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

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A novel, safe and sensitive method of spectrophotometric estimation in UV-region has been developed for the quantitative determination of paracetamol in its tablet formulation. The method have been developed and validated for the assay of paracetamol using Methanol and 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 and validated statistically.

Keywords: Spectrophotometric, Developed, Validated, Parameters, ICH [Q2 (R1)]

INTRODUCTION

Spectroscopy Methods[1, 2]
It is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analyses 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:

<table>
<thead>
<tr>
<th>Region</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Far (or vacuum)ultraviolet</td>
<td>10-200 nm</td>
</tr>
<tr>
<td>Near ultraviolet</td>
<td>200-400 nm</td>
</tr>
<tr>
<td>Visible</td>
<td>400-750 nm</td>
</tr>
<tr>
<td>Near infrared</td>
<td>0.75-2.2 μm</td>
</tr>
<tr>
<td>Mid infrared</td>
<td>2.5-50 μm</td>
</tr>
<tr>
<td>Far infrared</td>
<td>50-1000 μm</td>
</tr>
</tbody>
</table>

Ultraviolet-Visible spectrophotometry[3]
UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measures 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.

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

\[
A = a \cdot b \cdot c
\]

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%, 1cm)

\[
A = A_{1\%1\text{ cm}}bc
\]

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 (\(\lambda_{max}\)), where small error in setting the wavelength scale have little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal. 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%, 1cm) 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 absorbances 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.

\[
C_{test} = \frac{(A_{test} \times C_{std})}{A_{std}}
\]

Where \(C_{test}\) and \(C_{std}\) are the concentrations in the sample and standard.

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Received 15-09-2012; Accepted 27-11-2012
solutions respectively and Atest and Astd are the absorbances 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 spectrophotometry
- Solvent extraction method

INTRODUCTION TO PARACETAMOL

Paracetamol / Acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. The onset of analgesia is approximately 11 minutes after oral administration of paracetamol, and its half-life is 1–4 hours. Though acetaminophen is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity.

While generally safe for use at recommended doses (1,000 mg per single dose and up to 4,000 mg per day for adults), acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption.
Behera et al.: UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation

Paracetamol toxicity is the foremost cause of acute liver failure in the Western world, and accounts for most drug overdoses in the United States, the United Kingdom, Australia and New Zealand. \(^{(7)-(10)}\)

Table 3: Evaluation data of accuracy study

<table>
<thead>
<tr>
<th>% Recovery Level</th>
<th>% Recovery</th>
<th>Mean</th>
<th>% Recovery</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>98.62</td>
<td>98.62</td>
<td>0.0057735</td>
<td>0.005854</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>98.61</td>
<td>98.56</td>
<td>0.00316497</td>
<td>0.008279</td>
<td></td>
</tr>
<tr>
<td>125%</td>
<td>98.57</td>
<td>98.55</td>
<td>0.00693889</td>
<td>0.007041</td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.54</td>
<td>98.55</td>
<td>0.00942809</td>
<td>0.009566</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.13</td>
<td>99.75</td>
<td>0.15503083</td>
<td>0.015251</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.12</td>
<td>99.12</td>
<td>0.00707107</td>
<td>0.007134</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Evaluation data of solution stability study

<table>
<thead>
<tr>
<th>Time (Hrs.)</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.723</td>
<td>0.723</td>
</tr>
<tr>
<td>2</td>
<td>0.720</td>
<td>0.721</td>
</tr>
<tr>
<td>4</td>
<td>0.717</td>
<td>0.717</td>
</tr>
<tr>
<td>6</td>
<td>0.716</td>
<td>0.717</td>
</tr>
<tr>
<td>8</td>
<td>0.716</td>
<td>0.716</td>
</tr>
</tbody>
</table>

% Difference at (2hr) 0.414 0.276
% Difference at (4hr) 0.829 0.829
% Difference at (6hr) 0.968 0.829
% Difference at (8hr) 0.968 0.968

Table 5: Evaluation data of robustness study

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>242nm</th>
<th>243nm</th>
<th>244nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.65</td>
<td>0.653</td>
<td>0.657</td>
</tr>
<tr>
<td>2</td>
<td>0.65</td>
<td>0.653</td>
<td>0.656</td>
</tr>
<tr>
<td>3</td>
<td>0.651</td>
<td>0.653</td>
<td>0.656</td>
</tr>
<tr>
<td>4</td>
<td>0.651</td>
<td>0.653</td>
<td>0.657</td>
</tr>
<tr>
<td>5</td>
<td>0.651</td>
<td>0.654</td>
<td>0.657</td>
</tr>
<tr>
<td>6</td>
<td>0.652</td>
<td>0.654</td>
<td>0.657</td>
</tr>
<tr>
<td>Mean</td>
<td>0.650833</td>
<td>0.653333</td>
<td>0.6566667</td>
</tr>
<tr>
<td>SD</td>
<td>0.000753</td>
<td>0.0005164</td>
<td>0.0005164</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.115663</td>
<td>0.0790405</td>
<td>0.0786393</td>
</tr>
</tbody>
</table>

Table 6: Evaluation data of System Suitability Study

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>0.661</td>
</tr>
<tr>
<td>3</td>
<td>0.66</td>
</tr>
<tr>
<td>4</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>0.66</td>
</tr>
<tr>
<td>6</td>
<td>0.66</td>
</tr>
<tr>
<td>Average</td>
<td>0.66</td>
</tr>
<tr>
<td>SD</td>
<td>0.000408</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.061856</td>
</tr>
</tbody>
</table>

Mechanism of action: To date, the mechanism of action of paracetamol is not completely understood. The main mechanism proposed is the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2.\(^{(17)}\) While it has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is the high level of peroxides present in inflammatory lesions. However, in some circumstances, even peripheral anti-inflammatory activity comparable to other NSAIDs can be observed. An article\(^{(18)}\) in Nature Communications from researchers in London, UK and Lund, Sweden in November 2011 has found a hint to the analgesic mechanism of acetaminophen (paracetamol), being that the metabolites of acetaminophen e.g. NAPQI, act on TRPA1-receptors in the spinal cord to suppress the signal transduction from the superficial layers of the dorsal horn, to alleviate pain.

Metabolism: Paracetamol is metabolised primarily in the liver, into non-toxic products. Three metabolic pathways are notable:

- Glucuronidation is believed to account for 40% to two-thirds of the metabolism of paracetamol.\(^{(20)}\)
- Sulfation (sulfate conjugation) may account for 20–40%.\(^{(19)}\)
- N-hydroxylation and rearrangement, then GSH conjugation, accounts for less than 15%. The hepatic
cytochrome P450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite known as NAPQI (N-acetyl-p-benzo-quinone imine).(20) NAPQI is then irreversibly conjugated with the sulphhydryl groups of glutathione.(20)

All three pathways yield final products that are inactive, non-toxic, and eventually excreted by the kidneys. In the third pathway, however, the intermediate product NAPQI is toxic. NAPQI is primarily responsible for the toxic effects of paracetamol; this constitutes an example of toxication.

METHOD VALIDATION

- Validation is concerned with assuring that a measurement process produces valid measurements;
- 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 organisations 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)
- European Cooperation for Accreditation (EA)
- Food and Agriculture Organization (FAO)
- United States Food and Drug Administration (FDA)
- International Conference on Harmonization (ICH),

ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation(20): 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 formulae 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
- 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.

AIM OF PRESENT WORK

This work deals with the validation of the developed method for the assay of paracetamol 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 paracetamol tablet.
- Perform the validation for the method.

MATERIALS AND METHODS

Materials

Paracetamol standard of was provided by Torque Pharmaceuticals (P) Ltd. (India).
Paracetamol Tablets containing 500mg (Ranbaxy) , Acetronitrile HPL grade (Rankem), Methanol HPLC grade (Merck), potassium di hydrogen phosphate( Rankem) sodium hydroxide AR grade ( Rankem) and HPLC grade water were obtained from Spectrochem Pvt. Ltd., Mumbai (India).

Diluent Preparation

Methanol and Water (15:85, v/v) used as a diluent.

Blank Preparation

Diluent used as a blank.

Placebo Preparation

Weigh placebo powder accurately equivalent to 100mg of paracetamol into a 100 mL volumetric flask. Add to it 15mL diluent and sonicate for 5 minutes with shaking after that 50 mL of water was added and sonicate for 15 minutes with intermittent shaking, cool to room temperature and make up to the mark with the water. Fill the above solution through Whatman filter paper no-42. From that 1ml of filtrate
solution was taken into 100ml volumetric flask and volume was adjusted with diluent up to 100ml.

**Standard Preparation**

Weigh & transfer accurately about 10.0 mg of Paracetamol working standard in a 100 mL volumetric flask. Add to it, 15mL methanol, sonicate to dissolve in cool water then add 85 ml of water make up to the mark with water further dilute 5mL of this solution to 50 mL with diluent. Filter through Whathman filter paper no-42.

**Test Preparation**

20 tablets were weighed and determine the average weight, crushed into powdered and powdered equivalent to 100mg of paracetamol was weighed and taken into 100ml volumetric flask then 15ml of methanol was added and sonicate for 5 minutes after that 50 ml of water was added and sonicate for 15 minutes with intermittent shaking, cool to room temperature and make up to the mark with the water (1000 ppm). Filter the above solution through Whathman filter paper no-42. From that 1ml of filtrate solution was taken into 100ml volumetric flask and volume was adjusted with diluent up to 100ml (10 ppm).

**Instrumentation**

UV-Visible double beam spectrophotometer with matched quartz cells (1cm)
Model: Evolution 201
Make: Thermo Scientific, 81 Wyman Street Waltham, Massachusetts, U.S
Ultrasonic bath
Make : Enertech Electronics Private Limited.

**Calculation formula used:**

1. Calculation formula for % assay of Paracetamol

\[
\text{% Assay} = \frac{\text{Test. abs.} \times \text{wt. of Std.} \times \text{Dilutio of test}}{\text{Std. abs.} \times \text{Dilution of std.} \times \text{Wt of test} \times 100} \times \text{Potency} \times \text{Avg wt of tab.}
\]

2. Relative standard deviation = \frac{1}{\text{Mean}} \times 100

3. Recovery

\[
\text{% Recovery} = \frac{\text{Amount recover (mg)}}{\text{Amount added (mg)}} \times 100
\]

4. Amount Recover (mg) = \frac{\text{Abs. of test} \times \text{Wt of Std} \times \text{Potency}}{\text{Abs of Std} \times \text{Dilution of std} \times 100}

5. % Difference calculation for stability of Analytical Solution Study:

\[
\text{% Difference} = \frac{\text{Abs of std initial - abs of std at different time interval}}{\text{Abs of std initial}} \times 100
\]

**RESULTS AND DISCUSSION**

**Development and Optimization of the Spectrophotometric Method**

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of paracetamol, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, and other spectrophotometric conditions. Our preliminary trials using different composition of diluents consisting of Water : Methanol, (30:70) Water : Acetonitrile (20:80) , pH -6.8 phosphate buffer : Methanol (30:70), pH -6.8 phosphate buffer : Acetonitrile (30:70). By using diluent consisted of Methanol: Water (15:85, v/v) best result was obtained and degassed in an ultrasonic bath. Below figures represent the spectrums of blank, placebo, standard placebo + analyte and test preparation respectively.

**Selection of Wavelength:** Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank.

Paracetamol shows λ max at 243. The proposed analytical method is simple, accurate and reproducible.

**Method Validation**

**Specificity:** Scan separately blank, standard, placebo, placebo + analyte and test solution in near ultraviolet region (200 nm to 400 nm).There should not be any interference due to blank, placebo with analyte.

**Linearity:** Six points calibration curve were obtained in a concentration range from 0-150 ppm for Paracetamol. The response of the drug was found to be linear in the investigation concentration range and the linear regression
The present analytical method was validated as per ICH Q2B guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, robust and having stability indicating characteristics. The present analytical method can be used for its intended purpose.

REFERENCES


Source of support: Nil,
Conflict of interest: None Declared

December, 2012
International Journal of Chemical and Analytical Science, 2012, 3(12), 1656-1661