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Research Article

DEVELOPMENT AND VALIDATION OF RP - HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF FAMOTIDINE AND DOMPERIDONE IN PHARMACEUTICAL DOSAGE FORM

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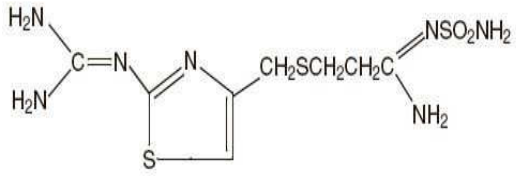
ABSTRACT

A simple, sensitive, and precise RP-High Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Famotidine (FAM) and Domperidone (DOM) combined dosage form (EMETEC) has been developed and validated. The components were well separated using Phenomenex-C_{18} (4.6 mm id, 250 mm, 5µm) column using Methanol: 0.1% ortho phosphoric acid in water (55:45% v/v) as mobile phase at a flow rate of 1.0 mL/min. The eluents were detected at 280 nm using UV detector. The retention time of FAM was found to be 1.69 min and that of DOM was 3.23 min. The linearity was observed between 2.5 to 50 µg/mL for both FAM and DOM. The marketed dosage form was analyzed by using the developed method. The percent content of FAM was 98.56±0.83 and of DOM was 99.36±0.60. The method was validated for system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits. The developed method was used for the stability studies (short, long and auto sampler) and forced degradation studies (acidic, alkaline, oxidative and photolytic). Both FAM and DOM were found to be stable in all conditions except alkaline conditions. This validated method can be used for the routine quality control testing of FAM and DOM combined dosage form.

Keywords: Famotidine, Domperidone, RP-HPLC.

INTRODUCTION

Chemically famotidine (FAM) is 3-([2-(diaminomethyleneamino) thiazol-4-yl] methylthio)-N-sulfamoylpropanimidamide[1] (**Fig. 1**) and is a H_2 receptor antagonist. They can inhibit histamine-gastrin and acetylcholine stimulated acid secretion; pepsin secretion also falls with the reduction in volume of gastric juice[2]. Domperidone (DOM) is chemically 5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl) propyl] piperidin-4-yl)-1H-benzo[d] imidazol-2(3H)-one[1] (**Fig. 2**) and is a D_2 receptor antagonist that acts centrally on the chemoreceptor trigger zone (CTZ) and also has a peripheral action on the gastrointestinal tract itself [2]. FAM is official in IP and USP. DOM is official in IP and BP. There is an immense need to develop a validated analytical method for the simultaneous estimation of FAM and DOM in pharmaceutical dosage forms. Several methods like HPLC[3-8], LC-MS[9-11], HPTLC[12] and UV-Visible spectrophotometric[13-17] have been reported for the quantitative determination of FAM, DOM in bulk, pharmaceutical and biological samples and in combination with other drugs. The literature review revealed that only HPTLC and UV-spectrophotometric methods were available and no RP-HPLC method was found for the simultaneous estimation of FAM and DOM combined dosage forms. Hence an attempt was made to develop and validate an analytical method for the simultaneous estimation of FAM and DOM combined dosage form using RP-HPLC method.



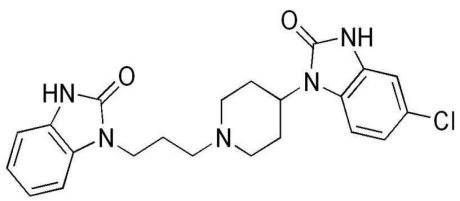


Fig. 1: Structure of Famotidine Fig. 2: Structure of Domperidone

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade methanol and other analytical grade reagents were purchased from Merck, India. Water - HPLC grade was prepared using Milli-Q water purification system. EMETEC tablets were purchased from local markets of Visakhapatnam. Class A Glassware is used throughout the experiment. Famotidine (FAM) and Domperidone (DOM) gift samples were obtained from Oyster labs ltd., Ambala Cantt.

Equipment & Chromatographic conditions

The chromatographic system consists of a Shimadzu class VP Binary pump LC-10ATvp Pump, SIL-10ADvp auto sampler, CTO-10Avp Column temperature oven, SPD-10Avp UV-Visible detector. All the components of the system are controlled using SCL-10Avp system controller. Data acquisition was done using LC Solutions version 1.23 software. Chromatographic separations were carried out using Phenomenex C_{18} (4.6 mm id, 250mm, 5µ) reverse phase column with a mobile phase consisting of Methanol and 0.1% orthophosphoric acid (55:45% v/v) at a flow rate of 1.0 mL/min. The eluents were monitored at 280 nm.

Preparation of solutions

Stock solution

10 mg of DOM and 10 mg of FAM were weighed separately and transferred into a 10 mL volumetric flask. The compounds are then dissolved separately in Methanol. The final equivalent concentrations of DOM and FAM are approximately 1000 µg/mL.

Mobile Phase

The HPLC grade solvents were used for the preparation of mobile phase. 0.1% OPA was prepared by dissolving 500 µL of OPA in 500 mL of water. Mobile phase was prepared by mixing 55 mL of methanol and 45 mL of 0.1% OPA. This mobile phase was filtered through 0.45 µ membrane filter and then it was sonicated for 30 min.

Calibration standards and Quality controls

Calibration standards of FAM and DOM were prepared at concentrations of 2.5, 5, 10, 20, 30, 40, 50µg/mL from a standard

solution of 1000µg/mL by appropriate dilution with mobile phase. Four quality control (QC) samples were prepared at concentrations of 2.5, 15, 25, 37.5µg/mL of FAM and DOM representing lower limit of quantification (LLOQ), low, medium, high concentrations of the linearity range were prepared from the standard solutions respectively.

Sample solutions

Twenty EMETEC tablets (20mg of FAM and 10mg of DOM) made into fine powder, an equivalent weight of powder containing 20mg of FAM and 10mg of DOM was accurately weighed and diluted with methanol in a 10 mL volumetric flask, the contents were shaken for ten minutes and then centrifuged. The clear supernatant liquid was sonicated in ultrasonic bath for 5 minutes and filtered through 0.45 µm membrane filter; final volume was made up with methanol. The sample solution was prepared within the linearity ranges of both the drugs using mobile phase.

Method Validation

The developed chromatographic method was validated for system suitability, linearity & range, specificity, selectivity, accuracy, precision, ruggedness and robustness as per ICH guidelines[18, 19].

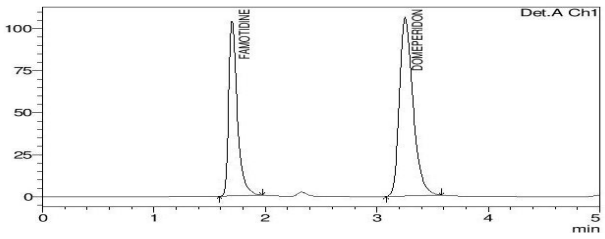


Fig. 3: Chromatogram of Famotidine and Domperidone

RESULTS AND DISCUSSION

Method Development and Optimization

The standard solutions of FAM and DOM were scanned in UV-Vis Spectrophotometer and the λ_{max} of FAM and DOM were found to be 268 nm and 294 nm respectively. The iso- absorptive point of the combined spectrum of both drugs at 280 nm was chosen for the detection of the drugs. Different permutations and combinations at different pH (3 to 11), using various columns (Hypersil-BDS-C18, Symmetry C18, Ymc C18, Sperisorb C18, Phenomenex C18, different buffers using ammonium acetate, ortho phosphoric acid, acetic acid & potassium dihydrogen phosphate along with acetonitrile and methanol were used as mobile phase for optimizing the method. Efficient separation with good resolution factors obtained with Phenomenex C_{18} column, methanol: 0.1 % OPA in water (55:45 v/v) as mobile phase, at a flow rate of 1 mL/min. Under these conditions FAM and DOM were eluted at 1.69 min and 3.23 min respectively with a run time of 5 min. A chromatogram of FAM and DOM was shown in **Fig. 3**.

Method Validation

System Suitability

The system suitability was assessed by six replicate analyses of the drugs at concentrations of 25µg/mL of FAM and DOM. The % CV of peak area and retention time for the both drugs FAM and DOM are within 2 % indicating the suitability of the system. Results are shown in Table 1.

Specificity

The specificity of the method is performed by separate injections of the blank, DOM, FAM and combined DOM and FAM samples. The specificity chromatogram was shown in **Fig. 4**, where the retention time of FAM does not interfere with the retention time of the DOM.

Linearity & Range

The calibration curve was constructed and evaluated by its correlation coefficient. The peak areas of FAM and DOM were linear in the range of 2.5 to 50µg/mL. Calibration curves of FAM and DOM were shown in **Fig. 5 & 6.** The linearity results were shown in **Table 2**.

Accuracy and Precision

Accuracy and precision studies were carried for the QC samples during the intra-day and inter-day runs and the obtained results were shown in **Table 3** and **Table 4**. All the data were within the acceptance criteria of 5% (except 10% for LLOQ).

Limit of detection and quantification

Limit of detection (LOD) and quantification (LOQ) were estimated from signal to noise ratio. The limit of detection (LOD) and quantification (LOQ) value for FAM and DOM were 0.3125µg/mL and 0.925µg/mL respectively. Chromatograms were shown in Fig. 7 and Fig. 8 Results are shown in Table 5.

Table 1: System suitability

	FAM		DOM	
	Retention Time (min)	**Peak Area**	**Retention Time (min)**	**Peak Area**
Mean (n=6)	1.695	585851	3.238	993073
S.D.	0.0122	47152	0.0075	133725.8
% CV	0.72	8.05	0.23	13.47

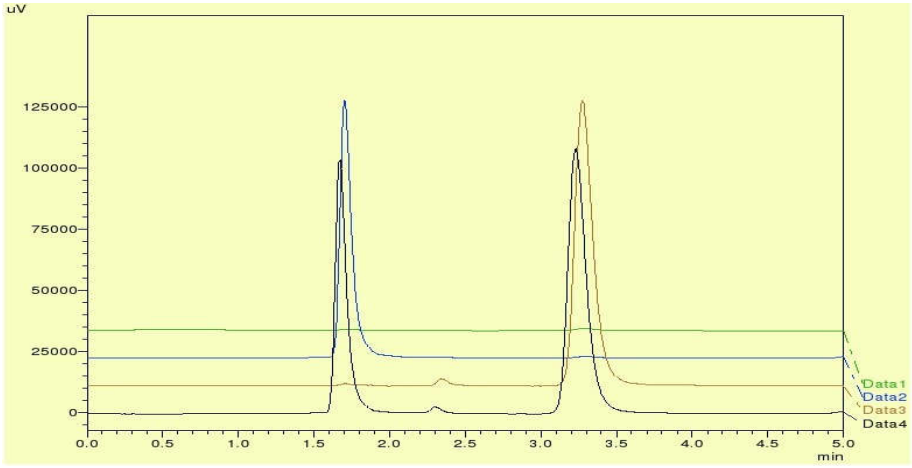


Fig. 4: Overlay chromatogram for specificity

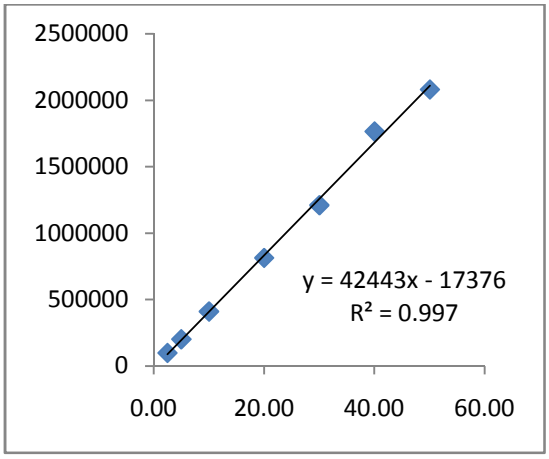


Fig. 5: linearity graph of Famotidine

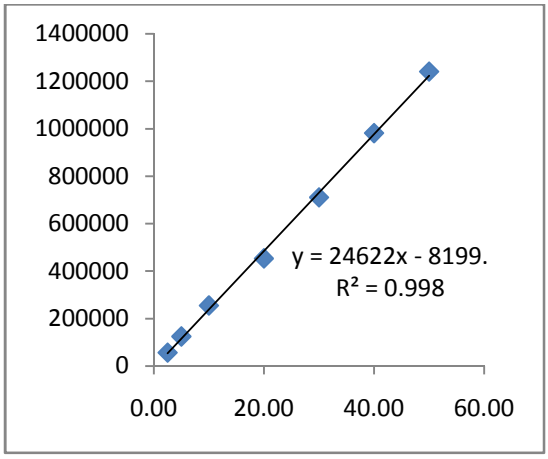


Fig. 6: linearity graph of Domperidone

Table 2: Results of regression analysis of the linearity data of FAM and DOM

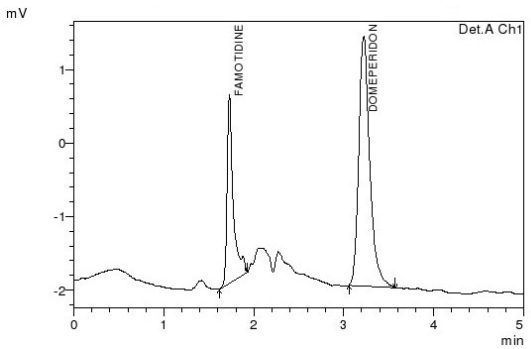
Parameters	FAM (mean of n=3)	DOM (mean of n=3)
Slope	24864 ± 107	42584 ± 116.4
Intercept	8280.8 ± 53	17434.3 ± 121.2
Correlation coefficient	0.9983 ± 0.0005	0.9972 ± 0.0007

Table 3: Intra and inter-day accuracy and precision of FAM

	LLOQ	LQC	MQC	HQC
INTRA-DAY				
Mean	2.6	14.12	25.6	36.36
SD	0.039	0.46	0.59	1.44
%RSD	1.51	3.32	2.30	3.95
Recovery (%)	105.4	94.15	102.76	97.24
INTER-DAY				
Mean	2.6	15.09	25.57	37.97
SD	0.09	0.5	0.47	0.36
%RSD	3.48	3.32	1.86	0.97
Recovery (%)	104.2	100.6	102.31	101.27

Table 4: Intra and inter-day accuracy and precision of DOM

	LLOQ	LQC	MQC	HQC
INTRA-DAY				
Mean	2.6	14.92	26.84	37.78
SD	0.122	0.327	0.84	1.66
%RSD	4.68	2.20	3.14	4.41
Recovery (%)	104.6	99.53	105.64	100.76
INTER-DAY				
Mean	2.63	15.37	25.57	37.8
SD	0.08	0.36	0.42	0.27
%RSD	3.04	2.39	1.64	0.74
Recovery (%)	105.26	102.48	102.31	100.8



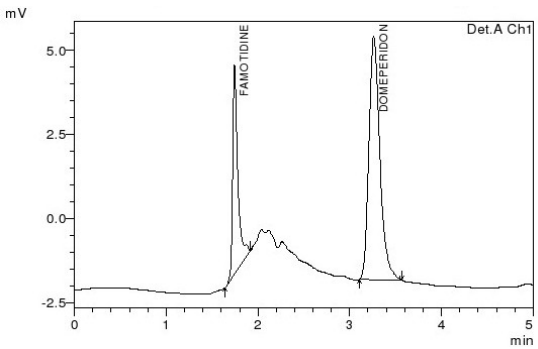


Fig. 7: chromatogram for LOD Fig. 8: chromatogram for LOQ

Table 5: LOD & LOQ of FAM and DOM

Drug name	Parameter	Peak area	Tailing factor	Theoretical plates
FAM	LOD	7462	1.7	7534
	LOQ	15303	1.7	10223
DOM	LOD	8952	1.2	8952
	LOQ	31040	1.3	9395

Ruggedness

The Ruggedness was determined by using the data obtained by the analysis performed by two different analysts. Each analyst prepared 5 samples of the same batch and the results obtained. Results are shown in **Table 6**.

Robustness

Robustness is the measure of method capacity to retain unaffected by deliberate small changes in the chromatographic conditions. The impact of flow rate (± 0.1) and effect of mobile phase composition (± 5%) was evaluated on the important system suitability factors such as retention time; theoretical plates and tailing factor were studied. Results were shown in Table 7.

Stability

Stability studies indicate that samples were stable when kept at bench top for 6 hours (short term), in auto sampler for 24 hours and when refrigerated at 4 for 30 days (long term). These stability studies results were given in **Table 8** and the percent ratios were within the acceptance range of 90 to 110 %.

Stress Testing

The stress studies involving acid, alkali, photolytic and oxidation revealed that FAM and DOM were not fully degraded. However in alkaline conditions (0.1N NaoH) DOM and FAM peaks were distorted. Except for alkaline conditions, the drugs content were within 90-110 % for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks. Results are shown in **Table 9**.

Application of method to dosage form

The developed method was used for the quantitative estimation of FAM and DOM in commercial dosage form EMETEC tablets. Each sample was analyzed in triplicate after extracting the drugs. None of the tablet ingredients were interfered with the analyte peak. Results are shown in **Table 10**.

Table 6: Ruggedness

	Drug name	Rt	Tailing factor	Theoretical plates	Resolution
Analyst 1	Famotidine	1.74	1.5	6611	-
	Domperidone	3.44	1.4	9312	12.1
Analyst 2	Famotidine	1.72	1.5	6629	
	Domperidone	3.42	1.4	9521	12

Table 7: Robustness studies of FAM and DOM

	Parameters	Variation	Rt	Tailing factor	Plate count
FAM	Flow rate	0.9 mL/min	1.93	1.5	7399
		1.1 mL/min	1.55	1.5	6001
	Mobile phase	50% organic phase	1.75	1.5	7015
DOM		60% organic phase	1.64	1.3	6606
	Flow rate	0.9 mL/min	3.8	1.4	9994
		1.1 mL/min	5.61	1.1	8317
	Mobile phase	50% organic phase	4.5	1.3	9719
		60% organic phase	2.4	1.3	9171

Table 8: Short term, long term and auto sampler stability of the FAM and DOM

Short term stability	FAM			DOM		
	Nominal concentrations (µg/mL)			Nominal concentrations (µg/mL)		
	15	25	37.5	15	25	37.5
%Recovery	102.12	101.35	100.36	102.34	100.23	100.56
SD	1.20	0.56	2.20	1.56	2.54	0.56
%RSD	1.18	0.57	2.18	2.46	4.61	3.74
Long term stability						
%Recovery	99.52	100.95	99.92	98.68	99.54	102.58
SD	2.56	1.09	2.31	1.35	1.74	0.86
%RSD	2.59	3.21	3.28	3.45	2.20	2.85
Auto sampler stability						
%Recovery	100.76	99.28	101.86	101.43	102.65	99.84
SD	1.56	1.65	2.45	0.86	1.45	1.65
%RSD	2.86	3.49	3..65	2.51	3.58	2.58

Table 9: Forced degradation studies

	FAM			DOM		
	Mean	SD	%RSD	Mean	SD	%RSD
Oxidation	104.32	0.65	2.68	96.36	2.7	2.63
Light	94.08	0.82	2.61	108.05	2.4	1.82
Acid	97.46	1.23	3.31	103.74	3.1	2.29
Alkaline	Peak distorted			Peak distorted		

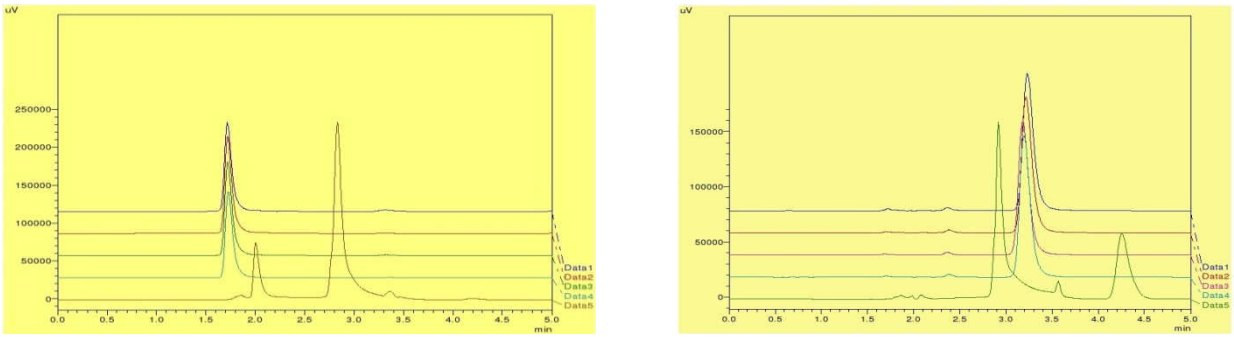


Fig. 9 & 10: chromatograms of FAM & DOM stress degradation

Table 10: Results of marketed product

Marketed formulation	Drug	Mean	SD	%RSD
EMETEC	FAM-20mg	98.56	0.83	0.85
	DOM-10mg	99.36	0.60	0.61

CONCLUSION

The new HPLC method was developed and validated for the simultaneous determination of FAM and DOM in combined pharmaceutical dosage form and was found to be accurate, precise, simple, economic, rapid and having good specificity, selectivity and stability. The developed method can be used for the routine analysis of the combined formulations.

ACKNOWLEDGEMENT

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