ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.13 | Issue 4 | Oct - Dec -2024

www.ijpar.com

DOI : https://doi.org/10.61096/ijpar.v13.iss4.2024.528-537

Research

Development and validation of rp-hplc method for the simultaneous estimation of magaldrate and simethicone in bulk And pharmaceutical dosage form

Ch. Navyani*, P. Vedavahini, R. Mounika, Dr. K. Shravankumar

[1]*Department Of Pharmaceutical Analysis, Samskruti College Of Pharmacy, Ghatkesar, Telangana. 501301.*

*Author for Correspondence: CH. Navyani
Email: cherupallynavyani8@gmail.com

Published on: 22 Oct 2024

Published by:
DrSriram Publications

Abstract

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Magaldrate and Simethicone in bulk and pharmaceutical formulations. Separation of Magaldrate and Simethicone was successfully achieved on a Agilent zorbax XDB C18 (150 mm×4.6 mm, 5µ) or equivalent in an isocratic mode utilizing Potassium dihydrogen phosphate (pH-4.8): Methanol (40:60) v/v at a flow rate of 1.0mL/min and eluate was monitored at 235nm, with a retention time of 1.694 and 3.234 minutes for Magaldrate and Simethicone. The developed method was validated and the response was found to be linear in the drug concentration range of 50µg/mLto150 µg/mL for Magaldrate and 50µg/mLto150µg/mL for Simethicone. The values of the slope and the correlation coefficient were found to be y=26615x+ 43142 and 0.999 for Magaldrate and y=5560x-1243 and 0.999 for Simethicone respectively. This method was found to be good percentage recovery for Magaldrate and Simethicone were found to be 100.00 and 100.31 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Magaldrate, Simethicone, High performance liquid chromatography, Validation.

INTRODUCTION

Chromatography

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

"] *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient.

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Chromatographic Process

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

Normal phase chromatography

Normal phase HPLC (NP-HPLC) was the first kind of HPLC chemistry used, and separate analytes, based on polarity. This method uses a polar stationary phase and a non-polar mobile phase, and is used when the analyte is fairly polar in nature. The polar analyte associates with and is retained by the polar stationary phase. Absorption strengths increase with increase in analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the elution time. The interaction strength not only depends on the functional groups in the analyte molecule, but also on stearic factors and structural isomers is often resolved from one another. Use of more polar solvents in the mobile phase will decrease the retention time of the analyte while more hydrophobic solvents tend to increase retention times. Particularly polar solvents in a mixture tend to deactivate the column by occupying the stationary phase surface.

Reversed phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention Time (R_t) is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. The energy released in this process is

proportional to the surface tension of the eluent (water: 73 erg/cm², methanol: 22 erg/cm²) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding less-polar solvent (MeOH, ACN) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically changing the polarity of the mobile phase during the course of the analysis.

Isocratic flow and gradient elution

A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning constant composition). The word was coined by Csaba Horvath, who was one of the pioneers of HPLC. The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; A is the "weak" solvent which allows the solute to elute only slowly, while B is the "strong" solvent which rapidly elutes the solutes from the column. In reverse-phase chromatography, solvent A is often water or an aqueous buffer, while B is an organic solvent miscible with water, such as acetonitrile, methanol, THF, or isopropanol.

Working Principle of HPLC

The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography. The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb Ultra Violet light, a UV-absorbance detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an Evaporative-Light-Scattering Detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a Mass Spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

Components of HPLC

- Solvent
- Solvent Delivery System (Pumps)
- Injector
- Column
- Detectors
- Recorder (Data Collection)

The most simplified way of explaining the cycle of operation, without taking into account the compressibility of the solvents, is as follows. From the moment when the outlet valve of cylinder a closes and its entrance valve open, the piston in A, moving backwards, sucks the eluent through the inlet check valve and the chamber fills. Meanwhile cylinder B is open and its piston moves forward to force the mobile phase towards the injector and the column. The volume displaced by piston B is half of that available in the chamber of piston A. With chamber A full, the entrance valve of a closes and the corresponding outlet valve opens. Piston a now advances and pushes out the contents of the chamber. Half of this volume is expelled directly towards the column, the other half serves to fill cylinder B as piston B retracts. A pulse absorber is located between the two cylinders (diagram courtesy of Agilent Technologies).

MATERIALS AND METHODS

Ortho-Phosphoric Acid-Finar, Acetonitrile- Merck, Methanol-Merck, Water -Lob Chemi, Potassium dihydrogen orthophosphate-Finar, Magaldrate- provided by Sura Pharma labs, Simethicone- provided by Sura Pharma labs.

METHODOLOGY

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Magaldrate and Simethicone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 1ml of the above Magaldrate and 1ml of Simethicone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Potassium dihydrogen phosphate: Methanol in proportion 40:60 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Agilent Zorbax XDB C18 (150 mm×4.6 mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : Ambient
Column : Agilent Zorbax XDB C18 (150 mm×4.6 mm, 5µ)
Mobile phase : Potassium dihydrogen phosphate: Methanol in proportion 40:60 v/v
Flow rate : 1ml/min
Wavelength : 235 nm
Injection volume : 10 µl
Run time : 8 min

Method validation

PREPARATION OF MOBILE PHASE

Preparation of Mobile Phase

Accurately measured 600 ml (60%) of Methanol, 400 ml of Phosphate buffer (40%) were mixed and degassed in digital ultra sonicater for 20 minutes and then filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized chromatographic method

Chromatographic parameters

Mobile phase : Potassium dihydrogen phosphate: Methanol (40:60)
Auto sample temperature : 25^{o}c
Injection volume : 10µL
Column : Agilent Zorbax XDB C18 (150 mm×4.6 mm, 5µ)
Detector wavelength : 235 nm
Flow rate : 1.0ml/min
Run time : 8 minutes

Procedure

Inject 10µL of standard, sample into chromatographic system and measure the areas for the Magaldrate and Simethicone peeks and calculate the % assay by using the formula.

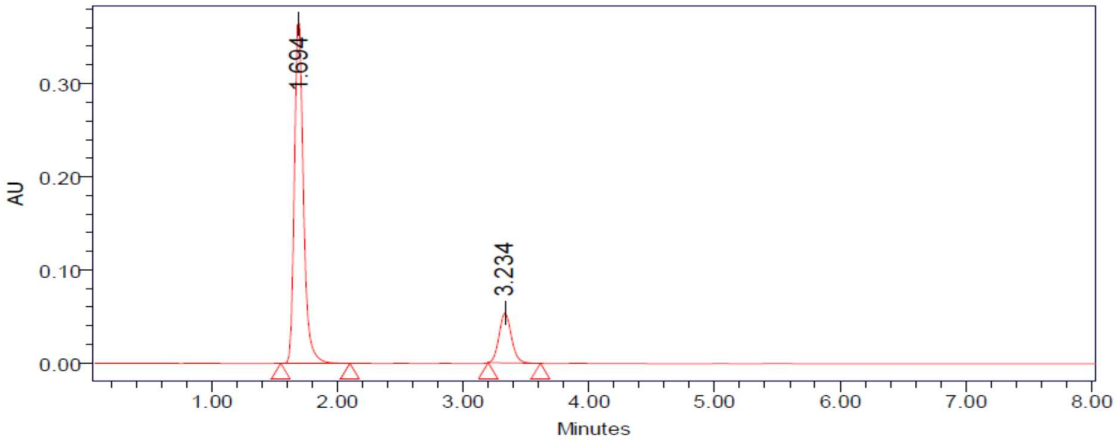

Fig 1: Typical Chromatogram for optimized method

Table 1: Results of optimized method Chromatogram

S. no	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1	MAG	1.694	2653415		1.67	4506
2	SIM	3.234	569858	4.99	1.52	5386

Peeks are well separated all the parameters are within the limits.

System suitability

Table 2: System suitability data of Magaldrate and Simethicone

parameter	Magaldrate	Simethicone	Acceptance criteria
Retention time	1.694	3.234	
Theoretical plates	4508	5387	>2500
Tailing factor	1.69	1.55	<2.00
% RSD	0.02	0.03	<2.00

Table 3: Standard Results of Magaldrate

S. no	Sample name	RT	Area	USP plate count	USP tailing
1	Injection 1	1.694	2653415	4639	1.61
2	Injection 2	1.689	2686577	4719	1.60
3	Injection 3	1.692	2678542	4654	1.62
4	Injection 4	1.688	2648574	4586	1.60
5	Injection 5	1.688	2653981	4557	1.63

Table 4: Standard Results of Simethicone

S. no	Sample name	RT	Area	USP plate count	USP tailing
1.	Injection 1	3.244	568957	5635	1.48
2.	Injection 2	3.238	563542	5709	1.48
3.	Injection 3	3.246	568748	5545	1.49
4.	Injection 4	3.265	569858	5443	1.47
5.	Injection 5	3.265	568474	5427	1.50

Results of system suitability study are summarized in the above table. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Specificity

Table 5: Specificity data for Magaldrate and Simethicone

S. no	Sample name	Magaldrate		Simethicone	
		Area	Rt	Area	Rt
1	Standard	2653415	2.176	569858	2.920
2	Sample	2678547	2.182	568424	2.923
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Chromatograms explain that retention time for standard, sample and commercial product of Magaldrate and Simethicone are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

Accuracy

Table 6: Accuracy data for Simethicone

S. no	Accuracy level	Sample name	Sample area	Rt
1	50%	1	281235	3.277
		2	282124	3.275
		3	279879	3.266
2	100%	1	559769	3.265
		2	561817	3.265
		3	554328	3.265
3	150%	1	837354	3.268
		2	832256	3.268
		3	836897	3.266

Table 7: Accuracy (%recovery) results of Simethicone

S. no	Accuracy Level	Sample name	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	50	50.358	100.716	100.66
		2	50	50.518	101.036	
		3	50	50.114	100.228	
2	100%	1	100	100.454	100.454	100.25
		2	100	100.822	100.822	
		3	100	99.475	99.475	
3	150%	1	150	150.379	100.252	100.03
		2	150	149.462	99.641	
		3	150	150.297	100.198	

Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount.
% Recovery was 100.00% for Magaldrate and 100.00% for Simethicone. All the results indicate that the method is highly accurate.

Precision

Table 8: Precision studies for Magaldrate and Simethicone

S. no	Intraday precision for Magaldrate			Intraday precision for Simethicone		
	Peak area	Mean peak area	%RSD	Peak area	Mean peak area	%RSD
1	2653415			567898		
2	2654514			568887		
3	2685475	2667028	0.556	569275	568727	0.137
4	2658426			569858		
5	2664858			568586		

6	2685479	567858

Results of variability were summarized in the above table. The %RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

Intermediate precision
Day 1

Table 9: Results of Intermediate precision for Magaldrate

S no	Name	Rt	Area	USP plate count	USP Tailing
1	Magaldrate	1.691	2758958	4586	1.69
2	Magaldrate	1.689	2757847	4571	1.68
3	Magaldrate	1.690	2758423	4522	1.69
4	Magaldrate	1.691	2754514	4563	1.68
5	Magaldrate	1.692	2758947	4528	1.69
6	Magaldrate	1.688	2753486	4579	1.69
Mean			2757029		
Std. Dev			2403.755		
% RSD			0.087186		

- *%RSD of five different sample solutions should not more than 2.*

Table 10: Results of Intermediate precision for Simethicone

S no	Name	Rt	Area	USP plate count	USP Tailing	USP Resolution
1	Simethicone	3.219	578958	5485	1.54	5.00
2	Simethicone	3.290	578478	5436	1.54	5.01
3	Simethicone	3.208	578546	5482	1.55	5.00
4	Simethicone	3.214	578562	5479	1.56	5.01
5	Simethicone	3.294	578457	5472	1.55	5.01
6	Simethicone	3.288	578984	5492	1.56	5.01
Mean			578664.2			
Std. Dev			241.0846			
% RSD			0.041662			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 11: Results of Intermediate precision Day 2 for Magaldrate

S no	Name	Rt	Area	USP plate count	USP Tailing
1	Magaldrate	1.688	2845125	4785	1.69
2	Magaldrate	1.688	2879854	4758	1.68
3	Magaldrate	1.688	2845741	4726	1.70
4	Magaldrate	1.691	2875698	4792	1.69
5	Magaldrate	1.687	2874571	4759	1.70
6	Magaldrate	1.688	2869563	4781	1.70
Mean			2865092		
Std. Dev			15578.11		
% RSD			0.543721		

- *%RSD of five different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision for Simethicone

S no	Name	Rt	Area	USP plate count	USP Tailing	USP Resolution
1	Simethicone	3.265	589654	5598	1.54	5.01
2	Simethicone	3.265	587454	5574	1.53	5.02
3	Simethicone	3.266	587683	5536	1.54	5.02
4	Simethicone	3.275	586984	5598	1.54	5.03

5	Simethicone	3.277	587482	5582	1.53	5.01
6	Simethicone	3.265	589658	5546	1.54	5.01
Mean			588152.5			
Std. Dev			1186.956			
% RSD			0.201811			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Linearity

Table 13: Linearity data for Magaldrate

S. no	Concentration (µg/ml)	Rt	Area
1.	50	1.689	1254871
2.	75	1.691	1895454
3.	100	1.692	2653415
4.	125	1.689	3258474
5.	150	1.688	3986547

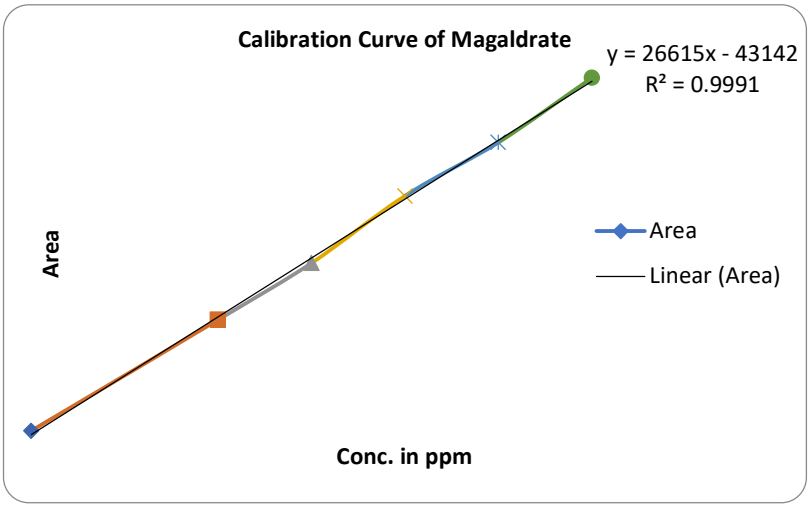

Fig 2: Linearity plot of Magaldrate

Table 14: Linearity data for Simethicone

S. no	Concentration (µg/ml)	Rt	Area
1.	50	3.203	269658
2.	75	3.299	418753
3.	100	3.294	559858
4.	125	3.290	695847
5.	150	3.288	828654

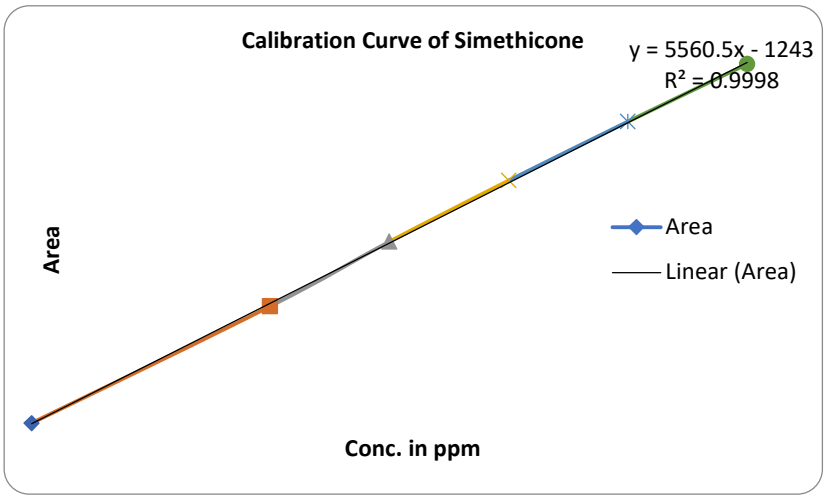

Fig 3: Linearity plot of Simethicone

A linear relationship between peak areas versus concentrations was observed for Magaldrate and Simethicone in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for Magaldrate and 1 for Simethicone which prove that the method is linear in the range of 50% to 150%.

Robustness

Table 15: Results for Robustness

Robustness data for Magaldrate

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	1.868	4052	1.60
Increased flow rate (1.2ml/min)	1.544	4941	1.49
Decreased temperature (20^0c)	1.731	4475	1.61
Increased temperature (30^0c)	1.675	4581	1.61

Robustness data for Simethicone

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	3.621	5230	1.45
Increased flow rate(1.2ml/min)	2.998	5828	1.41
Decreased temperature (20^0c)	6.242	5484	1.50
Increased temperature (30^0c)	2.302	5494	1.50

The results of robustness of the present method had shown that changes made in the flow and temperature did not produce significant changes in analytical results which were presented in the above table. As the changes are not significant we can say that the method is robust.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Magaldrate and Simethicone in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Magaldrate is practically insoluble in water and in alcohol, ethanol (96 per cent). It is soluble in dilute mineral acids. Simethicone is was found to be insoluble in water and in ethanol; soluble in carbon tetrachloride, benzene, chloroform, diethyl ether, practically insoluble in methanol and soluble in ethyl acetate and in methanol. Potassium dihydrogen phosphate: Methanol (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Magaldrate and Simethicone in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Samskruti college of pharmacy in Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES

1. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house meerut, 2004, P12-23.
2. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
3. John Adamovies, Chromatographic analysis of pharmaceutical, Marcel Dekker Inc. New York, 2nd ed, P.74, 5-15.
4. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, P.1.1-1.8, 2.566-2.570
5. D. A. Skoog. J. Holler, T.A. Nieman. Principle of instrumental analysis, 5th edition, Saunders college publishing, 1998, P.778-787.
6. Skoog, Holler, Nieman. Principals of instrumental analysis 5th ed, Harcourt publishers international company, 2001, P.543-554.
7. William Kemp. Organic spectroscopy, Palgrave, New York, 2005, P.7-10, 328-330
8. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and distributors, New Delhi (India), 2001, P.3-137.
9. Michael E, Schartz IS, Krull. Analytical method development and validation. 2004, P. 25-46.
10. R. Snyder, J. Kirkland, L. Glajch. Practical HPLC method development, 2nd ed, A Wiley international publication, 1997, P.235,266-268,351-353.653-600.686-695.
11. Basic education in analytical chemistry. Analytical science, 2001:17(1).
12. Method validation guidelines international onference on harmonization; GENEVA; 1996
13. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28
14. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, Pharmaceutical press, London, 2004, P.1109-1110, 1601-1602.
15. Klaus Florey, Analysis profile of drugs substances, Academic press, New York, 2005, P.406-435.