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Method development and validation for simultaneous estimation of domperidone maleate and cinnarizine by RP-HPLC

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ABSTRACT

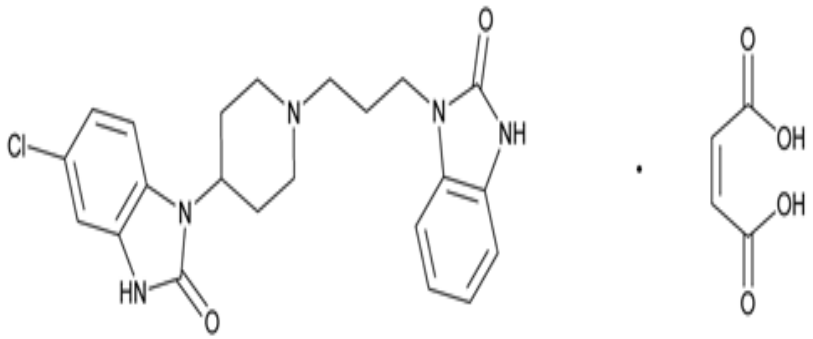
A validated RP-HPLC method has been developed for the determination of Domperidone maleate and Cinnarizine in tablet dosage form. This method is developed by using a Kromosil C_{18} column (250 cm length, 4.6 mm internal diameter and 5 µm particle size) and a mixture phosphate buffer pH 3.0, Acetonitrile and Methanol (40:40:20) as a mobile phase. The drug was quantified by a UV detector at 268nm. The method is linear for Domperidone maleate and Cinnarizine in range of 18 to 42 µg/ ml and 24 to 56 µg/ ml respectively. The Mean recovery of Domperidone maleate and Cinnarizine was found to be in the 99.73% to 99.23%. The Proposed method was found to be Linear, precise and accurate for the quantitative estimation of Domperidone maleate and Cinnarizine in tablets and can be used for commercial purposes.

Keywords: Domperidone maleate, Cinnarizine, HPLC, Validation.

DOMPERIDONE MALEATE

DESCRIPTION

Domperidone maleate is peripheral, specific blocker of dopamine receptors. Domperidone maleate is given in order to relieve nausea and vomiting; to increase the transit of food through the stomach.



STRUCTURE OF DOMPERIDONE MALEATE

IUPAC NAME

5-Chloro-1-[1-[3-(2-oxo-1, 3dihydrobenzoimidazol-1-yl) propyl] - 4- piperidyl]-1,3-dihydrobenzo imidazol- 2-one maleate

MOLECULAR FORMULA

$C_{22}H_{24}ClN_5O_2.C_4H_4O_4$

MOLECULAR WEIGHT: 541.99 g/mol

MECHANISM OF ACTION

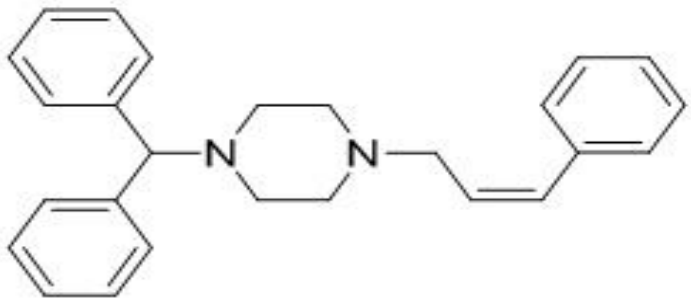
Domperidone maleate acts as a gastrointestinal emptying (delayed) adjunct and peristaltic stimulant. Domperidone maleate facilitates gastric emptying and decreases small bowel transit time by increasing esophageal and gastric peristalsis and by lowering esophageal sphincter pressure. The antiemetic properties of domperidone maleate are related to its dopamine receptor-blocking activity at both the chemoreceptor trigger zone and at the gastric level. It has strong affinities for the D2 and D3 dopamine receptors, which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which regulates nausea and vomiting.

INDICATION

Domperidone maleate is used for the treatment of emesis.

CINNARIZINE

Cinnarizine is a drug derivative of piperazine, and characterized as an antihistamine and a calcium channel blocker. It is more commonly prescribed for nausea and vomiting due to motion sickness or other sources such as chemotherapy, vertigo or Meniere's disease.



STRUCTURE OF CINNARIZINE

IUPAC NAME 1-(diphenylmethyl)-4-(3-phenylprop-2-en-1-yl) piperazine

MOLECULAR FORMULA: $C_{26}H_{28}N_2$

MOLECULAR WEIGHT: 368.5gms

CATEGORY

- Antiemetic
- Calcium Channel Blockers
- Anti-allergic agent

MECHANISM OF ACTION

Cinnarizine inhibits contractions of vascular smooth muscle cells by blocking L-type and T-type voltage gated calcium channels. Cinnarizine has also been implicated in binding to dopamine D2 receptors, histamine H1 receptors, and muscarinic acetylcholine receptors.

INDICATION

Cinnarizine is used for the treatment of allergic, vertigo or Meniere's disease.

EXPERIMENTAL EQUIPMENTS

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (Kromasil ODS, RP-18, 5µ, 250 mm × 4.6 mm) as

stationary phase. Thermo electron corporation double software), Ultrasonic cleaner, Shimadzu analytical balance AY-220,Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

MATERIALS

Pharmaceutically pure sample of Domperidone maleate and Cinnarizine were obtained as gift samples from Chandra Labs, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. HPLC-grade Acetonitrile was from Standard Reagents Pvt Ltd., and KH_2PO_4 (AR grade) was from Merck. A tablet formulation of Domperidone maleate and Cinnarizine (15 mg and 20 mg label claims) were procured from local market (Stugil, Johnson and Johnson Ltd., India).

CHROMATOGRAPHIC CONDITIONS

The sample separation was achieved on a C18 (5 µm, 25 cm X 4.6 mm i.d.) Kromasil ODS column, aided by mobile phase mixture of Phosphate Buffer buffer (30mM) pH: 3.0: Acetonitrile : Methanol (40: 20:40), that was filtered and degassed prior to use, at a flow rate of 1ml/min. Injection volume is 20 µl and detected at 268 nm at ambient temperatures.

PREPARATION OF MOBILE PHASE

BUFFER PREPARATION

Weigh accurately about 6.8 gms of KH_2PO_4 and dissolve with 500ml of HPLC Grade water than make up to 1000 ml with HPLC grade water then adjust the pH: 3.0 with Ortho-Phosphoric acid or Sodium hydroxide.

MOBILE PHASE

Then add 40 volumes of buffer, 20 volumes of Acetonitrile and 40 volumes of methanol solicited for 15 min and filtered through a 0.45 µ membrane filter.

ANALYSIS OF FORMULATION

PREPARATION OF STANDARD SOLUTION

A 15 mg of standard Domperidone maleate and 20 mg Cinnarizine were weighed and transferred to 100 ml of volumetric flask and dissolved in mobile phase. beam UV-visible spectrophotometer (vision pro-

The flask was shaken and volume was made up to mark with mobile phase to give a primary stock solution containing 15µg/ml Domperidone maleate and 20µg/ml of Cinnarizine. From the above solution 5ml of solution is pipette out into a 50 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 15µg/ml Domperidone maleate and 20µg/ml of Cinnarizine.

PREPARATION OF SAMPLE SOLUTION (TABLET FORMULATION)

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 15 mg Domperidone maleate and 20 mg Cinnarizine were accurately weighed and the powder was transferred to 100 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered by using Whitman filter no.41. from the above filtered clear solution 5ml of sample pipette out into a 50 ml volumetric flask volume made up to the mark with mobile phase to give a solution containing 15µg/ml Domperidone maleate and 20µg/ml of Cinnarizine.

RESULTS AND DISCUSSIONS

DETERMINATION OF WORKING WAVE LENGTH (ΛMAX)

10 mg of Domperidone maleate and Cinnarizine were weighed and dissolved in 25 ml methanol (400ppm). Prepare 20 µg /ml of solution by diluting 0.5ml to 10ml with methanol.

Method development work was started by taking UV-visible spectra from 400-200 nm of standard solutions (20µg/ml).

- UV Absorption spectrum of Domperidone maleate and Cinnarizine in Methanol was taken.
- The λmax of Domperidone maleate was found to be at 288 nm and λmax of Cinnarizine was found to be at 251 nm.

The isopiestic point of Domperidone maleate and of Cinnarizine was found to be at 268 nm. The U.V Graph shown in

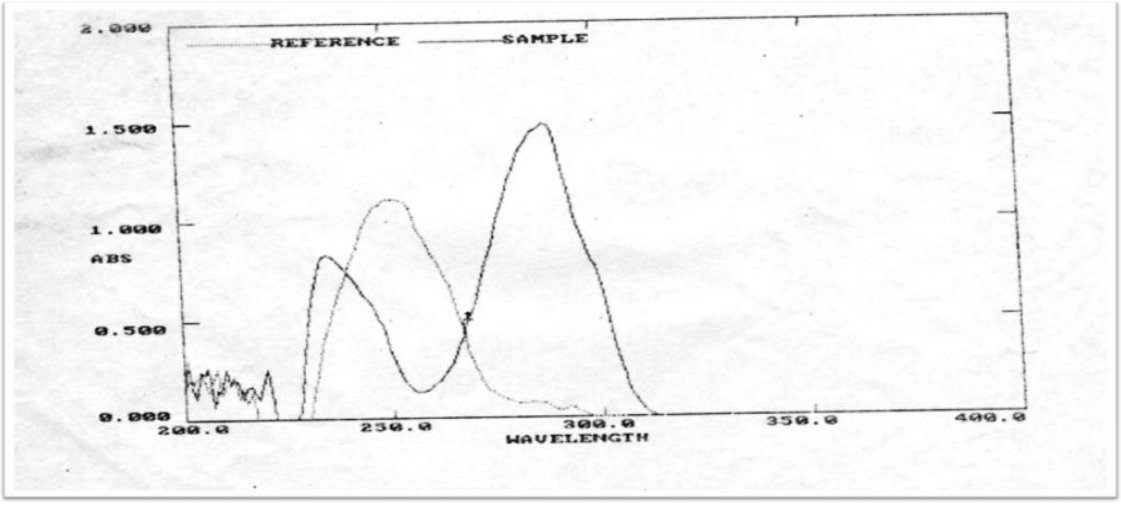


Figure 1: U.V Graph of Ambroxol HCl and Loratadine

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of Phosphate Buffer (30mM) pH: 3.0: Acetonitrile : Methanol (40:20:40) brought sharp and well resolved peaks. The chromatogram was shown in

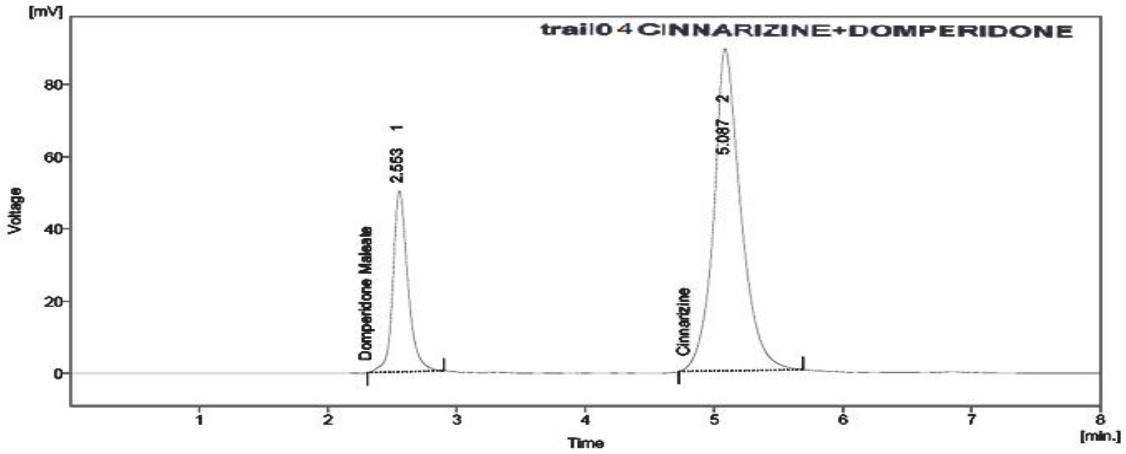


Fig.No.2

Figure 2: Chromatogram of Ambroxol HCl and Loratadine

METHOD VALIDATION

SYSTEM SUITABILITY

Weigh accurately about 15mg of Domperidone maleate and 20mg of Cinnarizine into a 100ml of clean and dry volumetric flask, add 70ml of diluents, shake and solicited to dissolve the content. Filter the solution through 0.45µm and make up the volume with diluents. Dilute 1ml of this solution in 10ml of diluents and prepare 6 replicate injections of standard.

SPECIFICITY (DIRECT COMPARISON METHOD)

The ability of the method to accurately measure the analytic response in the presence of all potential sample components, For specificity determination, all related substances of Domperidone maleate and

Cinnarizine solutions were prepared individually as per methodology and injected into HPLC to confirm the retention times. After that solutions of Domperidone maleate and Cinnarizine drug substances prepared in triplicate (control). Domperidone maleate and Cinnarizine drug substances spiked with all related substances prepared in triplicate (spiked samples) as per methodology and injected into HPLC to confirm any co-elution with Domperidone maleate and Cinnarizine peak from any of related substance peak and diluents.

LINEARITY AND RANGE

Weigh accurately about 15mg of Domperidone maleate and 20mg of Cinnarizine into a 100ml of clean and dry volumetric flask, add 70ml of diluents, shake and sonicated to dissolve the content, make up the volume with diluents. Filter the solution through 0.45µm. Dilute 0.6, 0.8. 1.2 and 1.4ml of this solution to10ml with diluents and prepare 5 different dilutions.

ACCURACY

Accuracy of the method was determined by recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120% for Domperidone maleate and Cinnarizine. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels.

PRECISION

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. Precision depends only on number of distribution of random errors and does not relate to the true or specified valve. The measure of precision is usually expressed in term of imprecision and computed as a deviation of the results.

METHOD PRECISION

Six sample solutions were prepared individually using single batch of Domperidone maleate and Cinnarizine as per test method and injected each solution into HPLC.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD and LOQ of Domperidone maleate and Cinnarizine were determined by calibration curve method. Solutions of both Domperidone maleate and Cinnarizine were prepared in the range of 18-42µg/ml and 24-56 µg/ml, respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration.

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

ROBUSTNESS

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like different flow rates and wavelengths. It provides an indication about variability of the method during normal conditions of laboratory.

RUGGEDNESS

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the assay by two different analysts, different column, on different day.

DISCUSSION

Development of new analytical methods for the determination of drugs in Pharmaceutical dosage forms is more important and is quite challenging problem. The developed and validated method was aimed to establish chromatographic conditions, capable of qualitative and quantitative determination of Domperidone maleate and Cinnarizine in tablets. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of Phosphate buffer (KH_2PO_4) pH3.0: Methanol: Acetonitrile (40:20:40v/v/v), with detection of 268 nm. Linearity (Fig.No.3; Table No.3) was observed in the range 18-42µg/ml (R^2=0.999) for Domperidone maleate and 24-56µg/ml (R^2=0.998) for Cinnarizine for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The percentage recoveries of Domperidone maleate and cinnarizine were 99.96% and 100.85% (Table

No.4).The method developed was robust (Table No. 6) against changes in flow rate, wavelength and rugged (Table No. 7) against changes in analyst, column and days. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2.The % purity of Domperidone maleate and Cinnarizine were 99.73% and 99.23%.

Table No. 1: System suitability of Domperidone maleate and Cinnarizine

Injection	Area for Domperidone maleate	Area for Cinnarizine
1	403.605	1315.84
2	401.825	1301.81
3	402.640	1313.11
4	405.499	1317.28
5	403.605	1315.84
6	402.412	1310.54
Mean	403.264	1312.403 744.87647.464744.8764744.8764744.8764
SD	1.297	5.722
%RSD	0.32	0.44
USP Plate count	2235	3136

Table No. 2: Specificity of Domperidone maleate and Cinnarizine

Domperidone maleate	Purity (%w/w)	Cinnarizine	Purity(%w/w)
Control-1	99.3	Control-1	99.2
Control-2	99.8	Control-2	99.5
Spiked sample-1	99.5	Spiked sample-1	99.8
Spiked sample-2	99.3	Spiked sample-2	99.3
Mean (control)	**99.55**	Mean (control)	**99.35**
SD	0.32	SD	0.31
%RSD	0.4	%RSD	0.31
Mean (spiked)	**99.4**	Mean (spiked)	**99.5**
SD	0.25	SD	0.24
%RSD	0.3	%RSD	0.24
% Difference	0.15	% Difference	0.2

Table No. 3: Linearity of Domperidone maleate and Cinnarizine

Domperidone maleate			Cinnarizine	
S. No	Con.mcg	Area	Con.mcg	Area
1	18	278.67	24	801.32
2	24	354.22	32	1059.31
3	30	415.49	40	1337.28
4	36	486.21	48	1588.90
5	42	560.16	56	1855.14

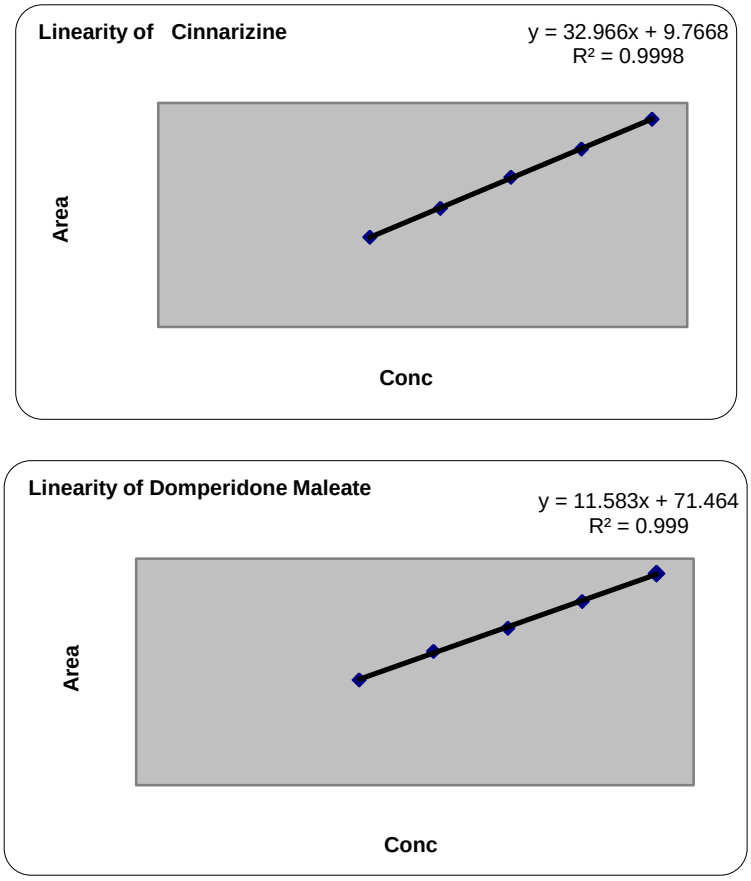


Fig. No.3: Linearity graphs of Cinnarizine and Domperidone maleate

Table No.4: Recovery results for Domperidone maleate and Cinnarizine

	Domperidone maleate		**Cinnarizine**		
Standard Area	12mcg	395.982	**Standard Area**	16mcg	1202.436
	15mcg	457.154		20mcg	1494.851
	18mcg	529.685		24mcg	1730.097

Accuracy of Domperidone maleate					**Average % Recovery**
Amount taken(mcg/ml)	**Amount added**	**Area**	**Average area**	**%Recovery**	
12mcg	3mcg	395.982	395.772	99.32	
		394.557			
		396.783			

15mcg	3mcg	455.355			99.96%
				99.92	
		457.154	456.793		
		457.511			
18mcg	3mcg	527.397			
		527.397		100.27	
		529.685	528.160		

Accuracy Cinnarizine					Average % Recovery
Amount taken(mcg/ml)	Amount added	Area	Average area	%Recovery	
16mcg	4mcg	1202.436			
		1201.922	1208.01	101.37	
		1219.691			
20mcg	4mcg	1487.546			
		1494.851	1489.60	101.26	100.85%
		1486.412			
24mcg	4mcg	1730.097			
		1730.097	1732.25	100.63	
		1736.576			

Table No.5: Method precision of Domperidone maleate and Cinnarizine

Injection	**Area for Domperidone maleate**	**Area for Cinnarizine**
1	403.605	1315.84
2	401.825	1301.81
3	402.640	1313.11
4	405.499	1317.28
5	403.605	1315.84
6	402.412	1310.54
Mean	403.264	1312.403 744.87647.464744.8764744.8764744.8764
SD	1.297	5.722
%RSD	0.32	0.44

Table No.6: Robustness study

	DOMPERIDONE MALEATE		CINNARIZINE	
Parameter	**Retention time(min)**	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	2.542	1.484	5.041	1.354
1.2 ml/min	2.100	1.500	4.017	1.275
Wavelength				
266nm	2.540	1.483	5.040	1.356
270nm	2.547	1.482	5.050	1.358

Table No.7: Ruggedness

DOMPERIDONE MALEATE	**%purity**	**CINNARIZINE**	**%purity**
Analyst 01	99.73	Analyst 01	99.90
Analyst 02	99.80	Analyst 02	99.80
%RSD	0.05%	%RSD	0.071%

Table No.8 LOD and LOQ

Domperidone maleate			**Cinnarizine**	
S. No	**Con.mcg**	**Area**	**Con.mcg**	**Area**
1	18	278.67	24	801.32
2	24	354.22	32	1059.31
3	30	415.49	40	1337.28
4	36	486.21	48	1588.90
5	42	560.16	56	1855.14
SD	9.474			12.684
Slope	11.58		32.96	

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Domperidone maleate and Cinnarizine was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries.

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