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**Original Article**

# Method development and validation of paracetamol in bulk and tablet formulation by UV-Visible spectroscopy

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## ABSTRACT

A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of Paracetamol in bulk forms and solid dosage formulations. The spectrophotometric detection was carried out at an absorption maximum of 200 nm using methanol as solvent. The method was validated for specificity, linearity, accuracy, precision, and robustness. The detector response for the Paracetamol was linear over the selected concentration range 1 to 7 µg/ml with a correlation coefficient of 0.999. The accuracy was between 99.92 & 100.94%. The precision (R.S.D.) among six sample preparations was 0.30% (Intraday) & 0.59 % (Interday). The LOD and LOQ are 0.480 and 1.457 µg/ml, respectively. The recovery of Paracetamol was about 100.264%. The results demonstrated that the excipients in the commercial tablets did not interfere with the method and can be conveniently employed for routine quality control analysis of Paracetamol in bulk drug, marketed tablets and other formulations.

**Keywords:** Paracetamol, Spectrophotometric and ICH Guidelines

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## INTRODUCTION

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers.

In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law. [1, 2, 3]

**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 concentrations. [4]

**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.

Paracetamol is also known as acetaminophen, is a medication use to treat pain and fever. It is typically used for mild to moderate pain relief. There is mixed evidence for its use to relief fever in children. It is often sold in combination with other medication, such as in much cold medication. Paracetamol is also used for severe pain, such as cancer pain and pain after surgery, in combination with opiod pain medication. It is typically used either mouth or rectal but it is also available by injection into a vein. Effects last between 2 to 4 hours. [5-10]

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)
- Cooperation on International Traceability in Analytical Chemistry(CITAC)
- European Cooperation for Accreditation (EA)
- Food and Agricultural Organization (FAO)
- United States Food and Drug Administration (FDA)
- International Conference on Harmonization (ICH

**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.

**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.

## EXPERIMENTAL

**Materials:** Paracetamol standard of was provided by scientific syndicate Pvt. Ltd. Paracetamol tablets containing 500 mg Paracetamol and the inactive ingredient used in drug matrix were obtained from market. Analytical grade methanol and water were obtained from Vijaya enterprises Pvt. Ltd., Hyderabad (India).

**Diluent preparation:** Methanol and water (15:85, v/v) used as a diluents

**Standard preparation:** 10 mg drug was dissolved in 15 ml methanol and was shaken well. Then 85 ml water was added to it to adjust the volume up to 100 ml (100 ppm). From that 5 ml was taken and volume was adjusted up to 50 ml with diluents.

**Test preparation:** 20 tablets were weighed and powdered. Powdered tablet equivalent to 100 mg of paracetamol was weighed and taken into 100 ml volumetric flask then 15 ml of methanol was added and shaken well to dissolve it after that 85 ml of water was added to adjust the volume up to 100 ml. From that 1 ml of solution was withdrawn and taken in 100 ml volumetric flask. The volume was adjusted with diluent up to 100 ml.

**Instrumentation:** UV-Visible double beam spectrophotometer with matched quartz cells (1 cm)
Make: Elico

## RESULTS AND DISCUSSION

**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 λmax at 200nm. The proposed analytical method is simple, accurate and reproducible

**Method validation**

**Linearity:** Seven points calibration curve were obtained in a concentration range from 1-7 ppm for Paracetamol. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.145x+0.283 with correlation coefficient 0.999.

**Table 1: Linearity, LOD & LOQ of paracetamol**

| CONC(µg/mL) | ABS | FOUND CONCENTRATION | %RECOVERY |
|---|---|---|---|
| 1 | 0.425 | 0.993 | 99.315 |
| 2 | 0.564 | 1.945 | 97.260 |
| 3 | 0.728 | 3.068 | 102.283 |
| 4 | 0.878 | 4.095 | 102.397 |
| 5 | 1.021 | 5.075 | 101.506 |
| 6 | 1.154 | 5.986 | 99.771 |
| 7 | 1.295 | 6.952 | 99.315 |
| MEAN (n=7) | | | 100.264 |
| SD | | | 1.881 |
| %RSD | | | 1.876 |
| SE OF INTERCEPT | | | 0.008007331 |
| SD OF INTERCEPT | | | 0.021275478 |
| LOD | | | 0.480 |
| LOQ | | | 1.457 |

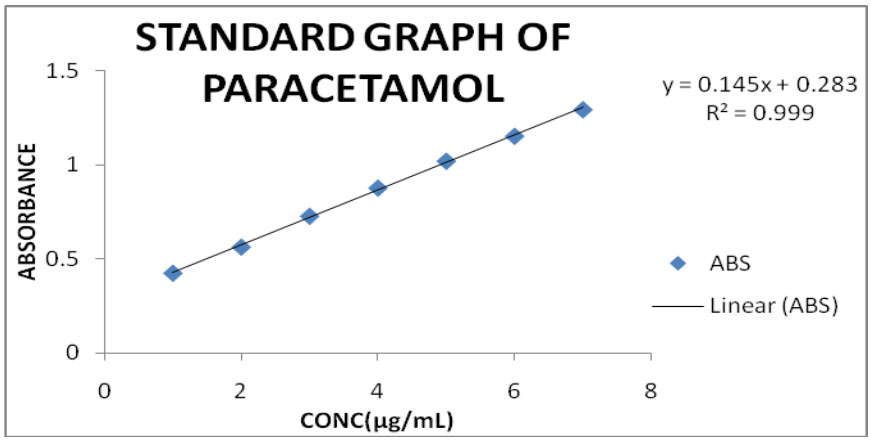


**Figure 1: Standard Graph of Paracetamol**

**Limit of detection:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact

value. LOD can be defined as the smallest level of analyte that gives a measurable response. The detection limit is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. It is usually determined by 3 ways.

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

The limit of detection may be expressed as

**LOD = 3.3 *(Standard error of the intercept/ Slope)**

**Limit of Quantitation:** The quantitation limit of an anlalytical procedure is the lowest amount of analyte in a sample which can be quantatively determined with suitable precision and accuracy. LOQ is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. It is usually determined by in 3 ways.

1. Based on Visual Evaluation
2. Based on Singal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

The limit of Quantitation be expressed as;

**LOQ = 10*(Standard error of the intercept/ Slope)**

LOD and LOQ were calculated according to the formula:

LOD=0.480µg/ml

LOQ=1.457µg/ml

**Assay:** Different brands from market have been received. Find out the % purity of each drug.

**Table 2: Assay of different formulations**

| TABLET CONC | ABS | FOUND CONC | RECOVERY % | | ASSAY |
|---|---|---|---|---|---|
| 5 | 1.038 | 4.904 | 98.095 | BRAND A | 98.095 |
| 5 | 1.046 | 4.775 | 95.510 | BRAND B | 95.510 |
| 5 | 0.840 | 4.920 | 98.503 | BRAND C | 98.503 |

**Precision:** Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis six times. Calculate the % assay, mean assay, % Deviation and % relative standard deviation and %RSD.

The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were 0.307% and 0.591%, respectively.

**Table 3: Precision of paracetamol**

| SAMPLE NO | **PRECISION** | |
|---|---|---|
| SET | INTRA DAY | INTER DAY |
| 1 | 100.498 | 100.124 |
| 2 | 100.217 | 100.041 |
| 3 | 99.878 | 99.865 |
| 4 | 99.912 | 99.014 |
| 5 | 100.614 | 99.872 |
| 6 | 100.415 | 100.855 |
| MEAN | 100.256 | 99.962 |
| SD | 0.308 | 0.590 |
| %RSD | 0.307 | 0.591 |

**Accuracy:** Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 50%, 100% and 150% is to be added in sample. The result shown that best recoveries (99.92-100.94%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

**Robustness:** The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. The result of robustness study of the developed assay method was established. The results shown that during all variance conditions,

assay value of the test preparation solution was not affected and it was accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

**Table 4: Accuracy of paracetamol**

| ACCURACY | | | | | | |
|---|---|---|---|---|---|---|
| %RECOVERY LEVEL | ABS | FOUND CONC | %RECOVERY | MEAN % RECOVERY | SD | %RSD |
| 50%(1.5µg/ml) | 0.498 | 1.482 | 98.850 | 99.923 | 0.957 | 0.957 |
| | 0.502 | 1.510 | 100.689 | | | |
| | 0.501 | 1.503 | 100.229 | | | |
| 100%(3.0µg/ml) | 0.717 | 2.993 | 99.770 | 99.693 | 1.036 | 1.039 |
| | 0.712 | 2.958 | 98.620 | | | |
| | 0.721 | 3.020 | 100.689 | | | |
| 150%(4.5µg/ml) | 0.941 | 4.537 | 100.842 | 100.945 | 0.468 | 0.463 |
| | 0.939 | 4.524 | 100.536 | | | |
| | 0.945 | 4.565 | 101.455 | | | |

**Table 5: Robustness of paracetamol**

| ROBUSTNESS | | | |
|---|---|---|---|
| SAMPLE NO | 199 nm | 200nm | 201nm |
| 1 | 0.493 | 0.454 | 0.426 |
| 2 | 0.474 | 0.453 | 0.424 |
| 3 | 0.471 | 0.451 | 0.422 |
| 4 | 0.485 | 0.455 | 0.425 |
| 5 | 0.477 | 0.452 | 0.419 |
| 6 | 0.482 | 0.449 | 0.423 |
| MEAN | 0.480 | 0.452 | 0.423 |
| SD | 0.008 | 0.00216 | 0.00248 |
| %RSD | 1.674 | 0.477 | 0.586 |

**System suitability:** A system suitability test of the spectrophometric system was performed before each validation run. Six replicate reading of standard preparation were taken and % RSD of standard reading were taken for same. Acceptance criteria for system suitability, % RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

**Table 6: System suitability study of paracetamol**

| SYSTEM SUITABILITY STUDY | |
|---|---|
| SAMPLE NO | ABS |
| 1 | 1.145 |
| 2 | 1.144 |
| 3 | 1.138 |
| 4 | 1.142 |
| 5 | 1.149 |
| 6 | 1.15 |
| AVG | 1.144 |
| SD | 0.0044 |
| %RSD | 0.389 |

## CONCLUSION

The analytical method developed on UV- Visible Spectrophotometer was simple, reliable, accurate and reproducible. The method eliminates extraction steps thus reduce analytical time, cost and minimize the extraction errors. Low cost, faster speed, and satisfactory precision and to assess unequivocally the analyte in the presence of components, which may be expected to be present, are the main features of this method. Method was successfully validated as per ICH guidelines and can be conveniently employed for routine quality control analysis of Paracetamol in bulk drug, marketed tablets and other formulations without any interference from excipients. The method was comparable to the existing methods in all respects.

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