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Research Article

STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM AND THEOPHYLLINE IN COMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

The scope of developing and validating a method is to ensure a suitable strategy for particular analysis which is more specific, accurate and precise. Here the main focus is on stability of drug. To develop and validate an accurate, precise, reliable and cost-effective stability indicating UV method for simultaneous estimation of Montelukast Sodium and Theophylline in combined pharmaceutical formulation. Montelukast sodium is used as antihistaminic and Theophylline is used as anti-asthmatic. The wavelength of maximum absorbance for Montelukast Sodium and Theophylline was 344nm and 273nm respectively. The UV method used for analysis was Q-Absorption ratio method, overlain spectra shows the isoabsorptive point at 255nm.The linear regression analysis data for the calibration plots showed good linear relationship with R^2=0.9999 and 0.9999 for Montelukast Sodium and Theophylline respectively at the concentration range of 4-40 µg/ml for Montelukast sodium and 4-28 µg/ml for Theophylline. The method was validated for accuracy, precision, specificity and robustness. The proposed developed stability indicating method can be applied for identification and quantitative determination of Montelukast sodium and Theophylline in bulk and drug formulation.

Keywords: Method development, validation, UV-Spectrophotometer, Montelukast Sodium, Theophylline.

INTRODUCTION

Montelukast

Montelukast sodium is 2–(1-((1-(3-(2- (7-chloroquinolin-2-yl) vinyl) phenyl) – 3 - (2-(2- hydroxypropan-2-yl) phenyl) propylthio) methyl) cyclopropyl) acetic acid[3]. It is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally[1]. Montelukast sodium is a CysLT1 antagonist; it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation[2]. The chemical structure of Montelukast sodium is shown in Fig 1.

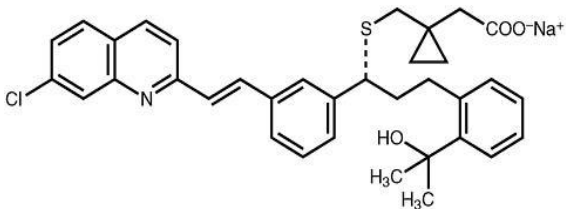

Figure 1: Structure of Montelukast Sodium

Theophylline

Theophylline is also known as1, 3-dimethylxanthine, is a methylxanthine drug used in therapy for respiratory diseases such as chronic obstructive diseases (COPD) and asthma under a variety of brand names[4]. Theophylline is structurally similar to theobromine and caffeine. It blocks the action of adenosine, an inhibitory neurotransmitter that induces sleep, contracts the smooth muscles and relaxes the cardiac muscle[5]. Theophylline is competitive nonselective phosphodiesterase inhibitor, which rises intracellular cAMP, activates PKA, inhibits TNF-alpha and inhibits leukotriene synthesis, reduces inflammation and innate immunity[8].The chemical structure of Theophylline is shown in Fig 2[6].

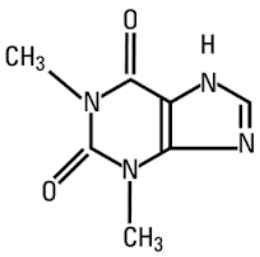

Figure 2: Structure of Theophylline

A literature survey revealed that few chromatographic and spectrophotometric methods are reported for determination of Montelukast sodium and Theophylline individually and with other drug combination. So, an attempt has been made to develop stability indicating analytical methods for simultaneous estimation of Montelukast sodium and Theophylline. The objective of work was to develop a method which is accurate, precise and sensitive stability indicating for estimation of drugs in their formulations.

MATERIALS AND METHODS

Instrument

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

Reagents and Chemicals

Reference Standards of Montelukast Sodium and Theophylline were purchased from yarrow chem products, Mumbai. The formulation (tablet) Telekast-t manufactured by Lupin Ltd. was procured from market. All other reagents were of analytical grade for Spectrophotometric method.

Preparation of Standard Solution of Montelukast sodium

Standard stock solution of Montelukast Sodium was prepared by dissolving accurately weighed quantity of 20 mg Montelukast Sodium was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to 100 ml. the final concentration becomes 200 µg/ml. [14]

Preparation of Standard Solution of Theophylline

Standard stock solution of Theophylline was prepared by dissolving accurately weighed quantity of 20 mg Theophylline was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to 100 ml. the final concentration becomes 200µg/ml. [14]

Preparation of tablet Solution

20 tablets were weighed and crushed to get powder. Tablet powder equivalent to 10mg Montelukast sodium and 400mg Theophylline was weighed and transferred to 100ml volumetric flask dissolved in methanol and volume made up to 100ml. And also, standard mixed solution was prepared by dissolving 10mg Montelukast sodium and 400mg Theophylline to 100ml methanol. [14]

Selection of Detection Wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm and the spectra were obtained. It was observed that Montelukast Sodium and Theophylline showed considerable absorbance at 344nm and 273nm respectively shown in Fig 4.

Overlain spectra of Montelukast sodium and Theophylline

Standard solutions of both the drugs Montelukast sodium and Theophylline (10 µg/ml) was prepared in methanol and scanned at the wavelength 200-400nm. From the overlain spectra the Iso-absorptive point was obtained at 255nm. Therefore, the wavelength selected for analysis of formulation is 255nm by using Q- absorption ratio method shown in Fig 5. The overlain spectra of Montelukast sodium and Theophylline exhibited λ_{max} of 344 nm and 273nm respectively. From the overlain spectra the isoabsorptive point was obtained at 255 nm. Therefore, the wavelength selected for analysis of formulation is 255nm by using Q- absorption ratio method.

Simultaneous equation method

Absorption maximas of Theophylline and Montelukast sodium are obtained at 273 nm and 344 nm respectively by using methanol as a solvent. Standard stock solution of 200 µg/ml both the drug was prepared separately in methanol. Series of serial diluations of both the drugs are prepared (4-40 µg/ml for Montelukast sodium and 4-28 µg/ml for Theophylline) in methanol. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were calculated. Drug concentrations in the sample was determined by using an equation:[14]

$$CX = A_1ay_2 – A_2ay1 / ax_1ay_2 – ax_2ay_1.$$
$$CY = A_1ax_2 – A_2ax_1 / ay_1ax_2 – ay_2ax_1.$$

where A_1 and A_2 are absorbance's of mixture at 273 nm and 344 nm respectively, ax_1 and ax_2 are absorptivities of Montelukast sodium at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of Theophylline at λ_1 and λ_2 respectively. Cx and Cy are concentrations of Montelukast sodium and Theophylline respectively.

Preparation of calibration curve

From the stock solution of Montelukast sodium and Theophylline (200µg/ml) prepare series of dilutions in the concentration range of 4-40 µg/ml for Montelukast sodium and 4-28 µg/ml for Theophylline. Absorbance of each dilution at their respective wavelengths (273 nm for Theophylline and 344 nm for Montelukast sodium) were recorded. Linearity was obtained by plotting the graph of concentration vs. absorbance. Fig 3.

Recovery study

For recovery study known amount of analyte (80%, 100 % and 120% of drug content) was added to the sample and absorbance's were recorded.

Method validation

For the validation of proposed analytical method, the parameters were taken into consideration are accuracy, precision, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), robustness. [11,12,13]

Linearity

From the plotted calibration curve and by measuring the absorbance's of the spectrum from serial dilutions of each drug at their respective wavelength (Montelukast sodium and Theophylline) the linearity was determined. Fig 4.

Accuracy

For the determination of accuracy concentrations corresponding to 80%, 100% and 120% are prepared from stock solution of Montelukast sodium and Theophylline. From the absorbance value of the spectrum difference between actual concentration and measured concentration of the analyte in the sample solution was calculated.

Precision

The intra-day and inter-day precision study of Montelukast sodium and Theophylline was carried out by estimating different concentrations of both the drugs (10,20,30,40,50,60µg/ml), six times on the same day as intra-day precision and six times on the six different days as inter-day precision. The % RSD was calculated.

LOD and LOQ

The LOD and LOQ of Montelukast sodium and Theophylline were estimated from the standard deviation of the response and slope of the caliberation curve, by using following equation,

$$LOD = 3.3 \times \sigma / S$$
$$LOQ = 10 \times \sigma / S$$

Where, σ is standard deviation and S is slope of the calibration curve.

Robustness

Robustness of proposed method was determined by analysis of 4µg/ml, 12µg/ml, 24µg/ml concentrations of standard formulations of Montelukast sodium and Theophylline by changing UV analyst and wavelength.

Stability Studies of Formulation

Stress degradation studies were carried under condition of Acid/ Base/ Neutral Hydrolysis, Oxidation, Dry heat and Photolysis. For each study, two samples were prepared: The blank subjected to stress in the same manner as the drug solution, working standard solution, sample solution of Montelukast Sodium and Theophylline was subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state. [7,15]

Preparation of Reagent Solution

Preparation of 0.1 N Hydrochloric Acid

A solution of 0.1 N hydrochloride acid was prepared by taking 0.86 ml concentrated hydrochloric acid in 100 ml volumetric flask and diluted up to mark with water.

Preparation of 0.1 N Sodium Hydroxide

A solution of 0.1 N Sodium Hydroxide was prepared by taking 0.4 gm sodium hydroxide powder /pellets in 100 ml volumetric flask and diluted up to mark with water.

Preparation of 3% Hydrogen Peroxide

A solution of 3% Hydrogen Peroxide was prepared by taking 10 ml Hydrogen Peroxide in 100 ml volumetric flask and diluted up to mark with water.

Acid hydrolysis

1ml working standard solution of Montelukast Sodium (200µg/ml) and Theophylline (200µg/ml) was mixed with 1 ml of 0.1 N HCL separately and volume make up to 10 ml with methanol in both test tube. (i.e. 20µg/ml). Solution was kept for overnight. Average 56.38 %Montelukast sodium was degraded and 9.15% Theophylline was degraded. Fig 6. [9]

Alkali hydrolysis

1 ml working standard solution of Montelukast Sodium (200µg/ml) was mixed with 1 ml of 0.1N NaOH and volume make up to 10 ml with water (i.e. 20µg/ml).Solution was kept for 6 hours. Average 5.144 % Montelukast sodium was degraded and 11.17% Theophylline was degraded. Fig 7. [9]

Oxidative hydrolytic degradation

1ml working standard solution of Formulation of Montelukast Sodium and Theophylline (200µg/ml) was exposed with 1 ml of 3% H_2O_2 and volume make up to 10 ml with water (i.e. 20µg/ml).Solution was kept for 6 hours. Average 12.82% Montelukast sodium and 24.14% of Theophylline was degraded. Fig 8. [9]

Neutral hydrolysis

1ml working standard solution of Montelukast Sodium and Theophylline (200µg/ml) was mixed with 9ml methanol each (i.e. 20µg/ml).Solution was kept for 6 hours. Average 52% of Montelukast sodium and 37.70% of Theophylline was degraded in Neutral hydrolysis. Fig 9. [9]

Degradation under Dry heat

Dry heat studies were performed by keeping Formulation Solution of Montelukast Sodium and Theophylline in oven at 80°C for 24 hrs.10mg was weighed & dissolved in 50ml methanol (200µg/ml). 1ml working standard solution from above solution (200µg/ml) was mixed with 9 ml methanol (i.e. 20µg/ml).Solution was kept for 6 hours. Average 10.0% of Montelukast sodium and 24.54 % of Theophylline was degraded. Fig 10. [9]

RESULTS AND DISCUSSION

The purpose of method development and validation is to ensure that the method under consideration is capable of giving reproducible and reliable result. The "Q" method is simple, easy and gives reproducible results. In this method binary mixture was prepared in laboratory from pure drugs. From the overlain spectra of Montelukast sodium and Theophylline the isoabsorptive point was found at 255nm where absorptivity of both drugs remains constant. Other wavelength was λmax of the Montelukast sodium (344 nm). In the method the admixture of both drugs simulated to marketed preparation, assayed and obtained results are statistically verified. The statistical parameter of formulation study signifies efficacy of the analytical method. The recovery studies were also carried out and data agreed and showed reproducibility of method and data were within the standard values reported in literature of other methods.

During method absorbance of standard solution remains constant proposed method was validated with various parameters like linearity, precision, accuracy, robustness and ruggedness. The data obtained in each parameter shows preciseness and accuracy of the method. The statistical parameters like standard deviation, % relative standard deviation was 0.34, 0.56 and 0.41, 0.71 respectively for Montelukast sodium and Theophylline. The recovery studies also carried out and were found 94.79% and 95.95% for Montelukast sodium and Theophylline respectively which agrees with the standard values reported in literature for other methods.

Table 1: Calibration curve parameters

Sr. No.	Parameter	Montelukast	Theophylline
1	Linearity range (µg/ml)	4-40 µg/ml	4-28 µg/ml
2	r^2	0.999	0.999
3	Intercept	0.022	0.028

Table 2: Linearity of Montelukast Sodium

Sr. No	Conc.(µg/ml)	Absorbance(nm)	Estimate Conc.	% Drug
1	4	0.101	4.13	82.77
2	8	0.196	7.77	92.77
3	12	0.353	11.66	97.77
4	16	0.402	16.21	98.05
5	20	0.510	20.52	98.11
6	24	0.617	23.77	99.25
Mean		**0.612**		**94.79**

Table 3: Linearity of Theophylline

Sr. No	Conc.(µg/ml)	Absorbance(nm)	Estimate Conc.	% Drug
1	4	0.201	3.83	87.77
2	8	0.396	7.97	94.71
3	12	0.633	11.66	97.81
4	16	0.797	15.91	98.05
5	20	1.010	19.92	98.11
6	24	1.217	23.77	99.25
Mean		**0.709**		**95.95**

Table 4: Validation parameter for Montelukast and Theophylline

Sr.no.	Parameter	Montelukast	Theophylline
1.	Linearity	4-40(µg/ml)	4-28(µg/ml)
2	Accuracy (80%)	96.45*	79.57
	Accuracy (100%)	99.17*	101.15
	Accuracy (120%)	120.14*	115.7
3.	LOD	0.27 µg/ml	0.35 µg/ml
4.	LOQ	5.27 µg/ml	7.14µg/ml
5.	Range	4-40 µg/ml	4-28 µg/ml
6.	Repeatibility	0.371	0.117

*-Mean of 6 Readings

Table 5: Precision study of Formulation

Sr. No	Conc. (µg/ml)	Absorbance (nm)		Estimate conc. (µg/ml)		% of Assay	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
1	10	0.347	0.358	9.37	9.67	93.78	*96.75*
2	20	0.704	0.703	19.02	19	95.13	95
3	30	1.073	1.073	29	29	96.66	96.66
4	40	1.487	1.48	40.18	40	100.47	100
5	50	1.843	1.83	49.81	49.45	99.62	98.91
6	60	2.241	2.232	60.56	60.32	100.94	100.54
Mean		**1.28**	**1.26**	-	-	**97.76**	**97.97**
SD		**0.071**	**0.069**	-	-	**2.99**	**2.17**
% RSD		**0.55**	**0.53**	-	-	**0.03**	**0.022**

Table 6: Summary of forced degradation study

SN	Stress Condition	%Degradation of Montalukast	%Recovered	%Degradation of Theophylline	%Recovered
1	Acid (0.1N HCL for Overnight)	56.38	43.62	9.15	90.85
2	Alkali (0.1N NaOH for 6hours)	5.14	94.86	11.17	88.83
3	Oxidative (10% H_2O_2 for 6hours)	12.82	87.18	24.14	75.86
4	Neutral hydrolysis	52.00	48	37.70	62.3
5	Degradation under dry heat (80°C for 24 hours)	10.0	90	24.54	75.46

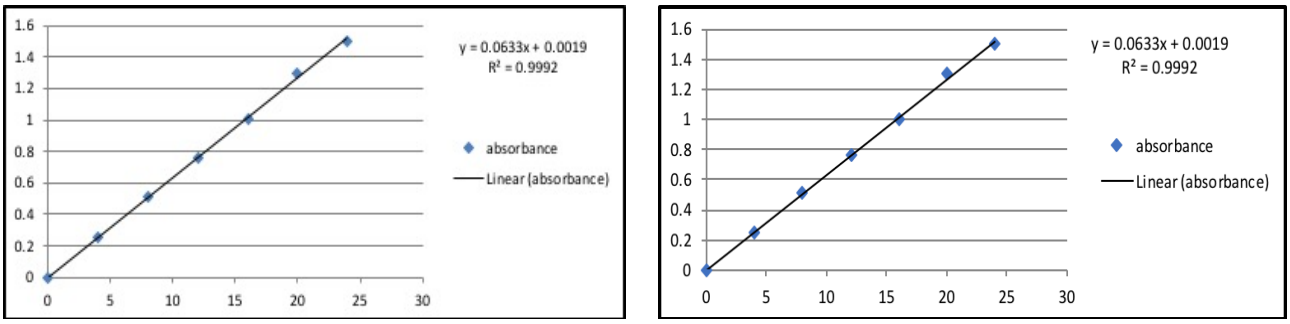

Fig 3: Calibration curve of Montelukast sodium and Theophylline

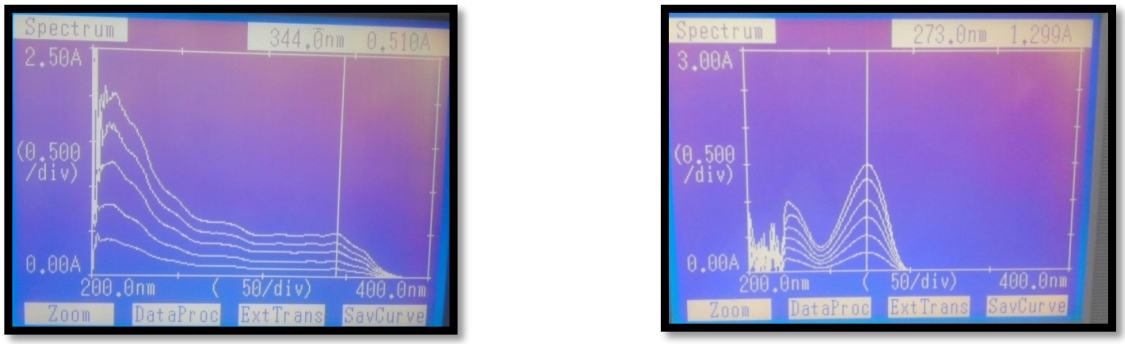

Fig 4: Linearity of Montelukast sodium and Theophylline

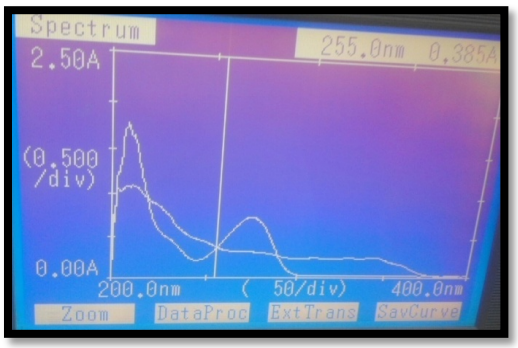

Fig 5: Overlay spectra of Montelukast sodium and Theophylline

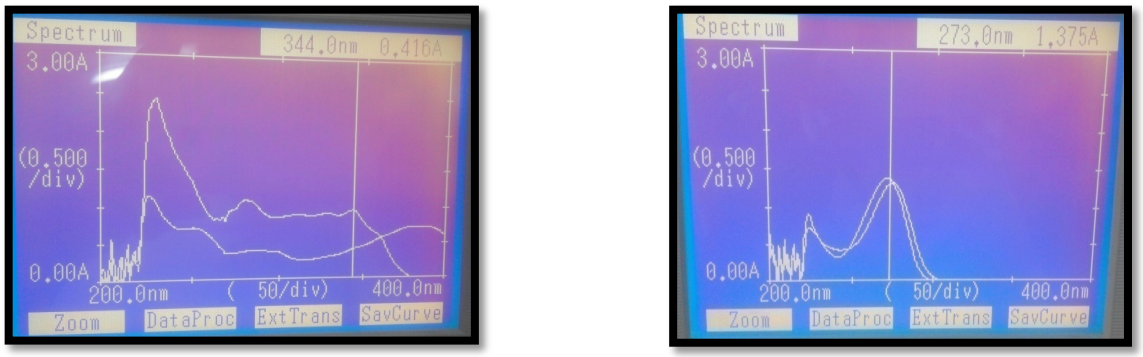

Fig 6: Overlay of Standard and acid treated Montelukast sodium and Theophylline (10µg/ml) respectively

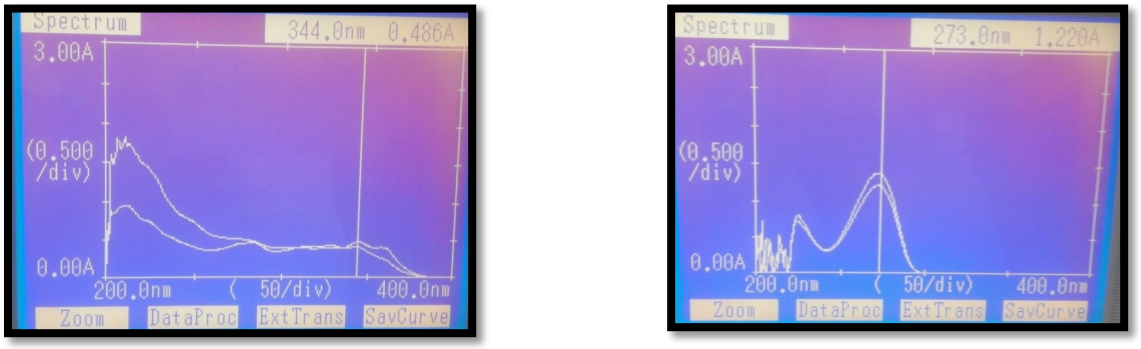

Fig 7: Overlay of Standard and **Alkali treated Montelukast sodium and Theophylline (10µg/ml) respectively**

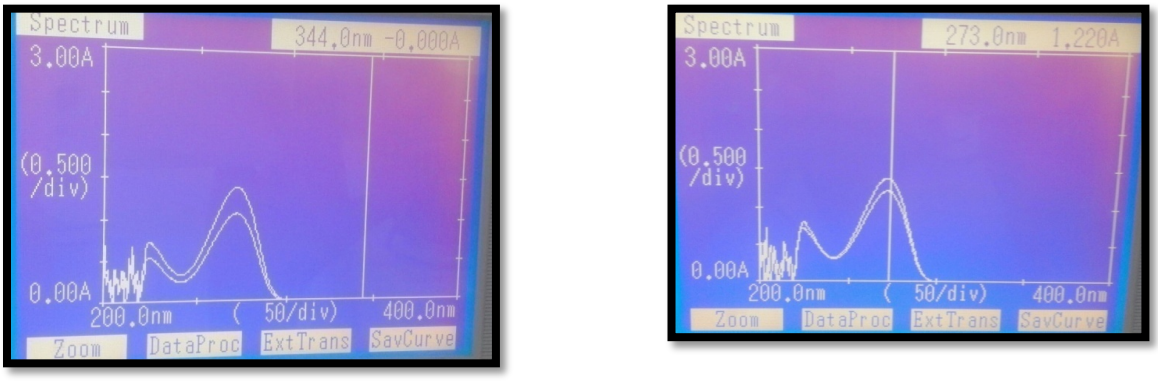

Fig 8: Overlay spectra of Standard formulation and hydrogen peroxide treated formulation at 344nm (10 µg/ml) and 273 nm (10 µg/ml) respectively

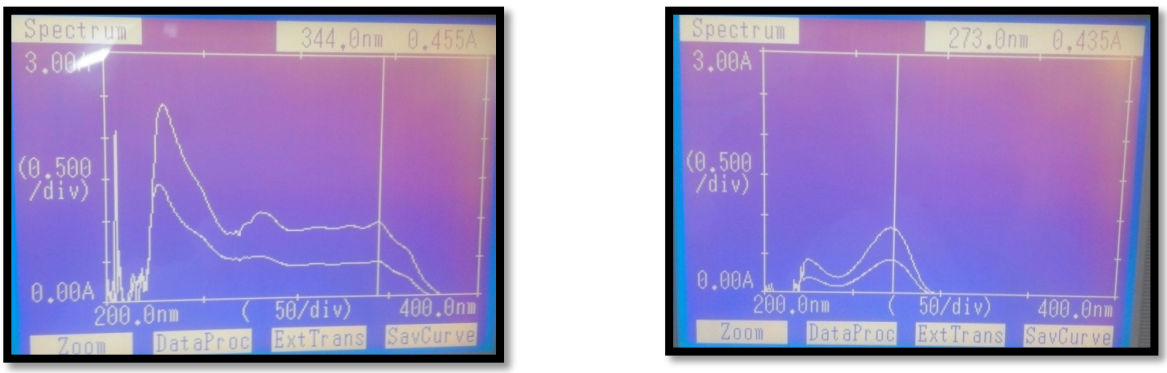

Fig 9: Overlay of standard and Neutral Montelukast sodium and Theophylline respectively (10 µg/ml)

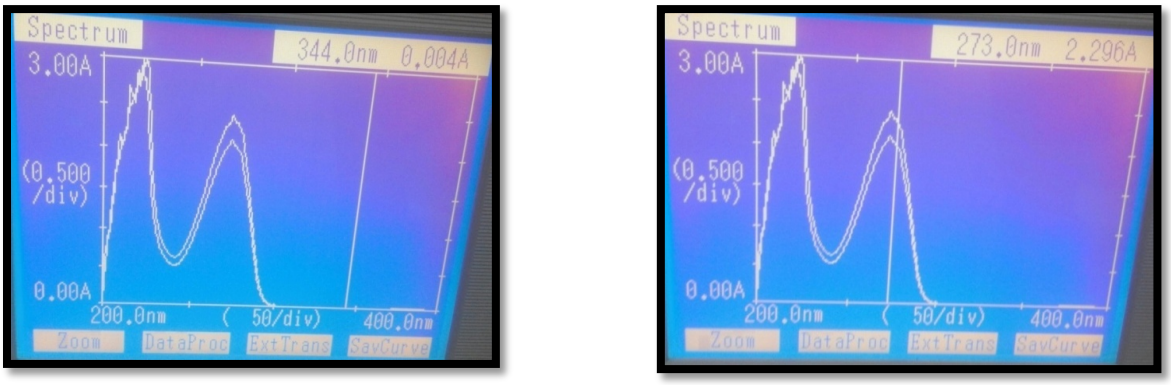

Fig 10: Overlay of Standard formulation and Dry Heat treated formulation at 344nm and 273 nm respectively (20 µg/ml)

Statistically parameters obtained through analysis of tablet formulation by proposed "Q" method shows application of method for routine analysis and its recovery study was carried at three levels as 80%, 100% and 120%. Stability studies was also carried out to validate the rigidness, reliability of method. The data obtained in stability study proves that method is not affected by minor change in PH of solvent, temperature of environment etc.

Therefore "Q" method is simple, reproducible and time saving which is recommended for estimation of both drugs from formulation simultaneously without any interference. Hence, procedure under consideration is statically sound and capable of giving reproducible and reliable result.

CONCLUSION

The present work deals with development and validation of stability indicating UV method for simultaneous estimation of Montelukast Sodium and Theophylline in combined pharmaceutical formulation. The study was performed by scanning the sample solutions of both the drugs (Montalukast Sodium and Theophylline) at 200-400 nm in the UV region. The maximum absorbance found at the wavelength 344 nm and 273 nm for Montelukast Sodium and Theophylline respectively using methanol as blank. The method was found to be linear in concentration range 4-40 µg/ml and 4-28 µg/ml for Montelukast Sodium and Theophylline respectively by plotting the graph of concentration vs. absorbance. From the overlain spectra of both the drugs the isoabsorptive point was obtained at 255nm. There was no interference so the "Q"- absorption ratio method was used for the analysis. And the wavelength used for this method are 344nm and 255nm. The statistical parameters like standard deviation, % relative standard deviation was 0.34, 0.56 and 0.41,0.71 respectively for Montelukast sodium and Theophylline. The recovery studies also carried out and were found 94.79% and 95.95% for Montelukast sodium and Theophylline respectively which agrees with the standard values reported in literature for other methods. Standard mixture was prepared in laboratory and it work by the proposed method. Statically parameters obtained through analysis of tablet formulation by proposed Q method shows application of method for routine analysis and its recovery study was carried at three levels i,e. 80%, 100% and 120%.The result of the formulation study shows methods sensitivity and reliability. The method validated for parameters such as linearity, range, precision, robustness and accuracy.

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