

SIMULTANEOUS ESTIMATION, VALIDATION, AND FORCED DEGRADATION STUDIES OF BETAHISTINE DIHYDROCHLORIDE AND DOMPERIDONE IN A PHARMACEUTICAL DOSAGE FORM USING RP-HPLC METHOD

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ABSTRACT

Objective: This study describes the stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous estimation of betahistine dihydrochloride and domperidone in pharmaceutical dosage forms.

Methods: The proposed RP-HPLC method was developed using Shimadzu Prominence-i LC-2030 HPLC system equipped with UV detector and chromatographic operation was carried on Shim-pack C18 (250 mm×4.6 mm, 5 μ) column at a flow rate of 1 ml/min and the run time was 10 min. The mobile phase consisted of methanol and water in the ratio of 80:20% v/v and eluents were scanned using a UV detector at 244 nm.

Results: The retention time of betahistine dihydrochloride and domperidone was found to be 2.3 and 3.6 min, respectively. A linearity response was observed in the concentration range of 9.6 μg/ml–22.4 μg/ml for betahistine dihydrochloride and 6–14 μg/ml for domperidone, respectively. Limit of detection and limit of quantification for betahistine dihydrochloride were 0.52 μg/ml and 1.58 μg/ml and for domperidone are 0.64 μg/ml and 1.94 μg/ml, respectively.

Conclusion: The stability-indicating method was developed by subjecting drugs to stress conditions such as acid and base hydrolysis, oxidation, photo and thermal degradation, and degraded products formed were resolved successfully from samples.

Keywords: Betahistine dihydrochloride, Domperidone, Reverse-phase high-performance liquid chromatography, Degradation, Validation.

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INTRODUCTION

Betahistine dihydrochloride (Fig. 1) is chemically N-methyl-2-pyridine-ethanamine, a well-known antvertigo drug [1]. It is official in Indian Pharmacopoeia [2], the British Pharmacopoeia [3], European Pharmacopoeia [4], and the United States Pharmacopoeia (USP) [5]. It possesses affinity as an antagonist of H₃ receptors and a weak affinity as an agonist for H₁ receptors. The active ingredient is a mainly a histamine agonist with no H₂ activity.

Domperidone (Fig. 2) 5-chloro-1-[1-[3-(2-Oxo-2,3-dihydro-1Hbenzimidazol-1-yl) propyl]-piperidin-4-yl]-1,3-dihydro-2H benzimidazol-2-one acts by selectively antagonizing the peripheral dopaminergic D₂ receptors in the gastrointestinal wall, thereby enhancing gastrointestinal peristalsis and motility and increasing lower esophageal sphincter tone. It is a dopamine (D₂) receptor antagonist. DMP is used for the treatment and prevention of acute nausea and vomiting of any cause, especially cytotoxic therapy and radiotherapy. According to Biopharmaceutical Classification System, DMP is classified under Class-II drugs which are poorly soluble and highly permeable [6].

Literature survey reveals high-performance liquid chromatography (HPLC) for estimation of betahistine dihydrochloride and domperidone and is not available for pharmaceutical dosage form. Therefore, the present study was carried out to develop novel, simple, precise, rapid, and cost-effective RP-HPLC method for the simultaneous estimation of betahistine dihydrochloride and domperidone in pharmaceutical dosage form [7,8].

METHODS

Betahistine dihydrochloride and domperidone standard were provided by Clearysynth Labs Ltd., Mumbai, Maharashtra, India, and Indoco

Remedies, Navi Mumbai, Maharashtra, India. Commercial tablet dosage form VERTISTAR PLUS was purchased from local markets. The HPLC-grade methanol and water were purchased from Thomas Fisher Scientific Pvt., Ltd., Powai, Mumbai, Maharashtra, India. Hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D. Fine Chemicals.

HPLC instrument

The chromatographic separation was carried out by Shimadzu prominence-i LC-2030 HPLC system equipped with UV detector and autosampler. The Software Lab solution was used for signal monitoring and processing, UV chamber has been used for photolytic degradation, and hot air oven was employed for thermal degradation.

Chromatographic conditions

The chromatographic separation of analytes was carried out using Shimadzu reverse-phase (RP)-HPLC system with Shim-pack GIST C18 (250×4.6 mm) column. The mobile phase consists of water and methanol in the ratio of 20:80% v/v, and column temperature was maintained at 40°C. The analytes were detected at 244 nm using UV detector. The run time was set at 10 min at a flow rate of 1 ml/min.

Preparation of standard stock solution

Standard stock solution of betahistine dihydrochloride and domperidone was prepared separately by dissolving 100 mg of betahistine dihydrochloride and 100 mg of domperidone in 100 ml of volumetric flask with water:methanol (20:80) as diluents and sonicated for 10 min. From the above solution, 0.16 ml of betahistine dihydrochloride and 0.1 ml of domperidone were transferred separately to 10 ml volumetric flask, made up the volume to get 16 μg/ml and 10 μg/ml of stock solution of betahistine dihydrochloride and domperidone, respectively.

Preparation of sample solution

10 tablets (Vertistar Plus tablet; 16 mg betahistine dihydrochloride and 10 mg domperidone) were weighed and the average weight of each tablet was calculated; then, the weight equivalent to 10 tablets was transferred into 100 ml volumetric flask; 50 ml of diluents was added and sonicated for 30 m; further, the volume made up with diluents and filtered. From the filtered solution, 1 ml was pipette out into 10 ml volumetric flask and sonicated for 10 min, and volume made up to 10 ml with diluents.

Forced degradation studies

Forced degradation studies [9,10] of the drug formulation were carried out by treating the drug samples under stress-induced conditions such as acid, base hydrolysis, oxidation, humidity, photodegradation, and thermal degradation. These studies help to know the inherent stability characteristic of the active molecule in drug product and possible degradation products.

Acid degradation

Acidic degradation was carried out by adding 5 ml of 1 N HCl to sample solutions and after 60 min neutralizing the mixture by adding 5 ml of 1 N NaOH.

Alkali degradation

Alkali degradation was carried out by adding 5 ml of 1 N NaOH and after 60 min neutralizing the mixture by adding 5 ml of 1 N HCl.

Oxidative degradation

Oxidative degradation was performed by exposing the drug to 5 ml of 10% (v/v) H₂O₂ for 60 min.

Photolytic degradation

Photolytic degradation was carried out by exposing the drug content to UV light inside a UV chamber for 2 days.

Thermal degradation

Thermal degradation was performed by placing the drug in an oven at 95°C for 24 h to study dry heat degradation.

Statistical analysis

To evaluate the contribution of each factor with different levels of responses, two-way analysis of variance was performed using GraphPad Prism 7.04 software.

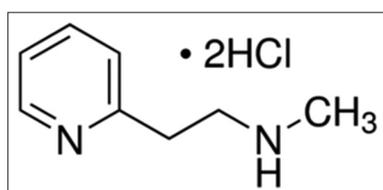


Fig. 1: Betahistine dihydrochloride

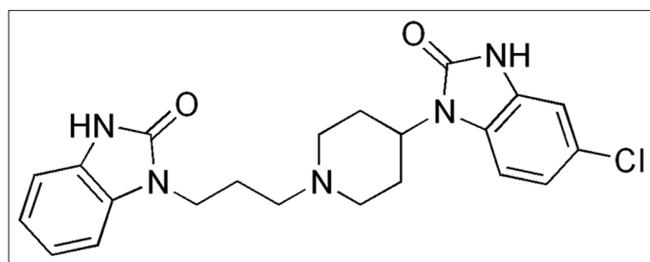


Fig. 2: Domperidone

RESULTS AND DISCUSSION

Method development

Using different columns, a series of trials was conducted, with different mobile phases to develop suitable RP-HPLC method for estimation of betahistine dihydrochloride and domperidone in tablet dosage form, and finally, a typical chromatogram was obtained with water and methanol in the ratio of 20:80% v/v. The chromatographic separation was performed on Shim-pack C-18 (250 mm×4.6 mm, 5 μ) column, on injecting 10 μL and the analytes were detected with UV detector at 244 nm. The retention time of betahistine dihydrochloride and domperidone was found to be 2.3 min and 3.6 min, respectively. The force degradation study was also carried out using developed method. The optimized conditions were given in Table 1.

Method validation

The validation was performed with above developed RP-HPLC method for simultaneous estimation of betahistine dihydrochloride and domperidone according to ICH guidelines. Various parameters were evaluated such as system suitability, precision, accuracy, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System suitability

System suitability was conducted to verify the acceptability of the resolution and repeatability of the system. System suitability was performed by injecting six replicate injections of the standard solution (100%) (Fig. 3) and parameters such as peak area, USP tailing, theoretical plates, retention time, and peak asymmetry were evaluated. The percentage relative standard deviation (RSD) was determined and reported within the limits (Table 2).

Accuracy

The accuracy of the proposed method was evaluated by calculating the recovery studies of the test drug at three different concentration levels (80%, 100%, and 120%) by the standard addition method. A known amount of betahistine dihydrochloride and domperidone was added to the pre-quantified sample solution, and three replicates of each concentration were injected into developing chromatographic conditions. The mean percentage recovery of betahistine dihydrochloride and domperidone was varied between 99.2 and 100.8% indicating that the developed method was found to be accurate (Table 3).

Precision

The precision of an analytical procedure may be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Optimized conditions
1	Column	Shim-pack C-18 (250 mm×4.6 mm, 5 μ) column
2	Mobile phase	Methanol and water 80:20
3	Flow rate	1 ml/min
4	Wavelength	UV detector 244 nm
5	Injection volume	10 μL
6	Temperature	40°C
7	Retention time	Betahistine dihydrochloride 2.3 min and domperidone 3.6 min

Table 2: System suitability parameters

S. No	Parameters	Betahistine dihydrochloride	Domperidone
1	Retention time	2.3	3.6
2	USP plate count	4890	2059
3	USP tailing	1.39	1.61

USP: United States Pharmacopeia

Table 3: Percentage recovery results of betahistine dihydrochloride and domperidone

S. No	Spiked	Percentage recovery		Mean percentage recovery	Betahistine dihydrochloride	Domperidone
		Betahistine dihydrochloride	Domperidone			
1	80%	99.12 99.78 99.56	99.23 99.37 99.16	99.3	0.34	0.10
2	100%	99.7 99.22 98.97	99.12 98.5 98.81	99.0	0.37	0.31
3	120%	101.09 100.54 100.90	101.36 100.05 100.45	100.7	0.27	0.66

prescribed conditions. The method precision and system precision studies were carried out by injecting six replicates of both standard and test solutions with the same concentration. The percentage RSD was calculated from the chromatograms, and the results obtained were within the limits of 2% and proposed method was found to be precise (Table 4).

Linearity

The linearity of the method was determined at different concentration levels ranging from 6 to 14 µg/ml of betahistine dihydrochloride and from 9.2 to 22.4 µg/ml of domperidone. All the concentrations were prepared and injected into the system. The linearity curve was constructed by plotting peak area versus concentration of the analyte. From the results obtained, the proposed method was found to be linear. The regression coefficient was found to be 0.998 and 0.998 for betahistine dihydrochloride and domperidone, respectively (Fig. 4).

LOD and LOQ

In the present study, the LOD and LOQ betahistine dihydrochloride and domperidone were evaluated based on the standard calibration curve method. LOD is performed to know the lowest concentration level of the analytes that give a measurable response. LOD and LOQ for betahistine dihydrochloride are 0.52 µg/ml and 1.58 µg/ml and for domperidone are 0.64 µg/ml and 1.94 µg/ml, respectively.

Robustness

Robustness of the proposed method has been evaluated by small deliberate changes in the system parameters such as flow rate, wavelength, and temperature. It was found that none of the above parameters caused alteration in the peak area, retention time, and USP tailing by small changes such as ±0.2 ml change in flow rate, ±2 nm wavelength, and ±2°C change in temperature. The percentage RSD was found to be within the limits and the method was found to be robust (Table 5).

Assay of marketed formulation

Analysis of the marketed formulation (Vertistar plus tablets: betahistine dihydrochloride is 16 mg and domperidone is 10 mg) was purchased from local markets. 10 tablets weighed and average weight was calculated; weight equivalent to 10 tablets was transferred into 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 30 min, and further volume was made up with diluents and filtered. From filtered solution, 1 ml was pipette out into 10 ml volumetric flask and made up to 10 ml with diluents. From the resulting solution, 10 µL was injected into HPLC system and peak areas were recorded. The percentage assay of the marketed formulation was found to be 98.7 for betahistine dihydrochloride and 98.6 for domperidone (Table 6).

Forced degradation studies

ICH degradation was attempted to various stress conditions such as acid hydrolysis (using 1 N HCl), base hydrolysis (using 1 N NaOH), oxidative hydrolysis (using 5% H₂O₂), thermal degradation (heated at

Table 4: Results of method precision for betahistine dihydrochloride and domperidone

S. No	Sample no	Betahistine dihydrochloride	Domperidone
		% assay	% assay
1	Injection 1	98.71	99.43
2	Injection 2	98.37	98.67
3	Injection 3	99.72	98.21
4	Injection 4	98.59	99.48
5	Injection 5	98.47	98.54
6	Injection 6	98.30	98.30
7	Average	98.70	98.77
8	SD	0.52	0.55
9	%RSD	0.53	0.56

SD: Standard deviation, RSD: Relative standard deviation

Table 5: Results of robustness

S. No.	Parameters	Betahistine dihydrochloride			Domperidone		
		RT	NTP	TF	RT	NTP	TF
1	Flow rate 0.9 ml	2.6	5670	1.4	4.0	1999	1.8
	Flow rate a 1.1 ml	2.1	4550	1.4	3.3	1899	1.7
2	Temperature 38	2.3	4951	1.5	3.6	2031	1.8
	Temperature 42	2.3	5008	1.4	3.6	1971	1.8
3	Wavelength 242	2.3	5036	1.4	3.6	2013	1.79
	Wavelength 246	2.3	5093	1.4	3.6	2043	1.78

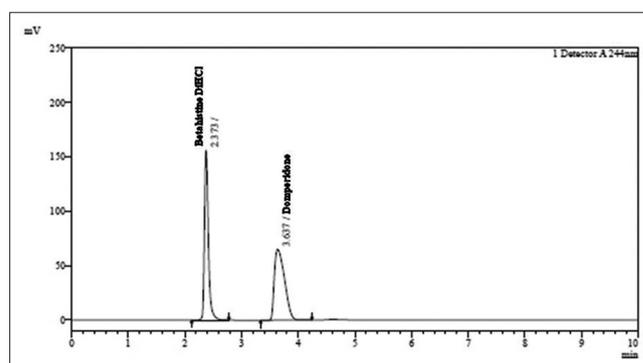


Fig. 3: Chromatogram of standard betahistine dihydrochloride and domperidone

100°C for 24 h), and photolytic degradation (using UV light inside a UV chamber for 24 h). The results of stress studies were shown in Table 7 (Figs. 5-10).

Table 6: Percent content of marketed formulation

S. No.	Tablet	Drug	Amount taken	Amount found	% assay
1	Vertistar plus (betahistine dihydrochloride 16 mg and domperidone 10 mg)	Betahistine dihydrochloride	16	15.79	98.68
		Domperidone	10	9.94	99.4

Table 7: Forced degradation studies of betahistine dihydrochloride and domperidone

S. No	Stress condition	Betahistine dihydrochloride		Domperidone	
		% assay	% difference w.r.t control	% assay	% difference w.r.t control
1	Control	98.6	NA	99.4	NA
2	Acid degradation	87.77	12.22	88.47	11.52
3	Base degradation	89.68	10.31	92.85	7.14
4	Oxidative degradation	95.69	4.30	96.68	3.31
5	Photolytic degradation	96.85	3.14	98.31	1.68
6	Thermal degradation	98.26	1.73	98.88	1.11

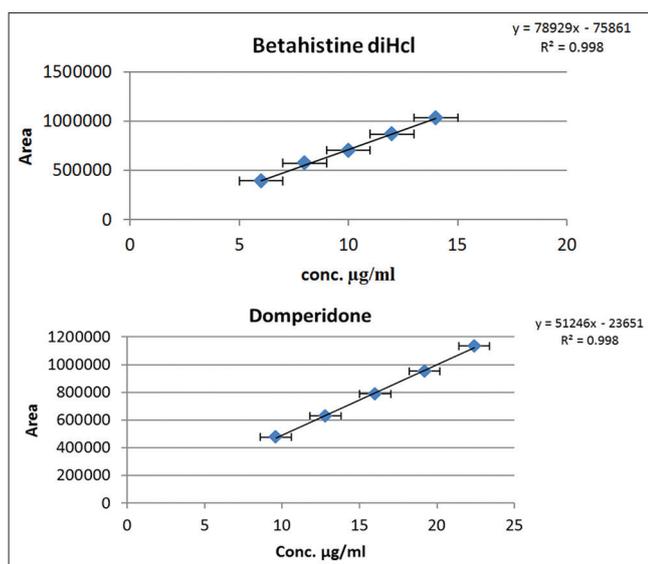


Fig. 4: Linearity graph of betahistine dihydrochloride and domperidone

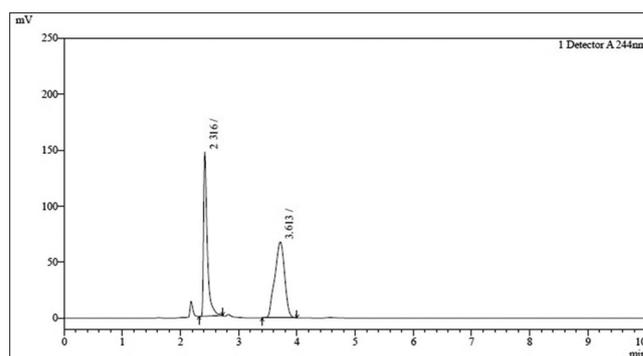


Fig. 6: Chromatogram of acid degradation

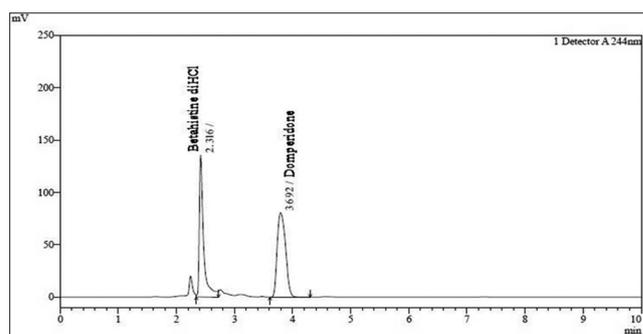


Fig. 7: Chromatogram of base degradation

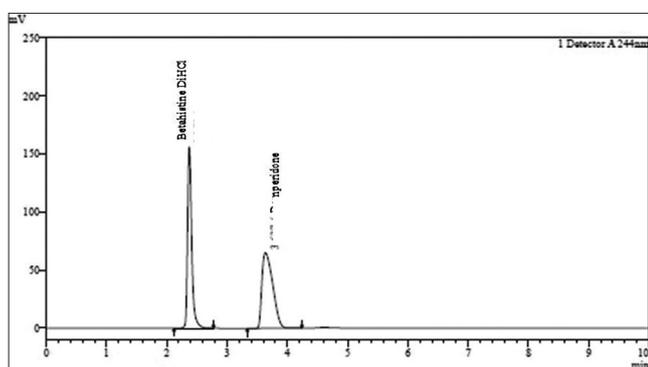


Fig. 5: Chromatogram of untreated tablet (sample)

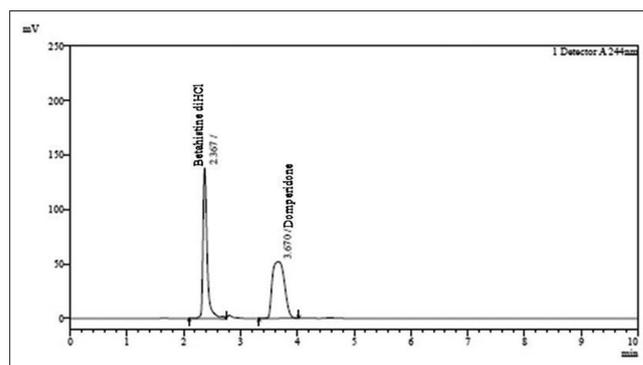


Fig. 8: Chromatogram of UV degradation

CONCLUSION

In the present study, a stability-indicating RP-HPLC method has been developed and validated for simultaneous estimation of betahistine dihydrochloride and domperidone in tablet dosage form. The validated method was successfully used for stress testing, analysis of betahistine dihydrochloride and domperidone. The proposed method was proved

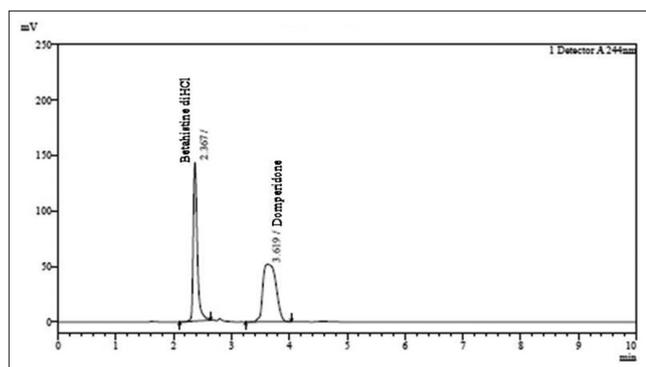


Fig. 9: Chromatograph of thermal degradation

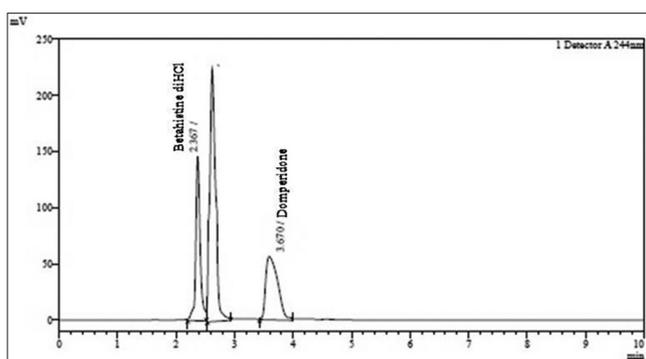


Fig. 10: Chromatograph of oxidative degradation

selective, accurate, precise, and rapid, and it can be used for the routine analysis of betahistine dihydrochloride and domperidone in formulation.

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AUTHOR'S CONTRIBUTION

We declare that this study was performed by the authors mentioned in this article and all liabilities relating to claims relating to the substance of this article will be borne by the authors. Mr. Rohan Mishra collected the data, analyzed the data, all the laboratory work performed, and wrote the introduction, discussion, and the material and method part. Mrs. Vaishali Mistry contributed to designing and conducting the study, also proofread the whole manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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