Research Article

Method Development and Validation of Montelukast in API and Pharmaceutical Dosage form by UV Spectrophotometry

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ABSTRACT

The present work is done to develop a new simple, rapid, specific, accurate and precise UV spectrophotometric method for Montelukast as API and in pharmaceutical dosage form. The validation of the proposed method was carried out according to the I.C.H guidelines. The wavelength maxima was found 270nm and calibration curves were obtained in the concentration range 5-45µg/ml for montelukast with good correlation coefficients (r2=0.9994.). The precisions of the new method for montelukast was less than the maximum allowable limit (%RSD <2.0) specified by the ICH. Therefore, the method was found to be an accurate, reproducible and sensitive for analysis of montelukast as standard, pharmaceutical dosage forms, and other routine analysis method.

Keywords: UV, Montelukast, Methanol, concentration range, correlation coefficient, Validation.

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INTRODUCTION

Montelukast is a leukotriene receptor antagonist used as part of an asthma therapy regimen, to prevent exercise induced bronchoconstriction, and to treat seasonal allergic rhinitis. [16] Montelukast (empirical formula $C_{35}H_{35}ClNO_3S$) is a highly selective leukotriene receptor antagonist that binds with high affinity to the cysteinyl leukotriene receptor for leukotrienes D4 and E4. These leukotrienes are excreted by various types of cells, such as mast cells, and are involved in the inflammatory process that may cause the signs and symptoms of asthma and allergic rhinitis. Leukotriene receptors are found in airway cells, such as macrophages and smooth muscle cells. When bound to leukotriene receptors, montelukast inhibits leukotriene physiologic effects (such as airway edema, smooth muscle contraction, and impairment of normal cellular activity) without exhibiting any agonist activity. [17]

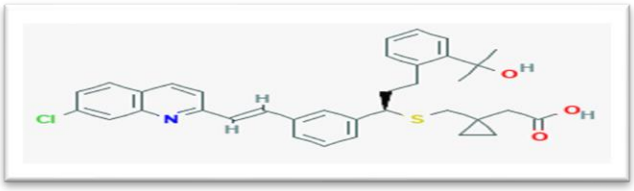

Figure 1: Molecular structure of Montelukast

MATERIALS AND METHODS

Instrument Used

The spectrophotometric analysis was carried out on Single beam UV spectrophotometer (GS2281) using 1cm quartz cell. The application software used for the obtaining the spectra was named CaryWin60 and is manufactured by Agilent Tech. All weighing was carried out on single pan Digital weighing balance (TX323L) manufactured by Shimadzu Instrument Pvt. Ltd.

Chemical Used

Montelukast with claimed purity was obtained as a gift sample from the Synokem Pharmaceuticals, Haridwar. Montair 10mg (Marketed formulation) was purchased from the local market. Methanol (analytical reagent grade) was obtained from central drug house Pvt. Ltd.

Method Development For Estimation of Montelukast by UV-Spectrophotometry

Preparation of Standard Stock Solution

Stock solutions of was prepared by transferring 50 mg of the drug in 50 ml volumetric flask and dissolved in 30 ml of methanol and the volume was made up to the mark with methanol. This standard stock solution contains 1000µg of drug per ml. 5 ml of this solution was transferred to additional 50 ml volumetric flask and further diluted up to 50 ml mark with methanol. This standard solution contains 100µg of drug per ml (working standard stock).

Selection of wavelength Maxima (λmax)

Pipette out 1 ml of working standard solution and transfer into 10 ml volumetric flask and the volume was made up to the mark with solvent to get the concentration 10 µg/ml. The resulted 10 µg/ml solution was scanned in UV-Spectrophotometer between 200-400 nm using methanol

as blank. The wavelength maxima were established at 270nm. The results are shown in figure 1.

Preparation of Calibration curve

Pipette out 0.5,1,1.5,2,2.5,3,3.5,4 and 4.5 ml working standard solution and transfer into nine separate 10 ml volumetric flasks and made the volume all of them to 10 ml with methanol to get the concentrations 5, 10, 15,20,25,30,35,40 and 45µg/ml respectively. Absorbance of the resultant solution was measured at 270nm using methanol as blank. A graph was plotted between the concentrations and their respective absorbance. The response of the drug was found linear in the entire investigational range of 5-45µg/ml. The calibration equation for montelukast the calibration curve equation obtained was found to be y = 0.0242x-0.0302 with 0.9994 correlation coefficient.

Repeatability

Pipette out 2, 2.5, 3ml standard solution and transfer into a series of nine, 10 ml volumetric flasks. Dilute it to 10 ml with methanol to get 20, 25, 30µg/ml solutions respectively. Absorbance of the resultant solutions was measured at 270nm using methanol as blank. The result obtained and summarized in the table 2.

Intra-Day Precision

Pipette out 2, 2.5 and 3ml working solution and transfer into separate 10 ml volumetric flasks and made up the volume to 10 ml with methanol to get the concentrations 20, 25 and 30µg/ml respectively. Absorbance of the resultant solutions was measured at 270nm using methanol as blank. Such three revisions were performed within a day at 3hrs interval. The result was summarized in the table 3.

Inter-Day Precision

Pipette out 2, 2.5 and 3ml working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with methanol to get solution of concentrations 20, 25 and 30µg/ml respectively. Absorbance of the resultant solutions was measured at 270nm using methanol as blank. Such three studies were performed for day one, day two, day three intervals. The result was summarized in the table 4.

Accuracy

Pipette out 2ml standard solution and transfer into 10 ml volumetric flasks. Nine such transfers were made. Spike three of volumetric flask with the solutions with 1.6ml of working solution (Prepared from Formulation) and dilute each to 10 ml with methanol to get 16µg/ml solutions. Spike another three of the solutions with 2ml of working solution and dilute each to 10 ml with methanol to get 20µg/ml solutions. Spike last three of the solutions with 2.4ml of working solution and dilute each to 10 ml with methanol to get 24µg/ml solutions. Absorbance of the resultant solutions was measured at 270nm using methanol as blank. The obtained results were summarized in the table 5.

Specificity

Specificity study was carried out by observing any interference in absorbance of drug in the presence of common excipients like starch, talc, lactose, magnesium stearate etc. Absorbance of 20µg/ml drug solution with and without excipients was measured at 270nm using methanol as blank. The results obtained were summarized in the table 6.

Limit of Detection (LOD)

The Detection Limit is defined as the lowest concentration of an analyte in a sample that can be detected.

The Detection Limit may be expressed as:

$$DL = 3.3\sigma / s$$

DL = 2.755909

Where,

σ = standard deviation of the response.

s = slope of the linearity curve.

Limit of Quantitation (LOQ)

The Quantitation Limit is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the analytical procedures.

The Quantitation Limit may be expressed as:

$$QL = 10\sigma / s$$

QL = 8.31524

Where,

σ = standard deviation of the response.

s = slope of the linearity curve.

Estimation of Montelukast in pharmaceutical dosage form (Montair 10mg)

Weigh 10 tablets and calculate the average weight of the tablets. Powered the tablets and weigh accurately a quantity of powdered containing about 50 mg of montelukast and transfer it into 50 ml volumetric flask and add 30 ml methanol, sonication for 10 minutes and made up the volume to 50 ml with solvent then mix and filter that solution. Taken 5 ml of the filtrate and made up the volume to 50 ml with methanol. Further dilute 1 ml of the resulting solution to 10 ml with methanol. Measure the absorbance of this resulting solution at 270nm. The above procedure was repeated for three times. The result obtained was summarized in the table 7.

RESULTS

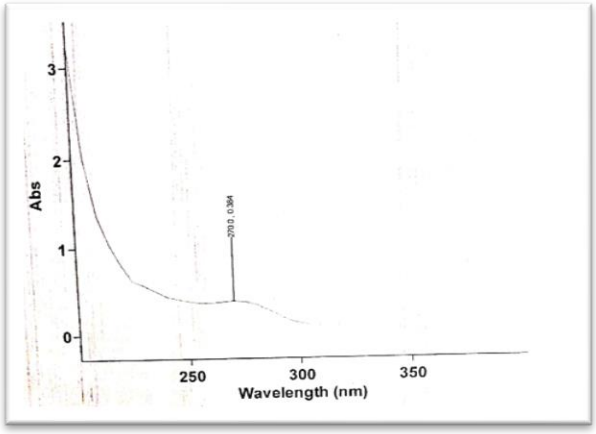

Figure 1: Wavelength maxima of Montelukast in Methanol

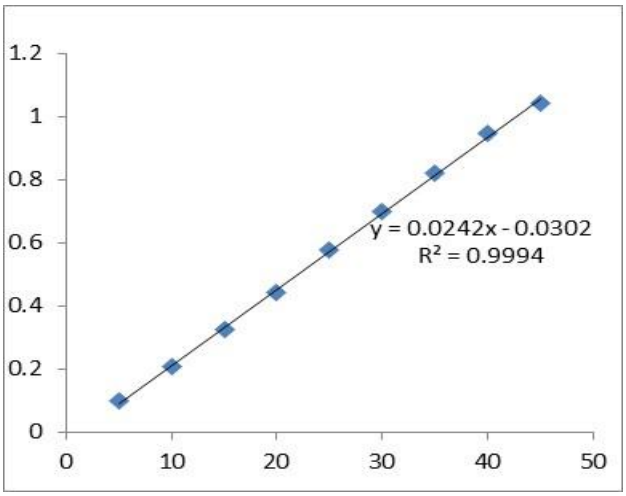

Figure 2: Calibration curve of Montelukast in Methanol

Table 1: Linearity, $E^{1\%}_{1\,CM}$, Absorptivity (L gm-1 cm-1), Molar Absorptivity (L mol-1 cm-1) of Montelukast

S. No	CONCENTRATION (µg/ml)	ABSORBANCE at 270nm	$E^{1\%}_{1cm}$	ABSORPTIVITY	MOLAR ABSORPTIVITY
1	5	0.0989	197.8	19.78	11606.42
2	10	0.2069	206.9	20.69	12128.13
3	15	0.3266	217.7	21.77	12761.20
4	20	0.4432	221.6	22.16	12989.82
5	25	0.5766	230.6	23.06	13517.38
6	30	0.7012	233.7	23.37	13699.09
7	35	0.8207	234.5	23.45	13745.99
8	40	0.9465	236.6	23.66	13869.09
9	45	1.0452	232.3	23.23	13617.03
		Mean	223.5	22.35	13103.79

Table 2: Study of Repeatability

Concentration (µg/ml)	Absorbance	Observed Concentration (µg/ml)	Mean Concentration	SD	%RSD
20µg/ml	0.4634 0.4610 0.4360	20.3µg/ml 20.2µg/ml 19.8µg/ml	20.1µg/ml	0.2645	1.3162
25µg/ml	0.5744 0.5722 0.5688	24.9µg/ml 24.8µg/ml 24.6µg/ml	24.76µg/ml	0.1529	0.5938
30µg/ml	0.6999 0.6871 0.6772	30.1µg/ml 29.6µg/ml 29.3µg/ml	29.66µg/ml	0.4044	1.3628
Mean					1.1062

Table 3: Study of Intra Day Precision

Conc. (µg/ml)	Absorbance			Observed Concentration (µg/ml)			Mean	SD	RSD
	0hrs	3hrs	6hrs	0hrs	3hrs	6hrs			
20µg/ml	0.4610	0.4640	0.4626	20.2µg/ml	20.3µg/ml	20.3µg/ml	20.26µg/ml	0.0583	0.2878
25µg/ml	0.5744	0.5728	0.5673	24.9µg/ml	24.8µg/ml	24.6µg/ml	24.75µg/ml	0.1541	0.6226
30µg/ml	0.6988	0.6871	0.6797	30.1µg/ml	29.6µg/ml	29.3µg/ml	29.66µg/ml	0.5844	1.9705
Mean									0.9603

Table 4: Study of Inter Day Precision

Conc. (µg/ml)	Absorbance			Observed Concentration (µg/ml)			Mean	SD	RSD
	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs			
20µg/ml	0.4640	0.4631	0.4365	20.3µg/ml	20.2µg/ml	19.8µg/ml	20.1µg/ml	0.2345	1.1667
25µg/ml	0.5744	0.5722	0.5673	24.9µg/ml	24.8µg/ml	24.6µg/ml	24.76µg/ml	0.1529	0.6178
30µg/ml	0.6984	0.6860	0.6780	30.1µg/ml	29.6µg/ml	29.3µg/ml	29.66µg/ml	0.4042	1.3628
Mean									1.0491

Table 5: Study of Accuracy

Recovery at	Nominal Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml).	% Recovery
80%	20+16=36µg/ml	0.8427	36.1	100.27%
80%	20+16=36µg/ml	0.8321	35.6	98.88%
80%	20+16=36µg/ml	0.8467	36.2	100.55%
100%	20+20=40µg/ml	0.9228	39.8	99.50%
100%	20+20=40µg/ml	0.9300	39.9	99.75%
100%	20+20=40µg/ml	0.9310	40	100%
120%	20+24=44µg/ml	1.0390	43.9	99.77%
120%	20+24=44µg/ml	1.0410	44	100%
120%	20+24=44µg/ml	1.0425	44.2	100.45%
Mean				99.91%

Table 6: Study of Specificity

Nominal Conc.(µg/ml)	Without Excipient		With Excipients		%Interference
	Absorbance	Observed Concentration	Absorbance	Observed concentration	
20µg/ml	0.4606	20.1µg/ml	0.4610	20.3	0.086
20µg/ml	0.4600	20µg/ml	0.4605	20.2	0.108
20µg/ml	0.4595	20µg/ml	0.4602	20.1	0.152
20µg/ml	0.4553	19.9µg/ml	0.4560	20	0.154
20µg/ml	0.4554	19.9µg/ml	0.4559	20	0.109
20µg/ml	0.4600	20µg/ml	0.4606	20.3	0.103
Mean					0.1186

Table7: %Assay of Montelukast in pharmaceutical dosage form (Montair, 10 mg)

S. No.	Absorbance	Nominal Conc. (µg/ml)	Observed Conc. (µg/ml)	Dilution Factor	Weight Taken (mg)	Average Weight (mg)	Label Claim (mg)	% Assay
1	0.4598	20µg/ml	20.1µg/ml	5000	246.8mg	144.7mg	10mg	101%
2	0.4545	20µg/ml	19.8µg/ml	5000	246.8mg	144.7mg	10mg	99%
3	0.4566	20µg/ml	20µg/ml	5000	246.8mg	144.7mg	10mg	100%
4	0.4553	20µg/ml	19.9µg/ml	5000	246.8mg	144.7mg	10mg	99.5%
Mean								99.87%

RESULTS AND DISCUSSION

The pronounced method has been developed and validated for, accuracy, repeatability, specificity and precision. The nominal concentration of standard solutions was approximately 5-45 µg/ml. The proposed method was found to be linear with a linear correlation coefficient 0.9994 of and the linear regression Equation, y = 0.0242x - 0.0302 (fig.3). The linearity of concentration levels at which can be reliably 5-45µg/ml. The mean recoveries were 99.91% method as accurate (Table 5). The repeatability, intraday, inter-day precision, specificity, and accuracy, (RSD) was less than 2%. Potency assay of dosage forms were performed by the proposed method. The brand products met the standard criteria with the new analytical method. Specificity is the ability of the reported method provides data on specificity for their estimation in the presence of formulation excipients. The absorbance obtained with the mixture of the excipients showed no interference with the absorbance of standard. The percent assays of Montelukast were found 99.87% in marketed product (Montair 10mg) in Table 7.

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