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STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION &VALIDATION OF LOPERAMIDE HYDROCHLORIDE & SIMETHICONE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive, specific, accurate RP-HPLC method was developed for the simultaneous estimation of Loperamide HCl and Simethicone in pharmaceutical dosage form. RP-HPLC separation was achieved on a Zodiac C18 (150 mm x 4.6 mm, 5µm) column and isocratic elution. The mobile phase composed of phosphate buffer: acetonitrile (pH 2.3) (70:30 v/v) [HPLC Grade] at flow rate 1ml/min with UV detection at 230 nm. Run time is 7min. The retention times of Loperamide HCl and Simethicone were found to be 2.776 min and 3.60 min respectively. Linearity was established for Loperamide HCl and Simethicone in the range of 0.201-3.018µg/ml and 12.420-186.300µg/ml respectively. System precision and method precision was found to be within the limits of the acceptance criteria. Relative standard deviation of Loperamide HCl and Simethicone for System precision was found to be 1.111 and 0.277 respectively and method precision was found to be 0.3 and 0.33 respectively. The percentage recoveries for Loperamide HCl and Simethicone were found to be in the range of 99.93-101.5% and 100.16-101.06% respectively. The forced degradation studies of Loperamide HCl and Simethicone were found to be within the acceptance criteria. This method can be successfully employed for simultaneous quantitative analysis of Loperamide HCl and Simethicone in bulk drugs and formulations. The results indicate that there is no interference from excipients for the proposed method, thus making the method simpler, less time consuming and suitable for routine estimation of Loperamide HCl and Simethicone tablet formulation.

KEYWORDS: Loperamide Hcl, Simethicone, RP- HPLC, Stress Degradation, Tablets.

INTRODUCTION

Loperamide HCl: Loperamide, is an anti-diarrheal agent. It has direct anti secretory effect on myentericopiate receptors in the gut.[1] Loperamide has minimal systemic availability (0.3%), with most of the drug being removed by first-pass metabolism,[2] Loperamide is a μ-opioid receptor agonist that works in the intestines.[3] which further supports a local action in the gut. The main objective of the study is to investigate the best suitable dosage form of Loperamide in combination with Simethicone and its method validation by HPLC. Loperamide, sold under the brand name **Imodium** among others,[4] is a medication used to decrease the frequency of diarrhea.[5] It is often used for this purpose in gastroenteritis, inflammatory bowel disease, and short bowel syndrome. It is not recommended for those with blood in the stool. Loperamide's safety in pregnancy is unclear, but there is no evidence of harm.[6] It appears to be safe in breastfeeding.[7] It is an opioid with no significant absorption from the gut and does not cross the blood brain barrier when used at normal doses.[8] Loperamide was first made in 1969 and used medically in 1976.[9] It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system.[10] Loperamide is available as an inexpensive generic medication.[4-14] Loperamide is effective for the treatment of a number of types of diarrhea.[15] Loperamide should not be used as the primary treatment in cases of bloody diarrhea, acute exacerbation of ulcerative colitis, or bacterial enterocolitis.[16] Loperamide is often compared to diphenoxylate. Recent studies suggest that Loperamide is more effective and has lower neural side effects.[17-20]

Simethicone

Simethicone is an orally administered anti-foaming agent used to reduce bloating, discomfort or pain caused by excessive gas mainly swallowed air, with small amounts of hydrogen and methane.[21,22] Simethicone is a mixture of polydimethylsiloxane and hydrated silica gel. Simethicone is an anti-foaming agent that decreases the surface tension of gas bubbles, causing them to combine into larger bubbles in the stomach that can be passed more easily. Simethicone does not reduce or prevent the formation of gas in the digestive tract; rather, it increases the rate at which it exits the body.[23] Simethicone can relieve pain caused by gas in the intestines by decreasing foaming, which then allows for easier passing of flatulence.

Simethicone is not absorbed by the body into the bloodstream and is therefore considered relatively safe. National Institutes of Health (NIH)[24] reports there are usually no side effects when Simethicone is taken as directed. Although Simethicone has also been promoted as a treatment for colic in babies, randomised controlled trials have not demonstrated efficacy for this use,[25] despite traditional views on the subject, colic does not appear to be caused by gas.[26,29]

MATERIALS AND METHODS

a. Instrumentation

To develop a liquid chromatographic method for simultaneous estimation of Loperamide HCl and Simethicone using a isocratic HPLC instrument on a Zodiac **C_{18}** column (150 mm x 4.6 mm, 5µm).

b. Chemical and solvents

The reference samples of Loperamide HCl and Simethicone (API) were obtained as gift samples from Cystron Pharmaceutical Laboratories, Vijayawada. The tablets (Imosec-M; Loperamide HCl-2mg, Simethione-125mg) were procured from the local market. Acetonitrile and orthophosphoric acid used were HPLC grade.

c. Preparation of buffer solution

To prepare buffer, 1ml of OrthoPhospharic acid was dissolved in 1L of Distilled water & Filter through 0.45µ membrane filter and degassed. (pH 2.3).

d. Preparation of mobile phase

The mixture of above buffer (pH 2.3) and Acetonitrile in the ratio of 70:30 v/v was prepared and used as mobile phase.

e. Preparation of Diluent

Methanol was used as diluent.

f. Preparation of Standard solution of the drug

Solution A: Accurately weighed 125mg of Simethicone working standard and taken into a 100ml volumetric flask. 70ml of the diluent was added and sonicated and then the volume was made up to 100 ml with diluent.

Solution B: Accurately weighed 50mg of Loperamide working standard and taken into a 100ml volumetric flask. 70ml of the diluent was added and sonicated and then the volume was made upto 100 ml with diluent.

Further 5mL of solution-A and 2ml solution-B were taken in a 50 mL Volumetric flask and the volume was made upto the mark with the diluent.

g. Preparation of Sample solution

Accurately transfer the contents of 20 tablets of Loperamide HCl and Simethicone, ground into a fine powder and calculate the average weight. Weigh and transfer the sample equivalent to 10mg of sample into a 100ml volumetric flask add about 70 mL of mobile phase as diluent, sonicate to dissolve it completely and filtered. Further 2 ml of the above solution was taken and diluted to 100 ml with the diluent to get the concentration of 2µg/ml and 125µg/ml of Loperamide HCl and Simethicone respectively.

Method Development and Optimization

These optimized conditions were followed for the simultaneous determination of Loperamide HCL and Simethicone in combined dosage forms. The Optimized chromatographic conditions were shown in Table.1.

Table. 1: Optimized chromatographic conditions.

S. No	Parameters	Conditions
1	Mobile phase	Buffer : acetonitrile (70:30 v/v)
2	Pump mode	Isocratic
3	Buffer	Orthophosphoric acid buffer
4	pH of buffer	2.3
5	Diluents	Methanol
6	Column	Zodiac $\mathbf{C_{18}}$ column (150 mm x 4.6 mm, 5µm)
7	Wavelength	230 nm
8	Injection volume	10 µl
9	Flow rate	1.0 ml/min
10	Run time	7 min

RESULTS

Validation

Linearity: The calibration graph shows that linear responses were obtained over the range of concentrations used in the assay procedure. The data demonstrate that the method have adequate sensitivity in the concentration of analyte regression analysis was done on the peak areas of the two drugs(y) v/s concentration(x).the linearity ranges of Loperamide

hydrochloride and Simethicone are 0.201-3.018(ug/ml) and 12.420-186.300(ug/ml). Results of Linearity studies are shown in Figure 1 to 3.

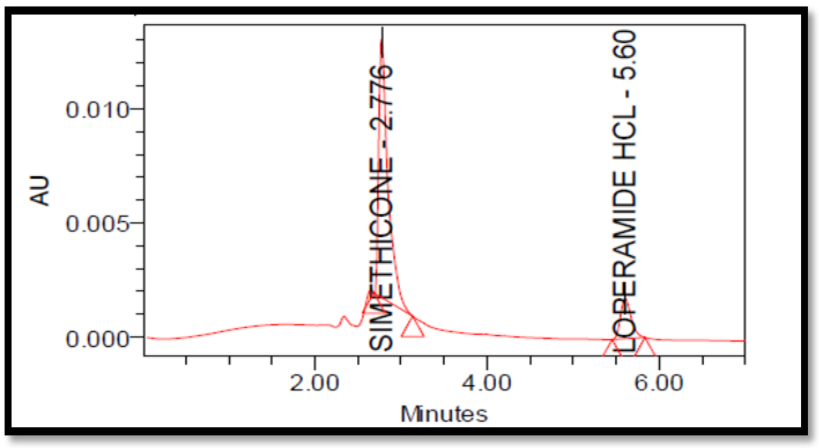

Fig. 1: Chromatogram for Linearity.

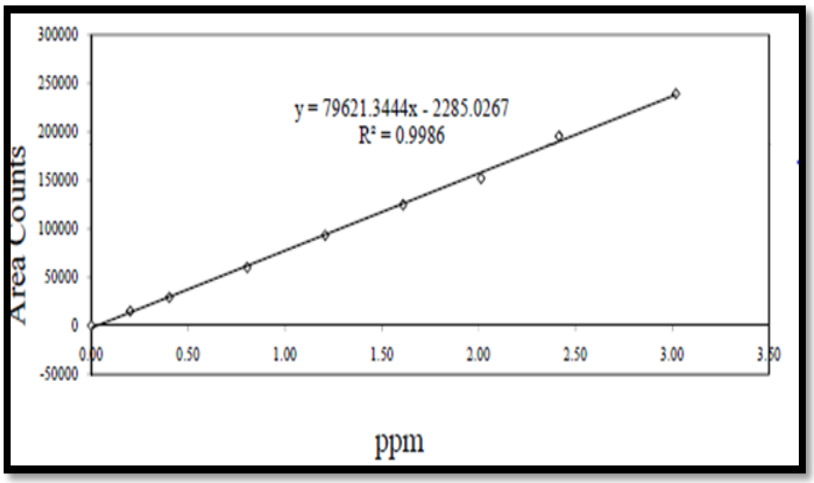

Fig. 2: Linearity Plot of Loperamide Hydrochloride.

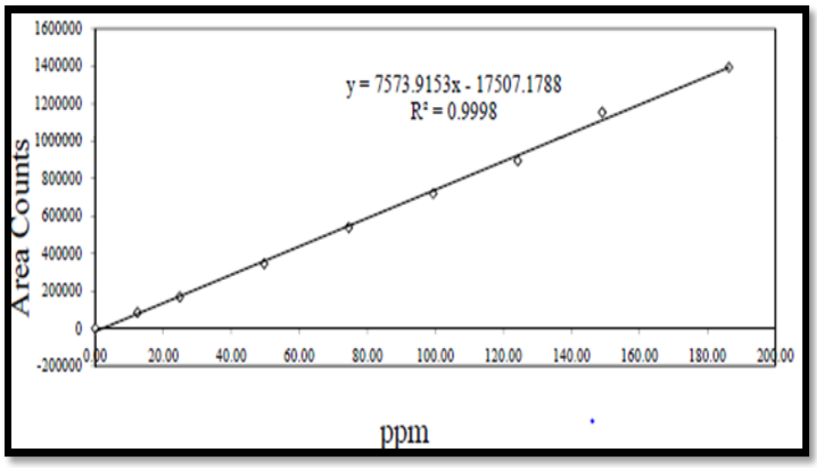

Fig. 3: Linearity Plot of Simethicone.

Accuracy: The present recoveries of the drug solutions were studied at three different concentration levels. The present individual recovery and the %RSD at each level were within the acceptable limits this indicates that the method is accurate. Results of Accuracy studies are shown in Table 2.And Figure 4 to 6.

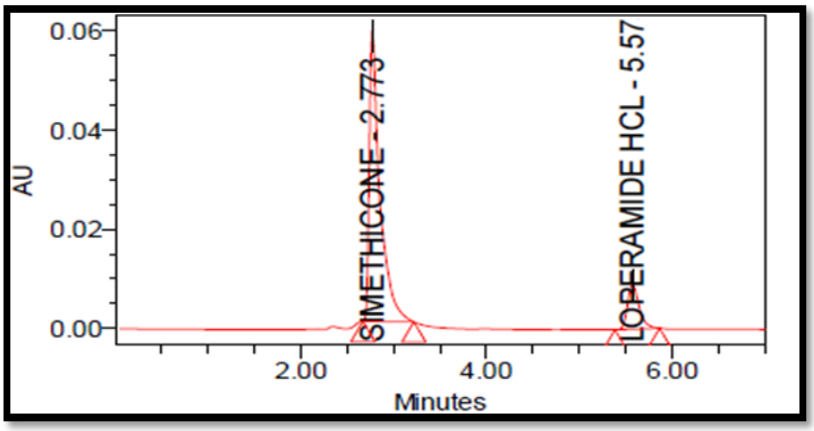

Fig. 4: Chromatogram for accuracy at 50% level.

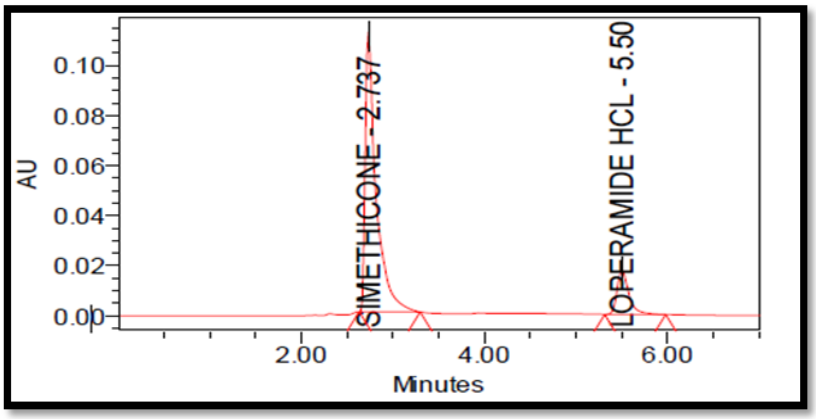

Fig. 5: Chromatogram for accuracy at 100% level.

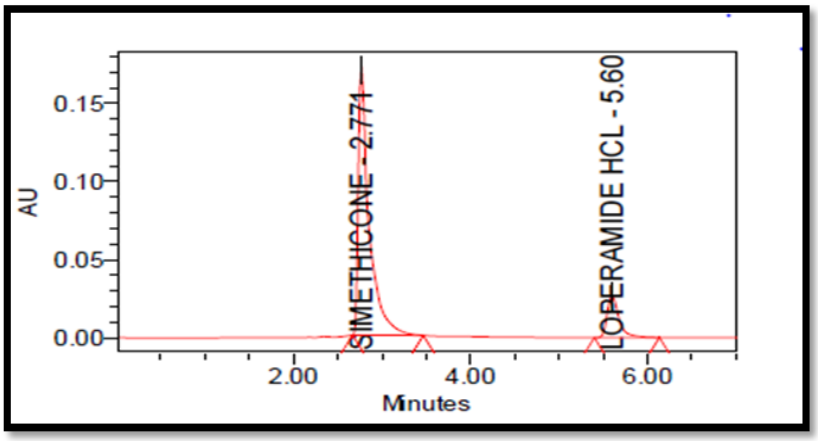

Fig. 6: Chromatogram for accuracy at 150% level.

Table. 2: Recovery study for Loperamide Hcl & simethicone.

loperamide Hcl					Simethicone			
Level %	**Actual API Added (mg)**	**Amount Recovered (mg)**	**% Recovery**	**Mean % Recovery**	**Actual API Added(mg)**	**Amount Recovered (mg)**	**% Recovery**	**Mean % Recovery**
50%	5	4.99	99.8	99.93	321	319.44	99.5	100.16
	5.2	5.21	100.2		320	322.27	100.7	
	5	4.99	99.8		318	318.85	100.3	
100%	10.1	10.25	101.5	101.5	625.3	627.84	100.4	100.26
	10.2	10.38	101.8		655	652.77	99.7	
	10	10.11	101.2		632	636.64	100.7	
150%	15.8	15.83	100.2	100.13	989	999.88	101.1	101.06
	15.7	15.63	99.6		972.5	980.15	100.8	
	15.8	15.89	100.6		985.5	1002.81	101.3	

Precision

The precision of the method was demonstrated by system and method precision studies. All the solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated and presented.

a. System precision

To study the system precision, six replicate mixed standard solution of Loperamide HCL and Simethicone were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.11 and 0.277 for Loperamide HCL and Simethicone respectively, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 3.And Figure 7.

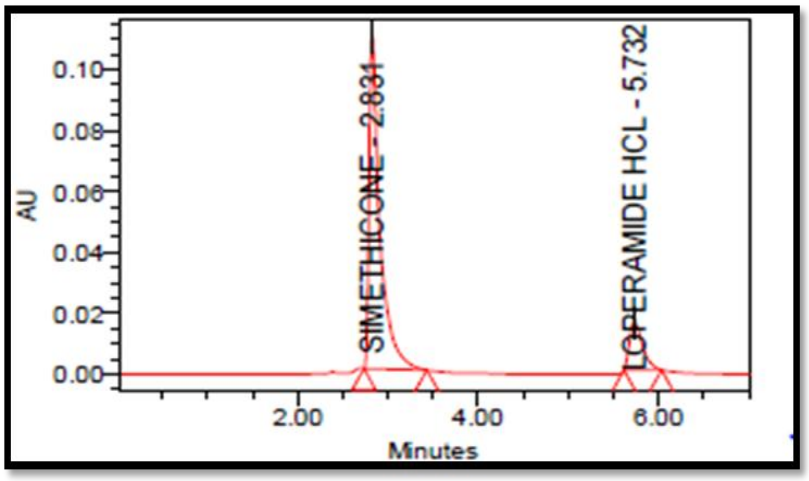

Fig.7: Chromatogram for System Precision.

Table. 3: System Precision for Loperamide Hcl & simethicone.

S. No	1	2	3	4	5	6	Mean	S.D	% RSD
Area of Loperamide Hcl	153288	185996	156051	155742	157952	157947	156158	1734.34	1.111
Area of Simethicone	902967	903489	903365	901868	909008	904941	904333	2503.55	0.277

b. Method precision

The method precision study was carried out on six preparations from the same tablet samples of and percent amount of both were calculated. The %RSD of the assay results of six preparations in method precision study was found to be 0.3 & 0.33 for Loperamide HCL and Simethicone respectively, which are well within the acceptance criteria of not more than 2.0. The results obtained for assay of Loperamide HCL and Simethicone were presented in Table 4. and Figure 8.

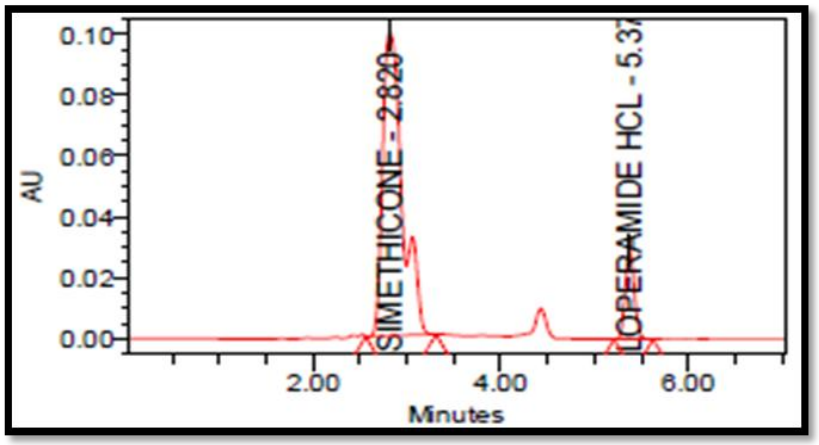

Fig. 8: Chromatogram for Method Precision.

Table 4: Method Precision for Loperamide Hcl & simethicone.

S. No	1	2	3	4	5	6	Mean	S.D	% RSD
Area of Loperamide Hcl	197604	199230	193469	194578	198535	199828	100.4	0.302	0.3
Area of Simethicone	1199320	1204519	119291	1250620	1250620	1452350	99.9	0.3027	0.33

Robustness: The robustness study was performed by slight modification in wavelength& mobile phase composition. Mixed samples of 2µg/mL Simethicone and 125µg/mL Loperamide Hcl respectively were analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the development method was robust in nature. The results of robustness study were shown in Table 5. Figures 9.to 14.

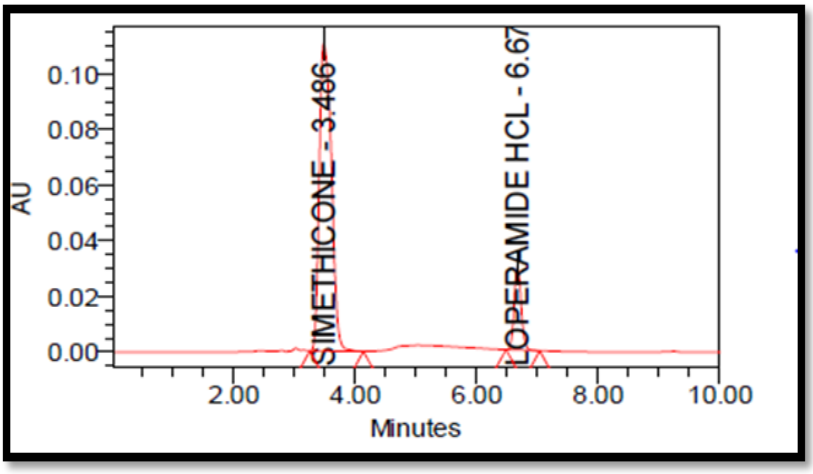

Fig. 9: chromatogram of effect of flow rate- 0.8ml/min.

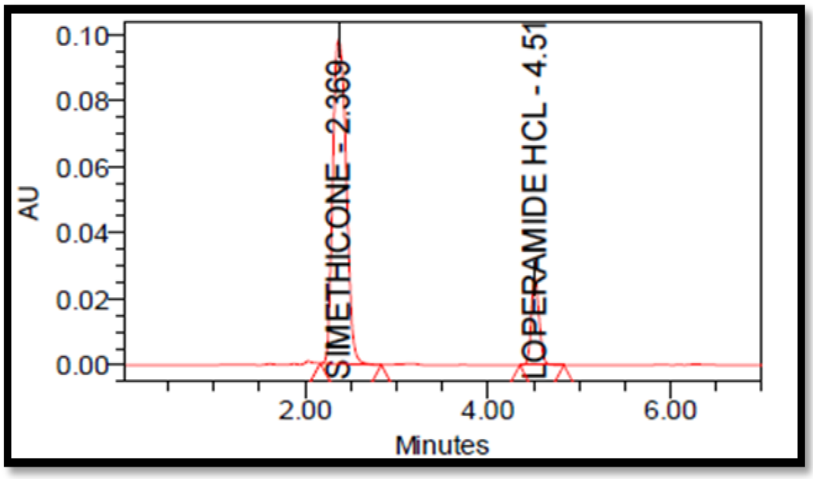

Fig. 10: chromatogram of effect of flow rate-1.2ml/min.

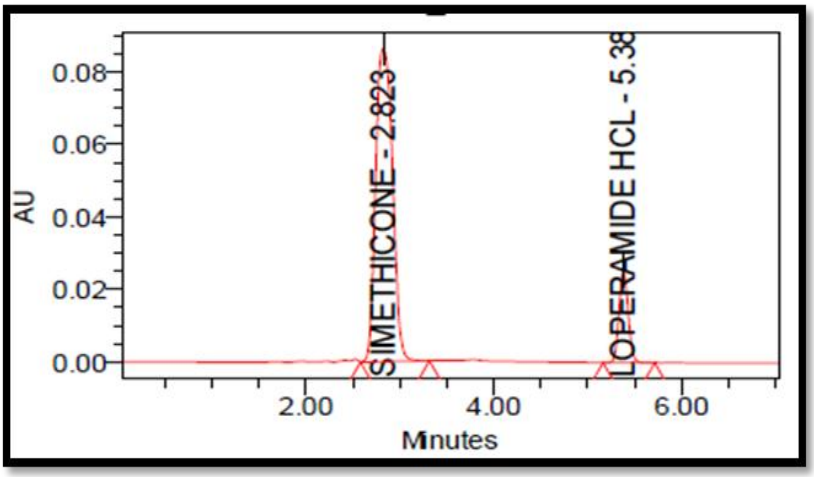

Fig. 11: Chromatogram of Effect of wave length.

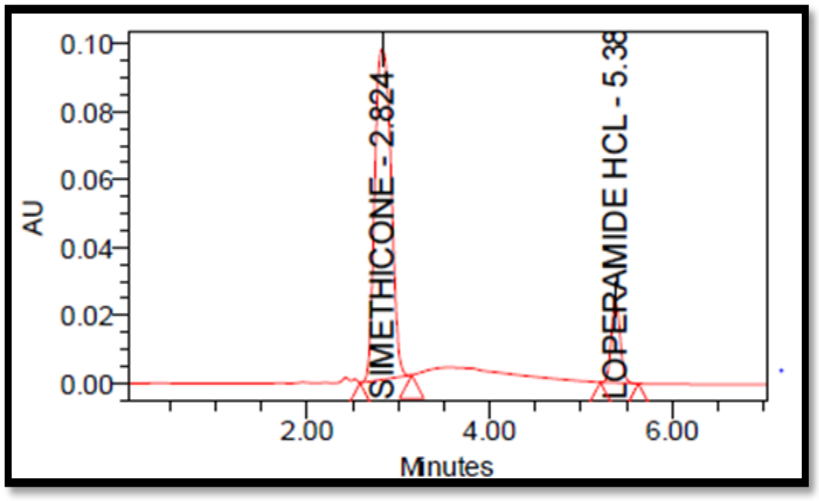

Fig. 12: Chromatogram of Effect of wave length.

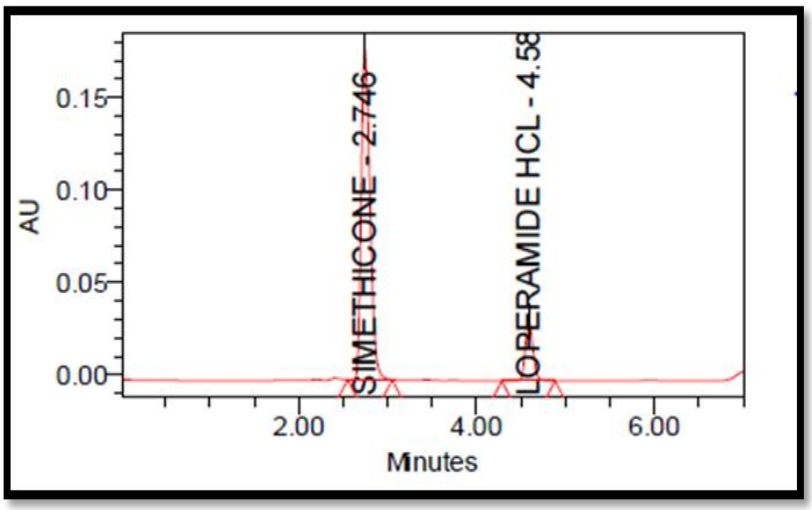

Fig. 13: Chromatogram of Effect of mobile phase (35:65).

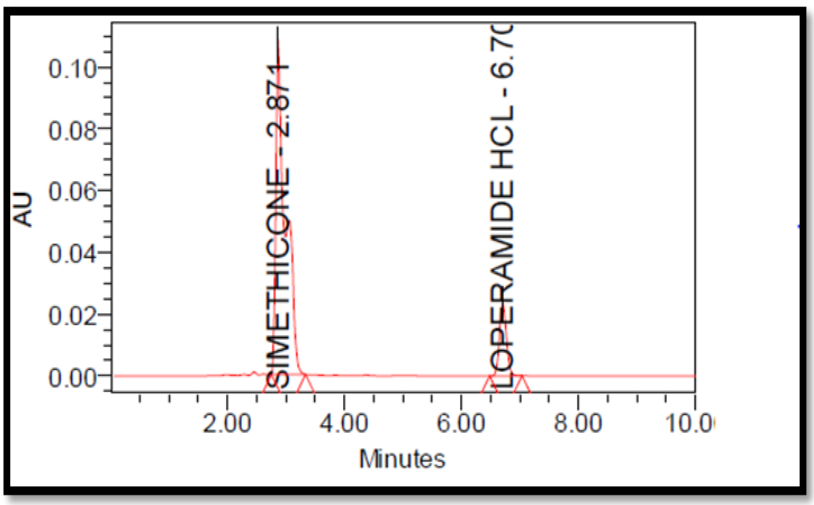

Fig. 14: Chromatogram of Effect of mobile phase (65:35).

Table. 5: Robustness Study for Loperamide Hcl & simethicone.

Loperamide Hcl		Simethicone	
Condition	**Mean area**	**Condition**	**Mean area**
Flow Plus -0.8 µl/min	158500	Flow Plus -0.8 µl/min	998419
Flow Minus- 1.2 µl/min	240543	Flow Minus- 1.2 µl/min	1525677
Wavelength at 232 nm	1172644	Wavelength at 226 nm	1068020
Wavelength at 228 nm	164691	Wavelength at 222 nm	1201064
Buffer : acetonitrile(35:65)	191804	Buffer: acetonitrile(55:45)	1185151
Buffer : acetonitrile(65:35)	191804	Buffer: acetonitrile(45:55)	1207109

System suitability

System suitability studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in table 6.

Table. 6: System Suitability Parameters.

Parameters	Retention time(min)	Area	Theoretical plates	Tailing factor
Loperamide Hcl	5.732	157947	6971	1.644
Simethicone	2.831	904941	4166	2.580

Assay

Estimation of Loperamide HCL and Simethicone in tablet dosage forms by the developed RP-HPLC method was carried out. The assay procedure was performed and the assay percentage was calculated. The results were shown in Table 7. and Figure 15.

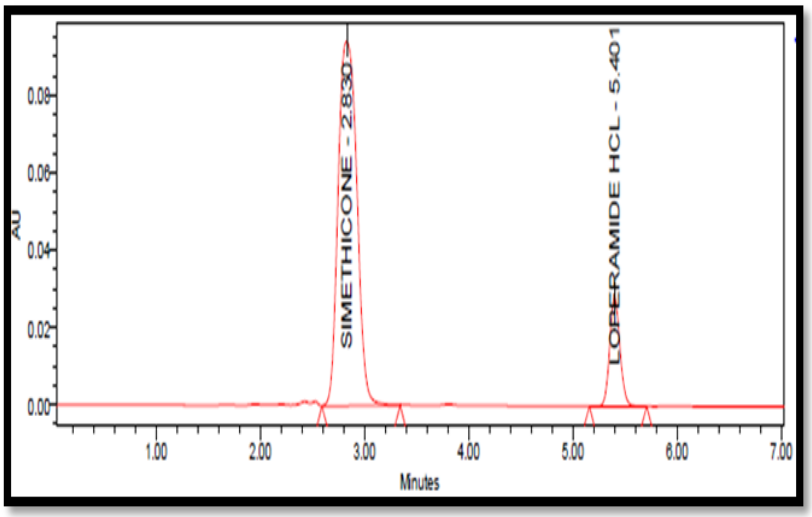

Fig. 15: Typical chromatogram of sample solution.

Table. 7: Summary of Assay results.

Drug	Label claim	Mean	% assay
Loperamide hydrochloride	2mg	194058	100.7
simethicone	125mg	1175512	99.8

Therefore, the propose method was simple, specific and sensitive and can be used for simultaneous analysis of Loperamide Hydrochloride and Simethicone in tablet dosage forms.

FORCED DERGRADATION STUDIES

Forced degradation studies have been carried out to conform that the drug molecules were stable throughout their shelf life and / or to confirm their resistance during stability studies or, any degradation product if found will not interfere with the peak of Loperamide HCL and Simethicone. In addition, the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali, acidic, neutral hydrolysis, heat or photolytic) for of the degradations. Results of degradation were presented in Table 8.

a. Acid stressed sample: To 1 ml of stock solution of LPH and SMT, 3 ml of 5N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 2µg/ml&125µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatogram was shown in Figure 16.

b. Alkali stressed sample: To 1 ml of stock solution of LPH and SMT, 3 ml of 5N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 2µg/ml&125µg/ml of LPH and SMT solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatogram was shown in Figure 17.

c. UV light exposed sample: The photochemical stability of the drug was also studied by exposing the 2µg/ml&125µg/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 2µg/ml and 125µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatogram was shown in Figure 18.

d. Oxidative stressed sample

Weighed powder equivalent to 2mg of LPH and125mg SMTand transferred into 100 ml volumetric flask, To 1 ml of stock solution of LPHand SMT, 5 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 2µg/ml&125µg/ml of LPH and SMT solution 10 µl of solution was injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatogram was shown in Figure 19.

e. Thermal stressed (dry heat) sample (in hot air oven)

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 2µg/ml &125µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The chromatogram was shown in Figure 20.

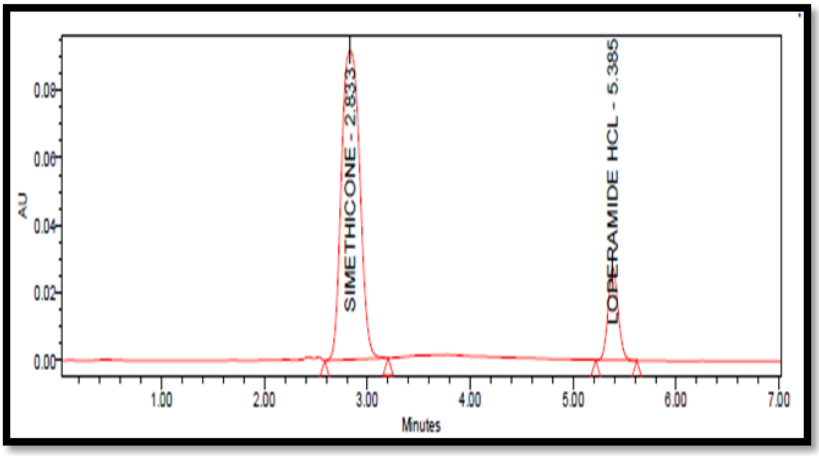

Fig. 16: Chromatogram of Acid Degradation.

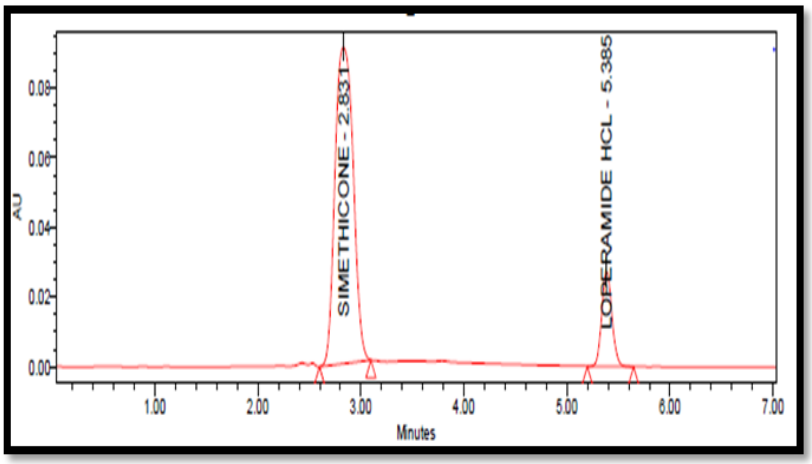

Fig. 17: Chromatogram of Alkali Degradation.

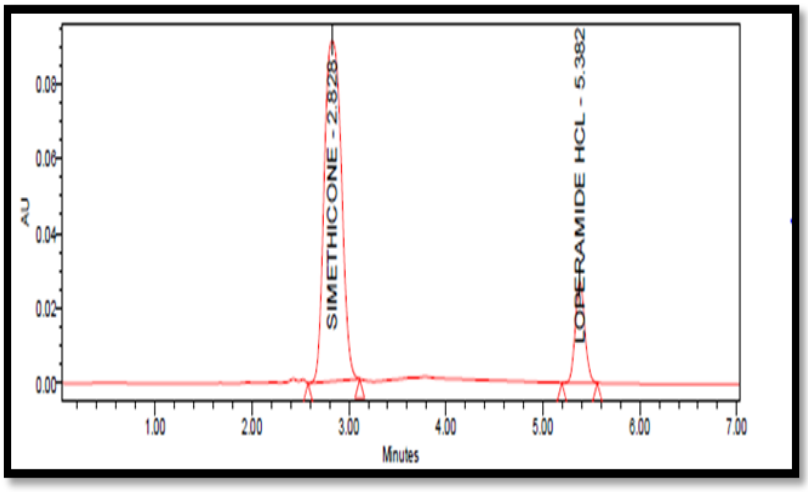

Fig. 18: Chromatogram of Photolytic Degradation.

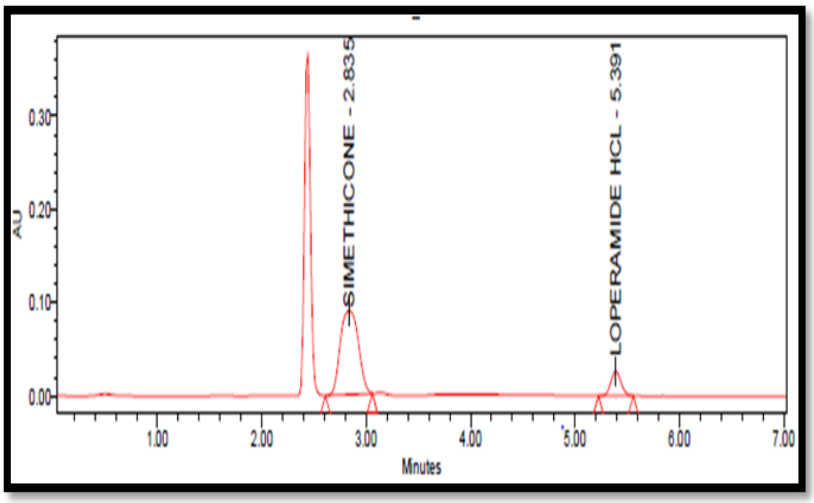

Fig. 19: Chromatogram of oxidative Degradation.

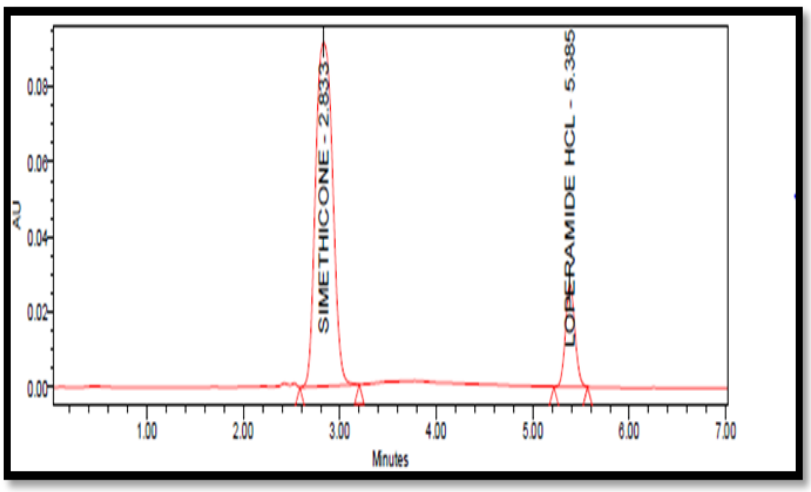

Fig. 20: Chromatogram of Thermal Degradation.

Table. 8: Degradation studies of Loperamide Hcl & simethicone.

Type of degradation	Loperamide Hcl			simethicone		
	Mean area	% purity	% degradation	Mean area	% purity	% degradation
Acid	186954	75.7	24.3	1130509	76.5	21.6
Alkali	187553	78.9	21.1	1116521	78.4	21.4
oxidative	177395	76.9	23.1	1085305	78.6	21.4
Thermal	185719	74.5	25.5	1130509	75.7	24.3
Photo lytic	185159	76.4	23.6	1119502	77.2	22.8

DISCUSSION

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the simultaneous analysis of Loperamide Hcl and Simethicone from tablet dosage forms. A non-polar C_{18} analytical chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of Loperamide Hcl and Simethicone. Mixtures of commonly used solvents like water, acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and acetonitrile in the ratio of 70:30 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention times of the of Loperamide Hcl and Simethicone were found to be 2.70 and 5.50 min respectively.

CONCLUSION

The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination of Loperamide HCl and Simethicone in bulk and its pharmaceutical dosage form. The developed method gave good resolution between Loperamide HCl and Simethicone with short analysis time (10 min). Hence, the method can be easily and conveniently adopted for routine analysis of Loperamide HCl and Simethicone in combined dosage forms.

ACKNOWLEDGEMENT

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