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Development and Validation of Analytical Method for Simultaneous Estimation of Montelukast Sodium and Doxycycline by UV-Visible Spectrophotometry

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Abstract: ***A two simple, accurate, reliable, precise, sensitive and economical spectrophotometric method has been developed for simultaneous estimation of Montelukast Sodium and Doxycycline in pharmaceutical formulations. In the first derivative UV method, methanol was used as a solvent and quantification was achieved at 288 nm for Montelukast sodium and 276 nm for Doxycycline over the concentration range of 5-30 µg/ml for Montelukast Sodium and 5-30 µg/ml for Doxycycline Respectively.The method linearity is observed in the concentration range of 5-30 µg/ml for Montelukast Sodium and 5- 30 µg/ml for Doxycycline. The correalation coefficient of drugs 0.999 for Montelukast Sodium and 0.996 for Doxycycline respectively . The Standard deviation of the Montelukast Sodium was 0.0061and the Doxycycline was 0.0051. The method validated according to ICH guidelines all validation parameter were studied for the proposed method like linearity, precision, LOD, LOQ. The % RSD for recovery study was found to be less than 2% indicating the precision of method. The results were validated statistically as per ICH Q2 R1 guidelines and it was discovered satisfactory. LOD and LOQ for Montelukast Sodium and Doxycycline were discovered to be 0.495µg / ml and 1.539µg / ml. and 0.142 µg/ml and 0.306 µg/ml respectively. Precision, accuracy, and robustness studies confirmed the method's reliability. The validated method is suitable for routine quality control and stability testing of combined Montelukast Sodium and Doxycycline formulations in the pharmaceutical industry. The above two methods were validated by performing analytical parameters which are mentioned under ICH guidelines and all parameters are within acceptable.***

Keywords: Montelukast Sodium, Doxycycline, First Derivative UV-Spectroscopy, Deviation, ICH Q2 R1, Validation

1. Introduction

Montelukast sodium ,[R–(E)]-1-[[[1-[3-[2-(7-chloro–2 quinolinyl)ethenyl]phenyl]–3–[2-(7-chloro-2-quinolinyl) ethenyl]phenyl]-3-[2-(1-hydroxy-1 methylethyl)phenyl]propyl]thio] an Leukotriene Receptor Antagonists It works by blocking the action of Leukotriene D4 in the lungs resulting in decreased inflammation and relaxation of smooth muscle. It's functions as a leukotriene receptor antagonist and consequently opposes the function of these inflammatory mediators; leukotrienes are produced by the immune system and serve to promote Bronchoconstriction, inflammation, microvascular permeability, and mucus secretion in Asthma and Chronic obstructive pulmonary diseases (COPD. Montelukast Sodium causes the inhibition of airway cysteinyl leukotriene receptors as demonstrated by the ability to inhibit bronchoconstriction due to the inhaled LTD_4 in asthmatics. [1, 2, 3]

Doxycycline, 4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydrotetracene-2-carboxamide is a broad-spectrum tetracycline antibiotic. It inhibits the synthesis of bacterial proteins by binding to the 30S ribosomal subunit, found in bacteria. This prevents the binding of t -RNA to m- RNA at the ribosomal subunit. Amino acids cannot be added to the polypeptide chains and new proteins cannot be made. This stops bacterial growth giving the immune system time to kill and remove the bacteria.[4,5,6]

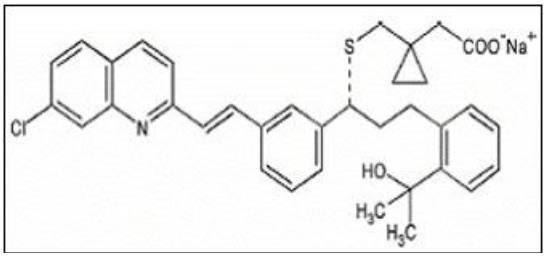

Figure 1: Montelukast Sodium

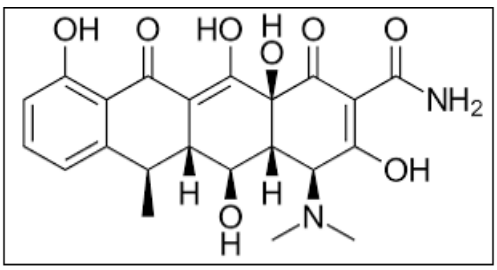

Figure 2: Doxycycline

2. Materials and Methods

2.1. Chemicals and Reagents

References drugs are Montelukast Sodium and. Doxycycline Hydrochloride were gifted by Madras

Pharmaceuticals Pvt. Ltd., Chennai and Montelukast Sodium was gifted by Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad. All the chemicals used were of analytical grade and HPLC grade procured from S d fine chem Ltd, Mumbai and Loba Chemie India Limited, Mumbai.

2.2. Instruments

Jasco V-630 Double Beam UV - Visible Spectrophotometer with a pair of 10 mm matched quartz cells. ELICO LI - 127 pH meter. SOLTEC - Sonica Ultrasonicator– Ultrasonic USB 400. Shimadzu, AUY – 220 Weighing balance are used.

2.3. Experimental Conditions

Preparation of stock solution:
10 mg of each Montelukast sodium and Doxycycline were weighed separately and transferred into two different 100 mL volumetric flasks. The drugs were dissolved in 10 mL of Methanol by sonication and adjusted the volume to 100 ml with distilled water to get a concentration of 100 µg/mL of Montelukast Sodium and Doxycycline. [5,6,8,9]

Preparation of Standard Solution:
From the stock solution of Montelukast Sodium and Doxycycline the standard solution of prepared in 5- 30 ug /ml concentration range for Montelukast Sodium and 5- 30 ug / ml concentration range of Doxycycline respectively.[5,6,8,9]

Preparation of sample solution :
The sample was prepared by taking 1 ml of each Montelukast Sodium and Doxycycline from stock solution in a 10 ml volumetric flask , mix well and make volume upto the mark using distilled water , then measure the absorbances of the sample solution at respective wavelength using double-beam UV- Visible spectrophotometer.[5,6,8,9]

3. Spectroscopic Method development:

3.1 Method A : Simultaneous Equation Method :

From the overlay spectra of the two drugs Montelukast Sodium and Doxycycline shows absorbance maxima at 288 and 276nm respectively. Working standard solution are analyzed in the concentration range of 5- 30 ug / ml for both Montelukast Sodium and Doxycycline respectively .[5,6]

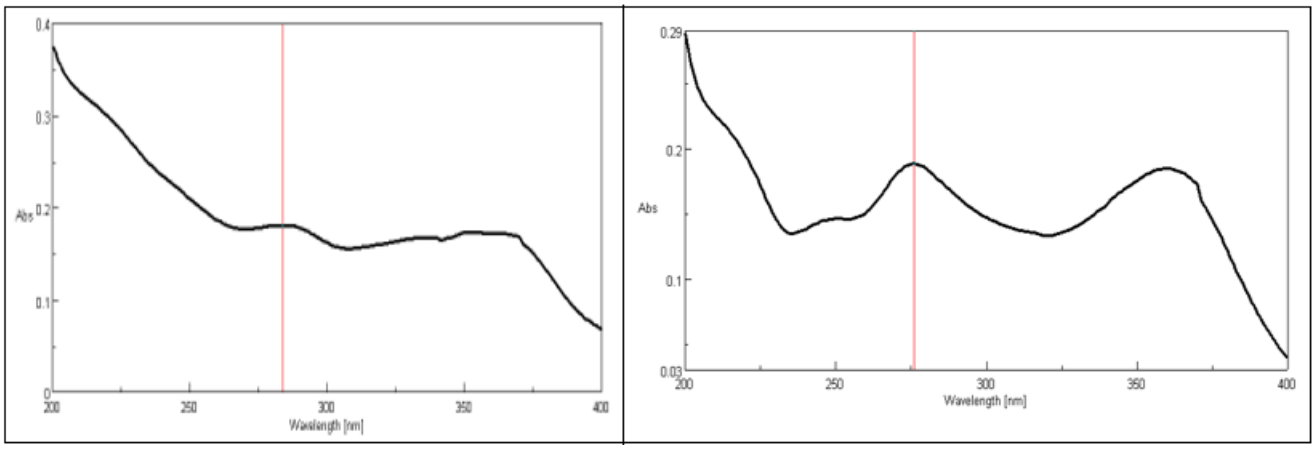

Figure 3: UV spectra of Montelukast Sodium & Doxycycline in distilled water at 288 nm and 276 nm (20ppm)

3.2 Method B : Absorbance Ratio Method

Absorbance ratio method is used as the absorbance at two selected wavelength , one of spectra of them isoabsorptive point and second λmax of any of the drug . From the overlay spectra of the two drugs Montelukast Sodium and Doxycycline shows isoabsorptive point at as 371nm and the second wavelength is 262nm . Working standard solution having the concentration range from 5- 30 ug / ml for Montelukast Sodium and 5- 30 ug/ ml for Doxycycline respectively .[5,6]

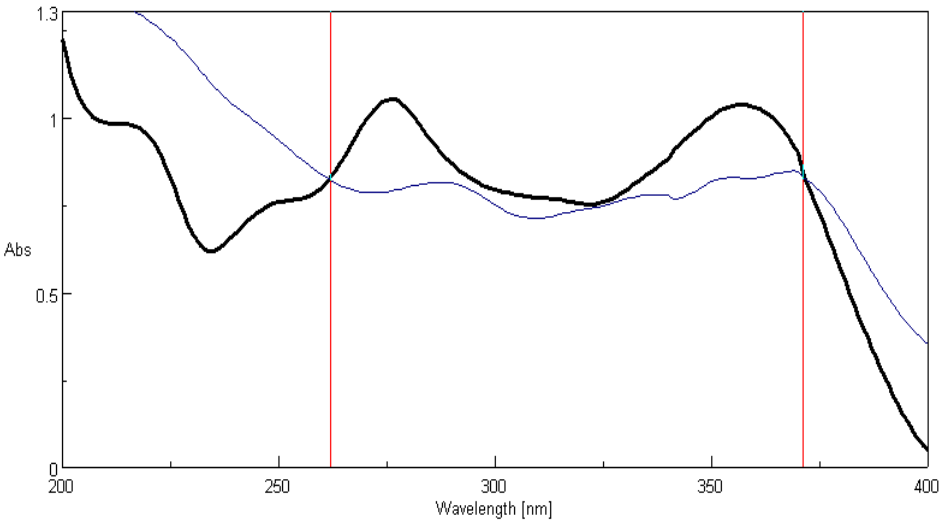

Figure 4: Overlain UV Absorption Spectra of Montelukast Sodium and Doxycycline shows Isoabsorptive point at 262nm in Distilled Water 25 PPM .

4. Results and Discussion

4.1 Linearity

The calibration curve were plotted for both the Montelukast Sodium and Doxycycline at 288nm and 276nm . Both drugs shows linearity and obey the beer's law in the concentration range of 5- 30 ug/ ml for Montelukast Sodium and 5- 30 ug/ml for Doxycycline . The correlation coefficients of calibration curve were found to be 0.999 for Montelukast Sodium and 0.996 for Doxycycline respectively .

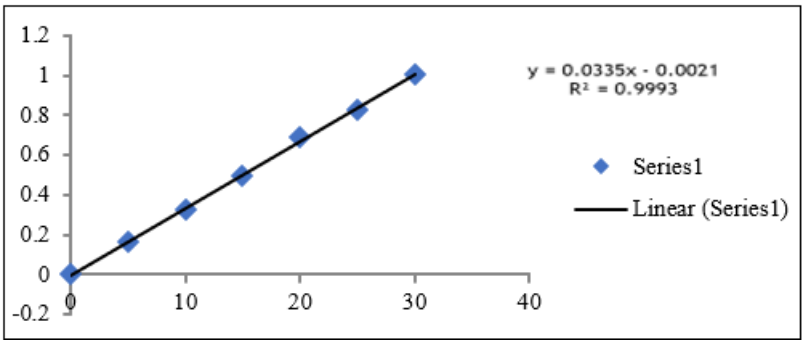

Figure 5: Calibration curve of Montelukast Sodium

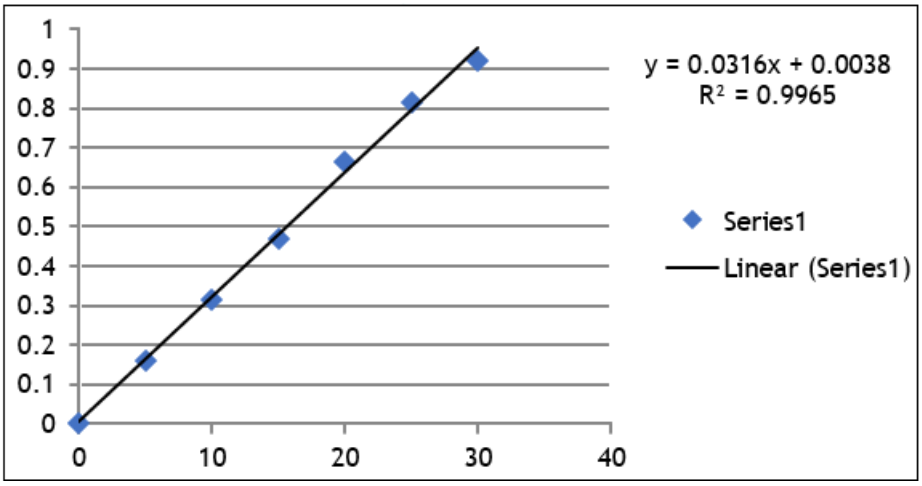

Figure 6: Calibration curve of Doxycycline

4.2. Accuracy and Precision

The recovery results confirm the method's correctness, which are within the range of 100 ± 2%. Studies on both intra-day and inter-day variance showed precision. Investigations conducted within a single day involved three consecutive injections of sample solutions. The percentage was used to evaluate precision. The % RSD for recovery study was found to be less than 2% indicating the precision of method. Inter-day precision tests, including three

repeated standard and sample solutions injections over successive days yielded %RSD values. The gathered data, which has a percentage RSD of less than 1.5%, shows how accurate the developed process is. The limits of detection (LoD) and quantification (LoQ) were also established. To calculate LoD, use the formula LoD = (3.3 x standard deviation/Slope of the calibration curve), which represents the lowest analyte concentration generating a detectable reaction LOD was found to be 0.423 and 0.106 at wavelength 288 nm and 276 nm, for Montelukast Sodium and Doxycycline respectively. The formula for LoQ, which represents the lowest accurately measurable. LOQ was found to be 0.626 and 0.379 at wavelength nm288 and 276 nm, for Montelukast Sodium and Doxycycline respectively.

Table 1: Intra- day Precision

Sr. No.	Concentration of Drug (µg/ml)	Absorbance		SD		%RSD	
		Montelukast Sodium	Doxycycline	Montelukast Sodium	Doxycycline	Montelukast Sodium	Doxycycline
1.	10 (n=3)	0.3300	0.3100	0.0043	0.00102	1.327	0.327
		0.3210	0.3114				
		0.3205	0.3125				
2.	20 (n=3)	0.6850	0.6641	0.0021	0.0012	0.306	0.180
		0.6812	0.6650				
		0.6863	0.6665				
3.	30 (n=3)	1.111	1.010	0.038	0.0037	0.035	0.036
		1.019	1.009				
		1.046	1.003				

Intra Day Precision:

Table 2: Inter Day Precision

Day	Concentration of Drug (µg/ml)	Absorbance		SD		%RSD	
		Montelukast Sodium	Doxycycline	Montelukast Sodium	Doxycycline	Montelukast Sodium	Doxycycline
1.	10 (n=3)	0.3219	0.3101	0.0061	0.0051	1.92	1.67
		0.3201	0.3019				
		0.3105	0.3005				
2.	20 (n=3)	0.6842	0.6542	0.0158	0.0011	0.21	0.17
		0.6831	0.6530				
		0.6811	0.6520				
3.	30 (n=3)	0.8670	0.9310	0.0036	0.0149	0.41	1.62
		0.8658	0.9107				
		0.8601	0.9019				

Table 3: Summary of Validation Parameters of UV-Spectroscopic Method for Simultaneous estimation of Montelukast sodium and Doxycycline

Parameters	Montelukast Sodium at 288nm	Doxycycline at 276nm
Beer's Law Range	5-30 (µg /ml)	5-30 (µg/ml)
Regression Equation (y= mx + c)	y = 0.0335+ (-0.0021)	y = 0.0316x + 0.0038
Slop (m)	0.0335	0.0316
Intercept (c)	-0.0021	0.0038
Correlation Coefficient (R^2)	R^2 = 0.999	R^2 = 0.996

5. Conclusion

The proposed method was found to be simple, rapid, accurate, selective and economical for simultaneous routine analysis of Montelukast sodium and Doxycycline in bulk and commercial dosage form. The developed method complies with ICH Q2 (R1) validation guidelines. This method can also be used for determination of content uniformity and dissolution profiling of this product. It can be also applied for the industrial as well as academic purpose.

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