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Research

Analytical Method Development And Validation For Simultaneous Estimation Of Ranitidine And Ondansetron In Pure And Its Tablet Dosage Form By Rp-Hplc

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Ranitidine and Ondansetron, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (50:50) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 244nm. The retention time of the Ondansetron and Ranitidine was 2.0, 4.0±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Ondansetron and 93.75-468.75µg/ml of Ranitidine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Ondansetron, Ranitidine, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc.

Table 1: Summary of Hyphenated separation techniques.[2]

Separation technique	Hyphenated mode
Liquid chromatography	Liquid chromatography-mass spectrometry(LC/MS) Liquid chromatography-Fourier-transform infrared Spectrometry(LC-FTIR) Liquid chromatography-nuclear magnetic resonance spectroscopy(LC/NMR) Liquid chromatography-inductively coupled plasma mass spectrometry(LC-ICPMS)
Gas chromatography	Gas chromatography-mass spectrometry(GC/MS) Gas chromatography-Fourier-transform infrared (GC-FTIR) Gas chromatography-FTIR-MS(GC-FTIR-MS)
Capillary electrophoresis	Capillary electrophoresis-mass spectrometry(CE/MS) Capillary electrophoresis- nuclear magnetic resonance spectroscopy(CE/NMR) Capillary electrophoresis-surface enhanced Raman spectrometry (TLC-SERS)
Thin layer chromatography(TLC)	Thin layer chromatography- mass spectrometry(TLC/MS) Thin layer chromatography- surface enhanced Raman spectrometry(TLC-SERS)
Superficial fluid chromatography/ extraction(SFC/SFE)	Superficial fluid extraction-capillary gas chromatography-mass spectrometry(SFE-CGC-MS) Superficial fluid-Fourier-transform infrared(SFC-FTIR)

INTRODUCTION TO HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance. [6]

MATERIALS AND METHODS

Ondansetron-Suralabs, Ranitidine-Suralabs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile-HPLC Merck, Triethylamine-Sura labs

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Ondansetron and Ranitidine 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 0.15ml of the Ondansetron and 2.81ml of the Ranitidine 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.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Hypersil C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (50:50v/v)
Flow rate : 1ml/min
Wavelength : 244 nm
Injection volume : 10 µl
Run time : 10 min

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 500 ml (50%) of Water, 500ml of Acetonitrile (50%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Water (50:50v/v)
Column : Hypersil C18 (4.6×250mm) 5µ
Column temperature : 40ºC
Wavelength : 235nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 8minutes

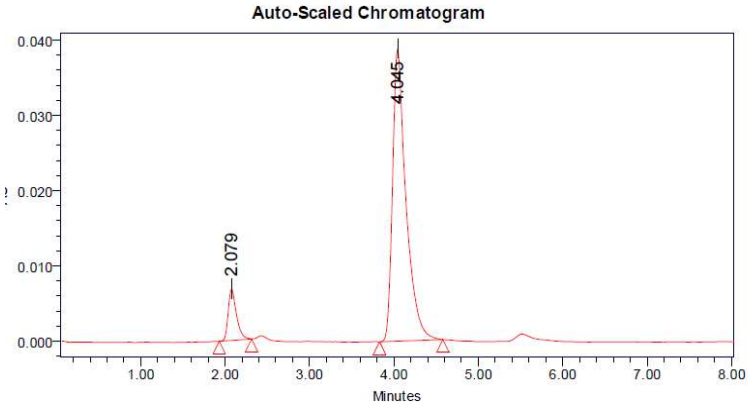

Fig 1: Optimized Chromatogram (Standard)

Table 2: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Ondansetron	2.079	46168	6841	1.33	4251
2	Ranitidine	4.045	429069	38885	1.59	5224

Assay (Standard)

Table 3: Peak results for assay standard of Ondansetron

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ondansetron	2.078	49569	6811	6945	1.51
2	Ondansetron	2.080	49649	6999	6149	1.57
3	Ondansetron	2.078	49731	6972	6473	1.49

4	Ondansetron	2.079	49479	6971	6190	1.49
5	Ondansetron	2.082	49684	6841	6294	1.49
Mean			49607			
Std. Dev.			107.963			
% RSD			0.217637			

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

Table 4: Peak results for assay standard of Ranitidine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ranitidine	4.041	423328	44147	7672	1.35
2	Ranitidine	4.033	423805	44538	7786	1.13
3	Ranitidine	4.050	423229	44964	5772	1.34
4	Ranitidine	4.045	423876	44959	5191	1.35
5	Ranitidine	4.032	423575	38885	5137	1.35
Mean			423559.5			
Std. Dev.			328.2606			
% RSD			0.0775			

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

Assay (Sample)

Table 5: Peak results for Assay sample of Ondansetron

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Ondansetron	2.078	46684	6918	1.34	5217	1
2	Ondansetron	2.079	46168	6841	1.33	5251	2
3	Ondansetron	2.077	46088	6851	1.37	7127	3

Table 6: Peak results for Assay sample of Ranitidine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Ranitidine	4.050	430575	39127	1.60	6197
2	Ranitidine	4.045	429069	38885	1.59	6224
3	Ranitidine	4.037	429543	38892	1.58	8203

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=429729/423559.5*10/120*120/0.199*99.7/100*1.1944/600*100

The % purity of Ondansetron and Ranitidine in pharmaceutical dosage form was found to be 100.1522%

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Table 7: Chromatographic Data For Linearity Study For Ondansetron

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	15065
66.6	10	31009
100	15	46166
133.3	20	60569
166.6	25	76862

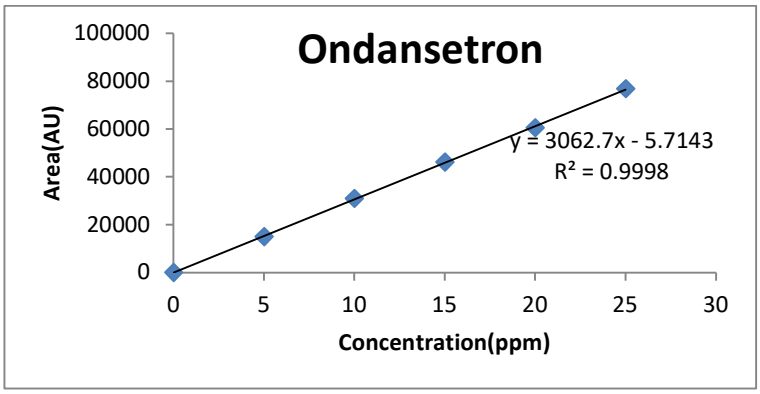

Fig 2: Chromatogram showing linearity level

Table 8: Chromatographic Data For Linearity Study For Ranitidine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	93.75	131289
66.6	187.5	284775
100	281.25	427559
133.3	375	555861
166.6	468.75	712514

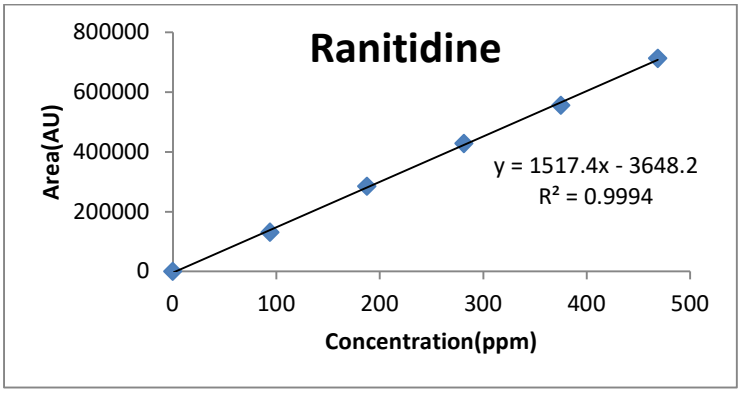

Fig 3: Chromatogram showing linearity level

REPEATABILTY

Table 9: Results of repeatability for Ondansetron:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ondansetron	2.077	46054	6784	4208	1.32
2	Ondansetron	2.076	46803	6867	6088	1.34
3	Ondansetron	2.076	46150	6766	4152	1.36
4	Ondansetron	2.077	46056	6715	4184	1.32
5	Ondansetron	2.074	46247	6746	4065	1.33
Mean			46262			
Std.dev			312.7099			
%RSD			0.675954			

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 10: Results of repeatability for Ranitidine

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ranitidine	4.031	427962	38634	5158	1.57
2	Ranitidine	4.024	429623	38673	5092	1.58
3	Ranitidine	4.019	427826	38246	5071	1.58
4	Ranitidine	4.016	427829	38310	5046	1.58
5	Ranitidine	4.014	429559	38181	5036	1.58
Mean			428559.8			
Std.dev			943.2246			
%RSD			0.220092			

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 11: Results of Intermediate precision day1 for Ondansetron

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate countt	USP Tailing
1	Ondansetron	2.075	46204	6673	5117	1.33
2	Ondansetron	2.074	46300	6735	5043	1.36
3	Ondansetron	2.075	46259	6652	5087	1.28
4	Ondansetron	2.075	46223	6667	5134	1.31
5	Ondansetron	2.075	46205	6674	5151	1.32
6	Ondansetron	2.074	46189	6703	5157	1.33
Mean			46230			
Std. Dev.			41.88556			
% RSD			0.090603			

%RSD of Six different sample solutions should not more than 2

Table 12: Results of Intermediate precision day1 for Ranitidine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate countt	USP Tailing
1	Ranitidine	4.013	428922	38004	7038	1.58
2	Ranitidine	4.011	428524	37935	7999	1.57
3	Ranitidine	4.010	427239	37850	7003	1.57
4	Ranitidine	4.008	427667	37780	7982	1.57
5	Ranitidine	4.006	427826	37824	7983	1.57
6	Ranitidine	4.006	427093	37970	7042	1.58
Mean			427878.5			
Std. Dev.			718.1952			
% RSD			0.16785			

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

DAY-2

Table 13: Results of Intermediate precision Day 2 for Ondansetron

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ondansetron	2.076	46803	6867	5149	1.57
2	Ondansetron	2.076	46056	6715	5190	1.13
3	Ondansetron	2.077	46252	6652	6088	1.58
4	Ondansetron	2.075	46205	6674	5184	1.58
5	Ondansetron	2.075	46940	7249	5087	1.57
6	Ondansetron	2.072	46727	6983	5151	1.57

Mean	46497.17
Std. Dev.	369.4739
% RSD	0.794616

%RSD of Six different sample solutions should not more than 2 Table:

Table 14: Results of Intermediate precision Day 2 for Ranitidine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ranitidine	4.024	429623	38673	6789	1.49
2	Ranitidine	4.024	427829	38310	5772	1.34
3	Ranitidine	4.016	427263	37850	5092	1.32
4	Ranitidine	4.010	427826	37824	6046	1.28
5	Ranitidine	4.006	421284	40752	6003	1.32
6	Ranitidine	4.008	421832	40281	6983	1.33
Mean			425942.8			
Std. Dev.			3492.681			
% RSD			0.819988			

- %RSD of Six different sample solutions should not more than 2

ACCURACY

Table 15: The accuracy results for Ondansetron

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	22938.33	7.5	7.3	99.88	100.166
100%	45426	15	14.7	98.89	
150%	70096.67	22.5	22.2	101	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 16: The accuracy results for Ranitidine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	140.6	140.2	99.7%	99%
100%	420697.7	281.25	281.1	99%	
150%	631550.7	421.8	421.4	99%	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 17: Results for Robustness -Ondansetron

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	46168	2.079	4251	1.33
Less Flow rate of 0.8mL/min	51177	2.29	5269	1.38
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	42190	1.890	5126	1.32
Less organic phase (about 5 % decrease in organic phase)	42402	1.885	5126	1.19
More organic phase (about 5 % Increase in organic phase)	42112	1.908	5854	1.36

Table 18: Results for Robustness-Ranitidine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	4.045	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.450	6328	1.58
More Flow rate of 1.0mL/min	392497	3.660	6217	1.54
Less organic phase (about 5 % decrease in organic phase)	391379	4.251	6996	1.61
More organic phase (about 5 % Increase in organic phase)	391703	3.239	6120	1.50

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ranitidine and Ondansetron 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. Ranitidine and Ondansetron was freely soluble in ethanol, methanol and sparingly soluble in water. Water and Acetonitrile (50:50) 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 Ranitidine and Ondansetron in bulk drug and in Pharmaceutical dosage forms.

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