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Analytical Method Development and Validation for the Estimation of Etodolac in Bulk and Tablet Dosage Form by Using UV Spectroscopy

Ram Sahay Sharma, Sonu Sharma*, Saurabh Sharma, Yogesh Matta, B. Shrivastava, Neha Arora

School of Pharmaceutical Sciences, Jaipur National University, Jagatpura, Jaipur, Rajasthan, Pin code: 302017, India

ABSTRACT

Introduction: Analytical chemistry is the study of the chemical composition of natural and artificial materials. Unlike other major sub disciplines of chemistry such as inorganic chemistry and organic chemistry, analytical chemistry is not restricted to any particular type of chemical compound or reaction.

Aim and **Objectives:** To develop and validate analytical method for estimation of etodolac in bulk and pharmaceutical dosage form using U.V spectroscopy. To study solubility characteristics of etodolac. To determine absorbance maxima for etodolac. To develop selective, precise and sensitive method for the development and estimation of etodolac in bulk and pharmaceutical dosage form.

Result and conclusion: Improvement and validation of two visible spectrophotometric methods applying methanol and water as reagents for etodolac determination in investigated formulations were successfully carried out. The near 100 % recoveries and low relative standard deviation values obtained, point to the suitability of the both modified and validated methods for determination of etodolac in human and veterinary medicine. It was concluded that the UV spectroscopic method could be used for simultaneous determination of trimethoprim immediate-release oral dosage forms. This method could be used for the analysis of active pharmaceutical ingredients in dissolution studies and for quality control purposes. The method is rapid, simple, and economic without the need of high cost investment.

Key words: Simultaneous, Determination Development, Estimation, Spectroscopy

INTRODUCTION

Spectroscopy : It is the measurement and interpretation of electromagnetic radiations absorbed or emitted when the molecules or atoms or ions of the sample undergo transition from one energy state (Ground state) to another (excited state). It is of two types:

Absorption Spectroscopy: Where absorption of electromagnetic radiation (EMR) takes place like: Colorimetry, UV spectroscopy, IR spectroscopy, etc.

Emission Spectroscopy: Where emission of radiation is being studied e.g. Fluorimetry, Flame Photometry.

***Corresponding Author:**
sonu.sharma283[at]gmail[dot]com

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UV – SPECTROSCOPY

It involves the extent of quantity of ultra-violet emission immersed by a matter in the resolution. The wavelength between 190–390 nm (practically 200–400 nm) is

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considered to be UV radiations/ region. Colored compounds absorb in visible range i.e. 400-800 nm. The assay of an absorbing substance can be carried out by using Standard absorptivity Value, Use of calibration graph, Single point standardisation., and Standard absorptive value. This procedure is adopted by official compendia for the stable substance that have reasonably broad absorption bands and which are practically unaffected by variation of instrumental parameters.

Use of Calibration Graph: the absorbance of a amount of standard solution of the allusion core at concentration surrounding model concentration is calculated and a calibration graph is constructed. The deliberation of the analyte in the sample solution is read from the graph as an attention analogous to absorbance of the solution.

$$C_{test} = A \times C \text{ test standard a standard.}$$

The use of UV and visible spectroscopy for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength. The analysis of mixtures of two or more components is facilitated by activity of absorbance. Other applications include measurement of absorption of complexes to establish their composition. All chromogenic compounds are not suitable for quantitative measurements, i.e. the choice of the system and procedure depends largely on the chemistry of the species to be determined.

The criteria for applying difference spectrophotometer to the assay of a substance in the presence of other absorbing substances are:

1. Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.
2. The absorbance of the interfering substances not altered by reagents.

Method Development: It requires a lot of effort, and there is a degree of doubt initially to whether the method will be successful. It involves working on various ideas simultaneously and then f inally picking one of those. Various steps involved in method development and validation are in the flow chart 1.

Method development plan definition
↓
Background information gathering
↓
Laboratory method development
↓
Generation of test procedure
↓
Methods validation protocol definition
↓
Laboratory methods validation
↓
Validated test method generation
↓
Validation report

Flow Chart 1: Method Development for etodolac

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Method Validation: The word validation originated from the Latin word validusmeaning strong, and suggests that something has been proved to be true, useful, and of an acceptable standard. Method validation can be defined as the process of proving that a particular developed analytical method is acceptable for its intended use. Validation is an important requirement in the practice of an analytical process. Method validation can be interpreted as the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with that the application requires. In connection with biotechnological synthesis of pharmaceutical drugs, validated methods for quantification of both the product and the substrate at different time intervals are essential for proper calculation of rate coefficients. The current trend is in the direction of phase-dependent methods development and validation. Analytical methods are progressively optimized, and a preliminary validation package is furnished as part of the end application before Phase I safety trials are initiated.

All analytical methods should be fully optimized and validation should be completed before the NDA is submitted at the end of Phase III studies. Method validation is a continuous process, and the final goal of validation of an analytical method is to ensure that every future measurement in routine analysis will be close enough to the unknown true value for the content of the analyte in the sample. Because of the ongoing advances in analytical chemistry technologies, analytical methods are updated over time; thus, validation and cross validation of methods become important for accurate interpretation of data collected over years. Validation is performed with a formal, approved, and signed methods validation protocol in quality assurance (QA) unit.

MATERIAL AND METHODS

Materials

Solubility of drugs in different solvents: Solubility of etodolac was observed by dissolving them in different solvents such as DMSO, Water, Dichloromethane, and Methanol. All chemicals used were of LR grade.

Determination of λ max: - λ max was determined by scanning the standard sample solution in UV range and the λ max was found to be 277 nm. Double beam UV-Visible Spectrophotometer UV- 1800, Shimazdu was used.

UV Spectrophotometric method for etodolac: In the present research work UV spectrophotometric methods have been developed for the estimation of etodolac from tablet formulation by UV Spectrophotometry.

Preparation of Standard Solution and Calibration Curve: - 10 mg of etodolac was accurately weighed and dissolved in methanol: water solution of strength 80:20 and then volume was made upto 100 ml. Solution was shaked to dissolve it completely. Solution was further dilute suitably to obtain Concentration range from 1- 40 µg/mL.

Preparation of Analysis of Tablet formulations: - 20 Tablets were accurately weighed and their average weight was determined. 10 mg of powder equivalent to etodolac was accurately weighed and add in a 100 mL of volumetric flask. Solution was shaked for 5 minutes to dissolve it completely. Solution was further diluted suitably to obtain final concentration of 10 µg/mL.

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Concentration of solvent and Wavelength selection: Solution of concentration of 1µg/mL, 5µg/mL, 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL was prepared. They were subjected to scanning from 200-400nm. For the absorbance value obtained the wavelength selected for the present work was 277 nm. The validated method undergoes quality control procedures for the further evaluation.

Method validation procedure: For validation the developed method is subjected to following studies:

- Precision/Reproducibility
- Accuracy
- Linearity
- Specificity/Selectivity
- Limit of detection
- Limit of quantization
- Robustness / Ruggedness

Validation

1. **Specificity:** Specificity is the ability of the analytical method to measure the response in presence of interferences. The UV spectra of drugs in sample solutions were found to be identical to the UV spectra obtained from standard solutions. No interference from formulation excipients was found, proves specificity of the proposed method.
2. **Accuracy:** To assess the accuracy of the proposed method, recovery experiment was performed at three different levels i.e. 75%, 100% and 125%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels and absorbance were recorded. The % recovery was then calculated by using formula.

% Recovery = [A - B/ C] X 100, Where,
A = Total amount of drug estimated
B = Amount of drug found on preanalysedbasis
C = Amount of Pure drug added

Precision

1. **Repeatability:** To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for three times with tablets formulation. The Standard deviation, Coefficient of variation and Relative standard deviation was calculated.
2. **Intermediate Precision (Inter-day and Intra-day precision):** The intra-day and inter-day precision was determined by assay of the tablet solution on the same day at different time intervals and on different days respectively**.**
3. **Linearity:** For etodolac appropriate dilutions of standard stock solutions were assayed as per the developed method. Etodolac has linearity between 1- 40 µg/mL. The linear regression equation for etodolac was show in Results.

RESULTS AND DISCUSSION

UV methods for ETODOLAC in bulk and solid dosage form.

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Table 1: Solubility results of the drug in different solvents

Solvent	ETODOLAC
Water	Partially Soluble
DMSO	Freely soluble
Methanol	Soluble

Validation

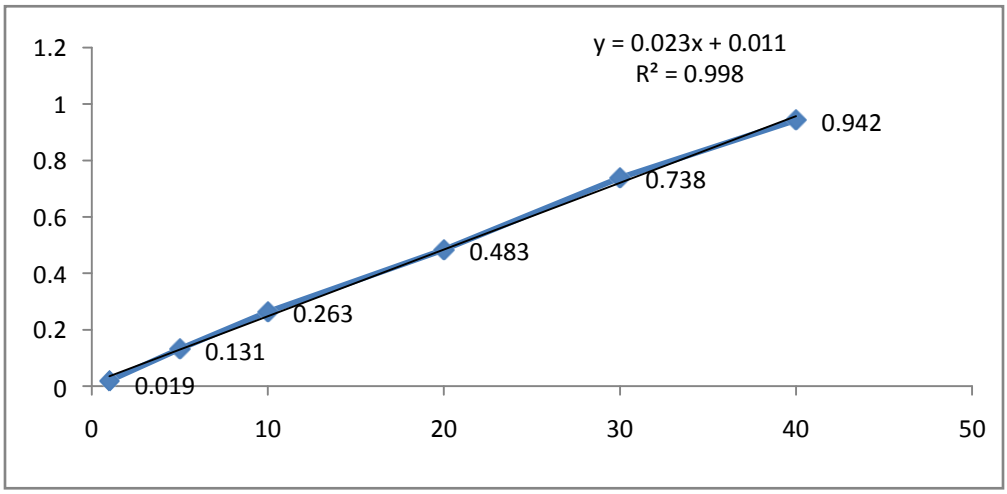

Figure 1: Linearity

Table 2: Linearity etodolac

Concentration (µg/mL)	Absorbance SD	%RSD Concentration (µg/mL)	Absorbance
1	0.0193	0.002	1.03
3	0.131	0.0005	0.381
5	0.263	0.0003	0.114
7	0.483	0.001	0.207
9	0.738	0.0015	0.203
12	0.942	0.0013	0.13

*Average of three Replicates

Table 3: Optical Characteristics of ETODOLAC Repeatability

Parameters	Values
λ max	277nm
Beer's law limit (µg/mL)	1-40µg/mL
Regression equation *	Y=0.023x+0.010
Correlation Coefficient (r^2)	0.998
Slope	0.023

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Table 4: Repeatability of etodolac

Sample No.	Absorbance	Average absorbance	SD
1	0.261		
	0.26	0.261	0.001
	0.262		
2	0.264		
	0.263	0.262	0.002
	0.259		
3	0.258		
	0.262	0.26	0.002
	0.261		
4	0.263		
	0.259	0.26	0.002
	0.258		

*Average of three Replicates

Intra-day: The %RSD was found to be >1% for intra-day.

Table 5: Intra-day precision data for etodolac

Drug	Concentration (µg/mL)	Absorbance	SD	%RSD
Etodolac	10	0.263	0.003	1.14
Etodolac	20	0.481	0.002	0.41
Etodolac	30	0.738	0.001	0.13

*Average of three Replicates

Inter-day: The %RSD was found to be >1% for interday.

Table 6: Interday precision data for etodolac

Drug	Concentration (µg/mL)	Absorbance	SD	%RSD
Etodolac	10	0.262	0.003	1.14
Etodolac	20	0.489	0.002	0.40
Etodolac	30	0.742	0.003	0.40

*Average of three Replicates

Ruggedness

A: Mean of six determinations

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Table 7: Results for Ruggedness

S. No.	Conc.	Etodolac	
		Analyst I	Analyst II
1	5 µg/Ml	0.483	0.482
2		0.482	0.479
3		0.478	0.481
4		0.479	0.482
5		0.485	0.480
6		0.482	0.481
Mean±SD		0.00015	0.0031
RSD		0.031	0.06

B: Ruggedness studies were carried out using different analysts.

Accuracy: Accuracy was performed and % Recovery was found to be 99% TO 100.4% for ETODOLAC.

Table 8: %Recovery data for etodolac

Recovery Level	Initial sample concentration (µg/mL)	Concentration of standard drug added (µg/mL)	Total concentration (µg/mL)	Absorbance	Amount of drug recovered (µg/mL)	%Recovery
75%	10	8	18	0.404	18	100%
				0.401	17.43	96.83%
				0.406	18.08	100.44%
1	10	10	20	0.45	20	100%
				0.451	20.04	100.2%
				0.452	20.08	100.4%
1.25	10	12	22	0.496	22	100%
				0.495	21.95	99.77%
				0.494	21.91	99.59%

*Average of three Replicates

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CONCLUSION

Improvement and validation of two visible spectrophotometric methods applying methanol and water as reagents for etodolac determination in investigated formulations were successfully carried out. The near 100 % recoveries and low relative standard deviation values obtained, point to the suitability of the both modified and validated methods for determination of etodolac in human and veterinary medicine. It was concluded that the UV spectroscopic method could be used for simultaneous determination of etodolac immediate-release oral dosage forms this method could be used for the analysis of active pharmaceutical ingredients in dissolution studies and for quality control purposes. The method is rapid, simple, and economic without the need of high cost investment**.**

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