ABSTRACT

Aim: The work mainly focuses on developing a robust UV-Visible spectroscopic method for qualitative and quantitative analysis of thymoquinone and this will open to many possibilities in exploiting the wonders of this bioactive molecule.

Study Design: UV-vis Spectrophotometric method development for thymoquinone.

Place and Duration of Study: Department of Pharmaceutics, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University Campus, Nagpur, India.

Methodology: To find out λmax and validate the developed method using Double beam UV – visible spectrophotometer module (JASCO V-630).

Results: The thymoquinone was found to be soluble in methanol. The absorption maximum (λmax) was found to be 252 nm. The good linearity was found to be within concentration range of 2-10 μg/ml with correlation coefficient (r²) >0.99 and regression equation of the curve was found to
be \( y = 0.0173x - 0.0394 \) at 252 nm. The precision (intra-day and inter-day) data represents good reproducibility with % RSD lower than 2.0% which assured that method is précised. Mean recovery value at different concentrations was found to be higher than 90%, indicates accuracy of the method. LOD and LOQ for thymoquinone were reported and were found to be 0.016 µg/ml and 0.0531µg/ml 252 nm. 

**Conclusion:** The developed method was found to be simple, specific, economic, reliable, accurate, precise, and reproducible used as a quality control tool for analysis of pure thymoquinone and thymoquinone in formulations.

**Keywords:** Thymoquinone; UV-vis spectrophotometric method; validation; accuracy; limit of detection; limit of quantification.

### 1. INTRODUCTION

Thymoquinone (TH) is the main active constituent of Black seed (*Nigella sativa*, family Ranunculaceae) plant oil and chemically known as 2-isopropyl-5-methyl-1,4-benzoquinone (Fig. 1) [1]. Thymoquinone has been traditionally used as an ancient folk medicine as it possesses various pharmacological activities like antioxidant, hepatic-protective effects against hepatotoxins [2], neuroprotective [3], anti-diabetic [4], anti-inflammatory, antimutagenic, and anticarcinogenic [5]. The previous studies have suggested that high end instruments like High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectroscopy (GC-MS) are the most commonly used techniques for the estimation of Thymoquinone (TH) [6]. But however these qualitative techniques are relatively time consuming and very expensive and requires skill. To overcome this constrains implantation of UV-visible spectrophotometry is more preferable for estimation of chemical entities as it is comparatively less time consuming and less tedious and simultaneously provide robust and accurate results. UV visible spectroscopy has emerged as most appealing technique in estimation of pharmacological moieties by the possible mechanism of absorbing the UV and visible light by the drug or chemicals and can be easily quantified as well as analysed or assayed [7]. Hence signifying the medicinal importance of thymoquinone it is important to develop a simple, rapid, cheap and effective UV-Visible spectrophotometric method for the estimation of thymoquinone. Hence this work mainly focuses on developing a robust UV-Visible spectroscopic method for qualitative and quantitative analysis of TH and this will open to many possibilities in exploiting the wonders of this bioactive molecule.

### 2. MATERIALS AND METHODS

#### 2.1 Materials

Thymoquinone was purchased from Sigma-Aldrich Corporation and Biotechnological company, Mumbai. Having purity of ≥ 99.0, certified. Methanol (Analytical Grade) was purchased from Merck Specialties Pvt. Ltd. Mumbai. All other chemical used were of either pharmaceutical or analytical grade.

#### 2.2 Method Development

##### 2.2.1 Instrument

The method development was done using Double beam UV – visible spectrophotometer module (JASCO V-630) having wavelength scanner from 600 nm to 190 nm. The silica quartz square cell cuvette with transmittance of 51.4±0.3% and having 3 cm length with 1 cm path length were used throughout the whole experiment.

##### 2.2.2 Preparation of standard stock solution

To assess the wavelength maximum absorption (\( \lambda_{max} \)) of thymoquinone, the standard stock solution (1000 µg/ml) of thymoquinone was prepared by weighing accurately 5 mg of pure drug into 5 ml volumetric flask and dissolved with a minimum quantity of methanol and final volume was made up to mark with methanol.

**Fig. 1. Chemical structure of Thymoquinone**
2.2.3 Preparation of working solution

The working stock solution was further diluted with methanol to get (10 μg/ml) concentration (1ml to 10 ml). The working stock solution of thymoquinone (100 μg/ml) was prepared by diluting 1 ml of standard stock solution to 10 ml with methanol.

2.2.4 Selection of (λmax)

The prepared solution was scanned between the wavelength regions of 200-400 nm against methanol as blank. The UV-vis spectra were shown (Fig. 2) and absorption curve showed characteristics absorption maxima at 252 nm. Hence (λmax) were selected for analysis of thymoquinone. From working stock solution, a series with a concentration range of 2-10 μg/ml at 252 nm for preparation of the calibration curve were obtained by further dilution of stock solution with methanol.

2.3 Validation of the Analytical Method

The developed analytical method was validated as per ICH guidelines and prepared different series of diluted solutions (2-10 μg/ml) were analysed for linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ).

2.3.1 Linearity and range

The linearity for this method at various concentrations of the range between 2-10 μg/ml and was analysed at 252 nm [Table 1]. The absorbance vs concentration plot for thymoquinone was found to be linear in (Fig. 3). R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV-vis method.

| Sr. No. | Concentration (μg/ml) | Absorbance  |
|---------|-----------------------|-------------|
| 1       | 2                     | 0.421±0.02  |
| 2       | 4                     | 0.718±0.12  |
| 3       | 6                     | 1.106±0.09  |
| 4       | 8                     | 1.459±0.31  |
| 5       | 10                    | 1.721±0.21  |

Data subjected to n=3

2.3.2 Precision

To assess the precision of the developed method it was subjected to repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was determined by analysing 10 μg/ml of thymoquinone at three different time points within a day. Similarly, Inter-day precision was performed with the same concentration of the test sample at three different points for three days, and average % RSD was calculated [8].

2.3.3 Accuracy

Accuracy suggests the nearness of the obtained results gained by the method to the true value. Validation of accuracy was analysed mainly by performing recovery studies by spiking pre – analysed sample solutions of thymoquinone with three different concentrations of 8 μg/ml, 10 μg/ml and 12 μg/ml and % recovery was computed [9].

2.3.4 LOD and LOQ

The lowest concentration of analyte in a sample that an analytical process can reliably differentiate from background levels is generally termed as LOD and the lowest concentration of calibration that can be measured acceptable accuracy and precision is LOQ. In this method, LOD and LOQ were based on a standard deviation of the response and slope of the calibration curve using following equations [8,9].

\[ \text{LOD} = 3.3 \frac{S}{M}; \text{LOQ} = 10 \frac{S}{M} \]  

(Eq. 1)

Where S is the standard deviation of the absorbance of the sample and M is the slope of the calibration curve.

3. RESULTS AND DISCUSSION

The thymoquinone was found to be soluble in methanol. The absorption maximum (λmax) was found to be 252 nm as shown in (Fig. 2). The good linearity was found to be within concentration range of 2-10 μg/ml (Tables 2, 3) with correlation coefficient (r²) >0.99 and regression equation of the curve was found to be 

\[ y = 0.0173x - 0.0394 \] at 252 nm.

The precision (intra-day and inter-day) data represents (Table 4) good reproducibility with % RSD lower than 2.0% which assured that method is précised. Mean recovery value (Table 4) at different concentrations was found to be higher
than 90%, indicates accuracy of the method. LOD and LOQ for thymoquinone were reported (Table 4) and were found to be 0.016 µg/ml and 0.0531 µg/ml at 252 nm.

![Fig. 2. λmax of Thymoquinone](image1.png)

![Fig. 3. Calibration curve of thymoquinone at 252 nm](image2.png)

**Table 2. Results of intra- and inter-day precision (n=6)**

| Concentration (µg/ml) | Intra-day | Inter-day |
|-----------------------|-----------|-----------|
|                       | Absorbance (nm)±SD | % RSD | Absorbance (nm)±SD | % RSD |
| 10                    | 1.731±0.0003 | 0.0975 | 1.799±0.0001 | 0.2311 |
| 10                    | 1.721±0.0004 | 1.061±0.0002 | 1.801±0.0023 | 1.861±0.0002 |

*RSD: Relative standard deviation*
### Table 3. Recovery study of Thymoquinone

| λmax (nm) | Level of recovery (%) | Amount spiked recovery (μg/ml) | Amount recovered (μg/ml) | Recovery (%) | Mean recovery |
|-----------|-----------------------|-------------------------------|------------------------|--------------|---------------|
|           | 80                    | 8                             | 7.21                   | 91.51        | 93.80         |
|           | 100                   | 10                            | 9.34                   | 92.13        |               |
|           | 120                   | 12                            | 11.14                  | 97.81        |               |

### Table 4. Validation parameters

| Validation parameters | Results                        |
|-----------------------|--------------------------------|
| λmax                  | 252 nm                         |
| Beer’s law range (μg/ml) | 2-10 μg/ml                    |
| Correlation coefficient ($r^2$) | 0.996                     |
| Slope (m)             | 0.1730                        |
| Intercept (c)         | 0.0039                        |
| Accuracy              | 93.00008                      |
| Precision (%RSD)      |                                |
| Intra-day             | 0.0975                        |
| Inter-day             | 0.2311                        |
| LOD (μg/ml)           | 0.016                         |
| LOQ (μg/ml)           | 0.0531                        |

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

### 4. CONCLUSION

The developed method was found to be simple, specific, economic, reliable, accurate, precise, and reproducible used as a quality control tool for analysis of pure thymoquinone and thymoquinone in formulations.

### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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