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Research Article

Analytical Method Development And Validation For The Simultaneous Estimation Of Aluminium Hydroxide And Simethicone By RP-HPLC Method

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ABSTRACT

In the present research, a new rapid, economical, simple, isocratic and cost-effective reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantification of simultaneous Aluminium hydroxide and Simethicone. The chromatographic parameters were successfully developed for the separation of Aluminium hydroxide and Simethicone by employing Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument utilized was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The analytical method was developed and validated as per the guidelines of ICH (ICH, Q2 (R1)). The linearity studies for Aluminium hydroxide and Simethicone was determine in different concentrations range from 1µg-5µg and 00µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999% mean recovery was observed at 100% and 100.5%, %RSD for repeatability was.0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively.

INTRODUCTION

A drug is a compound that have medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food or exclusively a food. There was no single, precise definition, as there are different meanings in drug control law, government regulations, medicine, and Colloquial usage. Pharmaceutical drugs are used for the determination of their quality assurance and quality control of bulk drugs and their formulations. Qualitative analysis provides the chemical identification of the sample, and establishment of the relative amount of one or more of the species or analytes in numerical terms. In Chromatography, Adsorption chromatography

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employs high-surface area particles that adsorb the solute molecules. Usually a polar compound such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are utilized in adsorption chromatography. In adsorption chromatography, adsorption process is defined by competition model and solvent interaction model. In partition chromatography, the solid support was coated with a liquid stationary phase; the relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can be either polar or non polar compound. If the stationary phase was polar and the mobile phase was non polar, it is known as normal phase partition chromatography. If the stationary phase was non polar and the mobile phase was polar, it is known as reverse-phase partition chromatography

METHOD DEVELOPMENT:

Selection of Detection wavelength:

10 mg of Aluminium hydroxide and Simethicone was placed in Phosphate buffer which is a mobile phase. That solution was scanned at 200-400 nm for determination of spectrum. The excessively spectrum was used for selection of wavelength for Aluminium hydroxide and Simethicone.

Selection of column:

Column is selected on the bases of solubility, polarity and chemical differences among Analytes [Column: Inertsil C18 (4.6 x 250mm, 5µm, Make: Waters)]

Selection of mobile phase:

Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) was used as mobile phase. The pH of the buffer should be 2 to 8.

Selection of flow rate:

Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry
4. Separation of impurities

Preparations and procedures:

Preparation of Phosphate buffer :(PH: 4.6):

Accurately 6.8 gm of KH2PO4 weighed and placed into a 1000ml beaker, dissolved and diluted with 1000ml with HPLC water, adjusted the pH to 4.6 with orthophosphoric acid.

Preparation of mobile phase:

300mL of pH 4.6 Phosphate buffer (30%), 700mL of ACN (70%) are mixed and degassed in ultrasonic water bath for 5 minutes. Then this mobile phase was filtered under vacuum filtration.

Preparation of the individual Aluminium hydroxide standard preparation:

10mg of Aluminium hydroxide was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF was added. Then the preparation was sonicated to dissolve completely and made the volume up to the mark with the diluant. Then 10 ml of the solution was pipette out into a 100 ml volumetric flask and was diluted up to the mark with diluant.

Preparation of the individual Simethicone standard preparation:

10mg of Simethicone was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF was added. Then the preparation was sonicated to dissolve completely and made volume up to the mark with the diluant. Then 10ml of solution was pipette out into a 100 ml volumetric flask and was diluted up to the mark.

Accuracy:

Preparation of standard solution (Aluminium hydroxide and Simethicone):

Accurately weighed 10mg of Simethicone and 10mg of Aluminium hydroxide and transferred into 100ml of clean dry volumetric flasks. 70ml of Diluent was added and sonicated to dissolve the drug completely and made volume up to the mark with the same solvent. Then 3ml of the solution

was pipette out into a 10ml volumetric flask and make up to the mark with diluent.

Preparation of Sample solutions:

For preparation of solution:

Accurately weighed 5mg of Simethicone and 5mg of Aluminium hydroxide and transferred into 100ml of clean dry volumetric flask. 7ml of diluent was added and sonicated to dissolve the drug completely and made volume up to the mark with the same solvent. Then 3ml of the Simethicone and Aluminium hydroxide solution were pipette out into a 10ml volumetric flask and diluted up to the mark with diluant.

Acceptance criteria

Correlation coefficient could be not less than 0.999

Linearity

Preparation of stock solution:

Procedure:

The solution was injected into the chromatographic system and the peak area was observed, a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was measured.

Acceptance criteria

Correlation coefficient could be not less than 0.999.

RESULTS AND DISCUSSION

WAVELENGTH DETECTION:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Aluminium hydroxide and Simethicone was obtained and the isobestic point of Aluminium hydroxide and Simethicone showed absorbance's maxima at 260 nm.

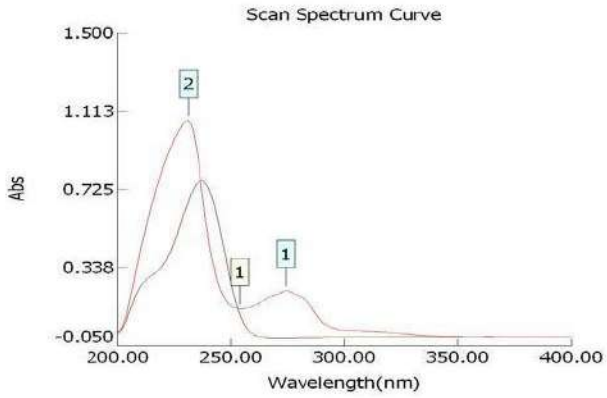

Fig. 01: Overlay spectrum of Aluminium hydroxide and Simethicone

Accuracy:

Accuracy study was performed for Aluminium hydroxide and Simethicon, solution injected in triplicate into chromatographic system results were shown in table 01, 02.

Table 01. Accuracy results of Simethicone

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

The accuracy results for Aluminium hydroxide

Table 2. Accuracy results of Aluminium hydroxide

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	**100.0%**
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

Precision

Repeatability

The precision studies were performed by injections of Aluminium hydroxide and Simethicone was injected in to chromatographic system. The area of standard injection was determined by calculation of % RSD.

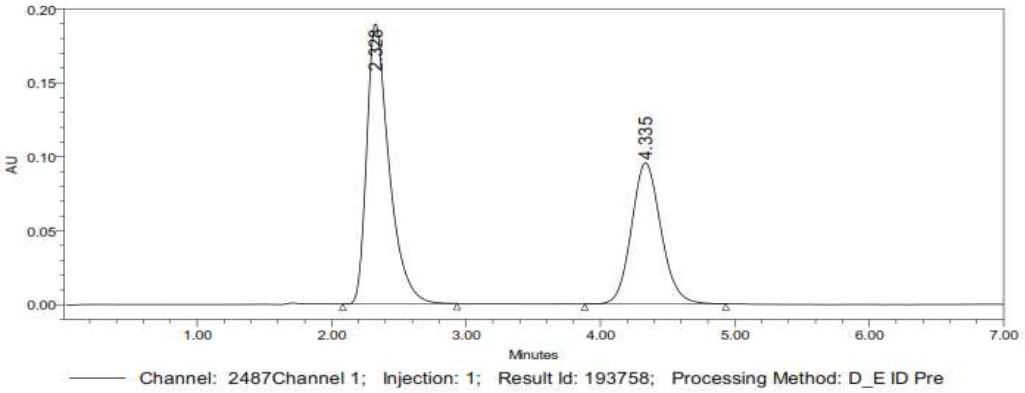

Fig 2. Chromatogram of Standard Injection

Name: Aluminium hydroxide

	Name	RT	Area	Height (µV)
1	Aluminium	2.328	2194758	189693
2	Aluminium	2.326	2195700	190025
3	Aluminium	2.327	2196191	189862
4	Aluminium	2.326	2195326	190700
5	Aluminium	2.331	2200951	189426
Mean			2196585	
Std. Dev.			2496.0	
% RSD			0.11	

Name: Simethicone

	Name	RT	Area	Height (µV)
1	Simethicone	4.335	1456296	95623
2	Simethicone	4.336	1457422	95150
3	Simethicone	4.334	1456513	95165
4	Simethicone	4.337	1454579	95298
5	Simethicone	4.340	1451483	95251
Mean			1455259	
Std. Dev.			2347.6	
% RSD			0.16	

Table: 03, 04 Ruggedness results of Simethicone& Aluminium hydroxide

Acceptance Criteria:

The % RSD for the area of five standard injections results could not be more than 2%.

Specificity:

The system suitability for specificity was performed to determine that there is any

interference of any impurities in retention time of analytical peak the studies was carried out by injecting blank, standard and sample solution results are shown in table no: 5, 6 respectively.

Sr. No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Aluminum hydroxide	2.237	7913799	394185	2632	1.8	5.23
2	Simethicone	4.342	1853381	162758	2614	1.6	

Table 5 Standard results of Simethicone& Aluminium hydroxide

Sr .No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Aluminum hydroxide	2.326	4726354	376488	2455	1.60	5.52
2	Simethiconeme	4.344	3122571	198418	2614	1.11	

Table 6. Sample results of Simethicone& Aluminium hydroxide

Detection of limit:

LOD's can be measured based on the standard deviation of the response (SD) and the slope of the calibration curve at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

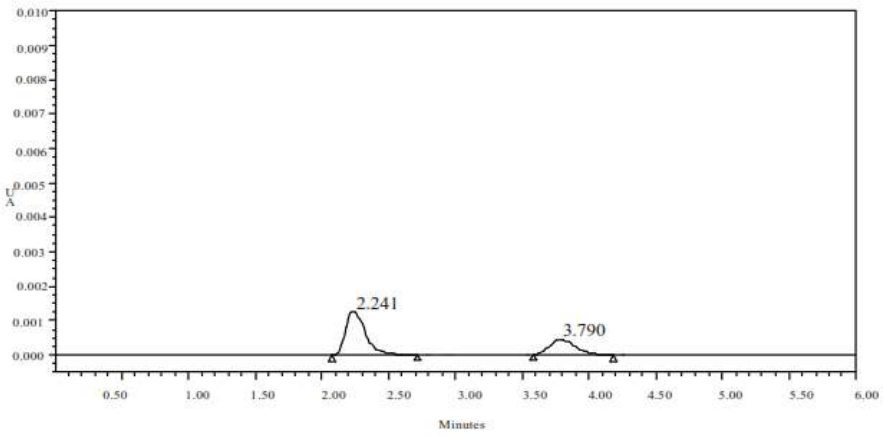

Fig. 3 Chromatogram of LOD

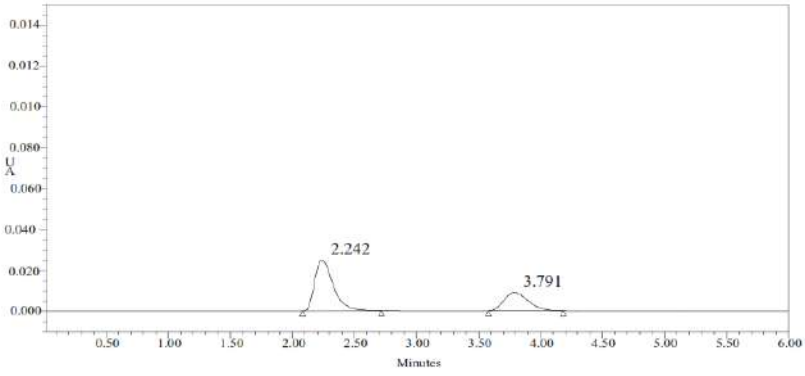

Fig.4. Chromatogram of LOQ

Linearity:

The linearity studies were determined for the concentration of 100ppm to 500ppm and 1ppm to 5ppm level each level was injected into chromatographic system.

	SampleName	Name	RT	Area	Height (µV)
1	Linearty 1	Aluminium	2.309	1810101	145957
2	Linearty 1	Simethicone	4.307	1164173	75128
3	Linearty 2	Aluminium	2.322	2044287	176935
4	Linearty 2	Simethicone	4.317	1342535	87703
5	Linearty 3	Aluminium	2.324	2367133	206622
6	Linearty 3	Simethicone	4.323	1555931	101999
7	Linearty 4	Aluminium	2.336	2602279	228576
8	Linearty 4	Simethicone	4.340	1777973	117084
9	Linearty 5	Aluminium	2.345	2869778	259346
10	Linearty 5	Simethicone	4.340	1942319	129409

Table 7 Linearity results of Aluminium hydroxide and Simethicone

Acceptance Criteria:

Correlation coefficient should be not less than 0.999

Plotting of calibration graphs:

Linearity peaks are plotted against Concentration

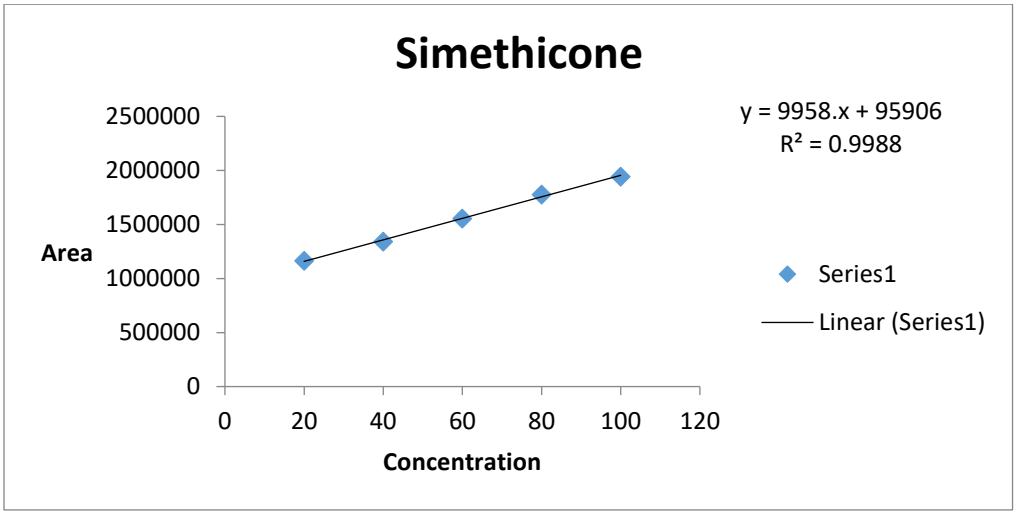

Fig. 5 Calibration curve of Simethicone

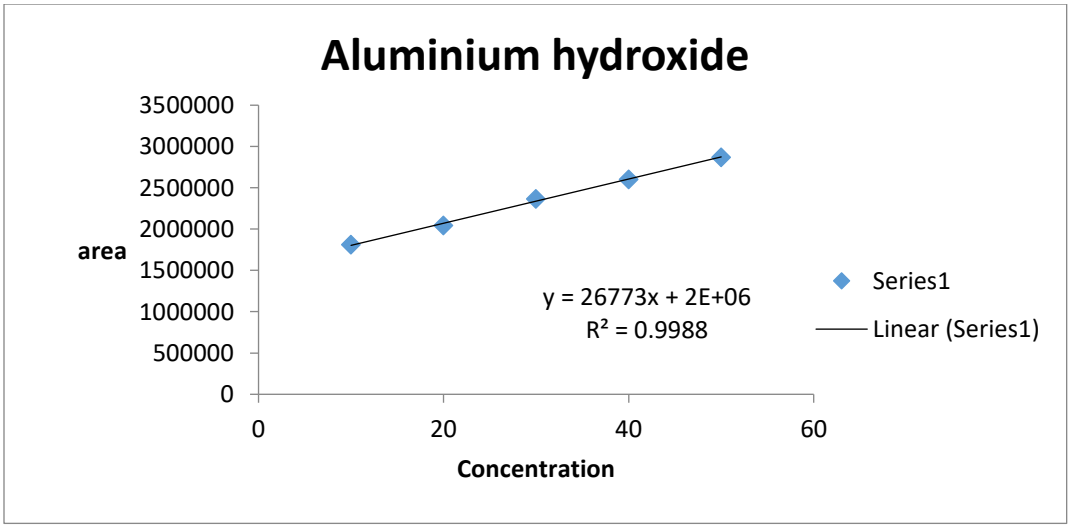

Fig.6 Calibration curve of Aluminium hydroxide

System suitability results for Simethicone:

	Changing c Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Plate count
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 8. System suitability results for Simethicone

System suitability results for Aluminium hydroxide :

	Changing Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Plate count
1	10% Less	883.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

Table 9. System suitability results for Aluminium hydroxide

CONCLUSION

A new method was developed for simultaneous estimation of Aluminium hydroxide and Simethicone by RP-HPLC method. The chromatographic parameters were successfully developed for the separation of Aluminium hydroxide and Simethicone by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v), detection wavelength was 255nm. WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2 were used for detection. The retention time was noted as 2.399mins and 3.907mins. The %purity of Aluminium hydroxide and Simethicone was noted as 100.7% and 101.4% respectively. The system suitability parameters for Aluminium hydroxide and Simethicone such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated as per the ICH of guidelines (ICH, Q2 (R1)). The linearity studies for Aluminium hydroxide and Simethicone was measured in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, %mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision studies were precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Aluminium hydroxide and Simethicone in bulk and Pharmaceutical dosage form.

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