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Research Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CINNARIZINE AND DOMPERIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for simultaneous estimation of cinnarizine (CIN) and domperidone (DOM) in tablet dosage form. The method was carried out on a C-18 (250mm × 4.6mm i.d, 5µm) column with a mobile phase consisting of methanol and acetonitrile in the ratio (70:30 v/v) and flow rate of 1.0 mL min^{-1}. The detection was carried out at 270 nm. The retention time for CIN and DOM were found to be 3.389 and 4.793 min respectively. The CIN and DOM followed linearity in the concentration range of 40-160 µg mL^{-1} and 45- 105 µg mL^{-1} with r^2= 0.999. The amount of both drugs estimated by the proposed method was found to be in good agreement with label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combination in the tablet formulations.
Keywords: Cinnarizine; domperidone; RP-HPLC, validation, C-18 column.

INTRODUCTION

Cinnarizine (CIN), IUPAC name 1-(diphenylmethyl)-4-(3-phenylprop-2-en-1-yl) piperazine, is an antihistaminic useful for treating motion sickness and. It is also effective for treating nausea and vomiting[1].The drug is official in Indian and British pharmacopoeia and estimated by potentiometric titration and liquid chromatography methods[2,3]. Literature survey revealed potentiometry titration and simple spectrophotometry methods for determination of CIN alone in pharmaceutical dosage forms as well as in biological fluids[4,5]. Further a RP-HPLC method is available for the determination of CIN in combination with other drugs[6]. Few RP–HPLC, UV-Spectrophotometric, methods have been studied for determination of CIN in bulk and in pharmaceutical formulations[7,8].

Domperidone (DOM), IUPAC name is 5-chloro-1-{1-[3-(2-oxo-2, 3-dihydro-1H-1, 3-benzodiazol-1-yl)propyl]piperidin-4-yl}-2, 3-dihydro-1H-1, 3-benzodiazol-2-one, is used to prevent feeling or being sick. It can also be used to treat stomach discomfort and nausea after eating meals[9]. In literature RP-HPLC and UV-spectrophotometric methods were reported for DOM alone[10,11]. The spectrophotometric, RP-HPLC and HPTLC methods for simultaneous estimation of CIN and domperidone maleate in bulk and dosage forms were found[12,13]. However no reports have been found in the literature for the simultaneous determination of CIN and DOM in pharmaceutical preparations. The present research describes a simple, rapid, precise and accurate gradient mixer RP-HPLC method for the simultaneous determination of CIN and DOM in the tablet dosage form and validation of the same as per the ICH guidelines.

MATERIALS AND METHODS

Chemicals

Cinnarizine and Domperidone were gratis samples of Alkem Laboratories, Mumbai, India. HPLC grade methanol and acetonitrile were purchased from Merck (India) Ltd., Mumbai, India. All other chemicals are of analytical grade from S.D. Fine Chemical Ltd., Worli, India. Tablet formulation (VERTIGIL) was purchased from Indian market, containing CIN (20 mg), DOM (15 mg) and manufactured by Cipla Pvt. Ltd., India.

Instrumentation

Analysis was performed on HPLC instrument equipped with UV detector, Rheodyne injector and C-18 column compartment with EX1600SM software (Cyber Lab Corporation, USA). Other equipment used in the study were, UV-Visible Spectrophotometer (Evolution-201; Thermo Scientific, India) and ultrasonic bath (Amkette Industries, India).

Chromatographic conditions

C-18 column (250 mm × 4.6 mm i.d., 5 µm) was used for chromatographic separation. The mobile phase composed of methanol and acetonitrile (70:30 v/v); at a flow rate of 1.0 mL min^{-1} with run time of 10 min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter (Millipore, USA) and degassed. The detection of both drugs was carried out at 270 nm.

Method development

Standard stock solutions of 1mg mL^{-1} each of CIN and DOM were prepared separately using mobile phase. The CIN stock solution was diluted with mobile phase to give working standard solutions containing 40-160 µg mL^{-1} concentrations. Similarly the DOM stock solution was diluted with mobile phase to give working standard solutions in the range 45-105 µg mL^{-1}. These standard solutions were injected into HPLC column to determine the retention time (RT) and peak areas. The linearity was determined separately for CIN and DOM by injecting six concentrations of both drugs prepared in mobile phase and calibration curves were constructed by plotting peak area against the respective concentrations.

Validation of method

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing CIN and DOM. The method precision was carried out the analyte six times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of CIN and DOM. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analysed sample solution of CIN and DOM; a known amount of standard drug powder of CIN and DOM were added at 80, 100 and 120 % level. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Specificity of the method was determined through study of resolution factor of drug peak from the nearest resolving peak. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ) and was determined using the formulae; LOD = 3.3 × ASD/S and LOQ = 10 × ASD/S, where, ASD is the average standard deviation and S is the slope of the line.

Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length, flow rate and change in mobile phase composition. The robustness of the method was studied for CIN and DOM. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 100 µg mL^{-1} of CIN and 75 µg mL^{-1} solution of DOM, respectively. The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied. Six replicates of the samples solutions (20 µL) were injected for quantitative analysis.

Stability of sample solution was established by the storage of sample solution at 25°C for 24 and 48hr Sample solution was reanalyzed after 24 and 48 hr time intervals, and assay was determined for the compounds CIN and DOM and compared against fresh sample.

Analysis of marketed tablet formulation

To determine the content of CIN and DOM in tablets (Brand name: VERTIGIL, label claim: CIN 20 mg, DOM 15 mg per tablet), twenty tablets were weighed; the mean weight was determined and finely powdered. An accurately weighed tablet powder equivalent to 20 mg of CIN and 15 mg DOM was transfer into 100 mL volumetric flask containing 25 mL of mobile phase and sonicated for 10min. After achieving complete solubility of the drugs, the volume was made up to the mark using mobile phase. The resulting solution was filtered through 0.45 µm membrane filter. From the filtrate, a 5 mL of solution was transferred into 10 mL volumetric flask and volume was made up to mark with mobile phase to obtain the concentrations of 100 µg mL^{-1} CIN and 75 µg mL^{-1} of DOM which were then subjected to proposed method and the amounts of CIN and DOM were determined using calibration curves.

RESULTS

The proposed chromatographic system was found suitable for effective separation and quantitation of CIN (RT 3.389 min) and DOM (RT 4.793 min) with good resolution, peak shapes and minimal tailing. The overlay UV spectra and typical chromatogram were shown in Figures 1 and 2.

Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.999 for CIN and DOM. The CIN and DOM have followed linearity in the concentration range of 40– 160 and 45-105 µg mL^{-1} respectively (Figure 3). Percent recoveries for CIN and DOM were 115 and 105%. %RSD for tablet analysis, recovery studies and intra and inter-day precision studies was less than 2. LOD and LOQ were found to be 8.4µg/mL for CIN and 6.27µg/mL for DOM.

The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 0.94-0.8 and 1.17-1.78 % respectively. As the RSD values were < 2%, the developed method was found to be precise (Table 1). The accuracy of the method studied at three different concentration levels i.e. 80, 100 and 120 % showed acceptable recoveries in the range of 95.55-105.65 % for CIN and 101.7-104.1 % for DOM (Table 2).

The LOD for CIN and DOM was found to be 0.26 and 0.1 µg/mL respectively. Further the LOQ for CIN and DOM was found to be 0.8 and 0.58µg/mL respectively. Robustness of the method was studied by making deliberate changes in the chromatographic conditions like flow rate (± 0.2 mL/min.), wavelength (± 5nm) and mobile phase composition (± 5%) The validation parameters were summarized in Table 3.

The results of robustness study of the developed method is validated by change in flow rate, change in wavelength and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4).

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2 %) indicating ruggedness of the method. The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and shown in Table 3.

Stability of sample solution was established by the storage of sample solution at 25°C for 24 and 48 hr. Sample solution was reanalyzed after 24 and 48 hr. time intervals, and assay was determined for the compounds (CNN and DOM) and compared against fresh sample. Sample solution did not show any appreciable change in assay value (%RSD <2) when stored at ambient temperature up to 48 hr.

Six replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of CIN and DOM estimated were found to 99.9 and 100 %, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results were shown in Table 5.

TABLE 1: PRECISION OF DEVELOPED METHOD

S.No.	Method precision				Inter-day precision			
	CIN		DOM		CIN		DOM	
	RT	Area	RT	Area	RT	Area	RT	Area
1	3.35	119823.6	4.70	146612.2	3.48	130830.5	4.92	166581.7
2	3.35	117010.4	4.71	148631.4	3.49	130737	5.05	173631
3	3.33	117753.7	4.69	147892.0	3.53	130614.4	5.15	166637.3
4	3.35	117844.4	4.70	149666.5	3.50	134543.1	4.95	172708.5
5	3.35	118717.4	4.71	146643.1	3.54	131746.8	5.00	168538.7
6	3.35	117433.9	4.71	146517.2	3.56	132856.7	5.02	172020.8
Mean	3.34	118409.4	4.70	147660.3	3.51	131888.08	5.01	170019.6
±SD	0.0134	1118.592	0.0089	7065.689	0.014	1553.90	0.081	3029.17
% RSD	0.4	0.94	0.19	0.8	0.41	1.17	1.6	1.78

TABLE 2: ACCURACY DATA

% level of recovery	Area	Amount present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml ±SD)	recovery % ±SD	%RSD
CIN						
80	208077 ±951	100	80	176.13 ±0.81	95.55 ±1.07	1.12
100	235549.2 ±347.9	100	100	199.47 ±0.29	99.47 ±0.28	0.28
120	266459.7 ±1458.7	100	120	225.6 ±1.27	105.65 ±1.23	1.16
DOM						
80	264749.7 ±1888.3	75	60	135.75 ±0.63	101.7 ±1.2	1.2
100	298603.4 ±1577.9	75	75	154.13 ±1.26	104.9 ±1.07	1.02
120	326663.2 ±1109.3	75	90	168.13 ±0.6	104.1 ±0.75	0.72

TABLE 3: VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	CIN	DOM
Range (µg/ml)	40-160	45-105
Slope	29566	39230
Intercept	y=29566x+29668	y=39230x+23589
Correlation coefficient (R^2)	0.9991	0.998
Retention time (min ±SD)	3.389 ±0.005	4.793 ±0.005
Precision (intra and inter-day) %RSD	< 2	< 2
Accuracy (%)	95.55-103.65	101.7-104
LOD (µg/ml)	0.26	0.1
LOQ (µg/ml)	0.8	0.58
Tailing Factor	1.50	1.61
Theoretical Plates	2726	4648
Resolution	5.21	

TABLE 4: INFLUENCE OF FLOW RATE, WAVELENGTH AND MOBILE PHASE COMPOSITION ON ANALYTICAL PAPRAMETERS

Parameter	CIN			DOM		
	RT	Area	Tailing	RT	Area	Tailing
Flow rate (±0.2 ml/min)						
0.8	4.19	147006.2	1.78	5.87	188218.1	1.9
1	3.38	119869.2	1.50	4.79	145782.0	1.61
1.2	2.72	99828.1	1.80	3.82	122860.7	1.56
Wavelength (±5nm)						
265	3.34	71094.2	1.77	4.69	314421.7	1.75
270	3.38	119869.2	1.50	4.79	145782.0	1.61
275	3.24	172492.6	1.71	4.55	77445.6	1.71
Mobile phase composition (±5%v/v)						
65 : 35	3.94	127153.02	1.64	5.51	167746.0	1.75
70 : 30	3.38	119869.2	1.50	4.79	145782.0	1.61
75 : 25	3.68	111657.6	1.41	5.20	144197.5	1.48

TABLE 5: ASSAY OF COMMERCIAL FORMULATION

Drug	Lable claim (mg/tablet)	Calculated value (mg ±SD/tablet)	% of assay
CIN	20	19.98 ±0.04	99.9
DOM	15	15 ±0.02	100

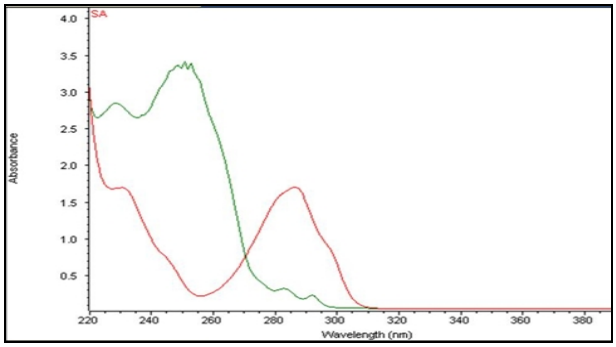

Figure 1. Overlay UV Spectra of standard CIN and DOM

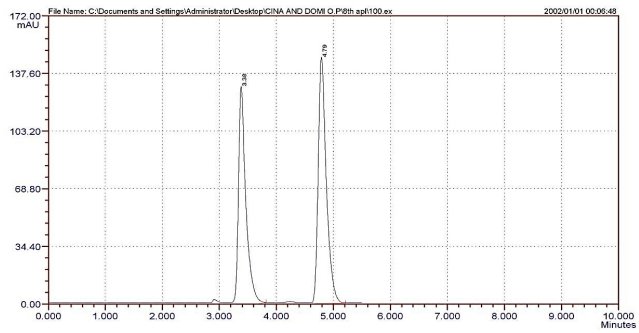

Figure 2. Typical HPLC chromatogram of CIN and DOM

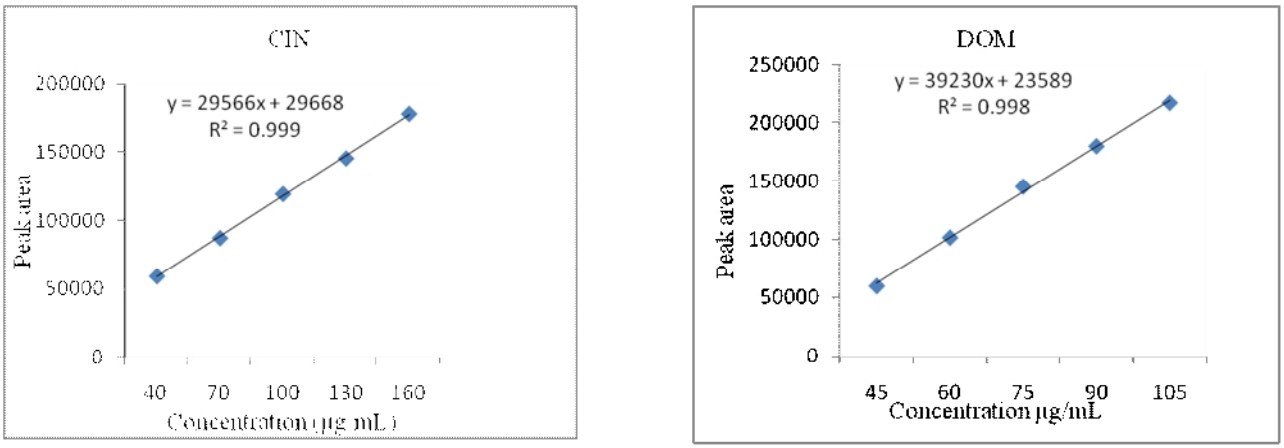

Figure 3. Calibration curves of CIN and DOM

DISCUSSION

The developed RP-HPLC method was found suitable for simultaneous estimation of CIN and DOM with good resolution, peak shapes and minimal tailing. The peak areas of the drugs were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.999 showed the stable linear detector response in different concentration ranges for both the drugs.

The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for CIN and DOM were 115 and 105% respectively indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of proposed method. The %RSD values of less than 2 for intra and inter-day variation studies indicated that the proposed method was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation in flow rate, wavelength and mobile phase ratio indicate that the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and % RSD was found to be less than 2 indicating the ruggedness of the proposed method.

The results from solution stability experiments confirmed that sample was stable up to 48 hr. during assay determination. The sample recoveries of CIN and DOM from the commercial tablet formulation were in good agreement with their respective label claim indicating that there was no interference from the commonly used tablets excipients.

CONCLUSION

The low standard deviation and % RSD calculated for the proposed method development and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of CIN and DOM in bulk and marketed formulation for routine quality control analysis.

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