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"Quantitative Analysis of Thymoquinone in Black Cumin Seeds using High-Performance Thin-Layer Chromatography"

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Abstract

Black cumin (*Nigella sativa*) seeds are a rich source of thymoquinone, a bioactive compound with numerous therapeutic properties. Accurate quantification of thymoquinone is crucial for quality control and standardization of black cumin seed-based products. This study presents a rapid and reliable high-performance thin-layer chromatography method for the quantitative analysis of thymoquinone in black cumin seeds. The method involves a simple extraction procedure followed by HPTLC separation and densitometric detection. Key parameters such as mobile phase composition, developing distance, and detection wavelength were optimized to achieve optimal separation and sensitivity. The developed HPTLC method demonstrated good linearity, precision, accuracy, and sensitivity, making it suitable for routine analysis of thymoquinone in black cumin seeds and related products.

Key Words: Nigella sativa, Thymoquinone, HPTLC, Quantitative analysis, Quality control

1. Introduction:

Black cumin seeds are widely used in traditional medicine for their diverse therapeutic properties, including anti-inflammatory, antioxidant, and antimicrobial activities. These beneficial effects are largely attributed to the presence of the bioactive compound thymoquinone, which is the major constituent of the essential oil extracted from the seeds. Accurate quantification of thymoquinone in black cumin seeds is crucial for standardizing herbal preparations and ensuring quality control. Here, we present a rapid and reliable high-performance thin-layer chromatography method for the quantitative analysis of thymoquinone in black cumin seeds. (Heikes et al., 2001) (Johri, 2011)

The cumin and caraway seeds are known to be rich in essential oils and have been extensively studied for their chemical composition and biological activities. A supercritical fluid extraction method coupled with gas chromatography has been developed for the quantification of volatile oils in ground cumin samples. (Heikes et al., 2001) However, HPTLC offers several advantages over conventional GC techniques, including simplified sample preparation, the ability to analyze multiple samples simultaneously, and lower

operational costs. (Johri, 2011) In this study, we optimize an HPTLC method for the determination of thymoquinone content in different samples of black cumin seeds. (Ishida et al., 2022)

Samples of black cumin seeds were obtained from various geographical locations and rigorously authenticated by a qualified botanist to ensure the accuracy and reliability of the study. The seeds were ground to a fine powder, and the thymoquinone content was extracted using an organic solvent. The extract was then spotted on pre-coated silica gel plates and developed using a suitable mobile phase. After drying, the plates were scanned densitometrical, and the thymoquinone content was quantified by comparing the peak areas with those of standard solutions. The proposed HPTLC method was validated for linearity, precision, accuracy, and robustness, ensuring its suitability for routine analysis of thymoquinone in black cumin seeds. (Abou Basha et al., 2013)

The results of this study demonstrate the applicability of HPTLC for the rapid and reliable quantification of thymoquinone in black cumin seeds. This technique can be effectively used for the quality control and standardization of herbal products containing black cumin seeds or their extracts. (Alam et al., 2013) (Hasrawati et al., 2021)

The results of this study indicate that the thymoquinone content in black cumin seeds varies significantly depending on the geographical origin and environmental factors. This information is crucial for the standardization of black cumin-based herbal products and the development of quality control protocols. Further research is warranted to investigate the factors influencing the accumulation of thymoquinone in black cumin seeds and to explore the potential of this bioactive compound for therapeutic applications.

In this study, we optimized an HPTLC method for the quantitative determination of thymoquinone in black cumin seed extracts. The method was validated for parameters such as linearity, precision, accuracy, and specificity. Cumin seeds were extracted with organic solvents, and the extracts were spotted on HPTLC plates. After development, the plates were scanned, and the thymoquinone content was quantified by comparing the peak areas with those of a standard solution.

The cumin and caraway seeds are rich sources of essential oils with diverse bioactivities that have been extensively studied. (Johri, 2011) Cumin seeds, in particular, are valued for their characteristic aroma and flavor, which are attributed to the presence of volatile oils. (Heikes et al., 2001)

The primary component of the essential oil from black cumin seeds is thymoquinone, which has demonstrated a wide range of pharmacological properties including anti-inflammatory, antioxidant, and antimicrobial activities. (Johri, 2011) (Ishida et al., 2022) Quantification of thymoquinone is critical for quality control and standardization of black cumin seed-based herbal products.

Supercritical fluid extraction coupled with gas chromatography has been used as a rapid and accurate method for quantifying volatile oils in cumin. (Heikes et al., 2001) However, this approach may not be feasible for routine analysis due to the specialized equipment required. High-performance thin-layer chromatography offers a simple, cost-effective, and efficient alternative for the quantification of thymoquinone in black cumin seeds. (Heikes et al., 2001)

In this study, we developed and validated a HPTLC method for the quantitative determination of thymoquinone in black cumin seeds. The method was optimized for sample extraction, chromatographic separation, and quantification using a validated standard compound. The developed HPTLC method was then applied to analyze the thymoquinone content in commercially available black cumin seed samples, providing insights into the quality and consistency of these herbal products.

2. Methodology

2.1 Materials and Reagents

Black Cumin Seeds: commercially available, Nigella sativa (Kalonji Seeds) was purchased from a local vender in Pune. Seeds were authenticated by Agarkar Research Institute, Pune, India (AUTH 23-91and stored in airtight containers at Room Temperature.

Thymoquinone Standard: The Marker Thymoquinone was purchased from Sigma-Aldrich, ≥99% purity].

Solvents: HPLC grade methanol, Toluene, Ethyl acetate, Formic acid was purchased from Sigma-Aldrich.

HPTLC Plates: pre-coated silica gel 60 F254 aluminum sheets, Merck. Plate was prewash with Methanol & heated at 1050C

2.2 Instrumentation and Software

Software: Server DESKTOP-5IHGUM1, version 3.2.23095.1

Instruments Used:

Linomat 5 (S/N: 150721)

TLC Scanner 3 (S/N: 151015)

TLC Visualizer 2 (S/N: 290326)

2.3 Extraction Process

2.3.1 Preparation of Nigella sativa Seeds:

Weigh 10gm of Nigella sativa seeds accurately and then grounded it into a coarse powder using mortar and pestle or grinder.

2.3.2 Soxhlet Extraction Setup:

The prepared seed powder was placed in a cotton or filter paper thimble. The thimble was placed in the main chamber of the Soxhlet extractor. A round-bottom flask is filled with the proper volume of dichloromethane DCM and attached to the Soxhlet extractor.The Soxhlet extractor was connected at the top with a condenser.

2.3.3 Extraction Process:

The round-bottom flask was put on the heating mantle and heated at the boiling point of DCM, 39.6°C.The DCM evaporated and went upwards into the condenser, cooled, and condensed into the liquid form. The condensed solvent then filled the Soxhlet chamber with seed powder and extracted the targeted chemicals. Once the Soxhlet chamber had been refilled, the solvent, now with the chemicals dissolved in it, was siphoned back into the boiling flask. This process was allowed to repeat for 6–24 hours.

2.3.4 Post-Extraction:

After the extraction time, the heating mantle was turned off and the apparatus was allowed to cool. The Soxhlet extractor was cautiously disassembled. The thimble with the residue of the extracted seed was removed. Then, the extracted chemicals together with the solvent were introduced to a rotary evaporator or evaporated over a water bath to evaporate the DCM. The concentrated extract was collected into an appropriate container and, if necessary, dried in a desiccator. Weigh out the final extract to determine yield and store the extract in an airtight, dark container to prevent degradation.

2.4 Chromatography Setup

Stationary Phase: HPTLC Silica gel 60 F_{254}.

Mobile Phase: Toluene: Ethyl acetate: Formic acid (8:2:0.3 v/v/v).

Application: Band application with Linomat 5.

Development: The plate is developed in a chamber saturated with the mobile phase for 20 minutes.

Drying: The developed plate is dried at room temperature.

2.5 Standard Solution Preparation

2.5.1 Standard Preparation:
10 mg of thymoquinone standard (STD) is dissolved in 10 ml of ethanol. The solution is sonicated for 10 minutes at 25°C. The solution is diluted to obtain a concentration of 1000 ppm. 1 ml of this solution is further diluted to 10 ml with ethanol to achieve a concentration of 100 ppm.

2.5.2 Sample Preparation:
10 mg of black cumin seed extract is dissolved in 10 ml of ethanol. The solution is sonicated for 10 minutes at 25°C. The solution is diluted to obtain a concentration of 1000 ppm. The extract is filtered using a syringe filter.

2.6 Application of sample
Using the Linomat 5 (S/N: 150721), the different solvent extract samples were applied with a bandwidth of 8 mm, spacing of 8 mm, and flow rate of 150 nL/sec maintained. Five to ten microliters of the sample were utilized.

2.7 HPTLC development
HPTLC plates were activated by pre-heating at 100°C 10 minutes before sample application. The mobile phase used in the experiment was chosen through empirical selection as a mixture of Toluene: Ethyl acetate: Formic acid (8:2:0.3 v/v/v). The plates were saturated in a tank containing the mobile phase for 20min. The plates were dried at room temperature.

2.8 Spectral Analysis of Thymoquinone Standard from (NS) Extract
The spectra were obtained by doing additional dilutions using methanol from the standard stock solution and scanning it over the 200–800 nm range. Both the medication and the marker were found to have significant absorbance at 260 nm. (as shown in fig. 1)

2.9 Detection and Quantification:
2.9.1 Visualization: Scan the plate using a TLC scanner at 260 nm.
2.9.2 Densitometry: The generated HPTLC plate was scanned using the TLC Scanner 3 at the designated wavelength of 260 nm, which is appropriate for thymoquinone detection. With peaks signifying several substances, including thymoquinone, the resultant densitogram makes precise quantification based on absorbance measurements obtained at this wavelength possible.

3. Result and Discussion

3.1 Spectral Analysis of Thymoquinone Standard from NS Extract
A UV-visible absorption spectra of a thymoquinone standard from an NS extract is shown in the image. For the purpose of detecting and characterizing chemical substances according to their absorbance at various wavelengths, this kind of spectral analysis is crucial. Figure 1 shows the absorbance of thymoquinone over a variety of wavelengths in the UV-visible spectrum. Critical information on the quantity and purity of thymoquinone in the extract is provided by the distinctive peaks and their corresponding absorbance values. The visible to ultraviolet (UV) light spectrum is represented by the wavelength values, which span 200–800 nm. With a notable absorption at 1.0 AU, the peak emerges at about 260 nm. Thymoquinone's principal absorption in the UV region is shown by the first peak at 210 nm, which also suggests the existence of conjugated double bonds and aromatic systems that are characteristic of Thymoquinone's molecular structure. Thymoquinone's presence is further supported by the secondary peak, or second peak, at 260 nm, which corresponds to the compound's recognized absorption properties.

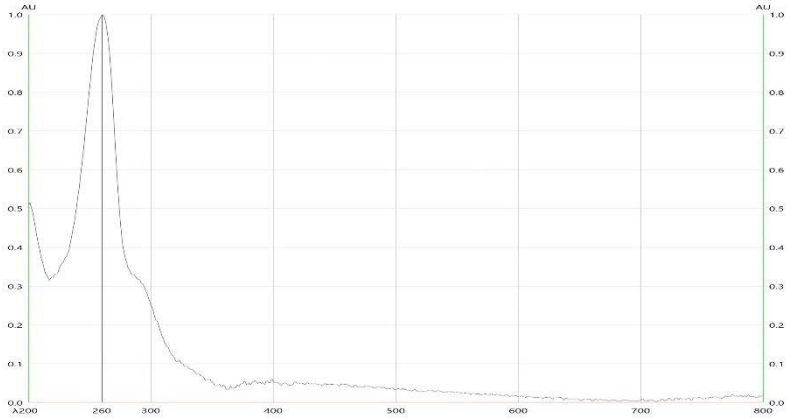

Figure 1: Spectra of Thymoquinone in NS extract

3.2. Thin Layer Chromatography (TLC) Analysis of Thymoquinone Standard from NS Extract

Figure 2 shows a Thin Layer Chromatography (TLC) plate seen at 254 nm under UV light. With Seven tracks—Six for the thymoquinone standard (STD) and one for the NS extract—the image shows how the components inside the samples were separated and identified. By contrasting the migratory patterns of the thymoquinone standard with the NS extract in Figure 2 , it is possible to determine the presence and purity of thymoquinone in the extract. A distinct band with an RF value of around 0.68 that matches the thymoquinone standard is shown on tracks 1 to 6. Thymoquinone may be present in the NS extract, as shown by a similar band that appears on track 7 at the same RF value 0.66. By comparing, this TLC technique successfully proves that thymoquinone is present in the NS extract.

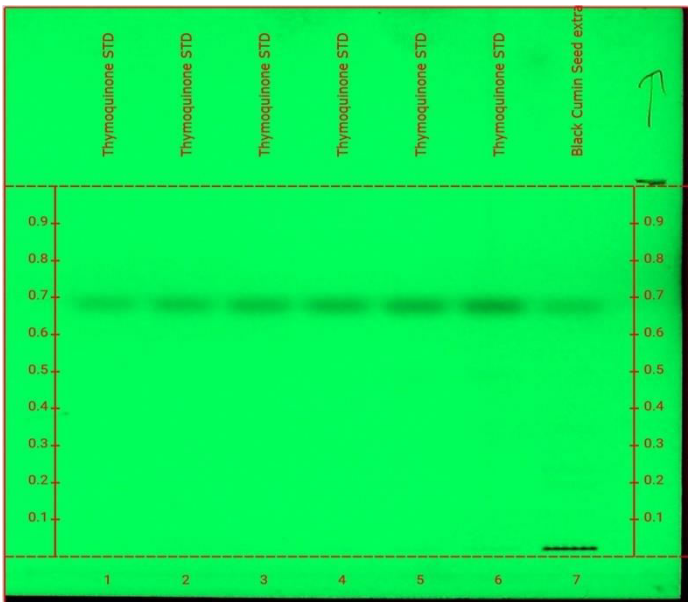

Figure 2: Thin Layer Chromatography (TLC) Analysis of Thymoquinone Standard and NS Extract

3.3 Description and Analysis of the Overlay Densitogram and 3D Densitogram

Thymoquinone is shown by the center peak in Figure 3, which is about at RF 0.68. The overlay densitogram in Figure 3 contrasts the chromatographic profiles of the Thymoquinone standard and the NS extract. The Y-axis displays the absorbance units (AU), while the X-axis shows the retention factor (RF), which is a range of 0.0 to 1.0. Thymoquinone is located in the center peak, at around RF 0.68. The close alignment of the peaks for the standard and the

extract with Rf 0.66 indicates the presence of thymoquinone in the (NS) extract. The anticipated range for Thymoquinone detection is indicated by the yellow dashed lines, indicating the accuracy and consistency of the employed chromatographic technology. The chromatographic profile of the Thymoquinone standard at five different doses is depicted in the 3D densitogram from Figure 4. The Retention Factor (RF), which ranges from 0.0 to 1.0, is represented by the X-axis. The Z-axis displays absorbance units (AU), while the Y-axis indicates the sample number or concentration. Every vertical peak in Figure 4 represents a distinct Thymoquinone concentration, which is regularly centered around an RF value of around 0.68. Thymoquinone detection has a high degree of linearity and repeatability, as seen by the peaks' consistent height and sharpness. This validates the chromatographic method's dependability for Thymoquinone quantitative analysis.

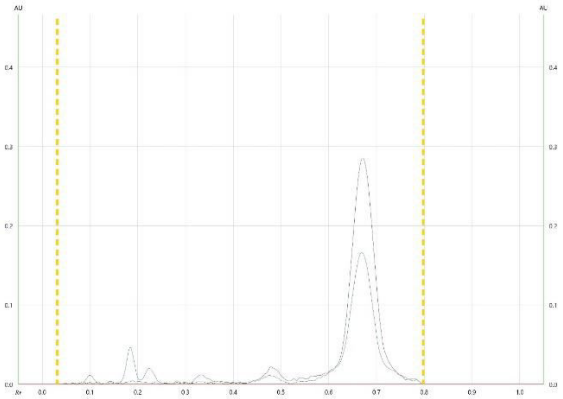

Figure 3: Overlay Densitogram of Thymoquinone and NS extract

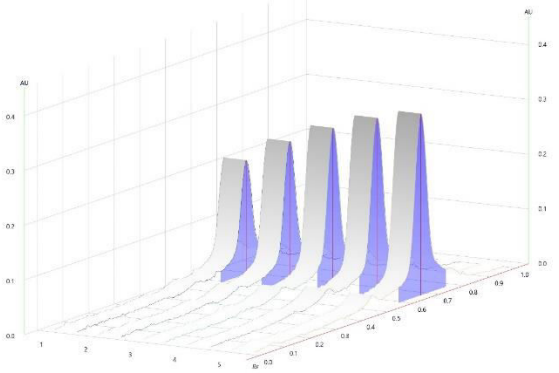

Figure 4: Densitogram of Thymoquinone Standard across Different Concentrations

3.4 Quantification of Thymoquinone from NS extract:

An HPTLC method for determining the concentration of Thymoquinone in an NS extract is described. The high correlation coefficient and calibration data in Figure 5 show that the method is dependable for quantifying Thymoquinone. The presence and concentration of Thymoquinone in the NS extract were confirmed by the identification and quantification of the particular peaks for Thymoquinone in both the sample and the reference standard. The report provides excellent precision and consistent results, validating the established HPTLC method. It was computed how much Thymoquinone was present in the NS Extract. 67.91 µg/mL was the average concentration that was discovered. The sample contained 679.1 µg in 10.000 mg of Thymoquinone.

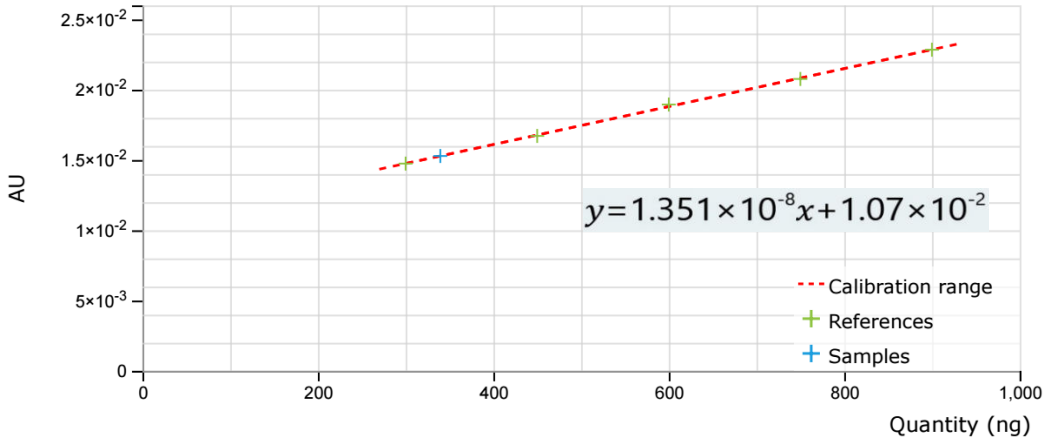

Figure 5: Area calibration for substance Thymoquinone STD at 260 nm

4. Conclusion:

In this work, a high-performance thin-layer chromatography (HPTLC) technique for the quantitative measurement of thymoquinone in black cumin seeds (Nigella sativa) was effectively developed and validated. The technique showed excellent consistency, sensitivity, and specificity, making it a dependable tool for thymoquinone measurement. Thymoquinone content was consistently found in all black cumin seed samples analyzed, demonstrating the method's resilience and suitability for use with a variety of seed batches and sources. Based on the study's findings, it can be concluded that the UV-visible absorption spectrum provides crucial information for thymoquinone identification and quantification in the NS extract. The detected peaks at 260 and 261 nm are consistent with the thymoquinone absorption characteristics that have been established, indicating that the extraction and isolation process was effective. Through TLC analysis, the thymoquinone content of the NS extract is satisfactorily demonstrated by comparison with the standard. The RF 0.66 value and fluorescence patterns that match thymoquinone identification with Rf 0.68 verify its identity, proving its effective extraction and its usage in pharmacological research. Overall, the safety and medicinal efficacy of black cumin seed products may be ensured by using this established HPTLC technique as a beneficial analytical tool for quality control and standardization. In order to improve yield and accuracy, future research might investigate the applicability of this technique to other herbal items containing thymoquinone and further refine the extraction and quantification.

References:

1. Heikes, D., Scott, B., &Gorzovalitis, N A. (2001, July 1). Quantitation of Volatile Oils in Ground Cumin by Supercritical Fluid Extraction and Gas Chromatography with Flame Ionization Detection. Oxford University Press, 84(4), 1130-1134. https://doi.org/10.1093/jaoac/84.4.1130
2. Johri, R. (2011, January 1). Cuminum cyminum and Carum carvi: An update. Medknow, 5(9), 63-63. https://doi.org/10.4103/0973-7847.79101 Ishida, M., Ohara, R., Miyagawa, F., Kikuzaki, H., Nishi, K., Onda, H., Yoshino, N., & Sugahara, T. (2022, June 25). Umbelliferose Isolated from Cuminum cyminum L. Seeds Inhibits Antigen-Induced Degranulation in Rat Basophilic Leukemia RBL-2H3 Cells. Multidisciplinary Digital Publishing Institute, 27(13), 4101-4101. https://doi.org/10.3390/molecules27134101
3. Basha, L I A., Rashed, M S., & Aboul-Enein, H Y. (2013, April 28). TLC Assay of Thymoquinone in Black Seed Oil (Nigella Sativa Linn) and Identification of Dithymoquinone and Thymol. https://www.tandfonline.com/doi/abs/10.1080/10826079508009224
4. Alam, P., Yusufoglu, H S., & Alam, A. (2013, December 1). HPTLC densitometric method for analysis of thymoquinone in Nigella sativa extracts and marketed formulations. Asian Pacific Journal of Tropical Disease Editorial Office, 3(6), 467-471. https://doi.org/10.1016/s2222-1808(13)60102-4
5. Hasrawati, A., Rizaldi, I., Febrianti, D., Mursyid, A M., & Bakri, N A. (2021, January 15). PREPARATION AND CHARACTERIZATION OF THYMOQUINONE NANOPARTICLES PEGYLATED AS DRUG DELIVERY SYSTEM. Society of Pharmaceutical Tecnocrats. https://doi.org/10.22270/ujpr.v5i6.506
6. Aboul-Enein, H Y., & Abou-Basha, L I. (1995, January 1). SIMPLE HPLC METHOD FOR THE DETERMINATION OF THYMOQUINONE IN BLACK SEED OIL (NIGELLA SATIVA LINN) Ghosheh, O., Houdi, A A., & Crooks, P A. (1999, April 1). High performance liquid chromatographic analysis of the pharmacologically active quinones and related

compounds in the oil of the black seed (Nigella sativa L.). Elsevier BV, 19(5), 757-762. https://doi.org/10.1016/s0731-7085(98)00300-8

7. Crooks, A L O O P O A G A A H P A. (1999, April 1). High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (Nigella sativa L.). https://www.sciencedirect.com/science/article/abs/pii/S0731708598003008
8. Alkharfy, K M., Ahmad, F J., Khan, R M A., & Al-Asmari, M. (2013, June 1). High-Performance Liquid Chromatography Of Thymoquinone In Rabbit Plasma And Its Application To Pharmacokinetics. Taylor & Francis, 36(16), 2242-2250. https://doi.org/10.1080/10826076.2012.717062
9. Pcrooks@pop.uky.edu, High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (Nigella sativa L.)..https://www.sciencedirect.com/science/article/pii/S0731708598003008
10. Habib, N., & Choudhry, S. (2021, April 20). HPLC Quantification of Thymoquinone Extracted from Nigella sativa L. (Ranunculaceae) Seeds and Antibacterial Activity of Its Extracts against Bacillus Species. Hindawi Publishing Corporation, 2021, 1-11. https://doi.org/10.1155/2021/6645680
11. Iqbal, M S., Ahmad, A., & Pandey, B. (2018, August 29). Solvent based optimization for extraction and stability of thymoquinone from Nigella sativa Linn. and its quantification using RP-HPLC. Springer Science+Business Media, 24(6), 1209-1219. https://doi.org/10.1007/s12298-018-0593-5
12. Bagul, M., Srinivasa, H., Padh, H., & Rajani, M. (2005, April 1). A rapid densitometric method for simultaneous quantification of gallic acid and ellagic acid in herbal raw materials using HPTLC. Wiley, 28(6), 581-584. https://doi.org/10.1002/jssc.200301695
13. Ishtikhar, M., Rabbani, G., Khan, S., & Khan, R H. (2015, January 1). Biophysical investigation of thymoquinone binding to 'N' and 'B' isoforms of human serum albumin: exploring the interaction mechanism and radical scavenging activity. Royal Society of Chemistry, 5(24), 18218-18232. https://doi.org/10.1039/c4ra09892g
14. Chen, J., Hong-mei, Z., Chu, V M., Jang, Y S., Son, J Y., Kim, Y H., Son, C G., Seol, I C., & Kang, J S. (2011, April 1). Quality control of a herbal medicinal preparation using high-performance liquid chromatographic and capillary electrophoretic methods. Elsevier BV, 55(1), 206-210. https://doi.org/10.1016/j.jpba.2010.12.022
15. K, S N S. (2015, January 1). The HPTLC Validated Method Development for the Quantification of Naringin from the Partially Purified Labisia Pumila Dichloromethane Extract. OMICS Publishing Group, 06(04). https://doi.org/10.4172/2157-7064.1000271
16. Nallamuthu, I., Parthasarathi, A., & Khanum, F. (2013, November 14). Thymoquinone-loaded PLGA nanoparticles: antioxidant and anti-microbial properties. , 2(12), 202-207. https://doi.org/10.3329/icpj.v2i12.17017
17. Jain, A., Sharma, T., Sharma, G., Khurana, R K., Katare, O P., & Singh, B. (2019, July 4). QbD-Driven Analytical Method Development and Validation for Raloxifene Hydrochloride in Pure Drug and Solid Oral Dosage Form. Taylor & Francis, 9(4), 463-477. https://doi.org/10.1080/22297928.2019.1624193
18. Hage, A., & Jadhav, A P. (2022, January 1). Development and Validation of High Performance Thin Layer Chromatography Method for Standardization of Polyherbal Formulation Using Mangiferin and Gallic Acid as Markers. Medknow, 84(4). https://doi.org/10.36468/pharmaceutical-sciences.1002