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# Applicability of QBD approach in Chemometric Estimation of Theophylline and Montelukast sodium from combined Dosage form by Spectrophotometric Method

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**ABSTRACT**

**Objective:** Regulatory agencies like ICH, USFDA recommended implementation of Quality by design (QbD) for various pharmaceutical developmentprocesses and insists to foster QbD based processes to enhance quality in the product and or process. QbDis a systematic process for development of various pharmaceutical processes including analytical methods and by applying Quality by design aids in ensuring the robustness of the method. QbD approached Chemo metric assisted absorption spectrophotometric analytical method was developed for the estimation of Theophylline(TPE) and Montelukast sodium (MKS) from their combined formulation.

**Materials and Method:**Simultaneous equation method and Q-absorbance method were selected from the nature of spectra, solvent 0.1 N NaOHwas utilized; and for method 275 nm and 329 nm was the wavelength for measurement of absorbance of TPEand MKS respectively. For Q method wavelength 259 nm was found isoabsorptive after repetitive measurement. Effect of input variables on spectrum characteristics were studied for selection of critical parameters and developed method was validated as per ICH Q 2 R1 regulatory guidelines. Linearity of the drugs was ascertained over the conc range 1-24 mcg/ml (microgram/ml) for TPE and 1-16 mcg/ml for MKS.

**Results and Discussion:**The percentage purity of assay was found 101.09% for TPE and 100.51% for MKS; and the precision study was shown acceptable data as SD data varied from 1.6293 to 3.4488 for TPEand from 1.0096 to 3.6778 for MKS.

**Conclusion:**The developed method is rigid, robust and efficient for the estimation of TPE and MKS from the combined formulation. QbD was applied to build rigid robust method through risk assessment at early stage and defining the design space at the later stage.

**Keywords:** Theophylline, Montelukast Sodium, QbD, ICH, Simultaneous equation method, Q-method,

## I. INTRODUCTION

Application of Quality by design concept in the development of pharmaceutical processes is monumental to ensure a predefined product quality. QBD concepts are elaborated in ICH guidelines Q8 (R2) (Pharmaceutical development), Q9 (Quality risk management), and Q10 (Pharmaceutical quality system) [1-3]shown in (Fig No 1). ICH guidelines Q8 (R2) defines QBD as a "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"[4]. QBD approachin analytical method summarizes a complete understanding of how the analytical technique attributes and operating conditions affect the analytical performance. Factors to study in analytical quality by design (AQbD) approach may include the type of analytical technique chosen, reagents used and instrument parameters.

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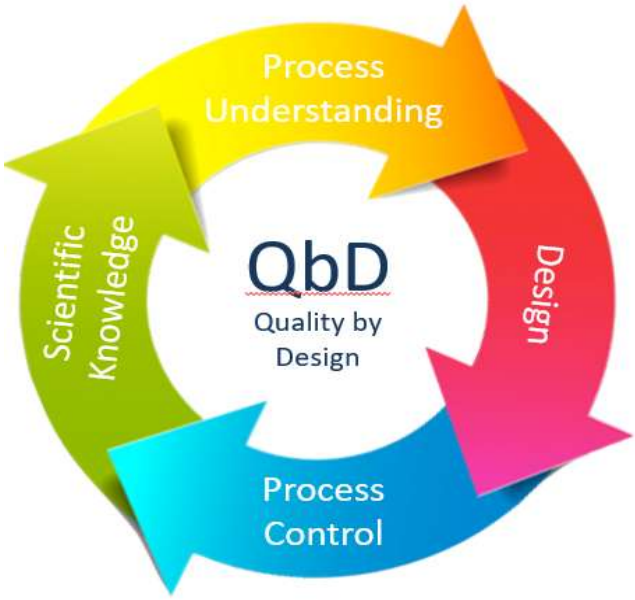


Fig No 1: Analytical QbD approach

There are similar advantages of applying QbD principles to analytical methods as to manufacturing processes and product[5]. A QbD approach can be beneficial in the development of suitable, robust, low cost and eco-friendly (eco-friendly solvent, chemicals) method which is applicable at any stage of the lifecycle of the product. Also some regulatory guidelines have mentioned flexibility of changing analytical method without revalidation if the AQbD approach has been implemented during analytical method development. The first stage of AQbD approach is to fix an analytical target profile (ATP) for the method. ATP defines the goal of the analytical method development process and it is the sign of method performance [6, 7].

Theophylline (TPE) 1, 3 dimethyl 3,7dihydro 1H purine 2,6 dione is xanthine bronchodilator[8]. Pharmacological action comprise relaxes bronchial smooth muscle relieves bronchospasm and has a stimulant effect on myocardium and CNS decreases peripheral resistance and venous pressure and causes diuresis, non-selective phosphodiesterase inhibitor[9], treatment of reversible airway obstructions[10, 11]..

Montelukast sodium (MKS) chemically Sodium [1-[[[(1 R)-1-(3-[(E) 2-(7-chloroquinoline-2-yl) ethenyl] phenyl)-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl] sulfanyl] methyl] cyclopropyl] acetate is an anti-asthmatic[8], leukotriene receptor antagonist. It can completely block the binding of CYSLT'S to receptor that they can inhibit the binding of inflammation mediator LTD4 [9, 11].

Various analytical methods have been reported for the estimation of TPE alone or in combination with other anti-asthamatic agents in pharmaceutical dosage form includes UV spectroscopic bioanalytical method [12], with other drug UV spectroscopic method [13], bio analytical HPTLC method [14],bio analyticalHPLC technique[15],HPLC analytical technique[16-18], stability indicating HPLC method [19]andLC-MS/MS bio analytical method [20]were reviewed.

Literature survey revealed that various analytical methods have been reported for estimation of MKS alone or along with other drug includes UV spectrophotometric method [21], HPTLC method [22, 23], RP-HPLCtechniques [24-26], stability indicating HPLC method [27], LC-MS-MS technique [28, 29], bio analytical HPLC method [30].

Theophylline and montelukast sodium are official in Indian and British Pharmacopoeia [8, 10]. Chemical structure of both these drugs is shown in (Fig No 2).

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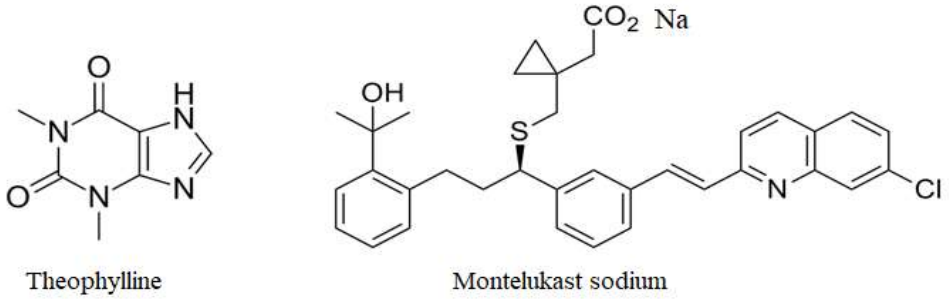


Fig. No 2: Chemical structure of drug molecule

For analytical method validation ICH Q2 (R1) has given various method performance characteristics for an analytical method. In development of UV-VIS spectrophotometric method, QbD approach[31, 32] was implemented with the study of the effect of method input variables on spectral shape, intensity of absorbance, and absorbance maxima $\lambda_{max}$ and critical parameters were selected for the proposed method and method was validated as per ICH guidelines Q2 (R1).

**Instrumentation**

Analysis was performed with a UV-1900i Shimadzu Double beam spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with 10 mm matched Quartz cells was used. Drugs were weighed on electronic balance 'Afcoset' (The Bombay Burmah Trading corpo Ltd) with accuracy ± 0.1 mg Model No. ER 200A and Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was usedfor degassing the solutions.

**Reagents and Chemicals**

Pharmaceutically pure samples of MKS from Morpean Laboratories Ltd, Baddi, HimachalPradesh, India and Theophylline from Pure & Cure Healthcare Pvt. Ltd. Ranipur, Haridwar, Uttrakhand., Sodium hydroxide Analytical Grade and laboratory distilled purified water was used as solvent and the commercial formulation containing theophylline 400 mg and montelukast sodium 10 mg was procured from the local market.

**Solvent selection**

TPEis soluble in water, alcohol, chloroform more soluble in hot water, freely soluble in alkaline hydroxide, dilHCl[11], other literature reported slightly soluble in water, sparingly soluble in alcohol dissolves in solution of alkaline hydroxides, in ammonia and in mineral acids[9, 10].MKS is freely soluble in ethanol, methanol, and water practically insoluble in acetonitrileand in dichloromethane[11].

Although the solubility of the procured both drugs were studied in water, 0.1 N HCl and 0.1 N NaOH separately; and found that in 0.1 N HClMKS was insoluble and in water sparingly solubility of MKS was observed. TPE was appreciably soluble in all three solvents, Hence 0.1 N NaOHwas selected as common solvent and medium to get resolved spectra for complete analysis. Solution with known conc of analyte was scanned in UV range of 400 nm to 200 nm. The recorded spectra in solvent are shown in Fig No 3 and 4. It was found that suitable solvent is 0.1 N NaOH with respect to average cost, robust and precise in producing result.

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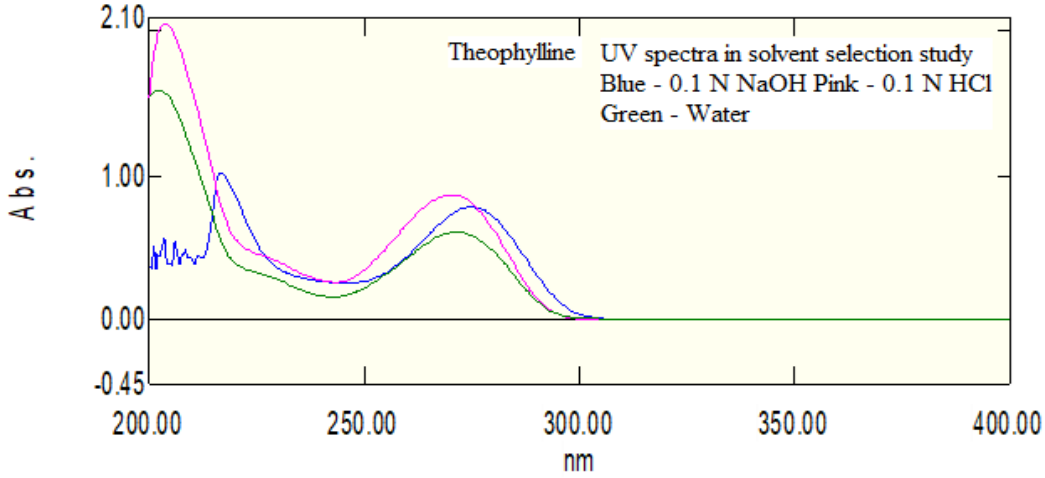


Fig No 3: UV spectra of Theophylline in solvents

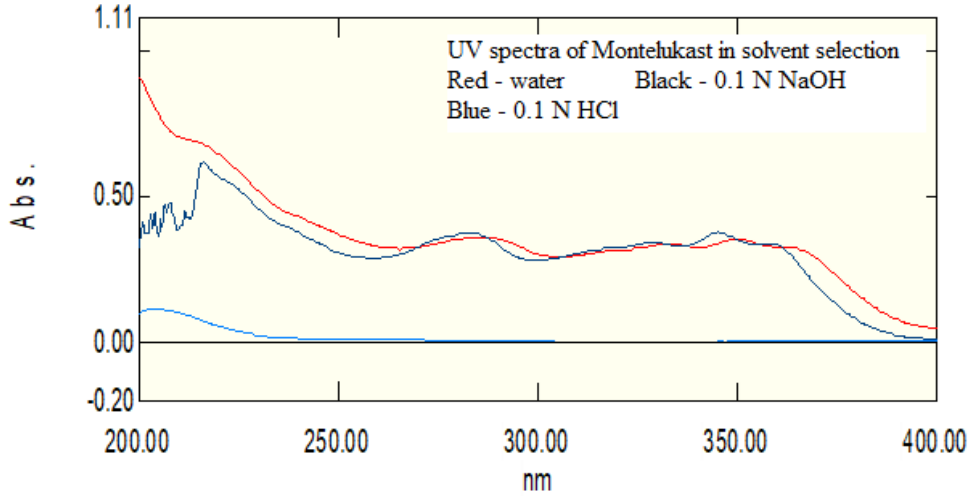


Fig No 4: UV spectra of Montelukast sodium in solvents

**Preparation of stock solutions and standard solutions**

10 mg each of drug TPE and MKS were separately and accurately weighed; and transferred into separate 25 ml volumetric flasks. Dissolved into 0.1 N NaOH and volume was made to 25 ml with solvent. Subsequent standard solution of each drug with conc12µg/ml was prepared by diluting aliquot 0.3 ml of stock solution to 10 ml into 10 ml capacity volumetric flask.

**Selection of wavelength and conc range**

Standard solution of TPE and MKSwith conc 12µg/ml was prepared and scanned in the spectrum mode from 400 nm to 200 nm.

From UV spectra (Fig No 5) it was found that TPE has measurable absorbance at 275 nm ($\lambda_{max}$)and interference was observed by MKS; similarly MKShas maximum absorbance at 329nm ($\lambda_{max}$) andnegligible interference by TPE. The wavelength 259nm was found where both drug has constant absorptivity; directed applicability of Q absorbance method. Chemometric method using simultaneous equation method and Q method was applied and which was reasonable remedy to overcome interference at each other's absorbance. From the nature of spectra to study linearity, working conc range 1 to 24µg/ml for TPE, 1 to 16µg/ml for MKS was selected. Also combined drug solution was prepared simulated to marketed formulation. Selected critical parameters based upon above discussion, observations were listed and by using these; method was validated as per ICH guidelines and by analysing marketed preparations.

**Experimental Method for estimation**

From the overlain spectra it was found that many approaches of multicomponent analysisare suitable for simultaneous estimation of both the

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drugs. Among of this simultaneous equation method and absorption ratio Q method were selected for estimation of TPE and MKS from the combined dosage form.

**Method-I: Simultaneous equation method**

TPE was shown absorbance at ($\lambda_{max}$)275 nm and MKS has maximum absorbance ($\lambda_{max}$) at 329 nm. The wavelength 275 and 329 nm was considered as 1 ($\lambda_1$) and 2 ($\lambda_2$) respectively. The equation A= abc was applied for x (TPE) and y (MKS) determination. On rearranging the 2 generated equations, the conc of x and y was calculated by following formula. Working standard solutions of TPEof conc 16µg /ml and MKSof conc8µg /ml were separately prepared and used for the method.

At 329 nm $A_s = A_1 + A_2$

$A_s = ax_2.b.C_x + ay_2.b.C_y$

On rearranging equation

$$Cx = \frac{A2\,.ay1 - A1.ay2}{ax2.ay1 - ax1.ay2}$$

$$Cy = \frac{As - ax2.Cx}{ay2}$$

Where Cx and Cy = Conc of TPE and MKS in sample solution

$A_1$ and $A_2$ = absorbance of sample solution at 1 and 2 wavelength

$ay_1$and$ay_2$ = absorptivity of MKS at 1 and 2 wavelength of standard solution

$ax_1$and$ax_2$ = absorptivity of TPE at 1 and 2 wavelength of standard solution

$A_s$ = absorbance of sample containing TPE and MKS at 329 nm

**Method-II Absorption ratio method**

The absorption ratio method is modification of simultaneous equation method. It is based upon fact that the ratio of absorbances at any two wavelengths is constant value independent of conc or pathlengths. Two different dilute solutions of same drug give the same absorption ratio $A_1$/ $A_2$. Two wavelengths are being selectedas $\lambda_1$(where absorptivity of both the drug remains constant)and $\lambda_2$($\lambda_{max}$of one of the drug). The wavelength at which two drugs show similar absorptivity is known as iso-absorptive point (shown in the figure). There should not interference of any other component like excipients, other drug except X and Y. From the overlain spectra of both drug iso absorptive point was found at 259nm.

The absorptivity of X Theophylline at $\lambda_1$and $\lambda_2$are $ax_1$and $ax_2$ respectively.

The absorptivity of Y Montelukast sodium at $\lambda_1$and $\lambda_2$are $ay_1$and $ay_2$ respectively.

$$Cx = \frac{Qm - Qy}{Qx - Qy}\,\frac{A}{a\,x1} \qquad Cy = \frac{Qm - Qx}{Qy - Qx}\,\frac{A}{aY1}$$

$$Q_m = \frac{A_2}{A_1} \qquad Q_X = \frac{ax_2}{ax_1} \qquad Q_Y = \frac{aY_2}{aY_1}$$

Where

Cx and $C_Y$ - are concentrations of TPE and MKSin sample solution respectively.

Qx - Ratio of absorptivity of TPE at 275 and 259 nm

$Q_Y$ - Ratio of absorptivity of MKS at 275 and 259 nm

Qm - Ratio of absorbance of sample solution at 275 and 259 nm

A - Absorbance of sample solution at Isobestic point

$a_{X1}$- Absorptivity of TPE at Isobestic point

$a_{Y1}$- Absorptivity of MKS at Isobestic point

**Validation of the Method**

Selected critical parameters should meet the performance characteristics of the analytical method so as to attain analytical target profile of the method. An ICH guideline Q2 R1 was applied to study methods performance with critical parameters in order to implement part of AQbD approach. The method was validated as per ICH guidelines

**System suitability**

System suitability is studied to demonstrate the suitability of the developed procedure under consideration for the analytical method. Six replicates of working standard solutions with conc16 and 8mcg/ml each of TPE and MKS were prepared separately and absorbance was recorded, and SD and % RSD of the response was calculated.

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**Linearity**

The linearity of an analytical method is its ability to obtain response i.e. absorbance which is directly proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range of 1-24µg/ml for TPE and 1-16µg/ml for MKS and scanned in 200 to 400 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at their respective wavelength; i.e. 275 for TPE and 329 nm for MKS in spectrum order. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

**Assay of formulation**

Assay was carried out by proposed methods and assay was validated by statistical parameters.

**Estimation of formulations by simultaneous equation and Q method**

Tablet powder equivalent to 80mg TPE and 2mg MKS was weighed and transferred into 100 ml volumetric flask. Dissolved into 0.1 N NaOH solution and volume was made to 100ml with solvent. Solution was filtered through what man filter paper and aliquots of solution were further diluted to obtain tablet solution. Solution was scanned in the range of 200 to 400 nm to obtain absorbance of tablet solution at 275 and 329 nm in spectrum order. Also absorbance was recorded at 259 and 329 nm to estimate drugs by Q method. Obtained absorbance were utilised to estimate unknown conc of formulation; and results were statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

**Precision of the method**

The precision study was carried out by performing assay of tablet six times; also the reproducibility in result was studied by inter day and intraday precision. Tablet solutions of TPE and MKS were prepared. Six replicates at each of intraday and interdaylevels were prepared absorbance measured at these wavelengths and % of conc, SD and RSD were calculated.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ of TPE and MKS by the proposed method were determined using calibration graph method and calculated as 3.3σ/s and 10 σ/s for LOD and LOQ respectively; σ is the standard deviation of calibration curve and s is the slope of regression line.

**Robustness and Ruggedness**

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

## II. RESULTS AND DISCUSSION

Method development comprises numerous steps; of which solvent selection, method for measurement selection are significant one. Uses of eco-friendly solvents have got remarkable weightage due to low cost, readily available and environmentally sound. Drugs underlying analysis must have appreciable solubility in the selected solvent. Chemical structure of the drug and physico-chemical properties available in the literature guides about use of appropriate solvent in the method. Solubility of TPEand MKS was studied in each solvent; and both drugs were shown maximum and consistent absorbance in 0.1 N NaOH solvent as compare to other solvent.

**System Suitability**

The absorbances of five replicates of standard solutions Fig No 5 (16 and 8µg/ml of TPE and MKS respectively) are reported in Table No 1. The SD was found for TPE and MKSwithin acceptable limit and meets the system suitability requirements indicates method was suitable for analysis.

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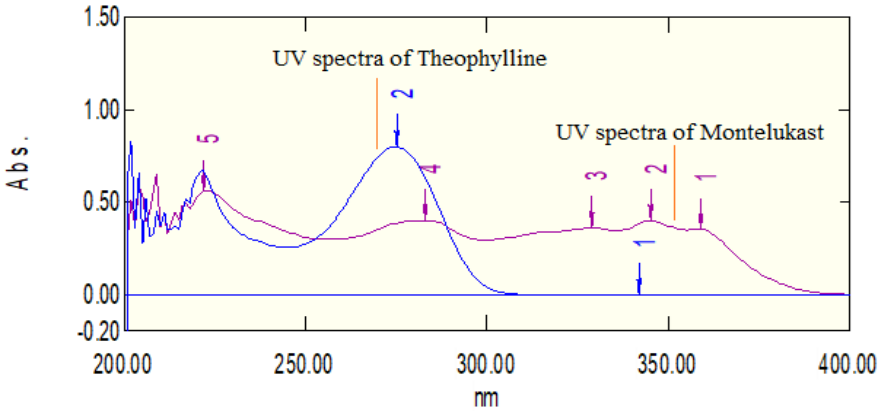


Fig No 5: Overlaid spectra of TPE and MKS

Table No 1: System suitability study of TPE and MKS

| Sr No | Conc in µg/ml | Absorbance of TPE 275 nm | Conc in µg/ml | Absorbance of MKS329 nm |
|---|---|---|---|---|
| 1 | 16µg/ml | 1.0073 | 8µg/ml | 0.3184 |
| 2 | 16µg/ml | 1.1100 | 8µg/ml | 0.2954 |
| 3 | 16µg/ml | 1.1115 | 8µg/ml | 0.3226 |
| 4 | 16µg/ml | 1.0492 | 8µg/ml | 0.2852 |
| 5 | 16µg/ml | 1.0859 | 8µg/ml | 0.3199 |
| | SD<br>RSD | 0.03975<br>3.7009 | SD<br>RSD | 0.01511<br>4.8971 |

**Linearity**

The overlay spectra obtained in linearity study was shown in Fig No 6 and 7 and the obtained calibration curve of both analytes was found to be linear in the selected conc range as shown in Fig No 8. The regression equation of line and its parameters slope, $r^2$ value and intercept are tabulated in Table No 2, which proved the linear relationship between conc and obtained response.

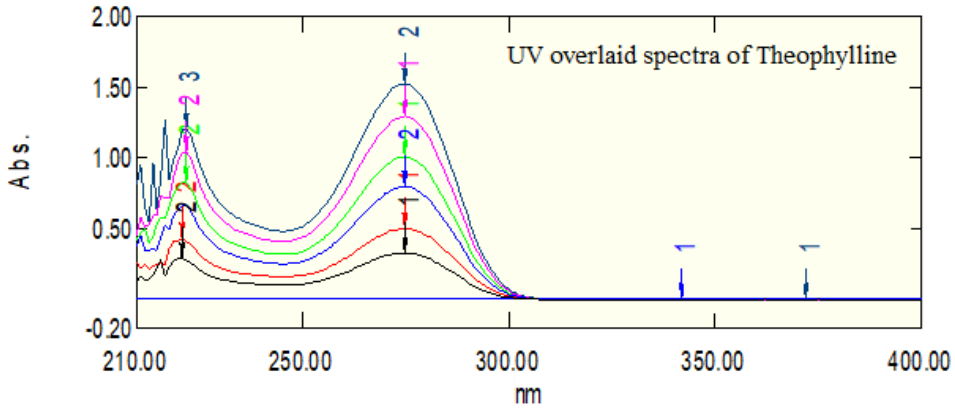


**Fig No 6: UV-VIS overlain spectra of TPE in linearity study**

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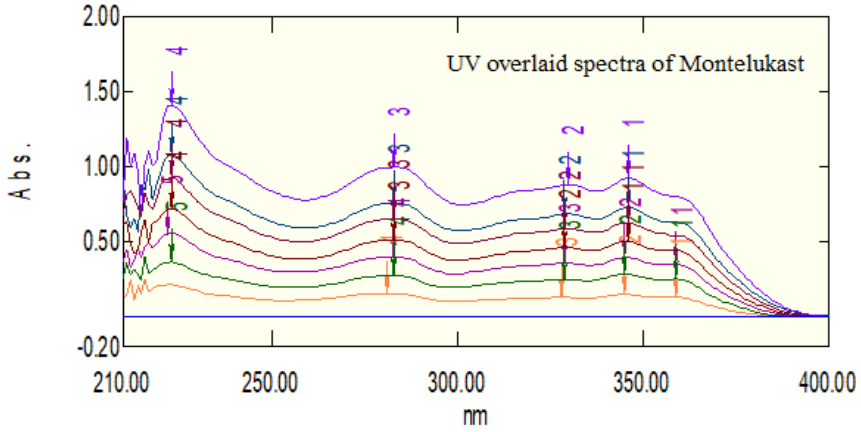
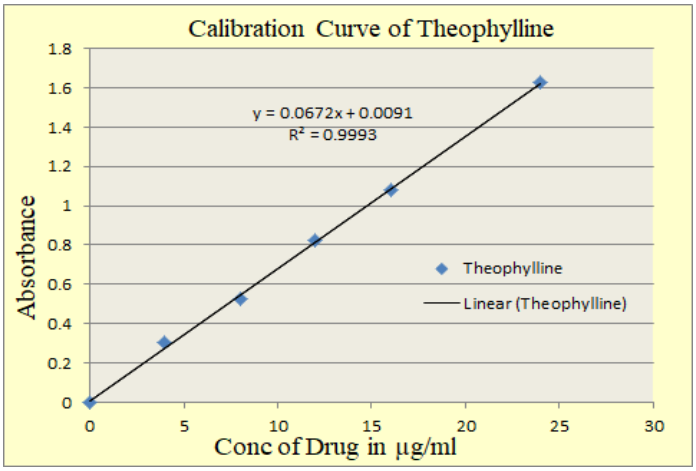



**Fig No 7: UV-VIS overlain spectra of MKS in linearity study**



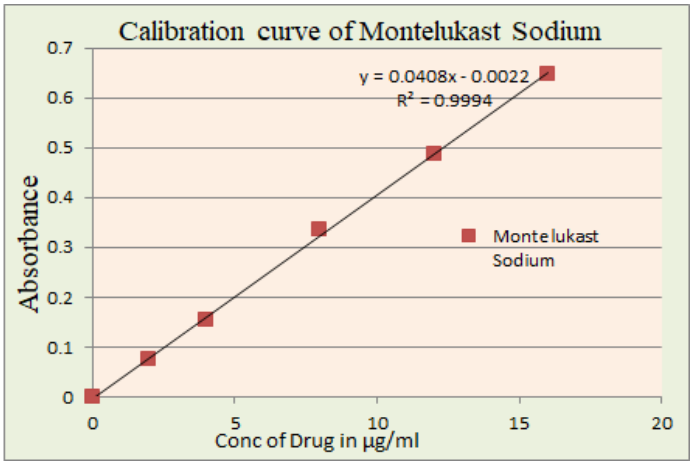




**Fig No 8: Calibration curve of Theophylline and Montelukast Sodium**

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**Table No 2: Parameters of regression equation obtained in Microsoft excel**

| **Parameters** | **TPE** | **MKS** |
|---|---|---|
| **Wavelength selected** | **275** | **329** |
| Conc range (µg/ml) | 1-24µg/ml | 1-16µg/ml |
| Correlation coefficient ($r^2$) | 0.9993 | 0.9994 |
| Regression equation(y = mx + c) | Y=0.0672x + 0.0091 | Y=0.0408x -0.0022 |

**Assay**

The assay was carried out by both these methods. The spectra of formulation was obtained and calculated % of nominal conc and SD, data was found within acceptable limits are summarized in Table No 3. The results indicated applicability of the method for estimation of Formulation.

**Table No 3: Results of assay of formulation by proposed method**

| Formulation | Drug | Label Claim (mg/Tablet) | Amount found/mg; n=6 | Drug Content % | Std Deviation | % RSD |
|---|---|---|---|---|---|---|
| **Method–I** | TPE | 400 mg | 404.36 mg | 101.09 | 1.4671 | 1.4516 |
| | MKS | 10 mg | 10.051 mg | 100.51 | 1.7016 | 1.6928 |
| **Method-II** | TPE | 400 mg | 404.46 mg | 101.116 | 1.7013 | 1.6826 |
| | MKS | 10 mg | 10.051 | 100.51 | 0.2332 | 0.2321 |

**Study of the method Precision**

The results of precision study are summarised in Table No 4, the obtained results were within acceptable limit; and methods precision was justified by calculating % of RSD. The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are shown in Fig No 9.

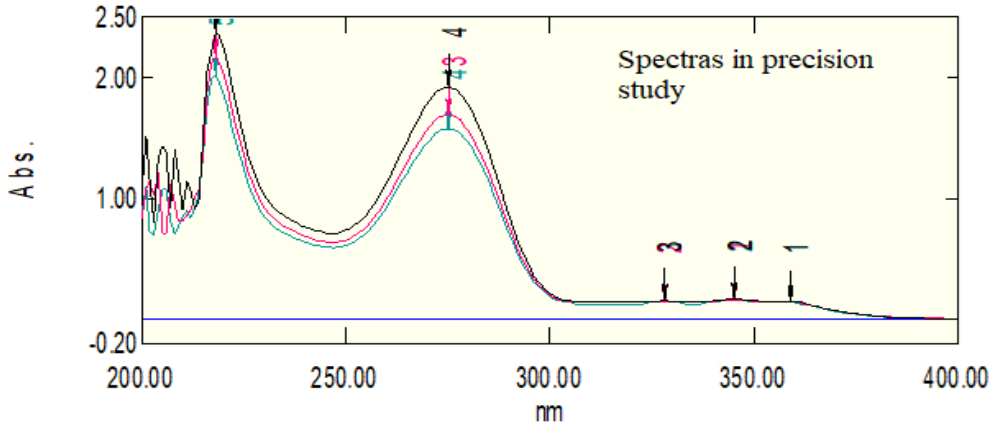


Fig No 9: Spectra of formulation obtained in precision study

Table No 4: Results of precision study in both the methods

| Sr. No. | Parameter | Level of study | Drug Name | Amount % | S.D. | % RSD |
|---|---|---|---|---|---|---|
| Method-I | Precision | Intraday Precision | TPE | 99.035 | 1.6293 | 1.6452 |
| | | | MKS | 102.545 | 1.3174 | 1.2841 |
| | | Interday precision | TPE | 105.058 | 3.4488 | 3.2828 |
| | | | MKS | 98.603 | 1.0096 | 1.0239 |
| Method-II | Precision | Intraday Precision | TPE | 98.263 | 2.7182 | 2.7384 |
| | | | MKS | 103.81 | 3.6778 | 3.5432 |
| | | Interday precision | TPE | 101.448 | 1.3272 | 1.3082 |
| | | | MKS | 97.11 | 0.8156 | 0.8407 |

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**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ of TPE and MKS by the proposed method were found within acceptable limit.

**Robustness and Ruggedness**

Robustness was studied and capacity of analytical procedure to measure analyte was remain unaffected by small but deliberate variations in method parameter like variation in the wavelength ± 1 nm, variation in the solvent strength by ± 0.1 %. The analytical method was found rugged during development; similarity the result was produced by performing the analysis by different analyst.

Table No 5: Results of LOD and LOQ

| Parameters | | MKS | TPE |
|---|---|---|---|
| LOD mcg/ml | | 0.3058 | 0.5985 |
| LOQ mcg/ml | | 1.1389 | 1.8136 |
| Robustness | (conc12 mcg/ml) (± 1 nm) | 328 (0.5871)<br>330 (0.5864) | 276 (0.7631)<br>278 (0.7619) |
| Ruggedness | Analyst 1 | SD ± 0.0271<br>RSD ± 4.6159 | SD ± 0.0153<br>RSD ± 5.8141 |
| | Analyst 2 | SD ± 0.0276<br>RSD ± 4.5418 | SD = 0.0147<br>RSD ± 5.6128 |

## III. CONCLUSION

The method was developed with eco-friendly and easily available aqueous 0.1 N NaOHsolvent. Theophylline and Montelukastsodiumwere estimated from the formulation by the method and satisfactory results were obtained. The Q method method was given reproducible results; however obtained results of both the methods were within acceptable limits given in the pharmacopoeia. The validated method is economical, precise, accurate, robust and reproducible hence can be routinely used for estimation of both the drugs from the dosage form.

**CONFLICT OF INTEREST**

All Authors declared that there is no conflict of interest

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