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# Analytical Method Development and Validation of Tramadol by Visible Spectroscopy: a Review

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Review Article

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## ABSTRACT

Tramadol is a halfway acting, paired pain relieving that is neither a sedative determined nor a non-steroidal mitigating drug and that was endorsed for use in the Assembled States in 1995. It is utilized to control moderate torment in perpetual torment settings, for example, osteoarthritis and postoperative cases. Utilized as a part of treatment as a racemic blend, the (+)-enantiomer pitifully ties to the μ-opioid receptor, and both enantiomers repress serotonin and norepinephrine reuptake. Tramadol's real dynamic metabolite, O-desmethyltramadol (ODT), demonstrates higher proclivity for the μ-opioid receptor and has double the pain relieving power of the guardian drug. The synergism of these impacts adds to tramadol's pain relieving properties with the (+)- enantiomer displaying 10-fold higher pain relieving action than the (−)- enantiomer. In spite of the fact that tramadol was at first thought to show low manhandle potential, Ortho-McNeil, the medication's producer, as of late reported a substantial number of unfavorable occasions credited to tramadol including misuse by opioid-dependent patients, hypersensitive responses, and seizures. The high number of antagonistic responses has provoked the organization to upgrade the endorsing data for the medication.

## INTRODUCTION

In developing a quantitative method for determining an unknown concentration of a given species by absorption spectroscopy [1,2], the first step is absorption band at which absorbance measurement are made when several absorption band of suitable absorptivity are present. The band selected should favor wavelength regions that correspond to relatively high output of the light source and high spectral sensitivity of the detector. (Usually λ max) [3,4].

The absorption band should not overlap with bands of the solvent or contaminants including excess reagent might be present in the sample.it is necessary that along the wavelength axis, the absorption spectrum of reagent ion

adduct should be well separated in at least from one place from absorption spectrum of reagent itself .Both the colour developing reagent and the absorbing product must be stable for a reasonable period of time [5-12].

A simple spectrophotometric method for determination of meclizine hydrochloride is developed based on the formation of coloured product with bromocresol as one method and phenol red as another method exhibiting maximum absorbances at 431 nm and 616 nm respectively [13-20].

Spectrophotometric parameters were established for the standardization of the method including statistical analysis of the drug. These methods have being successfully extended to the pharmaceutical formulation.

## PROCEDURE

### Shimadzu Ultra-Violet-Visible Double Beam Spectrophotometer Reagent

The reagent used is phenanthroline (0.5 ug/mL).

### Preparation of the Reagent Phenanthroline

Phenanthroline solution was prepared by dissolving 10 ug of phenanthroline in 10mL of water from the above solution 1 mL is taken and to it was diluted with 10 mL of water giving the concentrate of 100 µg/mL [21,22].

### Standard Drug Solution

Standard solution was prepared by dissolving 10 mg of tramadol in 10 mL of methanol and from the above solution 1mL of solution was taken and was diluted with distilled water giving the concentrate of 100 µg/mL.

1mL of 200 µg/mL of sample was mixed with 3 mL of phenanthroline and to this add 3mL of water.

### Linearity and sensitivity of the method

Spectrophotometry is a simple technique used to measure absorbance of solution .The sample is placed inside a cuvette which is rectangular prism shaped vessel. Light of a particular wavelength is directed to one side of the cuvette and the intensity of light reaching the detector is measured.

The transmittance, T of the solution is defined as the ratio of the intensities of transmitted beam, I to the intensity. $I_0$ of the incident beam;

Where; T-Transmittance

$I_o$ –intensity of initial light beam

I – intensity of transmitted light

If it is less than $I_o$, then obviously the sample has absorbed (absorbance of the sample – given the symbol A) some of the light [23-28].

Here the absorbance, A of a solution defined as

$A= -\log_{10}T$

The relationship between A (the absorbance) and the two intensities was explained by Beer's law states that for parallel beam of monochromatic radiation passing through homogenous solutions of equal path length the absorbance is proportional to the concentration.

Lambert's law states that for parallel beam of monochromatic radiation passing through a homogenous solution of equal concentrations the absorbance is proportional to the path length. The combination of two laws is Beer Lambert's law; it can be expressed as

**$A=abc$ vs $A=\acute{\varepsilon}bc= -\log_{10}T= - \log I/I_o=\log I_o/I$**

**A=** absorbance

**ε =** molar absorptivity with units of L $mol^{-1}$ $cm^{-1}$

b= path length of the sample (cuvette)

c= concentration of the compound in solution, expressed in mol $^{-1}$

The lambert beer's law however is valid only for diluted solutions. The limits for its validity differ from different materials. As a general rule, one can understand that every material showing absorption of up to 0.5 to 0.6 still obeys the lambert-beer's law [29-35].

## Molar absorptivity

The molar absorptivity coefficient, molar extinction coefficient or molar absorptivity, is measurement of how strongly a chemical species absorbs light at a given wavelength. IT is an intrinsic property of the species; the actual absorbance, A, of a sample is dependent on the path length land the concentration of the species via the Beer-Lambert law, A=ecb. The SI units for are $m^2/mol$, but in practice, they are usually taken as $M^{-1}cm^{-1}$ or L $mol^{-1}cm^{-1}$.

When c is in moles per litre, the constants is called the molar absorptivity and has the symbol £. Beers law limits and max values are expressed as ug/mL and litre $mole^{-1}cm^{-1}$ respectively [36-40].

## Sandell's sensitivity

Sandell's sensitivity refers to the number of mg of drug determined converted to the coloured product, which in a column solution of cross section $1cm^2$ shows and absorbance of 0.001 (expressed as $\mu gcm^{-2}$).

a=log x intensity of incident radiation/intensity of transmitted light the absorbance (a) is proportional to the concentration (c) of absorbing species if absorptivitivity 9a) and thickness of the medium (b) are constants.

## Limitations of Beer's Law

The law is not applicable for:

1. Highly concentrated solutions.
2. Solutions exhibiting stray fluoresce or suspensions may not strictly adhere to Beer's law.
3. If a dilute solution during measurement undergoes chemical reaction such as oxidation, reduction, hydrolysis, association, polymerization, then the law is not valid [41-45].

## Accuracy

The accuracy of a determination may be defined as the concordance between it and the true or most probable value. For analytical methods there are two possible ways of determining the accuracy. Absolute method and comparative method.

## Absolute Method

The test for accuracy of the method is carried out by taking varying amounts of the constituent and proceeding according to specified instructions. The difference between the mean of an adequate number of results and the amount of constituent actually present is usually expressed as parts per hundred [46-50].

Comparative Method: In the analysis of pharmaceutical formulation or solid laboratory prepared samples of desired composition, the content of the constituent sought has been determined by two or more methods of analysis .the agreement between at least two methods of essentially different character can usually be accepted as indicating the absence of an appreciable determinate error [51-60].

## Recovery (Standard Addition)

A known amount of the constituent being determined is added to the sample, which is analyzed for total amount of constituent present. The difference between the analyzed results for samples with or without the added constituent gives the recovery of the amount of added constituent. If the recovery is satisfactory, our confident in the accuracy of the procedure is enhanced [61-68].

## Precision

Precision may be defined as the concordance of a series of measurement of the same quantity. It refers to the agreement among a group as experiment results. Precise values may be in accurate. The precision terms usually one encounter is:

1. Mean
2. Standard Deviation
3. Average Deviation

Precision may be defined as the concordance of a series of measurement of the same quantity.

***Mean:*** Mean of a finite number of measurement like x1+x2+x3+.....xn is often designated as X.

Mean=x1+x2+x3+.....xn ÷ N

***Average deviation:*** The average deviation of measurement of asset is the mean of the difference of the individual measurements.

A.D= £xi- Mean ÷N=d

Where xi= individual measurements

N=number of measurements [69-75].

***Standard deviation:*** Standard deviation, s of an infinite set of individual data is theoretically the square root of the mean of the difference between the individual measures values xi and the mean of the infinite number of measurements X or μ.

$S= [£(Xi-\mu)^2/N]$

$S= [£(Xi-\mu)^2/N-1]$

The denominator is (N-1) rather than N When the number of values is small (less than 300. The square of the standard deviation is called the variance (s). A more accurate measure of the precision, known as the coefficient of variation is given by

C.V or %Relative Deviation =S ×100/Mean

### Parameter Fixation

The effect of various parameters affecting the absorption were studied by varying one parameter at a time and controlling all the others and observing the effect on the absorbance, the volume and strength of 1,10-phenanthroline-oxidant reagent ferric chloride, time and temperature required for color development, effect of solvents, time required for maximum color development and the stability of the colored species were studied [76-83].

The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation coefficient (R), obtained from different concentration and the results are summarized in **Table 1**.

**Table 1:** Optical characteristics and precision of the method.

| S.no | Parameter | Observation |
|---|---|---|
| 1 | Lamda max | 437 nm |
| 2 | Beers law range | 100-200 µg/mL |
| 3 | Molar extinction coefficient (litre $mole^{-1}$ $cm^{-1}$) | 0.0045 |
| 4 | Regression equation (y=mx+b) slope(m), intercept (b) | 0.005, 0.1153 |
| 5 | Correlation coefficient (R) | 0.993 |
| 6 | STANDARD deviation (S.D) | 0.025 |
| 7 | PRESISION (%RSD) | 2.64 |
| 8 | Standard error of estimate | 0.0263 |

### Spectral characteristics

In order to ascertain the wavelength of maximum absorption of the colored species obtained in each of the bulk sample and the formulations, specified amounts of tramadol were taken and the color was developed separately following the recommended procedure [84-88].The absorption spectrum of each colored species was scanned on spectrophotometer in the λ region of 400-800 nm against the corresponding reagent blank [89-96].

### Optical Characteristics

In order to test whether the colored species formed adhere to the beer's law, the absorbance at appropriate λ max (510) of a set of solutions containing varying amounts of tramadol and specified amounts of reagents were noted [97-100] The beer's law plots of the system are represented graphically in **Table 1**. All the optical characteristics were calculated and recorded in **Table 1**.

## DISCUSSION

The optimum conditions were established by varying one parameter at a time and keeping the other fixed and observing the effect on absorbance of chromogen. The optical characteristics such as ,absorption maxima Beer's law limits, Molar absorptivity and sandell's sensitivity RSD standard error of estimate etc. are represented in Table 1.The optical characteristics were calculated and results showed that the method was accurate and precise. The other ingredients and excipient usually present in the dosage forms did not interfere with the method.

### Precision

To find out the precision of the proposed method , absorbance values of the five replicates of a fixed amount of pure drug after developing the color as given in the recommended procedures were recorded. The %RSD is

presented in the **Table 1**.

### Accuracy

To determine the accuracy of the method, different amounts of the pure sample of the drug were taken and analysed by the proposed method.

### Recovery Studies

To ensure the accuracy and reproducibility of the results obtained. Recovery experiments were performed by adding known amounts of pure drug to the previously analyzed formulated samples were analyzed by the proposed method.

### Interference Studies

There was no interference of the additives in the proposed method.

## CONCLUSION

A sample visible spectrometer method for determination of Tramadol in pure and its dosage forms was developed. The absorbance of Phenanthroline was measured at maximum absorbance of 437 nm against the corresponding reagent blank (water). Various calculations have mentioned earlier. This method is found to be simple, precise, economic and less time consuming. The method has been statistically evaluated and the results obtained are accurate, precise, sensitive and free from the interferences of other additives present in the formulation.

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