

Simultaneous Estimation of Etophylline and Theophylline in Bulk and solid Dosage Form by Chemometric Assisted Spectrophotometric Methods

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Abstract: A simple UV-visible spectroscopic method was developed and Chemometric designs were applied for the simultaneous estimation of Etophylline (ETO), Theophylline (THEO) in bulk and solid dosage form. The spectroscopic method was developed by using methanol as solvent for the two drugs and the data generated from the spectra were mined by using Chemometric methods such as bi-linear regression analysis, Cramer's matrix method and Method of least squares. The wavelengths selected for all the above methods were 271 nm (wavelength of maximum absorption; λ_{max} of ETO), 273 nm (wavelength of maximum absorption; λ_{max} of THEO).

Results: The methods hold good linearity for ETO from 5-9 $\mu\text{g/ml}$, for THEO from 1-5 $\mu\text{g/ml}$ with regression coefficient values of 0.999/ 0.998 respectively. The RSD for interday and inter-day precision was found to be less than 2%. The percentage recovery and percentage assay was in the range 95-115% Of for Etophylline (ETO) and Theophylline (THEO) by all the methods.

Conclusion: The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the two drugs and moreover they are effective in minimizing the errors in analysis, simple and economical.

Keywords:- UV-visible spectroscopy, Etophylline, Theophylline.

I. Introduction

Chemo metrics is the science of extracting information from chemical systems by data-driven means. Chemometrics is inherently interdisciplinary, using methods frequently employed in core data-analytic disciplines such as multivariate statistics, applied mathematics, and computer science, in order to address problems in chemistry, biochemistry, medicine, biology and chemical engineering. (1) In this way, it mirrors other interdisciplinary fields, such as psychometrics(5) and chemo metrics can be applied to solve both descriptive and predictive problems in experimental natural sciences like in chemistry applications and their properties (6). These two chemo metrics techniques are used as analytical chemistry and the development of chemo metric methods. (2) Chemometrics can be described as the interaction of mathematical and statistical methods to chemical analysis and problems chemometric has developed because of the changes in the enormous data and in which data is obtained in chemistry along with the evaluation of new analytical Technique's along with the computer technology. (4)

The development of computer technology has been tremendously cause the wide growth of chemo metrics. (7) Majority of the methods can be utilized in the chemometrics depend upon the ability and capacity of computer to perform the large calculations. (8) In the analytical chemistry chemo metric techniques has its applications in spectroscopy like uv-visible spectroscopy, IR-spectroscopy, fluorescence, chromatograph like liquid chromatography and also various analytical chemistry like flow injection analysis in pharmaceutical preparations (10).

Chemo metrics methods are like:-

1. Bi-linear regression analysis.
2. Cramer's matrix method.
3. Method of least squares.

Principles of chemo metrics are as followed by:-

1. Chemometrics are use mathematical models for the connection, rationalization and interpretation of chemical data obtained by analysis.
2. These are include variability in the model, and handle the variability by means of distribution.
3. These are use statistical designs for planning sets of experiments when need to change conditions or optimizing etc. rather than changing only one factor and keeping others fixed.
4. Always to be use multivariate data analysis methods and show results as plots also perform the validation of mathematical model

1. **Etophylline**:-IUPAC name: -7-(2-Hydroxyethyl)-1, 3-dimethyl-3, 7-dihydro-1H-purine-2, 6-Dione;Mol.wt:-329.310g/mol.
2. **Theophylline** IUPAC name :- 1, 3-dimethyl-3, 7-dihydro-1H- purine-2, 6-dione;mol.wt:-180.16 g/mol

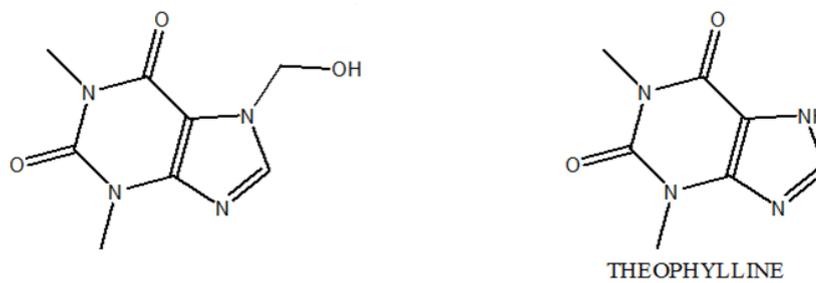


Fig:-1.Etophylline Structure like Theophylline (R = H) **fig:-2.Etophylline** (R = CH₃CH₂OH).

Etophylline and Theophylline are the non-selective phosphor diesterase inhibitor, anti-asthmatic agent used in the treatment of acute attacks or status asthmatic in conjunction with other drugs. Theophylline is respiratory smooth muscle relaxant, phosphor di-esterase inhibitor, bronchodilator agent and vasodilator agent. Chemically it is 1, 3-dimethyl-3, 7-dihydro-1H- purine-2, 6-dione.Literature survey revealed that Etophylline and Theophylline in combination with other drugs were simultaneously estimated by HPLC literature survey also revealed that HPLC and HPTLC methods have been reported for the simultaneous estimation of etophylline and theophylline in combined dosage forms. No UV spectrophotometric method has been reported for simultaneous determination of Etophylline and Theophylline in combined dosage form. The present article discusses the attempts made to develop simple, sensitive, reproducible and economical chemo metric methods for simultaneous determination of these drugs in combined dosage forms. Etophylline and theophylline both the drugs have same chromosphere and complete overlapping spectra Hence they cannot be quantified with any conventional UV method.by using different chemo metric methods.

II. Materials And Methods:

Instruments are used:

- Analytical balance
- UV-Visible spectrophotometer (Lab India -3072)

Data handling systems:

- UV-Win for the handling of spectrophotometer.
- Microsoft Excel.
- Easy Matrix Calculator Pro (Ver. 5.4) were used for resolving the data matrices.
- Chem diagram

Materials used:

These Working standard drugs are procured from Dr. Reddy s laboratory. Commercial formulation of drugs were purchased from local market. Methanol AR grade was procured from Merck (India) ltd, Mumbai.

PREPARATION STANDARED SOLUTIONS

Preparation of Etophylline standard solution:- 10mg of Etophylline (API) was dissolved in methanol and concentrations of 5,6,7,8,9µg/ml was prepared in 10ml of volume metric flask and make up with methanol. Observe in uv-visible spectroscopy at 273nm.

Preparation of Theophylline standard solution :10mg of theophylline (API) was dissolved in methanol and concentrations of 1,2,3,4,5µg/ml was prepared in 10ml of volume metric flask and make up with methanol. Observe in uv-visible spectroscopy at 271nm.

PREPARATION OF FORMULATION /TEST SOLUTION

Preparation of Etophylline, Theophylline solutions

By preparing the formulation based on the label claim take 24.2mg (formulation) was take and dissolved in 5ml of methanol then made up to 10ml with methanol (10 ml volumetric flask/1000µ/ml)then take into the above solution 0.1 ml then make up into 10 ml with methanol .take from above solution take 5ml then make up with methanol up to10 ml (5 conc) was observed in uv-visible spectroscopy at 200-400nm.

DESIGN OF CHEMOMETRIC MODELS:-

Chemometric models were designed for the developed spectrophotometric methods for the simultaneous estimation of etophylline (ETO) theophylline (THEO).

Linear Regression Component (LRC) method:-In this method three wavelengths were considered for the analysis of the component mixtures are like [ETO;THEO].The bi- linear regression equations were obtained by using the absorbance measured at three wavelengths against concentrations of standard solutions for each component. The slope values obtained from the linear regression analysis for each component were used for the formation of matrix set.

The wavelengths selected for analysis were 271 (λ_{max} of theo), 273 nm (λ_{max} of THEO).

Equations for the formation of matrix are:-

$$A_{mix1} = b_{x1}C_x + b_{y1}C_y + a_{xy1}$$

$$A_{mix2} = b_{x2}C_x + b_{y2}C_y + a_{xy2}$$

Where, A_{mix1} , A_{mix2} are the absorbance of the mixture of X, Y analytics at three wavelengths set. a_{xy1} , a_{xy2} are the sum of intercepts of the linear regression equation at the three wavelengths.

Conversion of equation into matrix form:

$$\begin{bmatrix} A_{mix1} - a_{xy1} \\ A_{mix2} - a_{xy2} \end{bmatrix} = \begin{bmatrix} b_{x1} & b_{y1} \\ b_{x2} & b_{y2} \end{bmatrix} \times \begin{bmatrix} C_x \\ C_y \end{bmatrix}$$

Cramer’s Matrix Method

Molar absorptivity (ϵ) values were calculated by using the absorbance measured at 271nm, 273 nm, for each compound in the bi mixture. The selected wavelength values were λ_{max} of ETO, and THEO respectively. By using absorptivity (ϵ) values, a system of equations with three unknowns is the bi mixture have been written as follows:

$$A_{m, 271} = \epsilon_{Te, 271} C_{Te} + \epsilon_{Et, 271} C_{Et}$$

$$A_{m, 273} = \epsilon_{te, 273} C_{Te} + \epsilon_{Et, 273} C_{Et}$$

Where A_m denotes the absorbance of the Two mixture and ϵ represents the values of molar absorptivity for the calculated, ETO, THEO respectively at 271nm, 273 nm. is the molar concentration of ETO,THEO.

The matrix simplifies and solves the system of equations with three unknowns as follows:

This matrix can be solved and each compound was determined by solving the following operations

(Δ = Determinant value of matrix)

$$\Delta = \begin{vmatrix} \epsilon_{Eto273} & \epsilon_{Theo273} \\ \epsilon_{Eto271} & \epsilon_{Theo271} \end{vmatrix}$$

$$\Delta_1 = \begin{vmatrix} A_{m271} & \epsilon_{Theo271} \\ A_{m273} & \epsilon_{Eto273} \end{vmatrix}$$

$$\Delta_2 = \begin{vmatrix} \epsilon_{Theo271} & A_{m271} \\ \epsilon_{Theo273} & A_{m273} \end{vmatrix}$$

By applying Cramer’s matrix rule the concentration ETO and THEO can be found by

$$C_{ETO} = \Delta_1 / \Delta \quad C_{THEO} = \Delta_2 / \Delta$$

Method of Least Squares

The standard stock solutions of ETO (8 μ g/ml) and THEO (2 μ g/ml) were measured at 250nm, 254nm, 258nm, 262nm, 266 nm, 270nm, 274nm, 278nm,) and their absorbance’s were recorded (acts as calibration set) and tabulated in MS- Excel. The individual drug absorbance of known concentrations of ETO and THEO were added and synthetic mixture (as validation set) was created and absorbance were recorded. Similarly the test sample was also measured at same wavelengths and absorbance’s were recorded and tabulated. By applying method of least squares using Solver add-in in MS-Excel, the actual concentration of ETO and THEO were predicted in test samples.

Validation of spectrophotometric method:

Linearity and range:-

The linearity of analytical method is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

The range of analytical procedure is the interval between the upper and lower concentrations of the sample for which the analytical procedure has a suitable level of Precision, Accuracy and Linearity.

Precision:

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Accuracy:

The accuracy of analytical procedure express the closeness or agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by adding known quantities of analyte (pure drug) to the drug product and applying the developed methods to determine the quantity of the drug present in the spiked sample. Samples were spiked with 75%100,125% level solutions of the standards and analysed. The experiment was performed triplicate (n=3). Percent recovery values were reported.

$$\text{Accuracy} = \frac{\text{Amount of Sample Conc. found} - \text{Amount of Test Conc. taken}}{\text{Amount of Standard Conc. added}} \times 100$$

Assay:

The commercial marketed formulation containing Etophylline of 8.7mg.and Theophylline of 2.3mg.The sample solution was treated same as standard solution. The resulting solution scanned under UV using methanol as blank.

$$\text{Percent Assay} = \frac{\text{Calculated qty of test sample(mg)}}{\text{Weight of test sample(mg)}} \times 100$$

III. Results And Discussion

1. Bi -linear regression analysis:

Table No.1: Absorbance of Etophylline at 273 nm, 271nm.

Conc. (µg/ml)	273nm	271 nm
5	0.253	0.051
6	0.331	0.062
7	0.348	0.073
8	0.443	0.082
9	0.473	0.094
Linear Equation	y = 0.055x+0.016	y = 0.010x -0.001
R ²	0.963	0.998

Table No.2 : Absorbance of Theophylline at 271 nm and 273 nm

Conc. (µg/ml)	271 nm	273 nm
1	0.072	0.009
2	0.117	0.018
3	0.184	0.032
4	0.186	0.040
5	0.356	0.049
Linear Equation	y = 0.063x +0.008	y = 0.010x+0.001
R ²	0.970	0.991

$$\begin{bmatrix} A_{mix1} - a_{xy1} \\ A_{mix2} - a_{xy2} \end{bmatrix} = \begin{bmatrix} b_{x1} & b_{y1} \\ b_{x2} & b_{y2} \end{bmatrix} \times \begin{bmatrix} C_x \\ C_y \end{bmatrix}$$

$$\begin{bmatrix} 0.443 & 0.024 \\ 0.135 & 0.002 \end{bmatrix} = \begin{bmatrix} 0.055 & 0.0063 \\ 0.010 & 0.010 \end{bmatrix} \times \begin{bmatrix} C_x \\ C_y \end{bmatrix}$$

$$\begin{bmatrix} C_x \\ C_y \end{bmatrix} = \begin{bmatrix} 8.269 \\ 2.731 \end{bmatrix}$$

The concentration of Etophylline (C_x), and Theophylline (C_y) present in the given formulation sample were found to be 8.26 µg/ml, and 2.731µg/ml.

2. Cramer’s matrix method:

$$A_{m, 271} = \epsilon_{Te, 271} C_{Te} + \epsilon_{Et, 271} C_{te}$$

$$A_{m, 273} = \epsilon_{te, 273} C_{Te} + \epsilon_{Et, 273} C_{te}$$

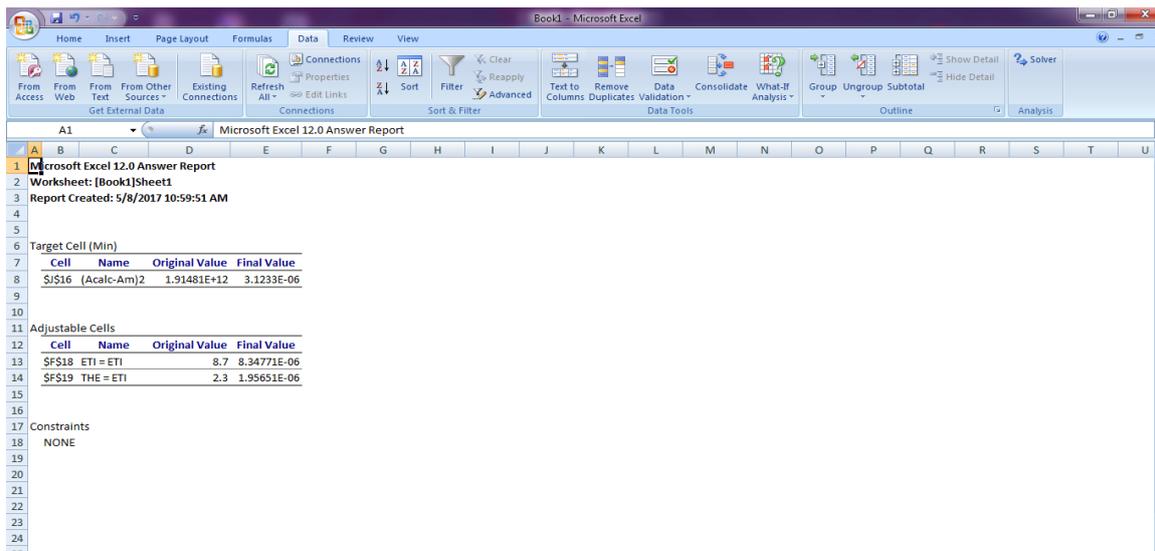


Fig No.4: Screen shot of solver report

The concentration of Ethophylline (C_x), Theophylline (C_y) present in the given formulation sample were found to be 8.7 μ g/ml, 2.3 μ g/ml respectively.

Table No.3:-Percentage assay for the three methods

	Actual concentration (μ g/mL)	TLR		CRM		MLS	
		Predicted concentration (μ g/mL)	Assay %	Predicted concentration (μ g/mL)	Assay %	Predicted concentration (μ g/mL)	Assay %
ETO	8.3	8.269	99.62%	8.426	100.5%	8.769	104.00%
THEO	2.5	2.731	109.24%	2.733	108%	2	80%

METHOD VALIDATION

Accuracy :-Table No:-5:-accuracy

DRUG	PERCENTAGE	% RECOVERY		
		Bi -linearity	FOR CRM	FOR MLS
ETO	85%	98.66	100.13	99.85
	100%	99.10	99.75	99.72
	115%	100.44	99.84	99.15
THEO	75%	98.89	100.44	99.12
	100%	99.16	100.50	100.26
	125%	100.26	99.86	98.98

Linearity and range :-

Table No. 6:-Linear equation parameters

Drug	Wave length nm	For TLRC Method			For Cramers matrix method(CRM)		
		Linear equation	R ²	RANGE μ g/mL	Linear equation	R ²	RANGE μ g/mL
ETO	271	y = 0.010x+0.001	0.998	5-9	y = 0.010x+0.001 y = 0.055x-0.016	0.998 0.968	5-9
	273	y = 0.055x-0.016	0.968				
THEO	271	y = 0.063x+0.008	0.979	1-5	y = 0.063x+0.008 y = 0.010x+0.001	0.979 0.991	1-5
	273	y = 0.010x+0.001	0.991				

PRECISION

Table No. 7: Percentage RSD for all the methods

DRUG	Concentration	Inter day precision (% RSD)			Intra day (% RSD)		
		Bi-LRC	CRM	MLS	Bi-LRC	CRM	MLS
ETO	6	1.1	1.7	1.5	1.7	1.3	1.2
	8	1.2	1.5	1.4	1.5	1.5	1.3
THEO	2	1.4	1.8	1.2	1.8	1.5	1.6
	4	1.2	1.6	1.3	1.7	1.7	1.5

The proposed spectrophotometric method was found to be linear and the data is presented in the Table No 7. The intra-day and inter-day precision values for both the chemo metric designs were presented in Table No 6 Accuracy was performed in terms of the Percent recovery values and the values for Etophylline, Theophylline by all the chemo metric designs were presented in Table No 5 The assay of the commercial formulation of the drugs were performed and their percentage assay values were presented in Table No 3.

IV. Conclusion

The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the three drugs and moreover they are effective in minimizing the errors in analysis, simple and economical. Finally it is concluded that the developed methods were simple and accurate can be used in routine analysis.

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Conflict Of Interest

This is a non-funding research work. There were no conflicts of interest.

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