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UV-Visible Spectrophotometric Method Development and Validation of Assay for Etophylline Tablet Formulation

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ABSTRACT

A novel, safe and sensitive method of spectrophotometric estimation in UV-region has been developed for the assay of Etophylline in its tablet formulation. The method have been developed and validated for the assay of Etophylline using water as diluents, which does not shows any interference in spectrophotometric estimations. All the parameters of the analysis were chosen according to ICH [Q2 (R1)] guideline.

Keywords: Spectrophotometric method Development, Validation, ICH [Q2 (R1)] guideline.

INTRODUCTION

Spectroscopy methods: It is the branch of science dealing with the study of interaction between matter and radiated energy [1, 2]. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 µm. The regions of electromagnetic spectrum are shown in table 1.

Table 1

Region	Wavelength
Far UV light	10-200nm
Near UV light	200-400nm
visible	400-750nm
Near Infra red	0.75-2.2µ
Mid Infra Red	2.5-50µm
Far Infra Red	50-1000µm

Ultraviolet-Visible spectrophotometer [3]: UV-Visible spectrophotometer is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law [4].

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λmax), where small error in setting the wavelength scale has little effect on measured absorbance. The assay of single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal procedures. They are, use of standard absorptivity value, calibration graph and single or double point standardization. In standard absorptive value method, the use of standard A (1%, 1 cm) or E values are used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. In calibration graph method, the absorbance of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. The single point standardization procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

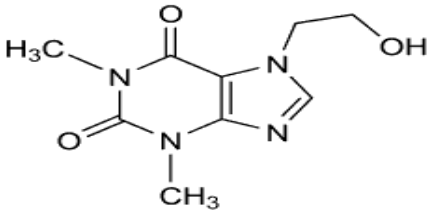
$$\mathbf{C}\text{test} = (\mathbf{A}\text{test} \times \mathbf{C}\text{std})/\mathbf{A}\text{std}$$

Where C_{test} and C_{std} are the concentrations in the sample and standard solutions respectively and Atest and Astd are the absorbance of the sample and standard solutions respectively. For assay of substance/s in multi component samples by spectrophotometer; the following methods are being used routinely, which includes.

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometer
- Solvent extraction method

Drug Profile of Etophylline: Etophylline [5, 6] is a non-selective phosphodiesterase inhibitor. It inhibits phosphodiesterase, which degrades cyclic nucleotides, hence increased amount of intra cellular C_{AMP} molecules causing smooth muscle relaxation. It causes blockade of adenosine receptors (which enhance release of histamine and other inflammatory mediator and bronchospasm). Overall effect of the etophylline1 is to produce bronchodilation by bronchial muscle relaxation. It is well absorbed orally, distributed in all tissues, crosses the placentas and is secreted in milk. It is 60% plasma protein bound. It is extensively metabolized in the liver by demethylation and oxidation. Only 10 % is excreted as unchanged drug, rest is excreted as changed metabolites in the urine. Steady plasma levels are obtained 1 –3 days after initiation of therapy after which half-life is 6 –8 hours. In neonates most of drug is excreted unchanged in urine and clearance is very slow. It is an odorless white crystalline powder, bitter and pungent, freely soluble in water; moderately soluble in ethanol; sparingly soluble in chloroform; practically insoluble in ether. It is a white crystalline powder having melting point 161° to 166° C.

Structure of Etophylline



IUPAC Name: 2-(1, 3-dimethyl-2, 6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)ethyl pyridine-3-carboxylate
Molecular Formula: $C_9H_{12}N_4O_3$
Molecular Weight: 224.2
Molecular Mass: 329.3107
Exact mass: 329.112403993
Partition Coefficient: Log *P* (octanol/water), −0.8.
Dose: Up to 1.5 g daily.
Storage: Store in a cool, dark place.
Shelf life: 2 years.

Method validation: Validation is concerned with assuring that a measurement process produces valid measurements [7]. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice. A measurement process producing valid measurements for an intended application is fit for purpose. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated before their introduction into routine use. Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and whenever the method is changed and the change is outside the original scope of the method. Nowadays, there are several international renowned organizations offering guidelines on method validation and related topics.

• American Society for Testing and Material (ASTM)
• Codex Committee on Methods of Analysis and Sampling (CCMAS)
• European Committee for Normalization (CEN)
• Cooperation on International Traceability in Analytical Chemistry (CITAC)
• European Cooperation for Accreditation (EA)
• Food and Agricultural Organization (FAO)
• United States Food and Drug Administration (FDA)
• International Conference on Harmonization (ICH).

ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation: The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation etc.

Types of Analytical Procedures to be validated: The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures
- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;

- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Furthermore revalidation may be necessary in the following circumstances:
- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;
- Changes in the analytical procedure.

MATERIALS AND METHODS

This work deals with the validation of the developed method for the assay of Etophylline [8, 9] from its dosage form (tablets). Hence, the method can be used for routine quality control analysis and also stability. The aim and scope of the proposed work are as under:
• To develop suitable spectrophotometric method for assay of Etophylline tablet.
• Perform the validation for the method.

Materials: Etophylline standard of was provided by Torque Pharmaceuticals (P) Ltd. (India). Etophylline tablets containing 500 mg Etophylline and the inactive ingredient used in drug matrix were obtained from market.

Solubility: Solubility of the drugs were studied using various solvents such as distilled water, Methanol, Ethanol, 0.1N HCL, 0.1N NaOH and Chloroform.

Determination of working wave length: In order to ascertain the wave length of maximum abruption (λ_{max}) of each drug, different solutions of the drugs (10 µg mL^{-1} to 100 µg mL^{-1}) in water were scanned using spectrophotometer within the wave length region of 200 – 400 nm against water.

Preparation of Stock Solutions: Standard stock solutions were prepared by dissolving 10 mg of each drug separately in 10 ml of water to get concentration of 1 mg mL^{-1} (1000 µg mL^{-1}) solutions.

Calibration Curve: The prepared stock solutions were further diluted with water to get working standard solutions of 100 µg/ml of the selected drugs. To construct Beer's law plot for pure drug, different aliquots of drugs were taken and diluted to 10 ml with water. The absorbance of each solution was measured at their respective $\lambda_{max\ 273nm}$ against water. The calibration curves were plotted by taking concentration of drug on x-axis and absorbance on y-axis.

Estimation of Drug in their Dosage Forms: Twenty tablets were weighed, average weight determined and crushed to fine powders. An accurately weighed sample equivalent to 10 mg of the drug was

transferred to a 100 mL volumetric flask. The drug was extracted four times by adding solvent in portions, 20 mL each and then volume was made up to the mark, using the same solvent. After appropriate dilution (within their linearity range), absorbance of each sample solution was recorded at respective λ_{max} and concentration of drugs in the samples were calculated.

Validation

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of bulk samples within the linearity range an added to the pre-analyzed formulation of concentration 30 µg/ml and from that percentage recovery values were calculated.

Precision: The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drugs within the Beer's range and finding out the absorbance by the proposed method. From the absorbance, Mean, Standard deviation and %RSD were calculated. Different parameters included for precision study were repeatability, intraday and interday precision.

Ruggedness: Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. These conditions included different analysts and different instruments. The data was subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Robustness: Robustness of the method was studied by deliberate variations of the analytical parameter such as solvent composition. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Detection Limit and Quantification Limit: Calibration curves were plotted by using concentration in the expected detection limit range (0.1-5 µg mL^{-1}) for each drug. The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection limit and quantification limits.

$$\text{Detection limit} = 3.3\ \sigma/s \text{ and Quantification limit} = 10\ \sigma\ /s$$

Where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

RESULTS AND DISCUSSION

Table .1 Calibration Table of the UV-Vis Spectrophotometric Method for Etophylline

Concentration (µg/ml)	Mean Absorbance (n=6)	Statistical Analysis
10	1.947	Mean = 2.078 SD=0.044 %RSD=2.117 R^2=0.999
20	2.048	
30	2.080	
40	2.089	
50	2.099	
60	2.096	
70	2.103	
80	2.101	
90	2.111	
100	2.114	

A calibration curve was plotted using these readings taking concentration on X-axis and absorbance on Y-axis.

Calibration curve of Etophylline: From the calibration curve it was found that it shows linearity in the range of 10-70 µg mL^{-1} with regression coefficient R^2=0.999

Table 2. Assay results of Etophylline Formulations

Sl No	Avg. Weight (in g)	Label claim (in mg)	Amount Present in each Tablet	% Recovery	Statistical Analysis
1	0.1862	5	4.97	99.4	Mean= 98.73 SD=0.71 % RSD = 0.72
2	0.1865	5	4.98	99.6	
3	0.1858	5	4.92	98.4	
4	0.1869	5	4.95	99.0	
5	0.1863	5	4.91	98.2	
6	0.1860	5	4.89	97.8	

Validation

Table 3. Accuracy Data of the UV-Vis Spectrophotometric Method for Etophylline

No of Preparations	Concentration (µg/ml)		% Recovery	Statistical Analysis		
	Amount Present in Formulation	Amount of drug Added		Mean	SD	%RSD
S_1 : 80%	30	24	99.34	99.61	0.38	0.38
S_2 : 80%	30	24	100.06			
S_3 : 80%	30	24	99.45			
S_4 : 100%	30	30	100.59	100.08	0.53	0.53
S_5 : 100%	30	30	99.52			
S_6 : 100%	30	30	100.05			
S_7 : 120%	30	36	101.04	100.05	0.97	0.97
S_8 : 120%	30	36	100.04			
S_9 : 120%	30	36	99.09			

Table 4. Precision Data showing repeatability of UV-Vis spectrophotometric method for Etophylline

Concentrations (µg/ml)	Absorbance	Calc. Amt (µg/ml)	Statistical Analysis
30	2.082	30.745	Mean = 30.021 SD = 0.433 %RSD = 1.442
30	2.080	30.054	
30	2.155	29.890	
30	2.531	29.454	
30	2.421	29.801	
30	2.336	30.181	

Table 5. Intraday Precision data of the UV-Vis Spectrophotometric Method for Etophylline

Conc. (µg/ml)	Abrobance1	Abrobance2	Abrobance3	Statistical Analysis
30	2.081	2.085	2.156	
30	2.062	2.068	2.032	
30	2.053	2.054	2.068	
30	2.029	2.047	2.045	Mean = 30.054
30	2.048	2.061	2.071	SD = 0.02
30	2.069	2.032	2.049	% RSD = 0.09
Mean	2.057	2.058	2.070	
Calc. Amt. (µg/ml)	30.021	30.078	30.063	

Table 6. Interday Precision data of the UV-Vis Spectrophotometric Method for Etophylline

Conc. (µg/ml)	Day1	Day2	Day3	Statistical Analysis
30	2.065	2.063	2.068	
30	2.032	2.065	2.082	
30	2.068	2.066	2.085	
30	2.064	2.061	2.073	Mean = 30.078
30	2.071	2.071	2.076	SD = 0.069
30	2.079	2.064	2.066	% RSD = 0.230
Mean	2.071	2.066	2.073	
Calc. Amt. (µg/ml)	30.063	30.018	30.154	

Table 7. Ruggedness Data of the UV-Vis Spectrophotometric Method by Different Analysis for Etophylline

Analyst 1				Analyst 2			
Conc. (mcg/ml)	**Abs.**	**Calc. Amt**	**Statistical Analysis**	**Conc. (mcg/ml)**	**Abs.**	**Calc. Amt**	**Statistical Analysis**
30	2.073	30.745		30	2.068	29.472	
30	2.074	30.054	Mean = 30.02	30	2.076	29.909	Mean = 30.01
30	2.063	29.890	SD=0.433	30	2.080	30.163	SD=0.460
30	2.067	29.454	% RSD = 1.44	30	2.075	30.036	% RSD = 1.534
30	2.075	29.803		30	2.077	30.745	
30	2.080	30.181		30	2.079	29.781	

Table 8. Ruggedness Data of the UV-Vis Spectrophotometric Method by Different Instruments for Etophylline

Instrument UV-1700				**Instrument UV-1800**			
Conc. (mcg/ml)	**Abs.**	**Calc. Amt**	**Statistical Analysis**	**Conc. (mcg/ml)**	**Abs.**	**Calc. Amt**	**Statistical Analysis**
30	2.063	29.472	Mean = 30.01 SD=0.46 % RSD = 1.53	30	2.073	30.745	Mean = 30.02 SD=0.43 % RSD = 1.44
30	2.065	29.909		30	2.074	30.054	
30	2.066	30.163		30	2.063	29.890	
30	2.061	30.036		30	2.067	29.454	
30	2.057	30.745		30	2.075	29.803	
30	2.064	29.781		30	2.076	30.181	

Table 9. Robustness Data of the UV-Vis Spectrophotometric Method by Different Solvent Composition for Etophylline

(92:08)				**(88:12)**			
Conc. (mcg/ml)	**Abs.**	**Calc. Amt**	**Statistical Analysis**	**Conc. (mcg/ml)**	**Abs.**	**Calc. Amt**	**Statistical Analysis**
30	2.072	31.109	Mean = 30.07 SD=0.557 % RSD = 1.853	30	2.070	30.745	Mean = 30.021 SD=0.433 % RSD = 1.443
30	2.066	30.163		30	2.066	30.054	
30	2.065	29.909		30	2.065	29.890	
30	2.064	29.781		30	2.062	29.454	
30	2.066	30.036		30	2.064	29.806	
30	2.063	29.472		30	2.066	30.181	

Limit of Detection (LOD): The LOD for Etophylline was found to be 0.237 µg mL^{-1}.

Limit of Quantitation (LOQ): The LOQ for Etophylline was found to be 0.718 µg mL^{-1}.

APPLICATIONS

The UV spectroscopic methods have been successfully developed for the drugs Etophylline [10, 11] in the present work and they can be routinely employed in Pharmaceutical Industries and Analytical Laboratories for their estimation in bulk and formulations.
The work will find its significant applicability in both academic and industrial establishments.

CONCLUSIONS

The ICH guidelines work with an objective to increase international harmonization of technical requirements to ensure that safe, effective and high quality medicines are developed and registered in the most efficient and cost-effective manner. The ICH guidelines which deals with "Stability Testing of new Drug Substances and Products" plays a vital role in establishing the stability of bulk drugs as well as the pharmaceutical formulations. The present work aimed at developing and validated a UV method for quantitative estimation of Etophylline.

From the results of method development it is found that the developed methods are simple, reliable, sensitive and accurate. The developed UV-Vis spectrophotometric method can be used routinely for rapid

and precise analysis of Etophylline [10, 11]. Present UV-Vis spectrophotometric method was utilized for solubility studies and drug content analysis.

For Etophylline, the present method was found to be linear from 100-100 µg mL^{-1} in UV-VIS analysis. The UV-VIS spectrophotometric method was found accurate with %RSD of 0.38-0.97, precise with %RSD of 1.443, rugged with %RSD of 1.44 – 1.54, robust with %RSD of 1.443 - 1.853. The interday precision was found out to be %RSD of 0.230 and 0.09. The LOD and LOQ were observed to be 0.237 and 0.718 µg mL^{-1} respectively.

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