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Stability indicating HPLC method development and validation for simultaneous determination of dimethicone and mosapride in bulk and tablet dosage form

Narottam Pal[1]*, A. Srinivasa Rao[2] and P. Ravikumar[3]

[1]*Assistant Professor, Department of Pharmaceutical Analysis, Bhaskar Pharmacy College (JB Group of Educational Institutions), Yenkapally (V), Moinabad (M), R.R.District, Hyderabad-500075*
[2]*Principal, Bhaskar Pharmacy College(JB Group of Educational Institutions), Yenkapally (V), Moinabad (M), R.R. District, Hyderabad-500075*
[3]*Manager, Bio-analytical Department, Aizant Drug Research Solution Pvt. Ltd, Hyderabad, India, PinCode-50014*

ABSTRACT

The present work is a method development and validation for the simultaneous determination as well as stability studies for the bulk and combined tablet formulation of Dimethicone and Mosapride by using reverse phase High performance liquid chromatography (HPLC) with isocratic elution where the stationary phase used was BDS column (250 mm, 4.6 mm, 5 µm), mobile phase was buffer (Ortho phosphoric acid 0.1%) and Acetonitrile at the ratio of 55:45. pH of the chromatographic system was maintained at 3.0, flow rate 1.2 ml/minute, eluent was monitored by PDA detector wavelength at 274 nm. Retention time was found to be 2.369 minutes and 3.433 minutes, correlation coefficient 0.999 and 0.998, LOD 0.207 and 0.002, LOQ 0.628 and 0.007 for Dimethicone and Mosapride respectively. Linearity range was designed 62.5 µg/ml to 375 µg/ml for Dimethicone and 2.5 µg/ml to 15 µg/ml for Mosapride. Accuracy study revealed percentage recovery 99.7%-100.87% and 100.02%-100.20%, repeatability results in terms of relative standard deviation (%RSD) 0.92 and 1.0 for Dimethicone and Mosapride respectively. The developed method was validated as per ICH guideline and was found to be an ideal one for regular analysis in the laboratory.

Key words: HPLC, isocratic elution, simultaneous, Dimethicone and Mosapride.

INTRODUCTION

Dimethicone belongs to group of compounds, more commonly known as Silicones, are chemically polymers in nature [1, 2]. Among all the polymers Poly dimethyl silicone is extensively used in pharmaceutical field and also known as Poly dimethyl siloxane. For better physico-chemical and pharmacological properties of such compounds Dimethicone is well accepted among the medical fraternity especially in gastrointestinal care. Physiologically Dimethicone coalesces the smaller bubbles of gas in the gastrointestinal tract and thereby help in releasing it easily. This compound is also used in personal care products as an anti-foaming agent, skin protect ant, and skin and hair conditioner [3]. Figure 1. Represents the structure of Dimethicone.

Mosapride is a selective agonist of 5HT4 receptor. These types of receptors are mainly distributed in lower gastrointestinal tract. Hence administration of the drug in our body improves the gastrointestinal motility in lower

tract. After metabolism of Mosapride the metabolite also finds act on the site as it is 5HT3 agonist and thereby increases overall intestinal motility. The direct benefit of the drug for a patient is like a reduced gastric emptying time [4, 5]. Chemically Mosapride is known as (RS)-4-amino-5-chloro-2-ethoxy-N-{[4-(4-fluorobenzyl) morpholin-2-yl] methyl}benzamide.

Figure 2.Represents the structure of Mosapride.

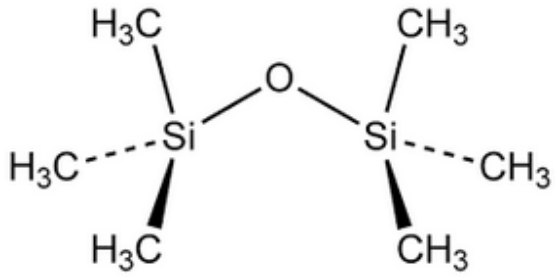

Figure 1: Structure of Dimethicone

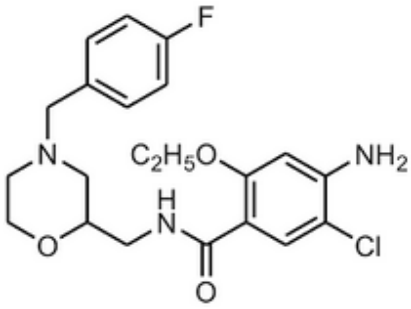

Figure 2: Structure of Mosapride

After going through literature [6-14] it was observed that from the time of launching of these drugs there have been continuous effort putting on establishing several methods for the determination of these compounds either individually or in different combinations. Authors like Hegazy M. A. et al innovated a spectrophotometric method for the determination of Mosapride combined with Pantoprazole. G.Manasa et al developed a HPLC method for Mosapride combined with Pantoprazole. Patel B. H. et al developed a HPLC method and TLC method for Mosapride combined with Rabeprazole, but no method was developed for the determination of combined formulation of Dimethicone and Mosapride. Hence, we made a whole hearted sincere attempt to come out with a simple method for the estimation of above said combination by High Performance Liquid Chromatography.

MATERIALS AND METHODS

Instruments: HPLC Waters 2695 Separations Module equipped with Quaternary pump with Auto sampler and Auto injector, PDA Detector 2996, Sonicator (Sartorius), Digital balance (Sartorius-M500P), PH meter (Thermo scientific).

Chemicals: All the chemicals and reagents were of analytical grade. Adequate amount of concentration was drawn while choosing various chemicals [15, 16]. Various materials were Reference standard, tablet samples, ortho phosphoric Acid (RFCL), Acetonitrile (Rankem), Methanol (Merck) and Milli Q water (Rankem).

Chromatographic Condition: Mobile Phase- Buffer : Acetonitrile (55:45), Stationary Phase- BDS column (250 mm, 4.6 mm, 5 µ), Flow rate- 1.2 ml/min, p^H 3.0, Temperature 30 ºC, Detecting wave length 274 nm.

Diluent: First dissolved in Methanol and made up the volume with the mixture of water and Acetonitrile (50:50).

Experimental:
Preparation of standard: Accurately Weighed and transferred 125 mg & 5 mg of Dimethicone and mosapride working Standards into 50 ml clean dry volumetric flasks, added $3/4^{th}$ volume of diluent, sonicated for 30 minutes and made up to the final volume with diluents. From the above stock solution, 1 ml was pipeted out in to a 10ml Volumetric flask and then made up to the final volume with diluent.

Preparation of sample: Ten tablets were weighed and calculated the average weight of each tablet then the weight equivalent to 125 mg & 5 mg of Dimethicone and mosapride was transferred into a 50mL volumetric flask, 30mL of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made the volume up to 10ml with diluent.
System suitability: The method was developed after varying many chromatographic parameters [17]. Dimethicone and Mosapride got eluted in reasonable time and were acceptable for regular analytical work when a C18 column

was used with mobile phase like aqueous and organic phases were taken at the ratio of fifty five and forty five. 150 mm column length was sufficient for considerable separation. Theoretical plates, tailing factor, resolution results also were satisfactory.

Validation Parameters: The method was validated as per ICH Guideline [18]. The validation parameters considered were accuracy, precision, intermediate precision, linearity, limit of detection, limit of quantification and robustness studies.
Accuracy: The accuracy of the methods was tested by calculating recoveries of Dimethicone and Mosapride by the standard addition method. Correct amounts of standard solutions of Dimethicone and Mosapride (each 50%, 100%, and 150%) were spiked to pre-quantified sample solutions. The amounts of each compounds recovered were estimated.

Precision: The instrumental repeatability or precision including intermediate precision was checked by repeatedly injecting six replicates containing Dimethicone (250 ppm) and Mosapride (10 ppm) at day one and day two. For each injection concentration representing areas were studied. Retention time, number of theoretical plates, peak symmetry, and peaks resolution were under observation.

Linearity: Linearity tests were conducted for both the molecules having concentration range-
Dimethicone : 62.5 ppm, 125 ppm, 187.5 ppm, 250 ppm, 312.5 ppm, 375 ppm

Mosapride: 2.5 ppm, 5 ppm, 7.5 ppm, 10 ppm, 12.5 ppm, 15 ppm. Concentration Vs area was used to build calibration curve. Results of correlation Coefficient (r), Slope of Regression, SD of Slope, Regression Intercept, %RSD of Intercept were under observation.

LOD and LOQ: Limit of detection and Limit of quantification were calculated by using following Equations. LOD = $3.3\times\sigma/S$ and LOQ = $10\times\sigma/S$, where σ indicates the standard deviation of y intercepts of regression lines and S indicates the slope of the calibration curve.

Robustness: Robustness test was performed by varying different chromatographic parameters like temperature, flow rate, mobile phase composition etc.

Assay of marketed formulation: The formulation (Tablet-MozaMPS) was purchased from local medical store. Ten tablets were taken, weighed, triturated to powder and collected a quantity equivalent to Dimethicone 125 mg and Mosapride 5 mg in a 50 ml volumetric flusk. Dissolved in diluent, sonicated and made the volume with diluent. Pipetted out 1 ml of the solution into a 10 ml volumetric flusk, made the volume with diluent. 20 µl was injected to the column and result was compared with standard in terms of area response.

Stability Studies: Force degradation studies [19] were conducted by providing different physico-chemical environment. The procedure for stability studies are described bellow. Figure 4 to Figure 9 represents the chromatograms due to stress degradation of the compounds.

Oxidative Degradation Studies: To 1 ml of stock solution of Dimethicone and Mosapride, 1 ml of 20% hydrogen peroxide (H_2O_2) was added. The solutions were kept for 30 min at 60^0c. The resultant solution was diluted to obtain 250µg/ml and 10µg/ml solution of Dimethicone and Mosapride respectively and 10 µl of the solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock solution Dimethicone and Mosapride, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 250µg/ml and 10µg/ml Dimethicone and Mosapride solution and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample in acidic environment.

Alkali Degradation Studies: To 1 ml of stock solution Dimethicone and Mosapride, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 250µg/ml and 10µg/ml Dimethicone and Mosapride solution respectively and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 250µg/ml&10µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo (UV) Stability Studies: The photochemical stability of the drug was also studied by exposing the 250µg/ml&10µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 250µg/ml&10µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral (water) Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 250µg/ml&10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS

Method development: To develop a new method for estimation and degradation studies several trials were conducted so that we can achieve most suitable chromatographic condition. The initial attempt was to employ as much low proportion of organic solvents for elution of the compounds. More part of aqueous solvents in mobile phase resulted in prolonging of retention time of both the compounds. Reasonable retention time, number of theoretical plates, value of tailing factors and all were found within the validation limit by using optimized Chromatographic condition. Figure 3 represents a typical chromatogram of Dimethicone and Mosapride.

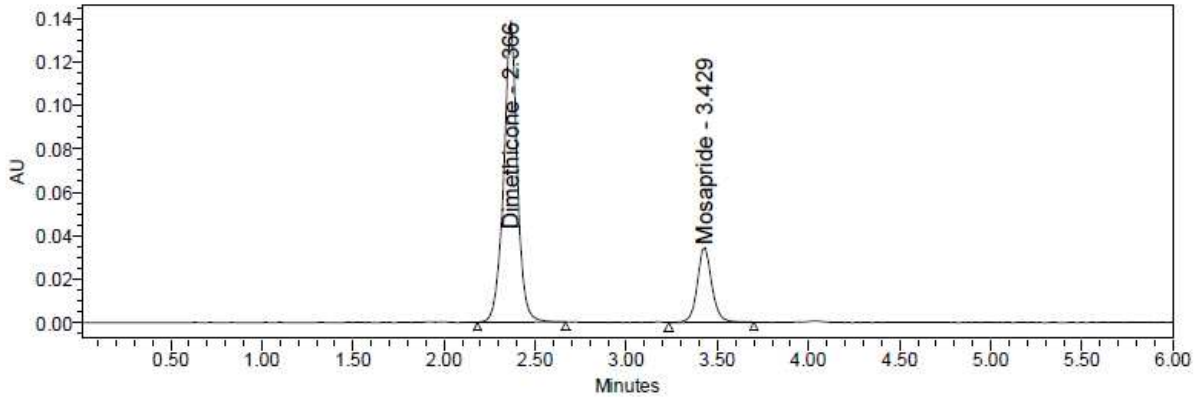

Figure 3: A typical chromatogram of Dimethicone and Mosapride

All the validation parameters were studied by using optimized chromatographic condition, results obtained were as follows-

Regression analysis: Test for linearity showed that mean value of Y intercept, slope, and correlation coefficient of Dimethicone was found to be 964, 2851, 0.999 at concentration range of 62.5 µg/ml to 375 µg/ml. The mean value of Y intercept, slope, and correlation coefficient of Mosapride was found to be 888, 20268, and 0.998 at concentration range of 2.5 µg/ml to 15 µg/ml.

Table 1 contains details of results for regression studies.

Accuracy Results: Recovery of Dimethicone and Mosapride standard 50%, 100% and 150% were 99.7, 100.26, 100.78 and 100.02, 100.14, 100.20 in terms of percentage respectively. Table 2. describes all the details of recovery studies

Precision: Intraday precision result in terms of mean area and %RSD for Dimethicone standard injection were – 720232 and 0.46. For Dimethicone sample injection results were 7214923 and 0.92. For Mosapride standard injection results were 190920 and 1.0, for Mosapride sample injection results were 191392 and 1.0.

Inter day precision result in terms of mean area and %RSD for Dimethicone sample injection were 716279.7 and 0.96.

Intraday precision result in terms of mean area and %RSD for Mosapride sample injection were 190174 and 1.2.

LOD, LOQ: For sensitivity test LOD of Dimethicone and Mosapride were 0.207 and 0.002. LOQ of Dimethicone and Mosapride were 0.628 and 0.007 respectively. Table 3 Represents results of precision and sensitivity studies.

Robustness Results: As far as robustness is concern, by varying different chromatographic parameters like reducing and increasing flow rate that is 1 ml/minute and 1.4 ml/minute of mobile phase the retention time was observed ranging from 2.363 minutes to 2.585 minutes and 3.422 minutes to 3.756 minutes for Dimethicone and Mosapride respectively.

Table 1: Regression Analysis of Calibration Curve

Parameters. (n=6)	**Dimethicone**	**Mosapride**
Linearity (µgm/ml)	62.5 - 375	2.5 – 15
Correlation Coefficient.(r)	0.999	0.998
Slope of Regression (mean)	2851	20268
SD of Slope	0.369	0.193
Regression Intercept (mean)	964	888
%RSD of Intercept	1.02	1.19

Table 2: Accuracy Results

Amount of Sample µg /ml (n=3)		**Amount of Standard µg /ml (n=3)**		**Amount recovered µg /ml**		**% recovered**	
Dimethi	Mosapri	Dimethi	Mosapri	Dimethi	Mosapri	Dimethi	Mosapri
250	10	125	5	124.63	5.001	99.7	100.02
250	10	250	10	250.65	10.104	100.26	100.14
250	10	375	15	378.26	15.03	100.78	100.2

Table 3: Precision and sensitivity

Parameters	**Dimethicone**	**Mosapride**
Retention time	2.368	3.433
LOD	0.207	0.002
LOQ	0.628	0.007
Accuracy%	99.7 – 100.87	100.02 – 100.20
Intraday precision RSD%	0.92	1.0
Interday precision RSD%	0.96	1.2

Table 4: Summary of Robustness Study

	Chromatographic Condition	**Retention Time (minutes)**	**USP Theoretical Plates**	**Asymmetric Factor**	**% Assay**
Dimethicone	Flowrate1.0 ml/min	2.585	4773	0.98	100.89
	Flowrate1.4 ml/min	2.363	4789	0.97	99.01
	Buffer : ACN (60:40)	2.333	4016	0.99	99.58
	Buffer : ACN (50:50)	2.362	4775	0.96	99.04
	Temperature (25^0c)	2.585	4778	0.97	100.11
	Temperature (35^0c)	2.365	4790	0.97	99.24
Mosapride	Flowrate1.0 ml/min	3.756	9950	1.07	100.88
	Flowrate1.4 ml/min	3.422	9666	1.07	99.14
	Buffer: ACN	3.346	9580	1.08	99.66
	Buffer: ACN	3.422	9487	1.08	100.22
	Temperature (25^0c)	3.756	9979	1.06	99.99
	Temperature (35^0c)	3.421	9696	1.09	99.36

Percentage recovery of drugs at the similar chromatographic conditions for Dimethicone and Mosapride it was found to be 100.89% and 99.01%, 100.88% and 99.14% respectively. Results of other parameters for both the drugs are mentioned in Table 4.

System suitability: Mean retention time for Dimethicone and Mosapride were 2.368 minutes and 3.433 minutes. Mean theoretical plates for Dimethicone and Mosapride were found to be 5055 and 9659. Asymmetric factors were 0.98 and 1.10, peak resolution was 7.43. Table 5 describes details of precision analysis.

Table 5: System suitability parameters

Parameters	Dimethicone	Mosapride
Retention time	2.368	3.433
%RSD of retention time	0.171	0.222
Theoretical plate	5055	9659
Asymmetric factor	0.98	1.1
Resolution	-----	7.43

Results of degradation studies: Stability studies were conducted in different physicochemical conditions like acid, alkali, peroxide, UV radiation, elevated temperature and neutral conditions. Solutions of the drugs shown that they were stable enough under stress environment. Very minor degradation was observed in acid, alkali and oxidative conditions. Figure 4 to 9 represent chromatogram after different stress condition. Table 6 to Table 11 describes results of force degradation.

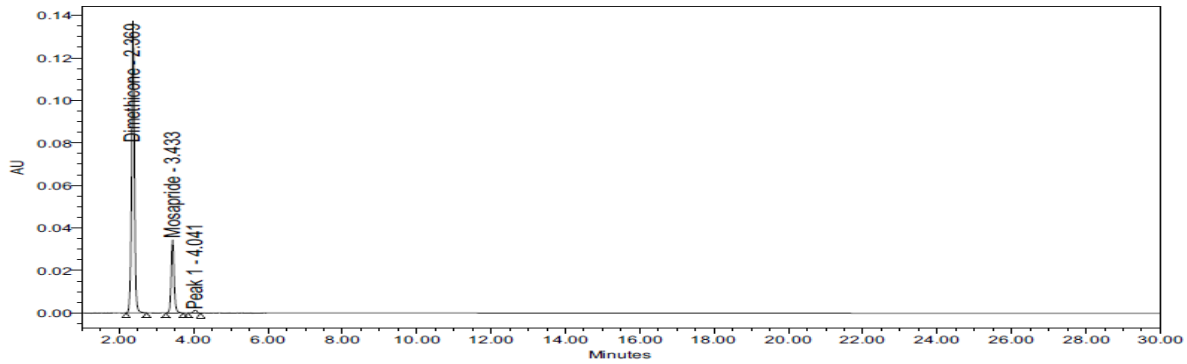

Figure 4: Chromatogram after acid stress

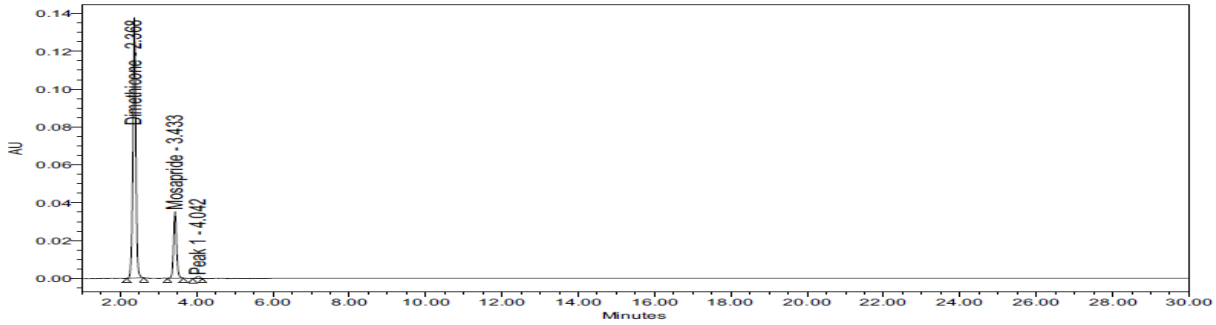

Figure 5: Chromatogram after alkali stress

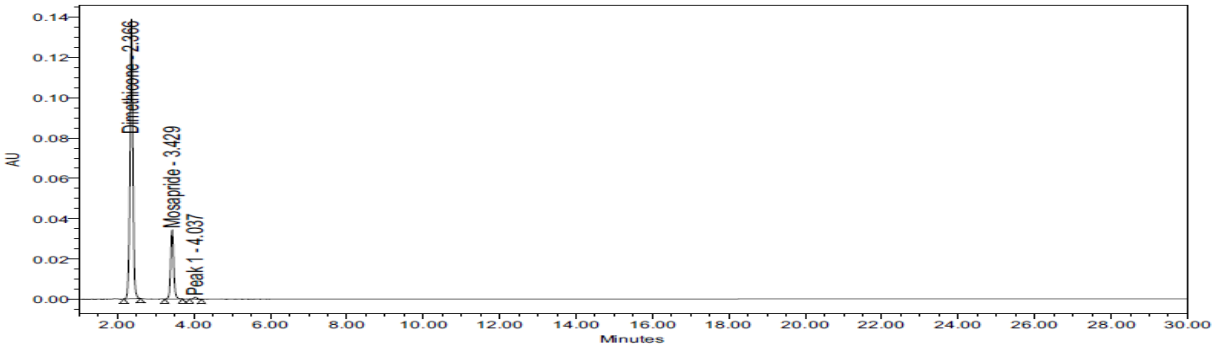

Figure 6: Chromatogram after oxidative stress

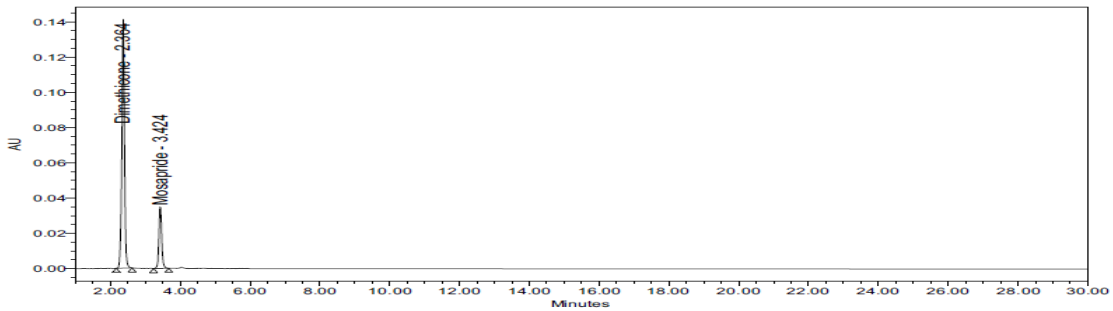

Figure 7: Chromatogram after thermal stress

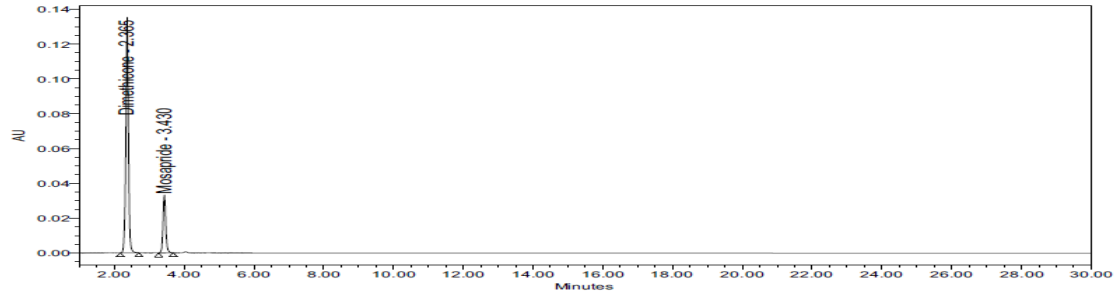

Figure 8: Chromatogram after UV radiation stress

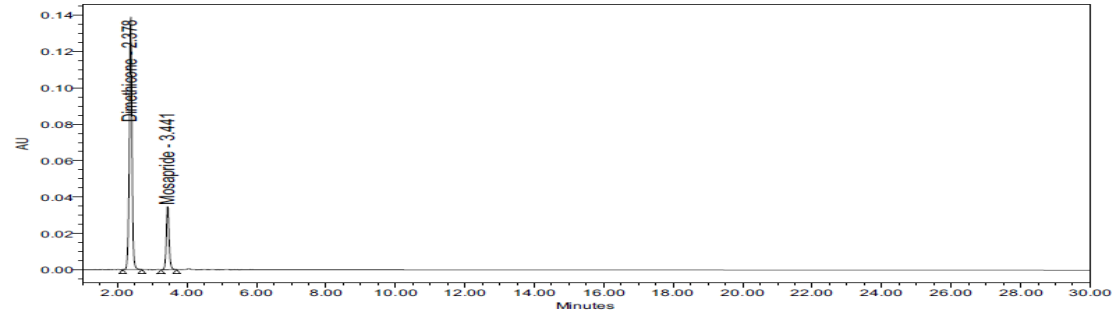

Figure 9: Chromatogram after nutral condition

Table 6: Acid Degradation Results

SL No.	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Dimethicone	2.369	694697	78.57	0.196	0.318	5158	1.0
2	Mosapride	3.433	177774	20.93	0.214	0.324	9136	1.1
3	Peak1	4.041	4484	0.50	4.372	5.090	11943	0.8

Table 7: Alkali Degradation Results

SL No.	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Dimethicone	2.368	696087	78.58	0.225	0.310	4960	1.0
2	Mosapride	3.433	181241	20.98	0.165	0.320	9109	1.1
3	Peak1	4.042	4013	0.44	4.013	4.720	8495	0.8

Table 8: Results of Oxidation Degradation

SL No.	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Dimethicone	2.366	712046	78.57	0.187	0.307	5113	1.0
2	Mosapride	3.429	184693	20.93	0.195	0.326	9672	1.1
3	Peak1	4.037	4472	0.49	4.127	4.950	13264	1.1

Table 9: Dry-heat Degradation Results

SL No.	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Dimethicone	2.364	710087	78.98	0.177	0.302	4929	1.0
2	Mosapride	3.424	187341	21.02	0.162	0.310	9671	1.1

Table 10: UV Degradation Results

SL No.	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Dimethicone	2.365	718866	79.14	0.218	0.320	5266	1.0
2	Mosapride	3.430	189532	20.86	0.227	0.320	9663	1.1

Table 11: Water Degradation Results

SL No.	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Dimethicone	2.378	720129	79.07	0.221	0.316	4932	1.0
2	Mosapride	3.441	190027	20.93	0.189	0.321	9759	1.1

DISCUSSION

Different compositions of mobile phase consisting of organic part acetonitrile and aqueous part Ortho phosphoric acid buffer ranging from 90 : 10 to 25 : 75 were tried in the validation study and the composition of 45 : 55 was selected as optimum since it gave best elution, acceptable retention time and least tailing. At pH value3.0 itself the elution was found to bemost suitable, while increased pH values did not bring significant variation in asymmetric factor. Flow rates ranging from 0.5 ml/minute to 1.5 ml/minute were observed and a flow rate of 1.2 ml/min was found to be ideal one to give a good separation time, peak resolution and shape.

CONCLUSION

The newly developed method was stability indicating, less time consuming, cost effective, highly accurate as results of recovery studies showed low value of percentage RSD, this also indicates that the method is precise and robust. The above said method is suitable to use for the estimation of combined formulation of Dimethicone and Mosapride on regular basis in laboratory.

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