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Analytical method development and validation of diclofenac sodium by UV-visible spectroscopy using AUC method

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Abstract
A simple, precise, accurate and economical UV visible spectrophotometric method has been developed for estimation of Diclofenac sodium drug by AUC method. The standard and sample solutions were prepared by using methanol as a solvent. Quantitative determination of the drug was performed at wavelength range 250-350 nm. The linearity was established over the concentration range of 10, 20, 30, 40&50 µg/ml for Diclofenac sodium with correlation coefficient value of 0.997. Precision studies showed that % relative standard deviation was within range of acceptable limits. The mean percentage recovery was found to be 99.38%. The proposed method has been validated as per ICH guidelines.

Keywords: diclofenac sodium, UV visible spectrophotometer, AUC, method validation

1. Introduction

Analytical Chemistry [1]

Analytical chemistry is a branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter.
It is mainly involved in the qualitative analysis or detection of compounds and quantitative analysis of the compounds. A qualitative method yields information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method, in contrast provides numerical information as to the relative amount of one or more of these components.
Analytical methods are classified into two categories; they are classical methods and instrumental methods

Spectroscopy [2, 3]

Spectroscopy is a branch of science dealing with the study of interactions of electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amount called quanta.
Spectroscopy is one of the most powerful tool available for the study of atomic and molecular structure and is used in analysis of a wide range of sample. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 µm

UV-Visible spectrophotometer [4, 5]

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states.

Fig 1: 2013 ELICO® SL 210 Double Beam UV-Visible Spectrophotometer

The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law.

Beer's law

It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration.

Lambert's law

It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law.

Beer-Lambert law

When beam of light is passed through a transparent cell

containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer Lambert law is expressed as

A=a b c

Where, A=absorbance or optical density,
a=absorptivity or extinction coefficient, b=path length of radiation through sample (cm), **c**=concentration of solute in solution. Both b and a are constant so a is directly proportional to the concentration c.

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

$$A=A\frac{1\%}{1cm}\ bc$$

Instrumentation [6]

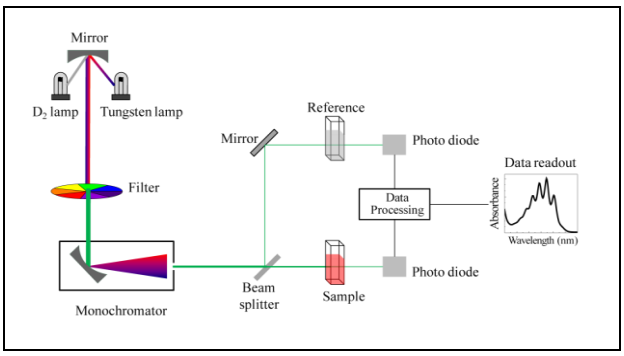


Fig 2: UV-Visible Instrumentation

Method Development [7]

Basic criteria for new method development of drug analysis

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations; Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantization of the drug in biological fluids may not be available, Analytical methods for a drug in combination with other drugs may not be available the existing analytical procedures may require expensive reagents and solvents.
- It may also involve cumbersome extraction and separation procedures and these may not be reliable

Steps involved in method development

1. Analyte standard characterization
2. Method requirements
3. Literature search and prior methodology
4. Choosing a method
5. Instrumental setup and initial studies
6. Optimization
7. Documentation of analytical figures of merit
8. Evaluation of method development with actual samples
9. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

Method Validations [13, 14]

Analytical Method Validation can be defined as (ICH) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

1. Specificity
2. Linearity
3. Accuracy
4. Precision
5. Detection Limit (LOD)
6. Quantitation Limit (LOQ)
7. Range

Table 1: Acceptance Criteria for the different characteristics of validation by ICH

Characteristics	Acceptance Criteria
Linearity	$r^2 \geq 0.99$, similar response ratios
Precision-System	RSD<2%
Precision-Method	RSD<2%
Accuracy	FDA 98-102%, EPA 50-150%
Specificity	No interference
Detection Limit	>2 times base line
Quantitative Limit	Signal-to-Noise=10:1
Range	Concentration where data can be reliably detected

2. Aim and Objective

The aim of the present work is to develop an analytical method and validation for Simultaneous estimation of Diclofenac sodium.

The objective of the proposed work is

- To develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Diclofenac sodium by using UV-Spectrophotometry.
- To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.
- To apply the developed method for the simultaneous estimation of Diclofenac sodium.

3. Plan of work

- Simultaneous Estimation of Diclofenac sodium will be done by UV spectrophotometry method.

Methodology

• Literature survey
• Procurement of drug sample and other chemicals
• Selection of wavelength (λ_{max})

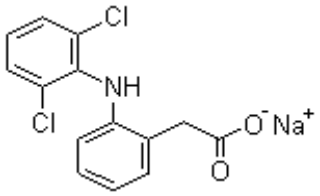
Method development by UV Spectrophotometry

1. Selection of preliminary UV Spectrophotometry.
a. Selection of solvent
b. Selection of wavelength
2. Analysis of laboratory mixture
3. Validation of proposed method
4. System Suitability Parameter
5. Linearity and Range
6. Precision
a. System Precision
b. Method Precision

7. Accuracy
8. Specificity

4. Drug profile-Diclofenac sodium

- **Chemical Name:** 2-[{2, 6-dichlorophenyl} amino] benzene acetic acid.
- **Category:** A non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions**.**
- **Chemical Structure**



- **Molecular Formula**:-$C_{14}H_{10}Cl_2NNaO_2$.
- **Molecular weight**: - 318.13.
- **Route:** Oral.
- **Brand:** Flector, Licart, Voltaren, Dicloran, Defnac, Artifin, voltra.
- **Pharmacokinetic Data**

Bioavailability-50–60%
Metabolism - 99% in liver
Elimination half-life - 3–6 hours
Excretion - Urine and bile (90%)

- **Mechanism of action:** anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of the transiently expressed prostaglandin-endoperoxide synthase-2 (PGES-2) also known as cycloxygenase-2 (COX-2). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis.

5. Experimental work and results

Choice of Solvent

A suitable solvent for ultraviolet Spectroscopy should meet the following requirements.

- It should not itself absorb radiations in the region under investigation.
- It should be less polar so that it has minimum interaction with the solute molecules.

Solvents which are transparent above 210 nm are n-hexane, cyclohexane, methanol, water and ether.

We choose Methanol as a solvent because it satisfy the above criteria an easy availability.

Method Development and Optimization of Spectrophotometric Conditions

Selection of Spectroscopy condition

Determination of Wavelength Range

For the selection of analytical wavelength range for area under curve method 10µg/ml solution of Diclofenac sodium was scanned in the spectrum mode from 200nm to 400nm against distilled water as blank. Wavelength range was selected around wavelength maxima (340nm).

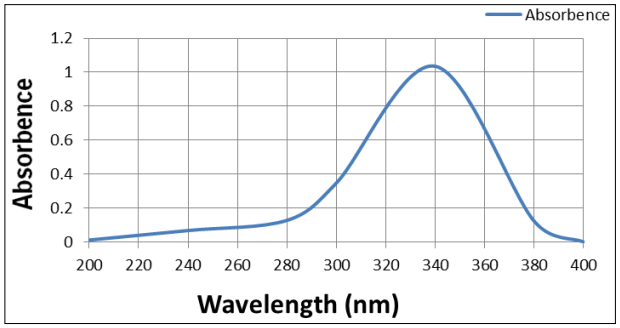


Fig 3: λmax of Diclofenac sodium (10µg/ml)

Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 200 to 350 nm.

Area calculation

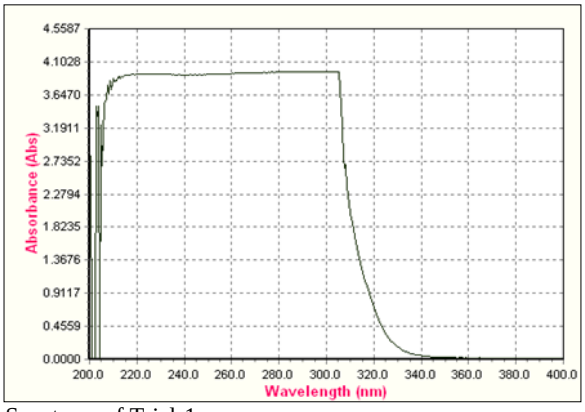
$$(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis λ1 and λ2 are wavelength range start and end point of curve region.

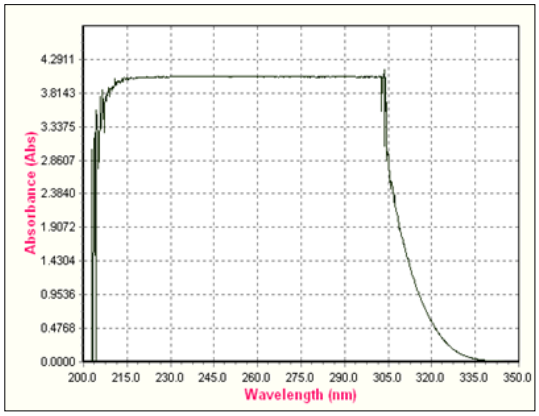
Method Development Trials

Table 2: Spectroscopy condition

Parameters	Description
Solvent	Methanol
Wavelength	200-350nm
Sample holders	A pair of 10 mm matched quartz cells
software	Spectra treats
Detector	Silicon Photo Diode
Readout device	Computer



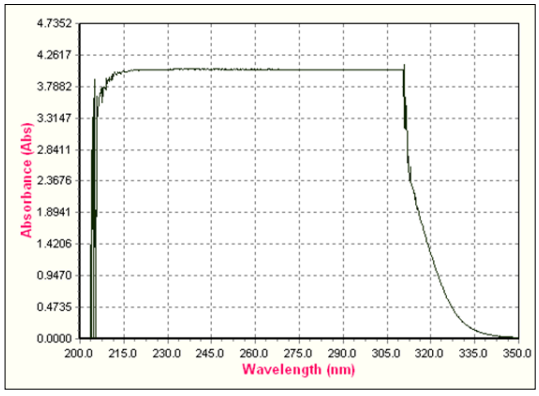
Spectrum of Trial-1



Spectrum of Trial- 2

Spectrum of Trial-3

Spectrum of Trial-4



Spectrum of Trial-5

Fig 4

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
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Accuracy

The accuracy for the analytical method was evaluated at 80%,100% and120% levels of 10µg/ml standard solution. Area under curve (AUC) was measured in wavelength range 300-350 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 3

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amount. Added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80%	30	12	42	99.13		
100%	30	15	45	100.77	99.38	1.8073
120%	30	18	48	99.26		

Precision

The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday precision was studied by integrating area of standard solution of 10µg/ml concentration at five independent series in the same day. Inter-day precision studies were performed by integrating area of standard solution of 10µg/ml concentration on three consequent days. The % RSD was calculated.

Table 4

Parameter	Intra-day	Inter-day
Sample sol conc	10	10
AUC (mean)	0.2181	0.3254

Linearity and Range

The linearity was determined by using working standard solutions between 10,20,30,40&50 µg/ml. The areas under curve (AUC) of these solutions were recorded. Calibration curve of area under curve to concentration plotted on excel sheet and linear regression was performed. The correlation coefficient, regration equation was calculated the response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.014x-0.004 with correlation coefficient 0.997.

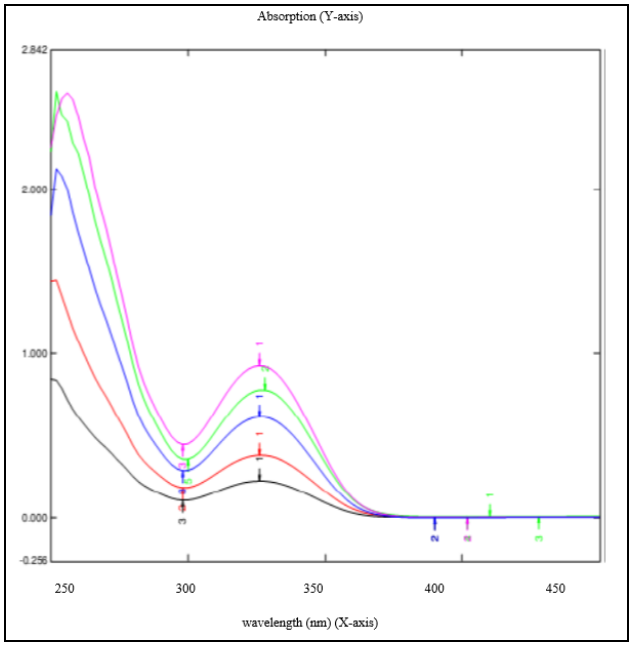


Fig 4: Linearity of Diclofenac sodium

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following

Formula LOD = 3.3 σ /S

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following

Formula LOQ = 10 σ/S

Where, σ is standard deviation of the response and S is the slope of the calibration curve.

LOD& LOQ of Diclofenac sodium found to be 0.9741µg/ml &2.9311/ml respectively.

Five sets of known concentrations (10-50µg/ml) were prepared and scanned. By using these spectras, regression equations were obtained. By taking average of slopes and standard deviation of y-intercept, LOD and LOQ were calculated. The values of LOD and LOQ are obtained.

Summary of validation parameters

Table 5

Parameter	Result
λ range	300-350nm
Regression Equation (y=mx+c)	y = 0.014x - 0.004
Measured wavelength	305nm
Linearity range	10-50µg/ml
Slope	0.014
Intercept	0.004
Correlation coefficient (R2)	0.997
Limit of Detection (LOD) µg/ml	0.9741
Limit of Quantitation (LOQ) µg/ml	2.9311
Accuracy (Mean % Recovery)	99.38%
Precision (%RSD)	1.8073

6. Conclusion

The UV spectroscopic AUC method for the analysis of Diclofenac sodium was found to be simple, precise, and accurate, rapid and economical. The developed method was validated in terms of accuracy, precision, linearity, LOD&LOQ and results will be validated statistically according to ICH guidelines.

From literature review and solubility analysis initial eluent was scanned with Silicon Photo Diode detector in system and it showed maximum absorbance at 340 nm. In this study, Area under curve, Area was integrated between wavelength ranges from 200 to 350 nm. The stock solution of Diclofenac sodium was prepared weighing & transferring, 100 mg of API to volumetric flask and add 100 ml of methanol. Then take from that 10ml and add to 100ml volumetric flask and make up with methanol to get final standard solution (10µg/ml). From stock solution, serial dilutions are prepared as 10, 20, 30, 40&50 µg/ml. Linearity Graph of Diclofenac sodium was plotted.

The linearity was established over the concentration range of 05,10,15,20&25µg/ml for Diclofenac sodium with correlation coefficient value of 0.997. Precision studies showed that % relative standard deviation was within range of acceptable limits. The mean percentage recovery was found to be 99.38%.The proposed method has been validated as per ICH guidelines.

The present analytical method was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate and having stability indicating characteristics. The present analytical method can be used for its intended purpose.

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