ISSN No:-2456-2165

Method Development and Validation of Simultaneous Estimation of Etofylline, Theophylline and Montelukast in Bulk and Tablet Dosage Form by First Order Derivative UV Spectrophotometric Method

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Abstract:- A rapid, economic, sensitive, simple and accurate first order derivative UV spectrophotometric method has been developed for estimation of Etofylline (ETO), Theophylline (THE) and Montelukast (MON) in bulk and tablet dosage form. The quantitation of selected wavelengths were 243 nm for ETO (zero cross for MON and THE), 286 nm for THE (zero cross for MON but shows absorbance in ETO) and 382 nm for MON (zero cross for ETO and THE). It obeyed beer's law in the concentration range of 46.2-231 µg/ ml, 13.8-69 µg/ ml and 2-10 µg/ ml for ETO, THE and MON respectively. The method was validated as per ICH guidelines.

Keywords:- *Etofylline, Theophylline, Montelukast, First order derivative method and Tablet dosage form.*

I. INTRODUCTION

Etophylline is used for relaxing and dilating the airway muscles. Theophylline is used to relaxes the smooth muscles and it is located in the bronchial airways and pulmonary blood vessels. Montelukast sodium is used to blocking the action of leukotriene D4 present in the lungs resulting to relaxation of smooth muscle and decreased inflammation [2,3.5,11,17,20] A comprehensive literature survey revealed that, methods have also been developed for the estimation of Etofylline, Theophylline and Montelukast in combination with other drugs simultaneously in bulk drugs and pharmaceutical formulations and in UV Spectroscopy [8,9,10,12,15,19] and RP-HPLC [1,4,13,14,16,18,21]. As per ICH norms the method was validated [6-7].

II. MATERIALS AND METHODS

➢ *Instrumentation*

The instrument used was Lab india UV/Visible spectrophotometer with spectral band width of 1 nm (Model UV- 3000).

➢ *Reagents and chemicals*

The tablet dosage form used was a Deriphyllin M tablet labeled to contain ETO 231 mg, THE 69 mg and MON 10 mg. Distilled water is used as a solvent.

➢ *Selection of solvent*

Selection of solvent in UV analysis is a key component of method development. Distilled water is used as a solvent for the estimation of Etofylline, Theophylline and Montelukast for the first order derivative method.

➢ *Preparation of standard stock solutions*

Standard stock solution of Etofylline (2310 µg/ml), Theophylline (690 µg/ml) and Montelukast (100 µg/ml) were prepared by dissolving 231 mg of Etofylline, 69 mg of Theophylline and 10 mg of Montelukast in 100 ml of distilled water in 100 ml clean volumetric flask, separately with vigorous shaking.

➢ *Selection of wavelengths for estimation*

In First order derivative method, solutions of Etofylline, Theophylline and Montelukast (10 µg/ml, each), were prepared separately from the dilution of standard stock solution with distilled water and scanned the spectra from 200 nm to 400 nm. The spectra was derivatized for first order. From the overlain spectra of these drugs, the wavelengths selected for quantification were 243.0 nm for Etofylline (Zero cross for MON and THE), 286.0 nm for Theophylline (Zero cross for MON but shows absorbance in ETO) and 382.0 nm for Montelukast (Zero cross for ETO and THE).

➢ *Preparation of calibration graph*

From the above standard stock solution, pipetted out and suitable distilled water was added so as to get the final concentration range of 46.2, 92.4, 138.6,184.8 and 231 µg/ml for ETO, 13.8, 27.6, 41.4, 55.2 and 69 µg/ml for THE and 2, 4, 6, 8, 10 µg/ml for MON. Absorbances were measured in the respective wavelengths.

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➢ *Quantification of tablet formulation*

Weighted Twenty tablets each containing 231 mg of ETO, 69 mg of THE and 10 mg of MON and calculated average weight then crushed to obtain fine powder. A quantity of powder equivalent to about 231 mg of ETO was transferred to a clean 100 ml standard flask; added 70 ml distilled water and sonicated for 15 min,further distilled water was added and made up to the mark. The resulting solution was mixed and filtrated. 0.4 ml was withdrawn and diluted to 10 ml using distilled water toget 92.4 µg/ml of ETO, 27.6 µg/ml of THE and 4 µg/ml for MON. The concentration of ETO, THE and MON were determined by measuring absorbance of sample solutionin first order derivative at 243.0, 286.0 and 382.0 nm, respectively. Concentration of ETO, THE and MON in the diluted solution was obtained from calibration curves. Amount of ETO, THE and MON in mg/tab was then calculated. This procedure was repeated six times.

➢ *Validation*

As per ICH guidelines the method was validated.

➢ *Accuracy*

Accuracy or recovery studies were carried out by standard addition method using three different levels (50%, 100% and 150%). The recovery studies result presented in Table 2.

➢ *Precision*

The reproducibility of developed method was determined by analyzing tablet at different intervals time (Intra-day) on same day in triplicates and on three different days (Inter-day). Coefficient of variance for intra-day assay precision was found to be 0.7360 (for Etofylline), 0.7471 (for Theophylline) and 0.0101 (for Montelukast). Inter-day assay precision coefficient of variance was found to be 0.7500 (for Etofylline), 0.7460 (for Theophylline) and 0.0112 (for Montelukast).

III. RESULTS AND DISCUSSION

The analysis of the new drug combinations such as Etofylline, Theophylline and Montelukast were taken for our studies. First order derivative method for estimation of multiple dosage form have advantages, that the method were consuming less time and also the usage of solvent is minimized. To ensure that the % purity in the combined dosage form of thedrug, the UV-spectroscopy was developed.

A accurate, simple, fast and precise first order derivative UV method was developed and validated. The solvent used was Distilled water for the estimation of ETO, THE and MON.

The standard solutions of 10 µg/ml of ETO, THE and MON in the solvent was prepared and scanned in the UV region of 200 nm to 400 nm by using distilled water. The zero order UV spectrum were derivatized into first order derivative UV spectrum. The first order derivative overlaid UV spectrum of ETO, THE and MON were recorded and the spectrum was shown in Figure 01. From the UV spectrum, 243 nm, 286 nm and 382 nm were chosen for the determination of ETO, THE and MON without any intervention. At 243 nm, the absorbance of MON and THE were zero, 286 nm, the absorbance of MON was zero but shows absorbance in ETO and 382 nm, the absorbance of ETO and THE were zero. For analysis of ETO, THE and MON, these three wavelengths were selected.

The aliquots of five different concentration ranging for ETO (46.2-231 µg/ml), THE (13.8-69 µg/ml) and MON (2-10 µg/ml) were prepared. The absorbances were measured at 243 nm, 286 nm and 382 nm in the first order derivative UV spectrum for ETO, THE and MON. The calibration graph was plotted using absorbance against concentration. Correlation coefficient, Sandell's sensitivity, LOD, LOQ, Molar absorptivity and Standard error are the examples for optical parameters and it was calculated for ETO, THE and MON drugs. The correlation coefficient of ETO, THE and MON was found to be about 0.9998, 0.9997 and 0.9998. Hence the calibration graphs was found to be linear.

Deriphyllin M tablet formulation containing ETO 231 mg, THE 69 mg and MON 10 mg was selected for estimation. From the linearity, the nominal concentration of ETO, THE and MON i.e. 92.4 µg/ml of ETO, 27.6 µg/ml of THE and 4 µg/ml of MON was prepared. The absorbances of the solutions were measured at 243 nm, 286 nm and 382 nm and the amount of 6 test solutions were determined. In tablet dosage form the % purity was found to be 100.14 ± 0.7300, 99.68 ± 0.7750 and 99.33 ± 0.8120 for ETO, THE and MON respectively. The % RSD values were found to be very less. Hence the method have good precision. The results are shown in Table-1.

Precision of the developed UV method was studied by making repeated analysis(Intraday and Interday). Intraday and interday analysis of % RSD values were found to be 0.7358 and 0.7510 for ETO, 0.7473 and 0.7462 for THE & 0.0101 and 0.0112 for MON. The results show the developed UV method was very high.

In ruggedness studies, the % RSD values for different Instrument 1 and 2 were found to be 0.4954 and 0.5146 for ETO, 0.6253 and 0.6348 for THE & 0.0001 and 0.0001 for MON. The % RSD values for different Analyst 1 and2 were found to be 0.5146 and 0.4954 for ETO, 0.6348 and 0.6253 for THE & 0.0001and 0.0101 for MON. Lower % RSD values indicated, method was more rugged.

Accuracy studies of the developed UV method was confirmed. The % recoverywas found from 99.80 to 100.38 % for ETO, 99.99 to 100.29 % for THE & 99.99 to 100.16 % for MON. Lower % RSD values indicated that the developed UV method was more accurate. The results was shown in Table-2.

ISSN No:-2456-2165

TABLE I: QUANTIFICATION OF DERIPHYLLIN M FORMULATION

Sample	Sample number	Label claim (mg/tab)	Amount present (mg/tab)*	% Purity* (% w/w)	% RSD
ETO	1 2 3 4 5 6	231 231 231 231 231 231	229.66 232.10 232.10 229.66 232.10 232.10	99.42 100.50 100.50 99.42 100.50 100.50	0.7310
THE	1 2 3 4 5 6	69 69 69 69 69 69	68.34 69.64 68.34 69.64 68.34 68.34	99.05 100.94 99.05 100.94 99.05 99.05	0.7752
MON	1 2 3 4 5 6	10 10 10 10 10 10	10 10 10 10 9.6 10	100.00 100.00 100.00 100.00 96.00 100.00	0.8121

*Mean of six observation

TABLE II: RECOVERY STUDY

Sample	% Conc.	Sample amount (µg/ml)	Amount spiked (µg/ml)*	Estimated Amount (µg/ml)*	Recovered Amount (µg/ml)*	Average % recovery
ETO	50 100 150	92.41 92.41 92.41	46.2 92.4 138.6	138.79 184.81 231.01	46.38 92.22 138.59	100.38 99.80 99.99
THE	50 100 150	27.63 27.63 27.63	13.8 27.6 41.4	41.43 55.23 69.03	13.79 27.62 41.52	99.99 99.99 100.29
MON	50 100 150	4.0 4.0 4.0	2.0 4.0 6.0	6.0 8.0 10.0	1.99 4.0 6.01	99.99 100.00 100.16

*Mean of three observation

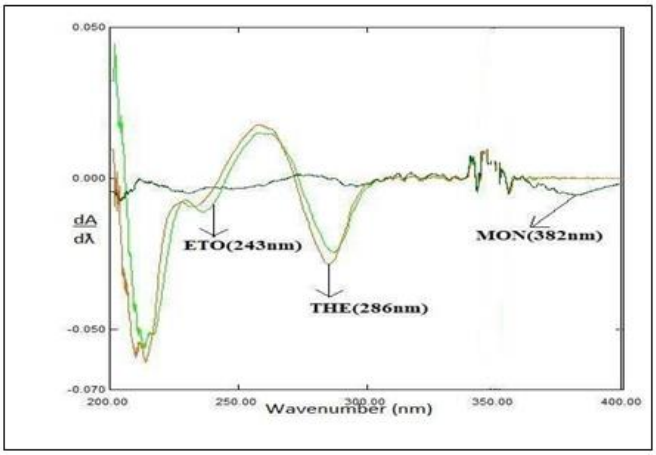

Fig 1. Overlain spectra of 10µg/ml of ETO, THE and MON by First order derivative UV Method

IV. CONCLUSION

The Developed and Validated First order derivative UV method employed here proved to be accurate, rapid , simple, economical and precise. These can be used for routine analysis of ETO, THE and MON in tablet dosage form instead of processing and analyzing each drug separately

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