution 0 indicates only one peak i.e. peak of DMH and peak shape of other i.e CNN was not proper.
2	50	8	0.2	8.32	
3	30	8	0.4	0	
4	40	6	0.3	18.37	
5	50	4	0.2	7.98	
6	30	4	0.4	0	
7	40	6	0.3	18.20	
8	30	4	0.2	0	
9	30	8	0.2	0	
10	50	4	0.4	16.20	
11	40	6	0.3	18.53	

column temperature and flow rate. pH of the mobile phase in the selected range has minimum impact on resolution.

- Counter plot for resolution indicates the impact of column temperature and pH, keeping the flow rate as 0.3 mL /min; for better resolution optimum column temperate is 40°C and pH is 6 (Figure 4).
- Design space for resolution indicates the temperature below 37.5 C does not give an ideal resolution of more than 8 and pH in the selected range of 4 to 8 has no impact on resolution. Optimized chromatographic conditions as per QbD are as shown in Figure 5. Further, the graph of predicted *vs* actual is linear, indicating the optimized parameters predicted based on the previous univariate studies matching with the output of DoE (Figure 6).

Method Validation

System Suitability Parameters

Resolution(peak separation) and repeatability of the proposed method are among the most important suitability parameters, including theoretical plates, tailing factor, retention time, and peak area investigated and the results are in Table 4.

Specificity

The analytical method is considered specific when the analytical method gives the analyte response in the presence of matrix of impurities, degradation products, or excipients. The specificity evaluated ensured no interference from forced degradation. Chromatogram showed a retention time of the DMH and CNN peaks for sample corresponding to that of the DMH and CNN peak for standard.

Linearity and Range

Linearity was studied at concentration levels from 10 to 150 µg.mL^{-1} for Cinnarizine and 40 to 300 µg.mL^{-1} for Dimenhydrinate. The calibration curve was in adherence to Beer's law over the concentration range for both the drugs. The regression equation obtained is Y = 2017x + 19.093 for CNN and Y = 646.15x -849.87, where Y is peak area and X is the concentration of CNN and with a correlation coefficient 0.999, as given in Table 5.

Precision

Precision was studied per the ICH guidelines for system precision, method precision, and intermediate precision. In

Table 3: ANNOVA Table

Analysis of variance table [Partial sum of squares - Type III]

Source	*Sum of Squares*	*df*	*Mean Square*	*F value*	*p-value*	*Remarks*
Model	365.7314	3	121.9105	3201.6171	<0.0001	Significant
A-Temperature	299.1458	1	299.1458	7856.1779	<0.0001	
C-Flow rate	33.2928	1	33.2928	874.3367	<0.0001	
AC	33.2928	1	33.2928	874.3367	<0.0001	
Lack of Fit	0.082	4	0.0205	0.2799	0.8712	Not significant

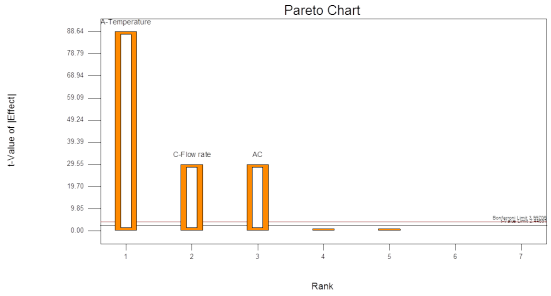


Figure 3: Pareto chart for resolution

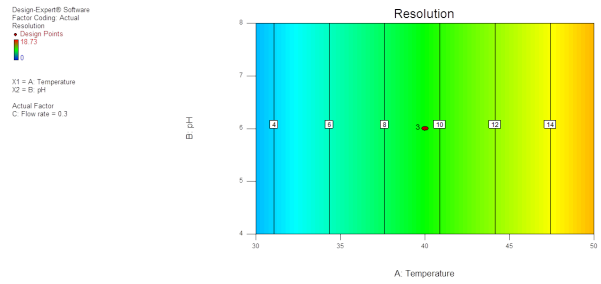
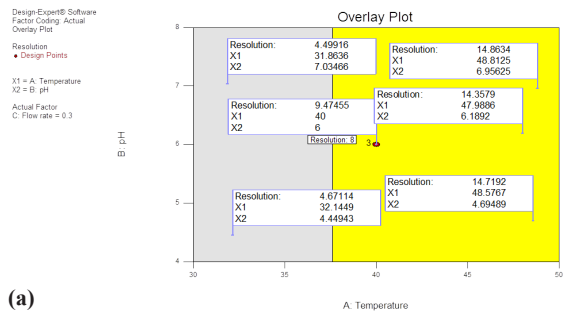
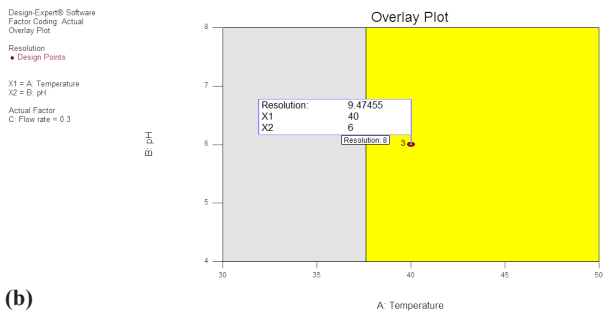


Figure 4: Counter plot for resolution



(a)



(b)

Figure 5: Overlay plot for (a) resolution with variation in temperature and pH; (b) Design space with optimized conditions

system precision, Six injections of the standard solution were injected as described under methodology. Table 6 and for method precision, Six sample solutions (n = 6) of Cinnarizine and Dimenhydrinate Tablets 20 mg/ 40 mg were injected using the optimized condition. The %RSD of method precision for Cinnarizine and Dimenhydrinate is 0.95% (Table 7).

Ruggedness (Intermediate precision)

Six sample solutions (n = 6) of Cinnarizine and Dimenhydrinate Tablets 20 mg/40 mg were made and injected into different column on a different day as described under methodology (Table 7). The results indicated that the UPLC method is precise and rugged for analysis of CNN and DMH in Tablets.

Accuracy

Standards solution of CNN and DMH at 50, 100, and 150% concentration were taken and added to suitable aliquots of tablet solution, in triplicate (total nine determinations for each drug) were mixed well to get final concentration of 100 $\mu g.mL^{-1}$ of CNN and 200 $\mu g.mL^{-1}$ of DMH and analyzed under optimized chromatographic conditions. Each Individual recovery is in the range of 98.0 to 102.0% for DMH and CNN. (Table 7). UPLC method was found to be accurate for determining Cinnarizine and Dimenhydrinate in Tablet formulation.

Stability of analytical solution in mobile phase

The standard and sample preparations of CNN and DMH Tablets 20 mg/40 mg were kept at room temperature for 48 hours and analyzed against freshly prepared standard preparations. The % correlation for standard and sample solution are within a limit up to 48 hours as shown in Table 8, so solutions were stable for 48 hours. No haziness or precipitation observed in the mobile phase up to 48 hours at room temperature.

Robustness

Chromatographic conditions varied deliberately (flow rate, pH, column temperature and composition/proportion of buffer and organic solvent in gradient program) as seen in the results obtained in Table 9, which shows that the developed method is robust over a given variation of method parameters.

Assay

Twenty tablets in the 10 mg CNN (20 mg DMH) ratio were analyzed using optimized chromatographic condition. The %Assay of the Label claim for CNN and DMH was found in the range of 98.0 to 102.0% with RSD of mean assay 0.552% for CNN and 0.564% for DMH.

Force Degradation Studies

Subjecting the analyte to stress testing can help identify the likely degradation products and the degradation pathways along with intrinsic stability of the molecule.

All degradation studies were performed at a concentration 100 ppm for Cinnarizine and 200 ppm of Dimenhydrinate. The %degradation was compared with the control sample chromatogram.

Acid and alkali-induced degradation

The chromatogram Figure 7 a shows that all the degradation products were well separated from Cinnarizine and Dimenhydrinate peaks with 10% degradation while the alkaline condition showed both the drugs with 12 to 15% degradation of both the drugs (Figure 7b).

Hydrogen Peroxide induced degradation

The sample was injected immediately. The chromatograms (Figure 7c) show no interference of minor degradation products with Cinnarizine and Dimenhydrinate peaks. Both the drugs are stable to oxidative stress.

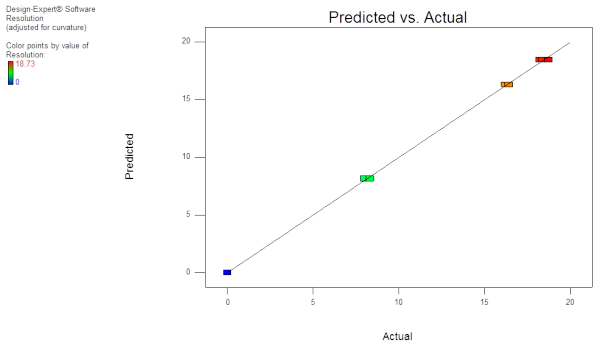


Figure 6: Graph of predicted vs actual optimized parameters

Table 4: System suitability parameter

		Results	
Parameter	*Acceptance Criteria*	*CNN*	*DMH*
Theoretical Plates for CNN & DMH peak in standard chromatogram	NLT 2000	Complies (more than 2000 for each injection)	Complies (more than 2000 for each injection)
Tailing for CNN and DMH peak in standard chromatogram	NMT 2.0	Complies (not more than 2.0 for each injection)	Complies (not more than 2.0 for each injection)
% RSD for CNN and DMH peak RT of replicate standard injections	NMT 1.0 %	0.39	0.84
% RSD for CNN and DMH peak areas of replicate standard injections	NMT 2.0 %	1.67	1.73

Conclusion: All system suitability parameters comply as per the acceptance criteria.

High Temperature/ Thermal (80°C) induced degradation.

The sample were subjected to exposure at 80°C for 72 Hours and analyzed as per methodology. The chromatogram Figure 7d shows that the drugs are thermally stable.

Photo-degradation

Sample and control sample (wrapped in Aluminum) were exposed to 1.2 million lux hours of light and UV Energy of 200 watt-hours/ Square meter. The chromatograms (Figure 7e) show that the drug product containing CNN and DMH is stable against photodegradation.

High Humidity (40°C and 75%RH) induced degradation.

Sample was exposed at 40°C/75% RH Humidity condition for 72 hours. The Figure 7f shows that both Cinnarizine and Dimenhydrinate are stable even in high humidity.

Also, peak purity was checked in applied stressed conditions and the results of % assay and % degradation as summarized in Table 10. The peak purity angle of the DimenhydrinateandCinnarizine peak in the chromatograms of the degradation sample solution is less than purity threshold. The peak purity data of CNN and DMH peak in every degradation sample shows that and Cinnarizine peak the Dimenhydrinate is similar to standard and no co-eluting peaks seen, suggesting that the method is specific for analysis of CNN and DMH in the presence of its degradation products-indicating that the method is stability-indicating and specific

Table 5: Table for Linearity for CNN and DMH

	CNN	*DMH*
Beers-Lambert's range	10–150 µg/mL	20–300 µg/mL
Slope	2017	647
Residual sum of squares (R^2)	0.99968	0.99962

*Mean of six determination (n = 6)

Table 6: System Precision- CNN and DMH

	CNN		*DMH*	
Standard	*RT*	*Area*	*RT*	*Area*
Inj. 1	6.878	214366	2.425	127245
Inj. 2	6.876	211325	2.426	128223
Inj. 3	6.879	219934	2.433	128896
Inj. 4	6.874	214632	2.426	129987
Inj. 5	6.878	219878	2.425	128965
Inj. 6	6.877	215476	2.429	129465
Mean*	6.877	215935	2.427	128796
SD	0.002	3381.17	0.003	963.17
% RSD	0.03	1.56	0.12	0.74

Mean of six determination (n=6)

Table 7: Method Precision, Ruggedness, Accuracy and %Assay for CNN and DMH

	*Method Precision (% Assay of the Label Claim)**		*Intermediate Method Precision and Ruggedness**		*Accuracy Level# % Recovery (100 ± 50%)*		*(% Assay of the Label Claim**	
	(CNN)	*(DMH)*	*(CNN)*	*(DMH)*	*(CNN)*	*(DMH)*	*(CNN)*	*(DMH)*
Mean	100.0	103.9	100.0	103.2	100.05	100.33	101.3	101.7
SD	0.952	0.991	0.732	1.067	0.567	0.305	0.558	0.785
RSD	0.950	0.950	0.730	1.030	0.570	0.300	0.552	0.564

*Mean of six determination (n=6)
#Mean of three determination (n=3)at every level

Table 8: Stability study of analyte solution in mobile phase

	Standard - CNN		*Standard - DMH*		*Standard - CNN*		*Standard - DMH*	
Time Hours	*Retention Time*	*% Difference with 0 Hours*	*Retention Time*	*% Difference with 0 Hours*	*% Content*	*% Correlation with 0 Hours*	*% Content*	*% Correlation with 0 Hours*
0	6.878	—	2.433	—	101.0	—	100.0	—
48	6.876	0.02	2.426	0.28	100.6	99.6	100.6	100.6
Time Hours	Sample - CNN		Sample - DMH		Sample - CNN		Sample – DMH	
0	6.879	—	2.429	—	101.3	—	98.0	—
48	6.874	0.07	2.426	0.12	101.7	100.4	98.6	100.6

Table 9: Robustness of UPLC method parameters-DMH and CNN

	DMH			*CNN*		
Parameter	*RT of Inj.*	*% RSD of RT*	*% RSD of Inj. area*	*RT of Inj.*	*% RSD of RT*	*% RSD of Inj. area*
Flow Rate ± 0.1mL/min (optimum: 0.3 mL/min)						
0.2 mL/min	3.092	0.1	0.44	6.675	0.2	0.31
0.3 mL/min	2.474	0.3	0.74	6.755	0.2	0.68
0.4 mL/min	1.583	0.7	0.28	5.944	0.1	0.52

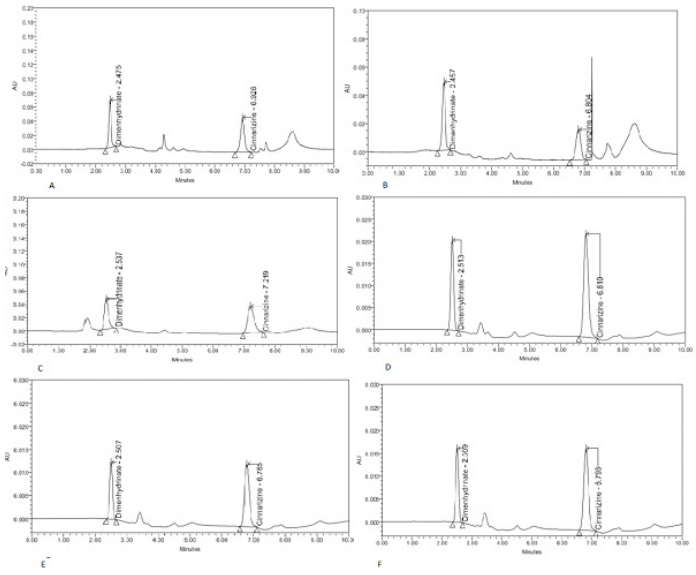


Figure 7: Forced degradation of sample solution in a) Acid induced b) Alkali induced c) Oxidative stress d) Thermal exposure e) Humidity f) Photolytic condition.

	DMH			*CNN*		
Parameter	*RT of Inj.*	*% RSD of RT*	*% RSD of Inj. area*	*RT of Inj.*	*% RSD of RT*	*% RSD of Inj. area*
pH of mobile phase ± 0.5 (optimum: pH 6.0)						
pH 5.5	2.475	0.4	0.50	6.761	1.0	0.74
pH 6.0	2.474	0.3	0.74	6.755	0.2	0.68
pH 6.5	2.472	0.0	0.68	6.758	0.7	1.25
Column temperature ± 5.0ºC (optimum: 40°C)						
35°C	2.073	0.2	1.07	7.128	0.3	0.46
40°C	2.474	0.3	0.74	6.755	0.2	0.68
45°C	2.047	0.1	0.13	6.896	0.1	0.31
Wavelength ± 5nm (optimum: 260 nm)						
255 nm	2.035	0.7	0.12	6.814	0.4	0.52
260 nm	2.474	0.3	0.74	6.755	0.2	0.68
265 nm	2.045	0.4	0.35	6.749	0.3	0.74

% RSD (n=3)with all the samples is well within 2, i.e., meeting acceptance criteria.

Table 10: Summary for Forced Degradation Studies for CNN and DMH

		Degradation Studies for CNN		*Degradation Studies for DMH*	
S. No.	*Degradation*	*% Assay*	*% Degradation*	*% Assay*	*% Degradation*
1	Control	98.3	-	99.7	-
2	Acid	88.5	9.8	90.5	9.2
3	Base	84.0	14.3	88.3	11.4
4	Peroxide	83.1	15.2	89.1	10.6
5	Thermal	94.2	4.1	93.7	6
6	Humidity	96.4		97.8	
7	Photolytic Control	98.6	No significant degradation	98.9	No significant degradation
8	Photolytic	97.9		98.2	

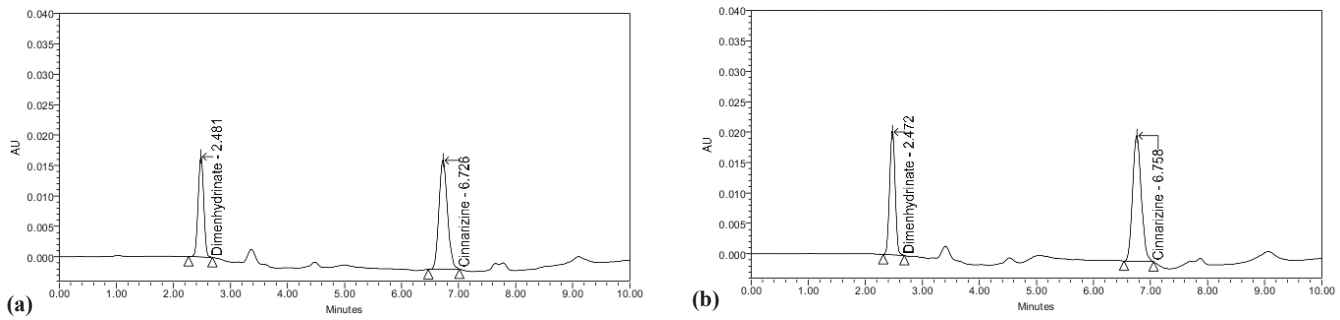


Figure 8: Chromatogram of Cinnarizine and Dimenhydrinate (a) Standard; (b) Sample

corelating further with the standard and sample retention time as seen in Figure 8.

CONCLUSION

The developed UPLC method has been validated as per ICH guidelines. The runtime for analysis was 10 minutes, wherein both the drugs were well separated. An approach of DoE was used for demonstrating the effects of column temperature, mobile phase pH and flow rate on the resolution between two main peaks.

The predicted values from the model equation (column temperature, pH of mobile phase and flow rate as 40°C, pH 6, and 0.3 mL/min, respectively). All variables had good agreement with observed values and were evaluated to achieve an optimal resolution, and the models were presented as 3D response surface plots. The results reveal that the Column temperature has a significant effect on resolution, whereas pH of mobile phase has less of an impact.

The Developed method can be used for routine quality control analysis to determine both the drugs CNN and DMH in bulk and tablet formulation.

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