Analytical method development and validation of ketoprofen tablet by UV spectrophotometer

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Abstract

**Precision:** The degree of agreement among individual test results when a method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or of repeatability (agreement under same condition) of the method.

**Linearity:** The ability of a method to produce results that is directly or indirectly proportional to the conc. of the analyte in samples within a given range.

**Range:** The interval between upper and lower level of analyte (including those levels) that has been shown to be determined with precision, accuracy and linearity using the method as written.

**Accuracy:** The closeness of test results obtained by method to the true value. It is a measure of the exactness of the method.

**Ruggedness:** The ruggedness of an analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of normal test conditions. Such as different laboratories, different analyst, different instruments, different lots of reagents different elapsed assay times, differently days at normal lab. Conditions etc. Intermediate precision is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method. Ultraviolet Visible spectrometric assay developed for the quantification of Ketoprofen was performed in methanol in the concentration of 10 mcg/ml. Single Point Standardization method was used for the quantitative analysis of drug. The drug obeys Lambert – Beer’s law in the concentration range of 5 mcg/ml. The absorbance maxima occur at 256 nm. The developed method was validated as per ICH norms. Single Point Standardization method involves simple calculations. The absorbance value at 256 nm was found to be around 0.291. The results obtained on the validation parameters of developed method meets the ICH requirements. It infers that the method was found to be simple, specific, precise, accurate, reproducible, reliable, linear and proportional (i.e.) it follows Lambert-Beer’s Law. The method was found to be rapid and economic. Hence it can be inferred that the above method was useful to be applied in routine laboratory analysis with a high degree of accuracy and precision.

**Keywords:** Ketoprofen, Analytical method development, Accuracy, Precision, Ruggedness, Robustness.

Introduction

Quality is important in every product or service, but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no second quality in drug. Quality control concept, which strives to produce perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The assurance of quality and reliability of pharmaceuticals together with their careful control are a more obligation arising from humanism towards sick human beings. In general terms pharmaceutical analysis comprises of those procedures necessary to determine the identity, strength, quality and purity of such articles. The raw material employed in the production of modern drugs and the water intermediates appearing during drug research development and synthesis, involves thousands of diverse organic compounds. So pharmaceutical analysis shall have firm grounding in basic organic analysis, in addition to special skill in the quality evaluation of drug products. All phrases of pharmaceutical control present problems of sampling without carefully concerned sampling plans, analysis is meaningless, pharmaceutical control of drug deals with batches rather than continuous process. Variation in the raw materials and the processing techniques, together with the nature of end use of the product add up to sampling challenge for the quality control specialists. Now a drug laboratory edging towards total quality control of a statistical basis gives promise of making pharmaceutical manufacture more efficient.
the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation.

Pharmacokinetics

Absorption
Ketoprofen is rapidly and well-absorbed orally, with peak plasma levels occurring within 0.5 to 2 hours.

Distribution
Ketoprofen is highly protein bound (96%).

Metabolism / elimination
Rapidly and extensively metabolized in the liver, primarily via conjugation to glucuronic acid. No active metabolites have been identified.

Experimental Part

Introduction to present study
Absorption spectrophotometry versatile technique frequently in pharmaceutical analysis. Many pharmaceutical substances can be determined by UV visible, fluorescence region of spectrum with greater accuracy and precision. In the presence study the assay of Ketoprofen was carried out by spectrophotometric method. All the chemicals and solvent used were of Analytical grade. In this spectrophotometric developed method a UV region having the maximum absorbance at 256 nm. The determination of drug was based on the measurement of the absorption at this wavelength. This method was statically validated and found precise, accurate and applicable to Ketoprofen.

Introduction
When a monochromatic light passes through absorption medium (solution of absorbing solute), the intensity of light decreases in relation to the distance travelled the solution and the concentration of the solution.

Definitions
A: Absorbance: log₁₀(I/T)

a: Absorptivity A _____________
Conc. (g/lit) X Path length (cm)

E: Molar Absorptivity: A _____________
Conc. (moles/ liter) X path length (cm)

Absorption
A graphic representation of absorbance, plotted against wavelength or function of wavelength.

Transmittance
The quotient of the radiant power transmitted by a specimen divided by radiant power incident up on the specimen.

For most of the systems the Absorptivity of a substance is constant independent of the intensity of the incident radiation, the internal cell length and the conc. May be determined photo metrically.

Preparation of standard stock solution
About 50.0mg of working standard of Ketoprofen was weighed accurately sufficient amount of methanol was added, sonicated to dissolved and diluted to 100 ml with methanol.

Absorption spectrum
A liquid of 2 ml from standard stock solution was pipette out to 100 ml volumetric flask and volume was making up with methanol. So that the final conc. 10 mcg/ml. The absorbance of resulting solution was scanned in the wavelength region between 220 nm to 400 nm against blank. Graphically represented as:

With reference to the absorption spectral data the maximum absorption was obtained at 256 nm, when scanned in the wavelength region between 220 nm to 400 nm.

Assay of tablet
20 tablets were weighed and the average weight of tablet was found out. The tablet powder containing Ketoprofen equivalent to 100 mg was accurately weighed, transferred in to 200 ml volumetric flask dissolved in methanol the volume was make up with methanol and filter through Whatman No. 41 filter paper. Reject few ml and collect the rest. Then further dilute 2 ml of the filtrate to 100 ml with methanol. The maximum absorbance was measured at 256 nm against blank. The experiment was repeated six times for the brand of the tablet. The absorbance of 10 mcg/ml conc. of working reference standard also measured at 256 nm. The result of brand tablets are present in the Table 1.

Standard dilution
50 mg of Ketoprofen→100 ml with methanol
2 ml of this soln→ 100 ml with methanol.

Test dilution
Tablet powder equivalent to 100 mg of Ketoprofen→ 200 ml with methanol
2 ml of above solution→100 ml with methanol

Calculation
Using the absorption of the standard and sample solution. The content of Ketoprofen present in the each tablet of average weight =Avg.Wt. (g).
Table 1: Data for assay of tablets

| S. No. | Average Wt. of tablets (gm.) | Weight of standard drug (mg.) | Weight of tablet powder (gm.) | Absorbance of standard drug | Absorbance of sample | Content of drug (mg.) | Average content of drug (mg.) |
|--------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------|----------------------|-----------------------------|
| 1      | 0.185                       | 50.0                        | 364.0                       | 0.568                       | 49.8                | 50.0                 | 49.9                        |
| 2      | 0.185                       | 50.0                        | 364.0                       | 0.567                       | 49.8                | 50.0                 | 49.9                        |
| 3      | 0.185                       | 50.0                        | 364.0                       | 0.568                       | 49.8                | 50.0                 | 49.9                        |
| 4      | 0.185                       | 50.0                        | 364.0                       | 0.569                       | 49.9                | 50.0                 | 49.9                        |
| 5      | 0.185                       | 50.0                        | 364.0                       | 0.570                       | 50.0                | 50.0                 | 49.9                        |
| 6      | 0.185                       | 50.0                        | 364.0                       | 0.570                       | 50.0                | 50.0                 | 49.9                        |

**Analytical validation**

The need for analytical method is well reflected in the following quotes.

“Sooner or later and it is usually sooner a set of analytical conditions that does gives satisfactory result appear to have been found. At this point, the investigator may freeze the procedure and proceed to accumulate some data for publication. Unfortunately, he is meticulous in adhering absolutely to this particular set of conditions never deviating from it. This holds true, not only for the conditions which are specifically written in the procedure, but also for various little habits of work which are faithfully followed every time. Under such circumstances, it is little wonder that repeated results show phenomenal agreement and it is so on basis of such results that the new procedure is offered to the quality control laboratory as routine tool.

**Types of validation**

**Prospective validation**

These is employed when historical data of product is not available or is not sufficient and in process and finished product testing are not adequate to ensure reproducibility or high degree of compliance to product likely attributes.

**Retrospective validation**

This provides trend of comparative results for review and evaluation of existing information for comparison when historical data is sufficient and reliable.

**Concurrent validation**

It verifies the quality characteristics of a particular batch and provide assurance that the same quality would be attained again when subsequent batches are manufactured and analyzed under similar conditions.

**Selection of analytical method**

The selected method must have the following parameters;

1. As simple as possible.
2. Most specific.
3. Most productive, economical and convenient.
4. As accurate and precise as required.
5. Multiple sources of key components should be avoided.
6. To be fully optimized before transfer for validation of its characteristics such as specificity, precision, linearity, accuracy, ruggedness etc.

**Analytical parameters to be validated**

1. Specificity
2. Precision
3. Linearity
4. Range
5. Accuracy
6. Ruggedness
7. Robustness

**Specificity**

The ability of a method to measure accurately and specifically the analyte (the constituent being tested or analyzed) in the presence of components that may be expected to be present in the sample. It is a measure of degree of interference in the analysis of complex sample mixtures such as analyte mixed with the formulation excipients or bulk drug substances containing degradation products, related chemical compounds etc.

**Precision**

The degree of agreement among individual test results when a method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or of repeatability (agreement under same condition) of the method.

**Linearity**

The ability of a method to produce results that is directly or indirectly proportional to the conc. of the analyte in samples within a given range.

**Accuracy**

The closeness of test results obtained by method to the true value. It is a measure of the exactness of the method.
Ruggedness
The ruggedness of an analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of normal test conditions. Such as different laboratories, different analyst, different instruments, different lots of reagents different elapsed assay times, differently days at normal lab. Conditions etc. Intermediate precision is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method.

Robustness
The robustness of an analytical procedure is a measure of its capacity of retain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Protocol for analytical method validation
Instrument condition:
Mode: Spectrometric
Range: 220 nm to 400 nm
Wavelength of detection: 256 nm
Measuring mode: Absorbance
Scan speed: Fast

Standard preparation:
Weigh accurately about 50 mg of Ketoprofen working standard in a 100 ml volumetric flask. Add 50 ml of methanol, sonicate to dissolve and dilute to volume with methanol. Further dilute 2.0 ml of this solution to 100 ml with methanol.

Sample preparation
Powder 20 tablets taken for average weight. Weigh accurately a quantity of tablet powder to 100 mg Ketoprofen and transfer in to a 200 ml volumetric flask. Add sufficient amount of methanol. Sonicate and dilute to volume with methanol. Filter through whatman filter paper no. 41. Reject first few ml and collect the rest. Further dilute 2 ml of filtrate to 100 ml with methanol.

Procedure
Measure the UV absorbance of standard and sample solution at 256 nm using methanol as blank.
Calculate the quantity in mg of Ketoprofen in the tablet taken by the formula:
= XXAvg.Wt.(g)

Specificity

Preparation of placebo solution
Transfer about 137 mg (equivalent to 1 tablet) of placebo in to 100 ml volumetric flask. Add sufficient amount of methanol, sonicate and dilute to volume with methanol. Filter through whatman filter paper no. 41. Reject few ml and collect the rest.

Standard preparation
Weigh accurately about 50 mg of Ketoprofen working standard in a 100 ml volumetric flask. Add 50 ml of methanol, sonicate to dissolve and dilute to volume with methanol. Further dilute 2.0 ml of this solution to 100 ml with methanol.

Procedure
Scan separately the Blank, Placebo and Standard solution in the range of 220 nm to 400 nm and measure absorbance at 256 nm. Record the spectrum.

Acceptance criteria
The absorbance at 256 nm of Blank and the placebo solution should be less than 0.005.

Precision

Standard preparation
Weigh accurately about 50 mg of Ketoprofen working standard in a 100 ml of volumetric flask. Add 50 ml of methanol, sonicate to dissolve and dilute to volume with methanol. Further dilute 2.0 ml of this solution to 100 ml with methanol.

Procedure
Scan the standard solution in replicates (6 times) in the range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectrum. Calculate % RSD of the absorbance at 256 nm from 6 replicates.
Prepare the sample solution as given in the analytical method in 6 replicates. Scan each sample solution in the range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectrum. Calculate the quantity in mg of Ketoprofen in the tablet in each preparation as given in the method. Calculate the % RSD of Assay of Ketoprofen.

Acceptance criteria
1. The RSD of absorbance at 256 nm from 6 replicates of standard solution should be less than 2.0%.
2. The RSD of assay of Ketoprofen from 6 assay preparation should be less than 2.0%

Linearity

Standard stock solution preparation
Weigh accurately about 50 mg of Ketoprofen working standard in a 100 ml volumetric flask. Add 50 ml of methanol, sonicate to dissolve and dilute to volume with methanol.
Dilute the standard stock solution as follows to get various linearity level solutions.
Level 1 solution: Dilute 1.0 ml of standard stock solution in a 100 ml volumetric flask and dilute volume with methanol. (This solution contains 50% of Ketoprofen with respective to the test solution conc.).
Level 2 solutions: Dilute 1.5 ml of standard stock solution in a 100 ml volumetric flask and dilute volume with methanol.
(This solution contains 75% of Ketoprofen with respective to test solution conc.)
Level 3 solutions: Dilute 1.8 ml of standard stock solution in a 100 ml volumetric flask and dilute with methanol. (This solution contains 90% of Ketoprofen with respective to the test solution conc.)
Level 4 solutions: Dilute 2.0 ml of standard stock solution in a 100 ml volumetric flask and dilute volume with methanol. (This solution contains 100% of Ketoprofen with respective to the test solution conc.)
Level 5 solutions: Dilute 2.5 ml of standard stock solution in a 100 ml volumetric flask and dilute volume with methanol. (This solution contains 125% of Ketoprofen with respective to the test solution conc.)
Level 6 solutions: Dilute 3.0 ml of standard stock solution in a 100 ml volumetric flask and dilute the volume with methanol. (This solution contains 150% of Ketoprofen with respective to the test solution conc.)

Procedure
Scan each level solution of Ketoprofen working standard in the range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectrum. Plot the linearity curve of Ketoprofen working standard conc. (mg/ml) against the absorbance at 256 nm and determine the linearity regression coefficient.

Acceptance criteria
1. The linearity regression coefficient should be more than 0.999.
2. The % Y intercept should be between + 2.0%.

Range

Procedure
Scan separately the linearity solution of 1st and 6th levels in replicates (6 times) in the range of 220 nm to 400 nm and measure the absorbance at 256 nm from 6 replicates of each level.

Acceptance criteria
The RSD of absorbance at 256 nm from 6 replicates of standard solution should be less than 2.0% for each level.

Accuracy

Preparation of sample stock solution
Powder 20 tablets taken for average weight. Weigh accurately a quantity of tablet powder to 100 mg of Ketoprofen and transfer in to a 200 ml volumetric flask. Add sufficient amount of methanol. Sonicate and dilute to volume with methanol. Filter through whatman filter paper no. 41. Reject first few ml and collect the rest.

Preparation of standard stock solution
Weigh accurately about 50 mg of Ketoprofen working standard in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Preparation of accuracy sample solution
Pipette out 2 ml of sample stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.
Level 1 solution: Pipette out 2 ml of sample stock solution and 1.0 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.
Level 2 solutions: Pipette out 2 ml of sample stock solution and 1.5 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.
Level 3 solutions: Pipette out 2 ml of sample stock solution and 1.8 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.
Level 4 solutions: Pipette out 2 ml of sample stock solution and 2.0 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.
Level 5 solutions: Pipette out 2 ml of sample stock solution and 2.5 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.
Level 6 solutions: Pipette out 2 ml of sample stock solution and 3.0 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Procedure
Scan the accuracy sample solution and each accuracy level solution in duplicate in the range of 220 nm to 400 nm and measure Absorbance at 256 nm. Calculate the % recovery for each level by using the formula.

Acceptance criteria
The recovery should be between 98% to 102% at each level.

Ruggedness
Perform the procedure as detailed in the specificity and precision study on a different day, with different chemist, with different instrument with freshly prepared standard solution and sample solution.
Scan the standard solution in replicates (6 times) in range of 220 nm to 400 nm and measure the absorbance at 256 nm record the spectrum. Calculate %RSD of the absorbance at 256 nm from 6 replicates.
Prepare 6 sample solutions as given in the method. Scan with sample solution in range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectrum. Calculated % of each preparation as given in the method and calculated in % RSD of assay.

Acceptance criteria
1. The absorbance at 256 nm of blank (methanol), placebo should be less than 0.005
2. The RSD of absorbance at 256 nm 6 replicates of standard solution.
3. The RSD of assay (6 preparations) should be less than 2.0 ml.

Robustness
Perform the procedure as detailed in the specificity and precision study on a with freely prepared standard and sample
solution by changing the wavelength to 258 nm instead of 256 nm. Scan the standard solution in replicates (6 times) in the range of 220 nm to 400 nm and measure the absorbance at 258 nm. Record the spectrum. Calculate % RSD of the absorbance at 258 nm from 6 replicates.

Prepare 6 sample solutions as given in the method. Scan with sample solution in the range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectrum. Calculate % of each preparation as given in the method and calculate in % RSD of assay.

**Acceptance criteria**
1. The absorbance at 256 nm of blank (methanol), placebo should be less than 0.005.
2. The RSD of absorbance at 256 nm from 6 replicates of standard solution should be less than 2.0%.
3. The RSD of assay (6 preparations) should be less than 2.0 ml.

**Specificity**
Instrument conditions:
Mode: Spectrum
Range: 220 nm to 400 nm
Wavelength: 256 nm
Measuring mode: Absorbance
Scan speed: Fast
Instrument: SHIMADZU UV 1800

**Preparation of placebo solution**
About 137.0 mg of placebo was weighed accurately; sufficient amount of methanol was added and warmed. Then the solution was cooled and made to 100 ml with methanol. Then the solution was filtered through whatman filter paper no 41. First few ml was rejected and the rest collected.

**Preparation of standard solution**
About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added, sonicated to dissolved and diluted to 100 ml with methanol. Further 2.0 ml of this solution was diluted to 100 ml with methanol. The absorbance of blank, placebo and working standard were measured at 256 nm against blank. The results are given in the table 2.

**Table 2: Data for specificity**

| S. No. | Test          | Absorbance at 256 nm |
|--------|---------------|----------------------|
| 1.     | Blank         | 0.000                |
| 2.     | Placebo       | 0.002                |
| 3.     | Working Standard | 0.577              |

**Precision**
Instrument conditions:
Mode: Spectrum
Range: 220 nm to 400 nm
Wavelength: 256 nm
Measuring mode: Absorbance
Scan speed: Fast
Instrument: SHIMADZU UV 1800

**Preparation of standard solution**
About 50.0 mg of working standard was weighed accurately sufficient amount of methanol was added sonicated to dissolved and diluted to 100 ml with methanol. Further 2.0 ml of this solution was diluted to 100 ml with methanol. Six replicate measurements were made by preparing dilution from the stock solution each time. The reading given below the table 3.

**Table 3: Data for precision of standard**

| Replicates | Absorbance of standard solution at 256 nm |
|------------|------------------------------------------|
| 1          | 0.578                                    |
| 2          | 0.578                                    |
| 3          | 0.578                                    |
| 4          | 0.579                                    |
| 5          | 0.579                                    |
| 6          | 0.579                                    |
| Mean       | 0.579                                    |
| (% )RSD    | 0.09                                     |

The % RSD was calculated by using the formula:

**Standard Deviation SD (σ)**

\[ \text{X} = \text{Mean (or) arithmetic average } \Sigma \frac{X}{N}. \]
\[ \Sigma = \text{Sum of } X - x. \]
\[ \bar{X} = \text{Observed value.} \]
\[ X - \bar{x} = \text{Deviation of value from the mean.} \]
\[ N = \text{Number of observations.} \]

For practical interpretation it is related to standard deviation in the following expression.

**Relative standard deviation (RSD) = X 1000 ppt**
Where
\[ X_i = \text{Individual values of X} \]
\[ N = \text{Number of replicates} \]
\[ Ppt = \text{Parts per thousand} \]

**Precision of tablet assay**

**Sample preparation**
Average weight was calculated for 20 tablets. Then the tablets were powderd and the powder equivalent to about 100 mg of Ketoprofen was weighed accurately and transferred in to 200 ml volumetric flask. Then sufficient amount methanol was added, warmed and cooled, sonicated to dissolve completely. Then the solution was diluted, made up to volume with methanol. The solution was filtered through whatman filter paper No. 41. First few ml was rejected and the rest was collected. The result of precision of tablet assay and the amount of drug is calculated as per the formula. The % RSD was calculated the readings were given in Table 4.
Table 4: Data for precision of tablet assay

| S. No. | Average Wt. of tablets (gm.) | Weight of standard drug (mg.) | Weight of tablet powder (gm.) | Absorbance of Content of drug (mg.) | Average content of drug (mg.) | (%) RSD |
|--------|-----------------------------|-------------------------------|------------------------------|-----------------------------------|-------------------------------|--------|
| 1      | 0.185                       | 50.0                          | 364.0                        | 0.578                             | 49.1                          | 0.44   |
| 2      |                             | 50.0                          | 364.0                        | 0.578                             |                               |        |
| 3      |                             | 50.0                          | 364.0                        | 0.579                             |                               |        |
| 4      |                             | 50.0                          | 364.0                        | 0.579                             |                               |        |
| 5      |                             | 50.0                          | 364.0                        | 0.579                             |                               |        |
| 6      |                             | 50.0                          | 364.0                        | 0.579                             |                               |        |

Product Name: Ketoprofen-50mg Tablet

Test assay

| Tablet No. | Absorbance | Precision-standard |
|------------|------------|--------------------|
| 1          | 0.578      |                    |
| 2          | 0.578      |                    |
| 3          | 0.578      |                    |
| 4          | 0.579      |                    |
| 5          | 0.579      |                    |
| 6          | 0.579      |                    |

Mean (X) = 0.580
SD 0.00
RSD (%) = 0.09

Limit = NMT 2.0%

Table 5: Data for linearity

| Level | Absorbance at 256 nm |
|-------|----------------------|
|       | (i) | (ii) | (iii) | Mean |
| 1     | 0.284 | 0.284 | 0.284 | 0.284 |
| 2     | 0.423 | 0.424 | 0.424 | 0.424 |
| 3     | 0.504 | 0.505 | 0.505 | 0.505 |
| 4     | 0.564 | 0.564 | 0.564 | 0.564 |
| 5     | 0.706 | 0.707 | 0.706 | 0.706 |
| 6     | 0.848 | 0.843 | 0.843 | 0.845 |

Linearity Regression Coefficient 1.0000

Range

| Level | % Y – Intercept |
|-------|-----------------|
|       | -0.35           |

Dilute the standard stock solution as follows to get various linearity level solutions.

Linearity Level 1 Solution: 1.0 ml of stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

Linearity Level 2 Solution: 1.5 ml of stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

Linearity Level 3 Solution: 1.8 ml of stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

Linearity Level 4 Solution: 2.0 ml of stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

Linearity Level 5 Solution: 2.5 ml of stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

Linearity Level 6 Solution: 3.0 ml of stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

Three replicate measurements were made by preparing dilution from the standard stock solution. Each the reading are given in the following Table 5. A graph is also plotted for the absorbance against concentration Graph 2.

Preparation of linearity standard stock solution

About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added sonicated to dissolve and diluted to 100 ml with methanol.
Scan speed: Fast
Instrument: SHIMADZU UV 1800

**Preparation of standard stock solution**

About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added sonicated to dissolve and diluted to 100 ml with methanol.

The standard stock solution was diluted as follows to get various concentration solutions.

**Lower concentration solution (5 mcg)**
1.0 ml of standard stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

**Higher concentration solution (15 mcg)**
3.0 ml of standard stock solution was added in a 100 ml volumetric flask and diluted to the volume with methanol.

**Procedure**

Lower concentration solution and Higher concentration solution was scanned separately. Replicates (6 times) for each level in the range of 220 nm to 400 nm and the absorbance was measured at 256 nm and the spectra recorded in table 6 and the calculated % RSD.

Table 6: Data for range

| S. No. | Absorbance at 256 nm |
|--------|----------------------|
|        | Concentration (5 mcg) | Concentration (15 mcg) |
| 1      | 0.281                | 0.840                |
| 2      | 0.281                | 0.840                |
| 3      | 0.282                | 0.842                |
| 4      | 0.282                | 0.842                |
| 5      | 0.282                | 0.843                |
| 6      | 0.283                | 0.843                |
| Mean   | 0.281                | 0.841                |
| (%) RSD| 0.27                 | 0.16                 |

**Accuracy**

Instrument conditions:
Mode: Spectrum
Range: 220 to 400 nm
Wavelength: 256 nm
Measuring mode: Absorbance
Scan speed: Fast
Instrument: SHIMADZU UV 1800

**Preparation of standard solution**

About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added sonicated to dissolve and diluted to 100 ml with methanol.

**Sample preparation**

Average weight was calculated for 20 tablets. Then the tablets were powdered and the powder equivalent to about 100 mg of Ketoprofen was weighed accurately and transferred in to 200 ml volumetric flask. Then sufficient amount methanol was added, warmed and cooled, sonicated to dissolve completely. Then the solution was diluted, made up to volume with methanol. The solution was filtered through whatman filter paper No. 41. First few ml was rejected and the rest was collected.

**Preparation of accuracy sample solution**

2ml of sample stock solution was pipette out in a 100 ml volumetric flask. Dissolved and diluted to volume with methanol.

Level 1 solution: Pipette out 2 ml of sample stock solution and 1.0 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Level 2 solutions: Pipette out 2 ml of sample stock solution and 1.5 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Level 3 solutions: Pipette out 2 ml of sample stock solution and 1.8 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Level 4 solutions: Pipette out 2 ml of sample stock solution and 2.0 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Level 5 solutions: Pipette out 2 ml of sample stock solution and 2.5 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

Level 6 solutions: Pipette out 2 ml of sample stock solution and 3.0 ml of standard stock solution in a 100 ml volumetric flask. Dissolve and dilute to volume with methanol.

**Procedure**

The sample solution and each level solutions prepared in accuracy study was scanned separately in duplicate in the range of 220 nm to 400 nm and the absorbance was measured for each solution at 256 nm. The spectra were recorded and the mean absorbance at 256 nm and the % Recovery was calculated for each level by using this formula.

Table 7: Data for accuracy

| Accuracy levels | Absorbance at 256 nm | Mean absorbance | Recovery (%) |
|-----------------|----------------------|-----------------|--------------|
|                 | Replicate-1 | Replicate-2     | Accuracy     | Corresponding Linearity level |     |
| Sample          | 0.560       | 0.559           | 0.559        | -                           | 100.0 |
| Level-1         | 0.843       | 0.843           | 0.843        | 0.284                       | 99.5  |
| Level-2         | 0.980       | 0.980           | 0.980        | 0.423                       | 100.0 |
| Level-3         | 1.063       | 1.064           | 1.063        | 0.504                       | 100.0 |
| Level-4         | 1.122       | 1.122           | 1.122        | 0.563                       | 99.2  |
| Level-5         | 1.260       | 1.260           | 1.260        | 0.701                       | 99.6  |
| Level-6         | 1.401       | 1.401           | 1.401        | 0.845                       |       |
Ruggedness
Instrument conditions
Mode: Spectrum
Range: 220 to 400 nm
Wavelength: 256 nm
Measuring mode: Absorbance
Scan speed: Fast
Instrument: SHIMADZU UV 1800

Specificity for ruggedness

Preparation of placebo solution
About 137.0 mg of placebo was weighed accurately; sufficient amount of methanol was added and warmed. Then the solution was cooled and made to 100 ml with methanol. Then the solution was filtered through whatman filter paper no 41. First few ml was rejected and the rest collected.

Preparation of standard solution
About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added sonicated to dissolve and diluted to 100 ml with methanol. Further 2.0 ml of this solution was diluted to 100 ml with methanol. Scan the solution in replicates (6 times) in the range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectra and calculated the % RSD. The reading given on the Table 9.

Table 9: Data for precision of standard for ruggedness

| S. No. | Absorbance of standard solution at 256 nm |
|--------|----------------------------------------|
| 1.     | 0.572                                  |
| 2.     | 0.572                                  |
| 3.     | 0.573                                  |
| 4.     | 0.573                                  |
| 5.     | 0.573                                  |
| 6.     | 0.573                                  |
| Mean   | 0.572                                  |
| RSD (%)| 0.09                                   |

Precision of tablet assay

Sample preparation
Average weight was calculated for 20 tablets. Then the tablets were powdered and the powder equivalent to about 100 mg of Ketoprofen was weighed accurately and transferred in to 200 ml volumetric flask. Then sufficient amount methanol was added, warmed and cooled, sonicated to dissolve completely. Then the solution was diluted, made up to volume with methanol. The solution was filtered through whatman filter paper No. 41. First few ml was rejected and the rest was collected. The result of precision of tablet assay and the amount of drug is calculated as per the formula. The % RSD was calculated the readings were given in Table 10.

Table 10: Data for precision of tablet assay for ruggedness

| S. No. | Average Wt. of tablets (gm.) | Weight of Standard drug (mg.) | Weight of Tablet Powder (gm.) | Absorbance of Standard | Content of drug (mg.) | Average Content of drug (mg.) | (%) RSD |
|--------|-------------------------------|-------------------------------|-------------------------------|------------------------|------------------------|-------------------------------|--------|
| 1      | 0.185                         | 50.0                          | 0.364                         | 0.572                  | 50.0                   | 50.0                          | 0.17   |
| 2      | 50.0                          | 0.364                         | 0.572                         | 0.572                  | 50.0                   | 50.0                          |        |
| 3      | 50.0                          | 0.364                         | 0.573                         | 0.564                  | 50.0                   | 50.0                          |        |
| 4      | 50.0                          | 0.364                         | 0.573                         | 0.554                  | 50.0                   | 50.0                          |        |
| 5      | 50.0                          | 0.364                         | 0.573                         | 0.555                  | 50.2                   | 50.2                          |        |
| 6      | 50.0                          | 0.364                         | 0.573                         | 0.565                  | 50.1                   | 50.1                          |        |
Robustness
Instrument conditions:
Mode: Spectrum
Range: 220 to 400 nm
Wavelength: 256 nm
Measuring mode: Absorbance
Scan speed: Fast
Instrument: VARIAN UV

Specificity for robustness

Preparation of placebo solution
About 137.0 mg of placebo was weighed accurately; sufficient amount of methanol was added and warmed. Then the solution was cooled and made to 100 ml with methanol. Then the solution was filtered through whatman filter paper no 41. First few ml was rejected and the rest collected.

Preparation of standard solution
About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added sonicated to dissolve and diluted to 100 ml with methanol. Further 2.0 ml of this solution was diluted to 100 ml with methanol. The absorbance of Blank, Placebo and working standard was measured at 256 nm against blank. The results are given in the Table 11.

Table 11: Data for specificity of robustness

| S. No. | Test         | Absorbance at 272 nm |
|-------|--------------|----------------------|
| 1     | Blank        | 0.000                |
| 2     | Placebo     | 0.001                |
| 3     | Working standard | 0.570              |

Precision for Robustness

Preparation of standard solution
About 50.0 mg of working standard was weighed accurately. Sufficient amount of methanol was added sonicated to dissolve and diluted to 100 ml with methanol. Further 2.0 ml of this solution was diluted to 100 ml with methanol. Scan the solution in replicates (6 times) in the range of 220 nm to 400 nm and measure the absorbance at 256 nm. Record the spectra and calculated the % RSD. The reading given on the Table 12.

Table 12: Data for precision of standard for robustness

| Replicates | Absorbance of standard solution at 272 nm |
|------------|------------------------------------------|
| 1.         | 0.569                                    |
| 2.         | 0.570                                    |
| 3.         | 0.570                                    |
| 4.         | 0.570                                    |
| 5.         | 0.571                                    |
| 6.         | 0.571                                    |
| Mean       | 0.570                                    |
| RSD (%)    | 0.13                                     |

Precision of tablet assay

Sample preparation
Average weight was calculated for 20 tablets. Then the tablets were powdered and the powder equivalent to about 100 mg of Ketoprofen was weighed accurately and transferred in to 200 ml volumetric flask. Then sufficient amount methanol was added, warmed and cooled, sonicated to dissolve completely. Then the solution was diluted, made up to volume with methanol. The solution was filtered through whatman filter paper No. 41. First few ml was rejected and the rest was collected. The result of precision of tablet assay and the amount of drug is calculated as per the formula. The % RSD was calculated the readings were given in Table 13.

Results and Discussion
Ketoprofen is a new non–steroidal anti cancer drug. It is not official in any pharmacopoeia as on the date. All the reagents used are A.R. Grade. All spectral measurements are made in SHIMADZU UV 1601 and VARIAN U.V with matched quartz cells and glass cell of 1 cm path length.

Specificity
The effect of excipients in the formulation had been studied to determine if they have any effect on absorbance. The dilution and absorbance was similar that of preparation. The absorbance measured at 256 nm. The data are given in the table 2. So that the method was concluded to be specific.

Table 13: Data for precision of tablet assay for robustness

| S. No. | Average Wt. of tablets (gm.) | Weight of Standard drug (mg.) | Weight of Tablet Powder (gm.) | Absorbance of Standard | Content of drug (mg.) | Average Content of drug (mg.) | (%) RSD |
|-------|-------------------------------|------------------------------|------------------------------|------------------------|----------------------|-------------------------------|--------|
| 1     | 0.185                         | 50.0                         | 364.0                        | 0.569                  | 50.3                  | 50.2                          | 0.39   |
| 2     |                               | 50.0                         | 364.0                        | 0.570                  | 50.3                  |                               |        |
| 3     |                               | 50.0                         | 364.0                        | 0.570                  | 50.3                  |                               |        |
| 4     |                               | 50.0                         | 364.0                        | 0.570                  | 49.9                  |                               |        |
| 5     |                               | 50.0                         | 364.0                        | 0.571                  | 50.4                  |                               |        |
| 6     |                               | 50.0                         | 364.0                        | 0.571                  | 50.5                  |                               |        |
Precision
It is the degree of reproducibility when the procedure was applied repeatedly to multiple homogeneous sampling. The precision of an analytical method is usually expressed as standard deviation. The precision study of this method was done on the data obtained from the Table 4. In the precision studies the standard deviation and related standard deviation of tablets of Ketoprofen was 0.12 and 0.25 respectively. So that the method was concluded to be precise.

Linearity
Beer’s law states the fraction of the monochromatic radiant energy absorbed on passing through a solution is directly proportional to the conc. of absorbance.

\[ \log_{10} = KC \]

Where,

- \( C \) = Concentration
- \( K \) = Proportionality constant
- \( I_o \) = Intensity of incident light
- \( I_t \) = Intensity of transmitted light

The absorbance spectral analysis showed that \( \lambda \) max at 256 nm. Beer’s law obeyed in the conc. range 5 mcg/ml to 15 mcg/ml drug concentration. The value obtained of linearity regression coefficient 1.0000 and % of Intercept- 0.35. The data for the linearity was shown in the Table 5. So that the method was concluded to be linear.

Range
Lower concentration solution and Higher concentration solution was scanned separately at 256 nm. The % RSD 0.27 and 0.16 respectively. So this method was complies with in the range. The data shown in the Table no-6.

Accuracy
The solution was prepared (150% to 250%) concentration of solution scanned at 256 nm and calculated the % recovery was 99.7%. So it complies with in the limit. The % recovery was calculated statically from the Table 7.

Ruggedness
The ruggedness of an analytical method was the degree of reproducibility of test results obtained by the analysis of same sample under a variety of normal condition. Such as different laboratories, different analyst, different instrument, different lots of reagents, different elapsed assay time, different days, at normal lab conditions. The result were shown in Table 8-10 and % RSD.

Robustness
The robustness of analytical procedure is a measure of capacity of retain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The estimation of the assay and specificity of the robustness sample preparation and standard preparation was developed as per usual procedure and absorbance at 272 nm results were shown in the Table no-11,12 and13 and % RSD.

Summary and Conclusion
Ultraviolet Visible spectrometric assay developed for the quantification of Ketoprofen was performed in methanol in the concentration of 10 mcg/ml.

Single Point Standardization method was used for the quantitative analysis of drug. The drug obeys Lambert – Beer’s law in the concentration range of 5 mcg/ml. The absorbance maxima occur at 256 nm. The developed method was validated as per ICH norms.

Single Point Standardization method involves simple calculations. The absorbance value at 256 nm was found to be around 0.291.

The results obtained on the validation parameters of developed method meets the ICH requirements. It infers that the method was found to be simple, specific, precise, accurate, reproducible, reliable, linear and proportional (i.e.) it follows Lambert-Beer’s Law. The method was found to be rapid and economic. Hence it can be inferred that the above method was useful to be applied in routine laboratory analysis with a high degree of accuracy and precision.

Conflict of Interest
None.

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None.

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