ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CINNARIZINE AND DIMENHYDRINATE IN COMBINED DOSAGE FORM

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ABSTRACT

In the present work, sensitive RP-HPLC method been developed for the quantitative estimation of Cinnarizine (CINNA) and Dimenhydrinate (DIMEN) individually as well as in combined dosage form. Methanol was selected as a common solvent for estimation of both the drugs. RP-HPLC method in which determination of CINNA and DIMEN was carried on a reverse phase C_{18} column using a mobile phase consisting of Methanol: Acetonitrile: 0.1% TEA (80: 10: 10 V/V/V) pH 7.8 adjusted with 0.5% Ortho phosphoric acid. The mobile phase was pumped at flow rate of 1.0 ml/min and the detection was carried out at 252 nm. The linearity was found to be in the range of 10-30 µg/ml and 20-60 µg/ml with (r^2=0.9989, and r^2=0.9967) for CINNA and DIMEN respectively. The peaks obtained were sharp having clear baseline separation with a retention time of 7.823 ± 0.0117 and 3.02 ± 0.0070 min for CINNA and DIMEN respectively. LOD for CINNA and DIMEN were found to be 0.5957 µg/ml and 0.9165 µg/ml respectively and LOQ for CINNA and DIMEN were found to be 1.8052 µg/ml and 2.7775 µg/ml respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords: Cinnarizine, Dimenhydrinate, Simultaneous Estimation, RP-HPLC, ICH.

INTRODUCTION

Cinnarizine, Chemically 1-(diphenyl methyl)-4-(3-phenyl prop-2-en-1-yl) piperazine [1]. An 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 [2-3].

Dimenhydrinate, Chemically 8-chloro-1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-ide; [2-(diphenyl methoxy)ethyl] dimethylazanium [4]. It is an over-the-counter drug used to prevent nausea and motion sickness. Dimenhydrinate is a salt of two drugs Diphenhydramine and 8-chlorotheophylline (a chlorinated derivative of theophylline) [5-6].
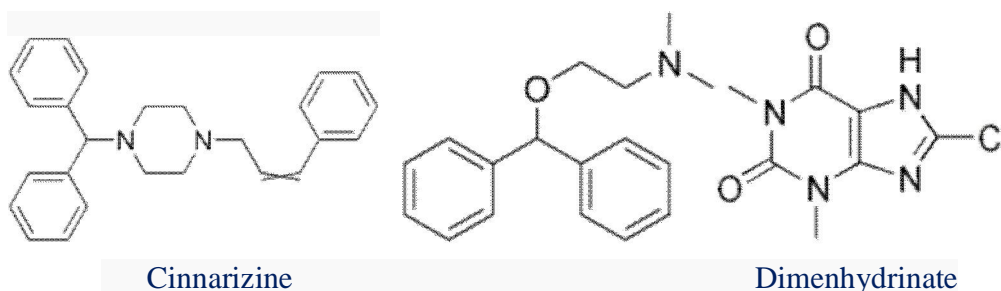
Arlevert 20 mg/40 mg Tablets contain two active substances Cinnarizine and Dimenhydrinate. Cinnarizine is official in BP [7] and EP [8], both of them includes Potentiometric titration for estimation of CINNA. Dimenhydrinate is official in BP [7], USP [9], EP [8] and JP 15 [10], which includes Potentiometric titration, Argentometric Titration and HPLC method for estimation of DIMEN. The combination of these two drugs is not official in any pharmacopoeia. Literature review [11-20] shows that numbers of analytical methods are available for estimation of both the drugs either alone or in combination with other drugs. Based on our current and ongoing referencing work, till date, we have not come across any official and reported analytical methods for simultaneous estimation of both the drugs in their combined dosage form. Therefore, the objective is to develop a RP-HPLC method for simultaneous estimation of Cinnarizine and Dimenhydrinate in their formulation and to validate the developed method according to ICH guidelines [21].

MATERIALS AND METHODS

Instruments:

a) Thermo Electron Corporation, HPLC system with auto sampler.

    i.   Liquid chromatography: Thermo LC.

    ii.   UV-Visible Detector: PDA, UV-2000.

    iii.   Column: Lichrosphere C_{18} column (250 X 4.6 mm, 5 µm).

    iv.   Auto sampler: AS 3000.

    v.   Software: SN 4000, chromquest.

b) CL 54+ Toshcon industries Pvt. Ltd, Digital pH meter.

c) Shimadzu UV-1800, UV-Visible double beam Spectrophotometer.

d) Shimadzu AUX-220, Electronic analytical balance.

e) Sonica Ultrasonic Cleaner, Sonicator.

Reagents and Chemicals:

a) Standard Cinnarizine (CINNA) and Dimenhydrinate (DIMEN) were kindly gifted by Vaibhav Analytical Laboratories, Ahmedabad, India.

b) Arlevert 20 mg/ 40 mg Tablets formulation was procured from local market of United Kingdom.

c) Methanol of HPLC grade (Rankem, RFCL chemicals Pvt. Ltd.)

d) Methanol (A.R. Grade - Chemco Chemicals Ltd.)

e) Acetonitrile of HPLC grade (Rankem, RFCL chemicals Pvt. Ltd.)

f) Water of HPLC grade (Rankem, RFCL chemicals Pvt. Ltd.)

g) Orthophosphoric acid (OPA) (Analytical reagent grade).

h) Triethylamine (TEA) (Analytical Reagent grade).

Methodology (RP-HPLC Method)

Preparation of combined stock solution of Cinnarizine and Dimenhydrinate:

CINNA (10 mg) and DIMEN (20 mg) were accurately weighed and transferred to a 100 ml volumetric flask, dissolved in sufficient quantity of methanol and then diluted to the mark with methanol. The solution contains 100 µg/ml of CINNA and 200 µg/ml of DIMEN. The final solution was labeled as Standard Solution (SS). The SS was filtered through 0.45 µm Nylon 66 (N66) 47 mm membrane filter paper and first few drops of filtrate were discarded.

Selection of wavelength for Detection:

An aliquot from stock solution of CINNA was transferred to a separate 10 ml volumetric flask and volume was adjusted to the mark with methanol to give the final concentration of 20 µg/ml for CINNA. An aliquot from stock solution of DIMEN was transferred to a separate 10 ml volumetric flask and volume was adjusted to the mark with methanol to give the final concentration of 40 µg/ml for DIMEN. An aliquot of 2 ml from the SS was transferred to a separate 10 ml volumetric flask and volume was adjusted to the mark with methanol to give the final concentration of 20 µg/ml for CINNA and 40 µg/ml for DIMEN. Each solution was scanned between 200-400 nm in a Shimadzu UV-1800, UV-

Visible double beam Spectrophotometer at a medium scanning speed. Overlain spectra of all the above solutions were taken which was used for the selection of wavelength for detection (Fig. 1).

Selection and preparation of Mobile phase:

Selection of mobile phase:

The standard solutions of CINNA and DIMEN were injected into the HPLC system and run in solvent system. Various compositions of the mobile phases was tried in order to find the best conditions for separation of CINNA and DIMEN. It was found that combination of methanol, acetonitrile and water gives satisfactory result. The mobile phase system was tried with different pH and using different flow rates. Finally, the optimal composition of the mobile phase was determined to be methanol: acetonitrile: 0.1% TEA (80: 10: 10 V/V/V) pH 7.8 ± 0.05 adjusted with 0.5% OPA and flow rate adjust at 1.0 ml/min.

Preparation of the optimized mobile phase:

The optimized mobile phase was prepared by mixing 400 ml of methanol (HPLC grade), 50 ml of acetonitrile (HPLC grade) and 50 ml of 0.1% TEA (AR grade) in water (HPLC grade). Than mobile phase was filtered through 0.45µm Nylon 66 (N66) 47 mm membrane filter paper. After filtration it was ultrasonicated for 20 minute on ultrasonicator. Finally pH 7.8 ± 0.05 was adjusted with 0.5% OPA (AR grade).

Chromatographic conditions:

Stationary phase: Lichrosphere C_{18} column (250 X 4.6 mm, 5 µm).

Mobile phase: Methanol: Acetonitrile: 0.1% TEA (80: 10: 10 V/V/V).

pH: pH of mobile phase was adjusted to 7.8 ± 0.05 using 0.5% OPA.

Flow rate: 1.0 ml/min.

Temperature: $26 \pm 2^{o}C$.

Wavelength: 252 nm.

Injection volume: 20 µl.

Run time: 10 min.

Pressure: 720 - 790 PSI

Preparation of the calibration curve:

Aliquots of 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml and 3.0 ml from the SS were transferred to a series of 10 ml volumetric flasks and volume was adjusted to the mark with HPLC grade methanol to get the concentrations of CINNA in the range of 10-30 µg/ml (10, 15, 20, 25 and 30 µg/ml) and of DIMEN in the range of 20-60 µg/ml (20, 30, 40, 50 and 60 µg/ml). The diluted solutions were filtered through 0.45 µm Nylon 66 (N66) 47 mm membrane filter. Chromatograms for each of the above solutions were recorded using the same chromatographic conditions as described above. Peak areas were recorded and a plot of peak area against respective concentration was plotted for CINNA and DIMEN (Table 1 and Table 2, Fig.6 and Fig. 7). The straight line equations and correlation coefficients for CINNA and DIMEN were determined (Table 3).

Method validation:

Linearity and Range (n = 5):

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 10-30 µg/ml (10, 15, 20, 25 and 30 µg/ml) for CINNA and 20-60 µg/ml (20, 30, 40, 50 and 60 µg/ml) for DIMEN (Fig. 5). The plot of peak area against concentration was plotted. Correlation coefficient and regression line equations for CINNA and DIMEN were calculated. Linearity range was established through consideration of required practical range and according to each drug concentration present in the pharmaceutical product, to give accurate, precise and linear results**.**

Accuracy (n = 3):

It was carried out to determine the suitability and reliability of the proposed method. Accuracy was determined by calculating the % Recovery of CINNA and DIMEN from the marketed formulation by the standard addition method in which, known amounts of standards powders of CINNA and DIMEN at 50%, 100% and 150% levels were added to the pre-analyzed samples. The recovered amounts of CINNA and DIMEN were calculated at each level and % Recovery was reported.

Precision:

Repeatability (n = 6):

For the repeatability study, the SS was utilized. From the SS, an aliquot of 2.0 ml was transferred to a separate 10 ml volumetric flask and diluted up to mark with HPLC grade methanol such that it gives the concentration of 20 µg/ml of CINNA and 40 µg/ml of DIMEN. The solution was injected into the system. The peak areas of CINNA and DIMEN were observed. The procedure was repeated six times and % CV was calculated.

Intraday Precision (n = 3):

From the SS aliquots of 1.0 ml, 2.0 ml and 3.0 ml were transferred to separate 10 ml volumetric flasks and diluted up to the mark with HPLC grade methanol to give the concentration of 10, 20 and 30 µg/ml for CINNA and 20, 40 and 60 µg/ml for DIMEN. The solutions were injected into the HPLC system and analyzed three times on the same day and % CV was calculated.

Interday Precision (n = 3):

From the SS aliquots of 1.0 ml, 2.0 ml and 3.0 ml were transferred to separate 10 ml volumetric flasks and diluted up to the mark with HPLC grade methanol to give the concentration of 10, 20 and 30 µg/ml for CINNA and 20, 40 and 60 µg/ml for DIMEN. The solutions were injected into the HPLC system, analyzed on three different days and % CV was calculated.

Specificity:

In the case of assay, demonstration of specificity is required to show that the procedure is unaffected by the presence of impurities or excipients. Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparison of the chromatograms of

a) Standard sample solutions of CINNA and DIMEN

b) Blank (mobile phase) and

c) Sample solution of CINNA and DIMEN.

LOD and LOQ:

The LOD and LOQ were estimated from the set of 5 calibration curves.

They were calculated as, LOD = $3.3 \times$ (SD/Slope) and LOQ = $10 \times$ (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Estimation of Cinnarizine and Dimenhydrinate in the marketed formulation by the proposed method (n = 5):

Twenty tablets were weighed and finely powdered. The powder equivalent to 10 mg of CINNA and 20 mg of DIMEN was weighed accurately and mixed, diluted with methanol (50 ml) in 100ml volumetric flask, kept in ultrasonic water bath for 10 min to get optimum dissolution of the active ingredients and diluted up to mark with methanol (100 μg/ml of CINNA and 200 μg/ml of DIMEN). The final solution was filtered using 0.45 μm Nylon 66 (N66) 47 mm membrane filter paper and first few drops of filtrate were discarded. 2 ml of aliquot of this solution was diluted to 10 ml with HPLC grade Methanol (20 μg/ml of CINNA and 40 μg/ml of DIMEN). The peak areas of CINNA and DIMEN were obtained from the chromatogram and utilized for estimation the concentration of each drug was calculated using equation of regression line (Table 6).

RESULTS AND DISCUSSION

The mobile phase used consisted of Methanol: Acetonitrile: Triethylamine (0.1% v/v) in the proportion of 80:10:10, v/v/v and the pH of mobile phase was adjusted to 7.8 ± 0.05 using 0.5% v/v Orthophosphoric acid. The peaks were well resolved with a resolution factor of 8.13. The estimation was carried out at 252 nm using a UV detector keeping the flow rate of 1.0 ml/min and injection volume 20 μl. Results of the validation of the above method indicate that the method was linear in the range of 10-30 μg/ml for CINNA and 20-60 μg/ml for DIMEN. The data for all validation parameters are mentioned in Table 5. The % recoveries for CINNA and DIMEN obtained in the accuracy study were 99.57 – 99.86% and 98.53 - 100.27% respectively. The results of the precision study indicate that the proposed method showed good repeatability for CINNA and DIMEN with a % CV of 0.23 and 0.18 respectively. The % CV from the intraday precision data were found to be 0.15 - 0.41 for CINNA and 0.16 - 0.50 for DIMEN. Similarly % CV from the interday precision data were found to be 0.28 – 0.60 for CINNA and 0.35 – 0.60 for DIMEN. The LOD for CINNA and DIMEN was found to be 0.5957 μg/ml and 0.9165 μg/ml respectively. Similarly LOQ for CINNA and DIMEN was found to be 1.8052 μg/ml and

2.7775 μg/ml respectively. The % assay results of 98.89% for CINNA and 98.02% for DIMEN indicate that the developed method was successfully utilized for the estimation of CINNA and DIMEN in their combined dosage form.
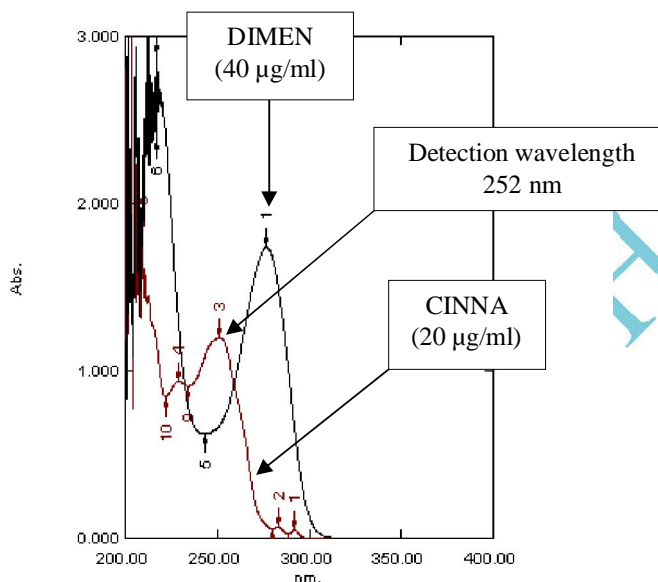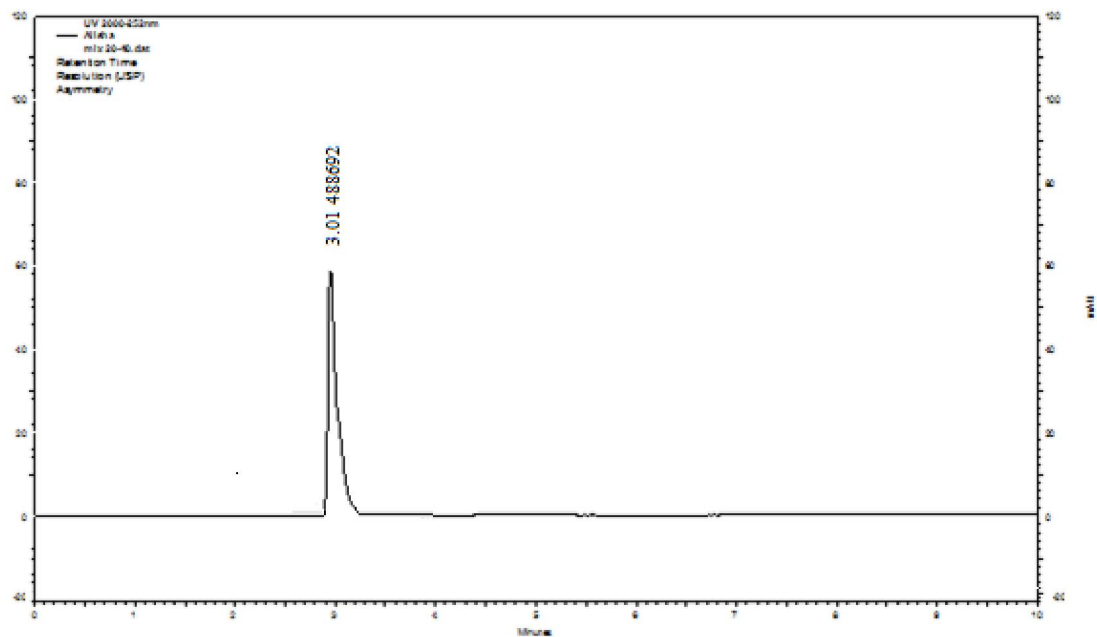

Figure 1
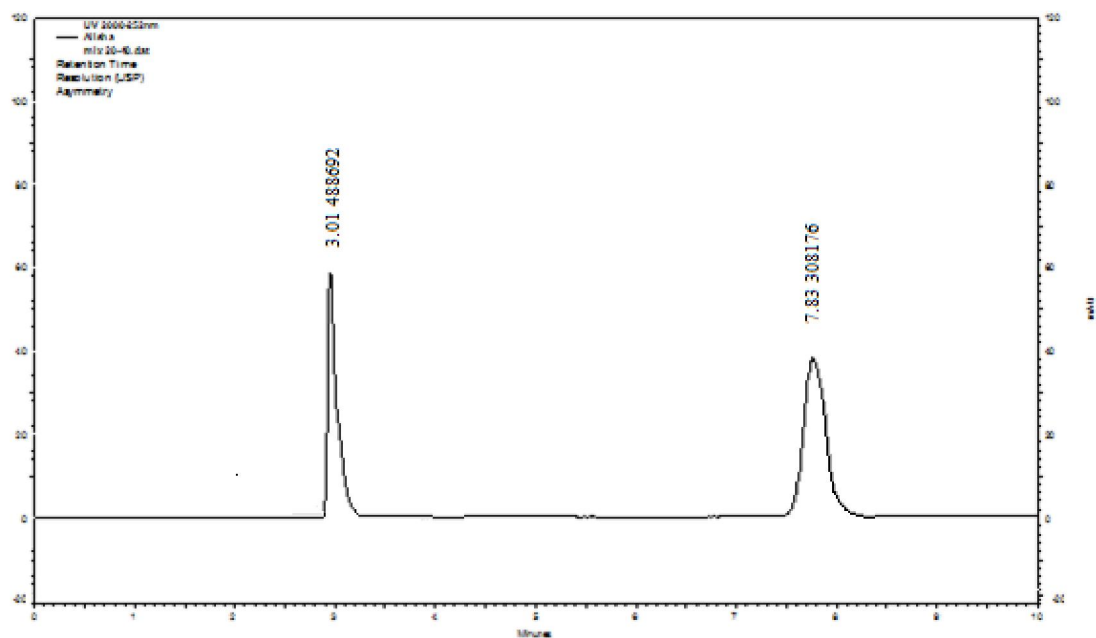Determination of wavelength for detection
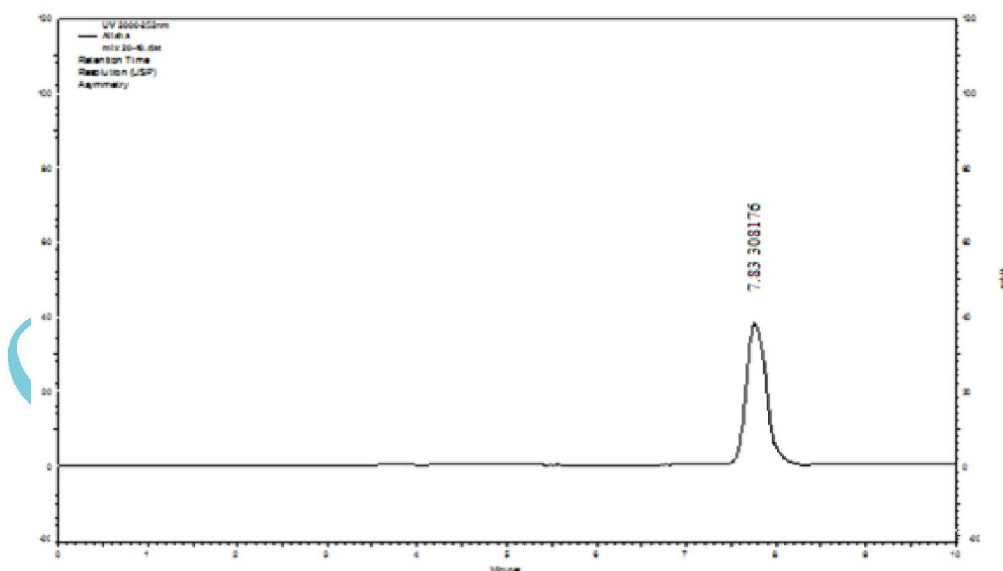
Figure 2
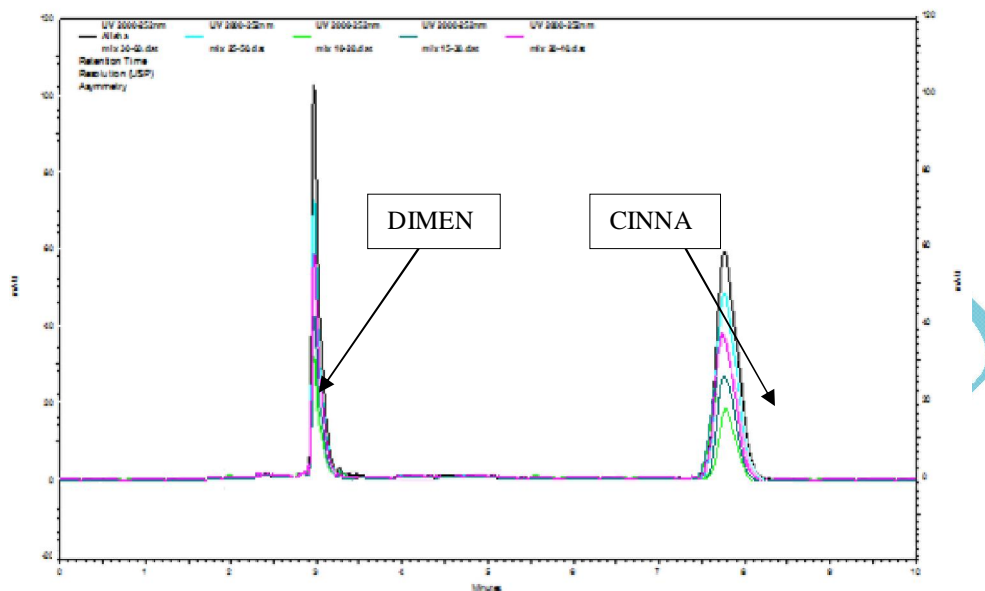Chromatogram of CINNA (20 μg/ml)
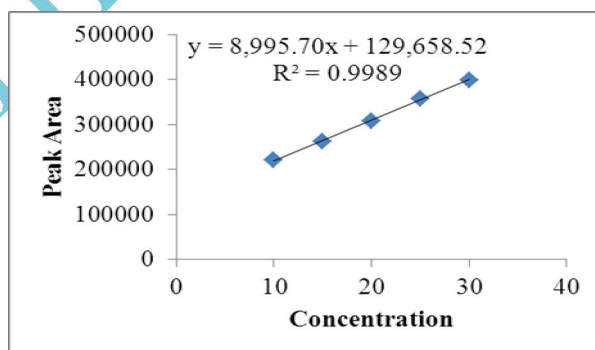
Figure 3
Chromatogram of DIMEN (40 µg/ml)

Figure 4
Chromatogram of binary mixture 20 µg/ml CINNA and 40 µg/ml DIMEN

Figure 5
Chromatogram of overlain spectra of CINNA and DIMEN

TABLE 1: LINEARITY DATA FOR CINNA

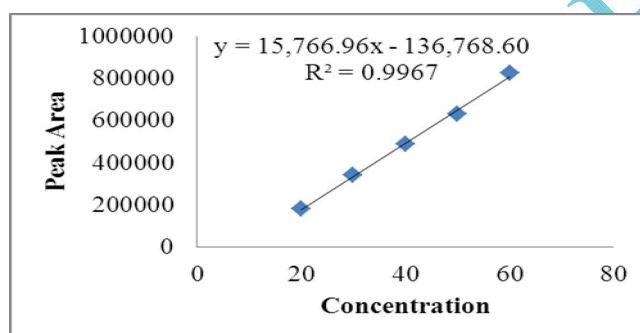| Conc. (μg/ml)	Area Mean± SD	% CV
10	221650 ± 979.94	0.44
15	261828 ± 1543.21	0.58
20	308176 ± 1965.64	0.63
25	357505 ± 3018.29	0.84
30	398704 ± 8716.36	0.42

Figure 6
Calibration curve for CINNA (10-30 μg/ml)

TABLE 2: LINEARITY DATA FOR DIMEN

Conc. (μg/ml)	Area Mean ± SD	% CV
20	181216 ± 628.79	0.34
30	343274 ± 2432.28	0.70
40	488692 ± 2584.46	0.52
50	630335 ± 5728.38	0.90
60	826034 ± 5338.10	0.64

Figure 7
Calibration curve for DIMEN (20-60 μg/ml)

TABLE 3: DATA OF REGRESSION ANALYSIS OF CINNA AND DIMEN

Drug	Straight line equation of Calibration curve	Correlation coefficient
CINNA	Y = 8,995.70x + 129,658.52	0.9989
DIMEN	Y = 15,766.96x - 136,768.60	0.9967

TABLE 4: SYSTEM SUITABILITY PARAMETERS FOR CINNA AND DIMEN

SYSTEM SUITABILITY PARAMETERS	DRUG	
	CINNA	DIMEN
Retention time (min ± SD) (n=5)	7.83 ± 0.0117	3.02 ± 0.070
Tailing factor (T)	1.0174	1.1282
Number of theoretical plates (N)	2858.6	3716
Resolution (R)	8.1338	

TABLE 5: SUMMARY OF VALIDATION PARAMETERS

PARAMETER	CINNA	DIMEN
Linearity	10-30 μg/ml	20-60 μg/ml
Accuracy (% Recovery) (n=3)	99.57 – 99.86 %	98.53 - 100.27 %
Precision (% CV)		
Repeatability (n=6)	0.23	0.18
Intraday (n=3)	0.15 - 0.41	0.16 - 0.50
Interday (n=3)	0.28 – 0.60	0.35 – 0.60
LOD (μg/ml)	0.5957	0.9165
LOQ (μg/ml)	1.8052	2.7775

TABLE 6: ANALYSIS OF MARKETED FORMULATION (N = 5)

DRUG	ACTUAL CONC. (mg)	CONC. FOUND (mg) ± SD	% CV	% PURITY
CINNA	20	19.77 ± 0.0601	0.30	98.89%
DIMEN	40	39.21 ± 0.1729	0.44	98.02%

CONCLUSION

The developed RP-HPLC method was found to be simple, rapid, accurate, sensitive and specific methods for the estimation of CINNA and DIMEN. The % assay results of 98.89% for CINNA and 98.02% for DIMEN indicate that the developed method was successfully utilized for the estimation of CINNA and DIMEN in their combined dosage form in routine analysis.

ACKNOWLEDGEMENT

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