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# Spectroscopic methods for the simultaneous estimation of theophylline and furosemide

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**ABSTRACT**

*The simple, sensitive and specific U.V. spectrophotometric methods were developed for simultaneous determination of theophylline (THE) and furosemide (FUR) in synthetic mixture. Spectrophotometric studies were carried out using double beam JASCO U.V. spectrophotometer with methanol as solvent. The present work includes two methods: method A- simultaneous equation method and method B-absorbance ratio method. Wavelength selected are 272 nm for THE and 275 nm for FUR respectively. In both the methods linearity were observed in the concentration range of 5-30 µg/ml and 5-35 µg/ml for THE and FUR, with good correlation coefficient 0.999 and 0.9988 respectively. Method B involved formation of absorbance equation at isobestic point (251 nm). THE and FUR are used for the treatment of asthma and congestive heart failure. Both methods showed high sensitivity with reproducibility in result.*

**Keywords**: Furosemide, Q- Absorbance, Simultaneous equation, Theophylline.

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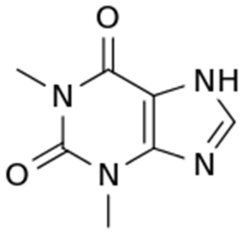
## INTRODUCTION

Furosemide (FUR) is a loop diuretic used in the treatment of congestive heart failure and edema [1,2]. Like other loop diuretics, (FUR) acts by blocking the $Na^+$-$K^+$-$2Cl^-$ symporter in the thick ascending limb of the loop of henle, decreasing the sodium reabsorption from the tubular fluid resulting in increased water secretion into the tubule and hence reducing the blood pressure [2]. It is an official drug in Indian and British Pharmacopiea. Few analytical methods by RP-HPLC and spectrophotometry have been reported for the estimation of FUR [3].Theophylline (THE), also known as 1,3-Dimethylxanthine, is a methyl xanthine drug used in therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma. As a member of the xanthine family, it bears structural and pharmacological similarity to caffeine [3,4]. It is an official drug in Indian and British Pharmacopiea. There are very few analytical methods reported for the estimation of theophylline, which includes HPLC, Spectrophotometry [4].

The combination of FUR and THE is very useful in the treatment of heart failure and asthma [5].On literature survey, it was found that not a single method reported for the simultaneous estimation of FUR and THE. And no method is available in the pharmacopoeias. In view of the need for suitable methods for routine analysis in combination, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in synthetic mixture.

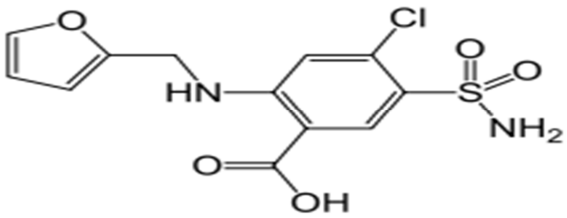
**DRUG PROFILE**
**Theophylline**



IUPAC Name:1,3-Dimethyl-7H-purine-2,6-dione, Molecular formula:$C_7H_8N_4O_2$, Molecular weight:180.164 g/mol, Description:It is a white powder, Solubility: Freely soluble in methanol, sparingly soluble in water, Category: Anti-asthmatic drug [6].

Theophylline (THE) is competitive nonselective phosphodiesterase inhibitor. which raises intracellular cAMP, activates PKA, inhibits TNF-alpha and inhibits leukotriene synthesis, and reduces inflammation and innate immunity [6]. Nonselective adenosine receptor antagonist, antagonizing A1, A2, and A3 receptors almost equally, which explains many of its cardiac effects. THE has been shown to inhibit TGF-beta-mediated conversion of pulmonary fibroblasts into myofibroblasts in COPD and asthma via cAMP-PKA pathway and suppresses COL1 mRNA, which codes for the protein collagen [9].

**Furosemide**



IUPAC Name: 4-Chloro-N-furfuryl-5-sulphamoyl anthranilic acid, Molecular formula:$C_{12}H_{11}ClN_2O_5S$, Molecular weight: 330.75 g/mol, Description: It is a white or slightly yellow crystalline powder, Solubility: Freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol and practically insoluble in water, Category: Diuretic drug [8,9].

Like other loop diuretics, furosemide acts by inhibiting NKCC2, the luminal Na-K-2Cl symporter in the thick ascending limb of the loop of henle. The action on the distal tubules is independent of any inhibitory effect on carbonic anhydrase or aldosterone,[8,9]. It also abolishes the corticomedullary osmotic gradient and blocks negative, as well as positive, free water clearance. It is a potent diuretic that inhibits the active reabsorption of chloride in the diluting segment of the loop of henle, thus preventing the reabsorption of sodium, which passively follows chloride. Additionally, FUR is a non-competitive subtype specific blocker of GABA-A receptors [10].

## MATERIALS AND METHODS

**Instrumentation**
For the present study JASCO double beam UV/Visible spectrophotometer (model-V630) was used with slit width at 1.8 nm and spectra manager software version 1.5,was used. Pair of 10 mm matched quartz cells was used to measure absorbance of solution. Weighing were done on electronic balance (Model ShimadzuAUW-220D), Ultrasonicator model 5.5L150H were used.

**Reagents and chemicals**
All analytical grade reagents were used.

**Preparation of standard stock solutions and calibration curve**
Standard stock solutions of both (THE) and (FUR) were prepared by dissolving 10 mg of THE and 10 mg of FUR separately in 20 ml of methanol in 100 ml volumetric flasks. Final volume was made up to 100 ml with methanol to

get working standard solution of each 100 µg/ml. These stock solution were used to prepare series of solution with concentration 5-40 µg/ml of THE and FUR respectively for both methods.

**Determination of absorption maxima**:
By appropriate dilution of standard stock solution of THE and FUR with methanol, solutions containing 20 µg/ml of THE and 20 µg/ml of FUR were scanned separately in the range of 200- 400 nm. Wavelength of maximum absorption was determined for both the drugs. THE showed maximum absorbance at 272 nm ($\lambda_1$) and FUR at275 nm($\lambda_2$)

**Method A: Simultaneous equation method**
THE and FUR showed absorbance maxima at 272 nm ($\lambda_1$) and 275 nm ($\lambda_2$) respectively. The absorbancewere measured at the selected wavelength and absorptivity (A1%,1cm) for both the drugs at both wavelengths were determined. The calibration curves for THE and FUR were plotted in the concentration range of 5-40 µg/ml. The linearity was observed in the concentration range of 5-30 µg/ml for THE and 5-35 µg/ml for FUR. The concentrations of drugs in sample solution weredetermined by using the following formula**,**

Preliminary calculations and assumptions
1) The absorptivity of THE at $\lambda_1$ and $\lambda_2$, $ax_1$and $ax_2$ respectively. 2) The absorptivity of FUR at $\lambda_1$ and $\lambda_2$, $ay_1$ and $ay_2$ respectively. 3) The absorbance of diluted sample at $\lambda_1$ and $\lambda_2$, A1and A2 respectively. 4) Let $C_x$ and $C_y$ be the concentrations of THE and FUR respectively in diluted sample.

The two equations were constructed based upon the fact that at $\lambda_1$ and $\lambda_2$ the absorbance of the mixture is the sum of individual absorbance of THE and FUR.

At $\lambda_1$, $A_1 = ax_1bc_x + ay_1bc_y$…… (1) At $\lambda_2$, $A_2 = ax_2bc_x + ay_2bc_y$…… (2)

Where, $A_1$ and $A_2$ are absorbance of mixture at 272 nm and 275 nm respectively.

**Method B: Absorbance ratio (Q – Absorbance) method**
It uses the ratio of absorbance at two selected wavelengths, one which is an iso-absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that THE and FUR show an iso-absorptive point at 251 nm ($A_1$). The second wavelength used is 275 nm ($A_2$), which is the λ-max of FUR. Concentrations of THE and FUR were determined using following equations and calibration curve was plotted at both wavelength.

The concentration of two drugs in the mixture can be calculated using following equations.
$C_x = (Q_m-Q_y).\ A_1/\ (Q_x-Q_y).\ ax_1$, $C_y = (Q_m-Q_y).\ A_1/\ (Q_y-Q_x).\ ay_1$, Where, $Q_m = A_2/\ A_1$, $Q_x = ax_2/\ ax_1$, $Q_y = ay_2/\ ay_1$.Where, $A_1$ and $A_2$ are absorbance's of mixture at 272 nm and 275 nm respectively $ax_1$ and $ax_2$ are absorptivity of THE at $\lambda_1$ and $\lambda_2$ respectively and $ay_1$ and $ay_2$ are absorptivity of FUR at $\lambda_1$ and $\lambda_2$ respectively. $C_x$ and $C_y$ are concentrations of THE and FUR respectively.

**Preparation of synthetic mixture-**Synthetic mixture of THE and FUR were prepared by dissolving 10 mg of THE and 10 mg of FUR separately in 20 ml of methanol in 100 ml volumetric flasks. Final volume was made up to 100 ml with methanol to get working standard solution of each 100 µg/ml, from these stock solution pipette out 1 ml THE solution and 1 ml FUR solution in 10 ml volumetric flask and make up volume with methanol to get working standard solution of 20 µg/ml.

**Validation:** The method was validated according to ICH guidelines to study linearity, precision, sensitivity, LOQ and LOD.

**Linearity:** The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of THE and FUR. For both the methods, the Beer's law was obeyed in the concentration range of 5-30 µg/ml and 5-35 µg/ml for THE and FUR respectively. The correlation coefficient was found to be 0.999 at 272 nm for THE and 0.9988 at 275 nm for FUR.

**Precision:** Precision of these methods was checked by analysing the samples at three different time intervals of the same day (intraday precision) as well as on different days (inter-day precision).

**Limit of detection and limit of quantitation:** LOD and LOQ are calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

## RESULT AND DISCUSSION

The combination of Theophylline (THE) and Furosemide (FUR) is play vital role in the treatment of patient suffering two disease at a time like asthma and hypertension. Hence the present work provide very simple and accurate method for simultaneous estimation of THE and FUR. In simultaneous equation method, wavelengths selected for analysis were 272 nm for THE and 275 nm for FUR. In Q-method, wavelength selected for analysis was 251 nm.In both the methods linearity were observed in the concentration range of 5-30 µg/ml and 5-35 µg/ml for THE and FUR, with good correlation coefficient 0.999 and 0.9988 respectively. Both method are validated according to ICH guidelines and all validation parameters were studied for the proposed method, like linearity, precision, sensitivity.

**Table-1: Optical characteristics of theophylline and furosemide**

| Optical characteristics | Theophylline | Furosemide |
|---|---|---|
| Wavelength (nm) | 272 nm | 275 nm |
| Beer lambert's law limit (µg/ml) | 5-30 µg/ml | 5-35 µgml |
| Regression equation (y=mx+c) | Y = 0.0696x+ 0.0341 | Y=0.0649x+0.0244 |
| Slope (m) | 0.0696 | 0.0649 |
| Intercept (c) | 0.0341 | 0.0244 |
| Correlation coefficient ( $R^2$) | 0.999 | 0.9988 |

**Table-2- Repeatability study of theophylline for proposed method**

| Conc. µg/ml | Simultaneous equation method | | Q-absorbance ratio method | |
|---|---|---|---|---|
| | 272 nm | 275 nm | 251 nm | 275 nm |
| 20 | 1.1984 | 1.0191 | 0.5891 | 1.4234 |
| 20 | 1.1886 | 1.0183 | 0.5891 | 1.4232 |
| 20 | 1.1979 | 1.0198 | 0.5883 | 1.4234 |
| Mean | 1.1949 | 1.0190 | 0.5888 | 1.4233 |
| SD | 0.0005519 | 0.000751 | 0.000462 | 0.000115 |
| %RSD | 0.04618 | 0.07369 | 0.07846 | 0.08079 |

*Limit: % RSD for area NMT 2.0%*

**Table-3- Repeatability study of furosemide for proposed method**

| Conc. µg/ml | Simultaneous equation method | | Q-absorbance ratio method | |
|---|---|---|---|---|
| | 275nm | 272nm | 251nm | 275nm |
| 20 | 1.3281 | 1.3376 | 0.4100 | 1.2201 |
| 20 | 1.3282 | 1.3372 | 0.4100 | 1.2203 |
| 20 | 1.3281 | 1.3372 | 0.4102 | 1.2201 |
| Mean | 1.3281 | 1.3373 | 0.4100 | 1.2201 |
| SD | 0.0003451 | 0.0002309 | 0.0001147 | 0.0001154 |
| %RSD | 0.02598 | 0.017266 | 0.02797 | 0.09458 |

*Limit: % RSD for area NMT 2.0%*

**Table-4 – Intra-day and inter-day precision data for simultaneous equation method**

| Conc. (µg/ml) | Intra-day and inter-day precision data for theophylline | | | |
|---|---|---|---|---|
| | Intra-day precision | | Inter-day precision | |
| | Absorbance | | Absorbance | |
| | 272 nm | 275 nm | 272 nm | 275 nm |
| 10 | 0.6328 | 0.5102 | 0.6325 | 0.5114 |
| 15 | 0.8997 | 0.8120 | 0.8997 | 0.8121 |
| 20 | 1.1984 | 1.0192 | 1.1983 | 1.0193 |
| | Intra-day and inter-day precision data for furosemide | | | |
| 10 | 0.7022 | 0.7016 | 0.7022 | 0.7017 |
| 15 | 0.9843 | 1.0354 | 0.9841 | 1.0355 |
| 20 | 1.3281 | 1.3376 | 1.3283 | 1.3378 |

*Limit: % RSD for area NMT 2.0%*

**Table-5 – Intra-day and inter-day precision data for Q-absorbance ratio method**

| Conc. (µg/ml) | Intra-day and inter-day precision data for theophylline | | | |
|---|---|---|---|---|
| | Intra-day precision | | Inter-day precision | |
| | Absorbance | | Absorbance | |
| | 251nm | 275nm | 251nm | 275nm |
| 10 | 0.2712 | 0.5527 | 0.2716 | 0.5531 |
| 15 | 0.3573 | 0.7902 | 0.3572 | 0.7910 |
| 20 | 0.5891 | 1.4234 | 0.5891 | 1.4233 |
| | Intra-day and inter-day precision data for furosemide | | | |
| 10 | 0.2202 | 0.6998 | 0.2203 | 0.6997 |
| 15 | 0.3160 | 0.9733 | 0.3161 | 0.9734 |
| 20 | 0.4100 | 1.2201 | 0.4100 | 1.2200 |

*Limit: % RSD for area NMT 2.0%*

**Table-6- Limit of detection and limit of quantification**

| Parameters | Simultaneous equation Method | | | |
|---|---|---|---|---|
| | THE | | FUR | |
| | 272 nm | 275 nm | 272nm | 275nm |
| LOD (µg/ml) | 0.1880 | 0.1458 | 0.1833 | 0.1160 |
| LOQ (µg/ml) | 0.5698 | 0.4418 | 0.5555 | 0.3517 |

**Table-7- Limit of detection and limit of quantification**

| Parameters | Q-absorbance ratio method | | | |
|---|---|---|---|---|
| | THE | | FUR | |
| | 251 nm | 275 nm | 251 nm | 275 nm |
| LOD (µg/ml) | 0.5285 | 0.1388 | 0.6333 | 0.1316 |
| LOQ (µg/ml) | 1.6016 | 0.4206 | 1.9211 | 0.3990 |

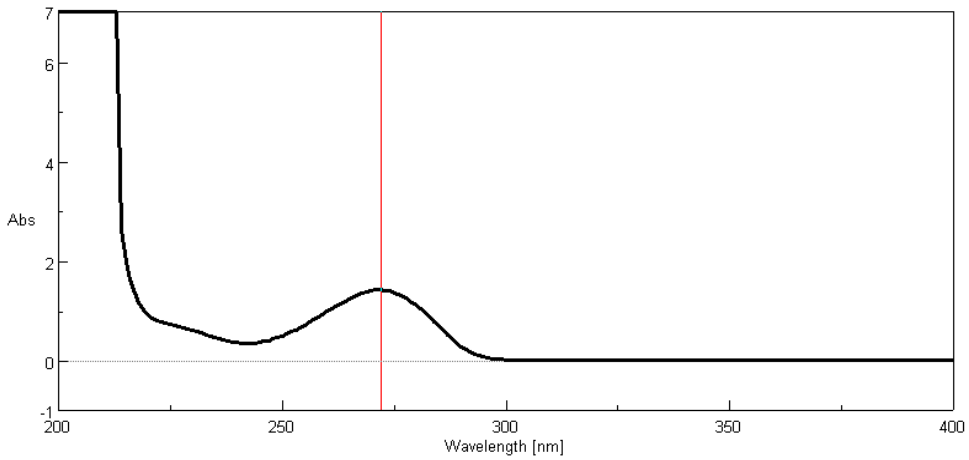



**Fig-1: Spectra of THE in methanol**

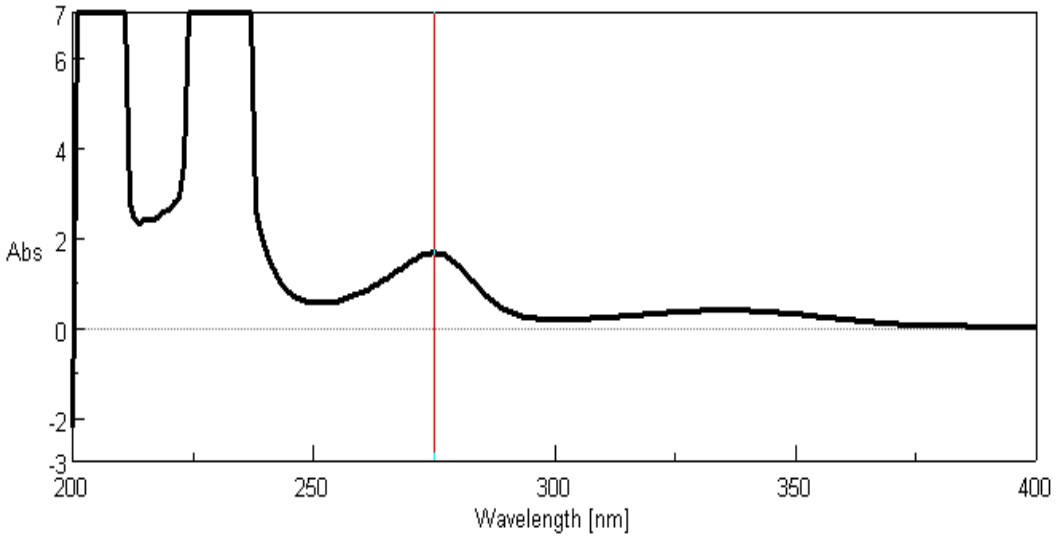



**Fig-2: Spectra of FUR in methanol**

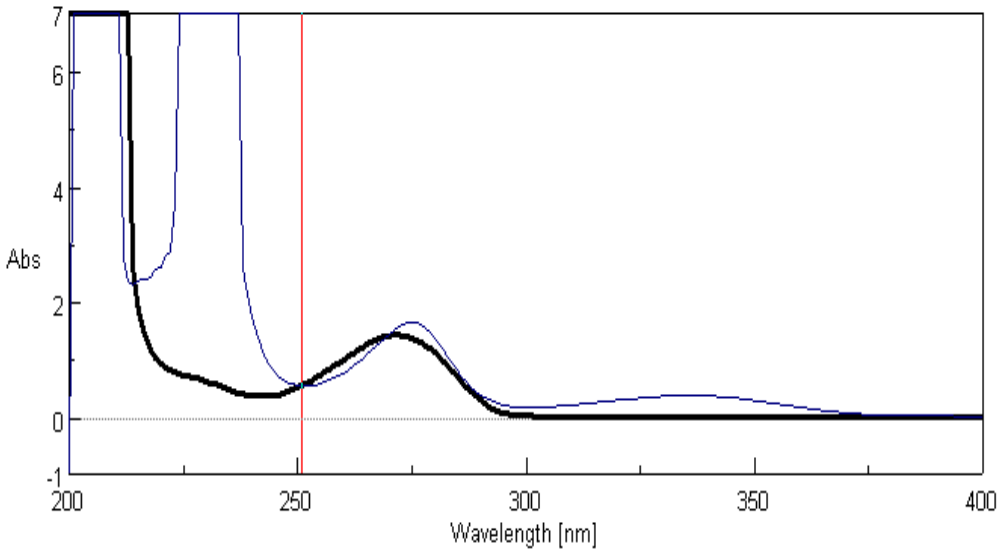



**Fig-3: Overlay spectra of THE and FUR**

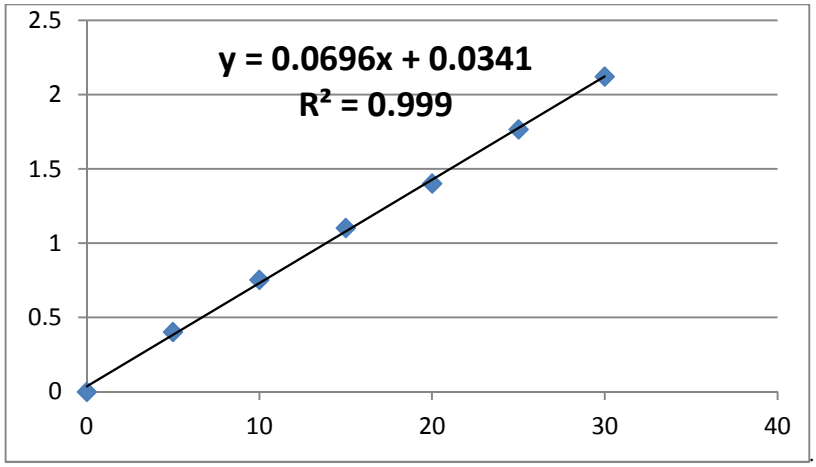



**Fig-4: Standard calibration plot of THE at 275 nm in methanol**

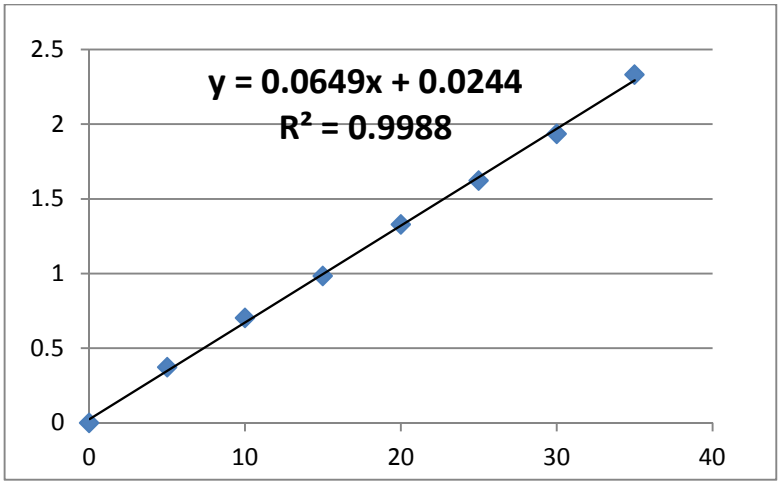



**Fig-5: Standard calibration plot of FUR at 275 nm in methanol**

## CONCLUSION

The two spectrophotometric methods were developed and validated as per ICH guidelines. These validated methods are new, rapid, accurate, precise, sensitive, and reproducible. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods.

Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of theophylline and furosemide in bulk and formulation.

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