Linearity and standard curve

Calibration curves of THQ were constructed by using the analyte peak area versus nominal concentrations of the analytes. The linearity of the method was determined by analysis of standard plots associated with a six-point standard calibration curve of 0.07-12 µg/ml. The data were subjected to statistical analysis using a linear-regression model. Least squares linear regression analysis of the data gave slope, intercept and correlation coefficient data. Curves were best fitted using a least square linear regression model $y = mx + b$, weighted by $1/x^2$, in which y is the peak area ratio, m is slope of the calibration curve, b is the y-axis intercept of the calibration curve, and x is the analyte concentration. Back calculations were made from these curves to determine the concentration of THQ in each calibration standard, and the resulting calculated parameters were used to determine concentrations of analyte in quality control or unknown samples in plant extracts and formulations. The correlation coefficient $R^2>0.98$ was desirable for all the calibration curves.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantitation (LOQ) were determined by standard deviation (S y/x) method. For the determination of LOD and LOQ, blank sample was injected in triplicate to the chromatograph, and then peak area of this blank was recorded. The LOD and LOQ were determined using the slope of the calibration curve and $S_{y/x}$ of the blank sample by following formulae: $LOD = 3.3\times S_{y/x}/S$ and $LOQ = 10\times S_{y/x}/S$; where $S_{y/x}$ is the standard deviation of the blank response and S is the slope of the calibration curve.

Accuracy as recovery

Recovery was determined by standard addition method. The pre analyzed samples of THQ (10 µg/mL) were spiked with the extra 0, 50, 100, and 150% of the standard THQ, and the mixtures were reanalyzed by the proposed method. The percent (%) recovery of samples, percentage relative standard deviation (% RSD), and standard error were calculated at each concentration level.

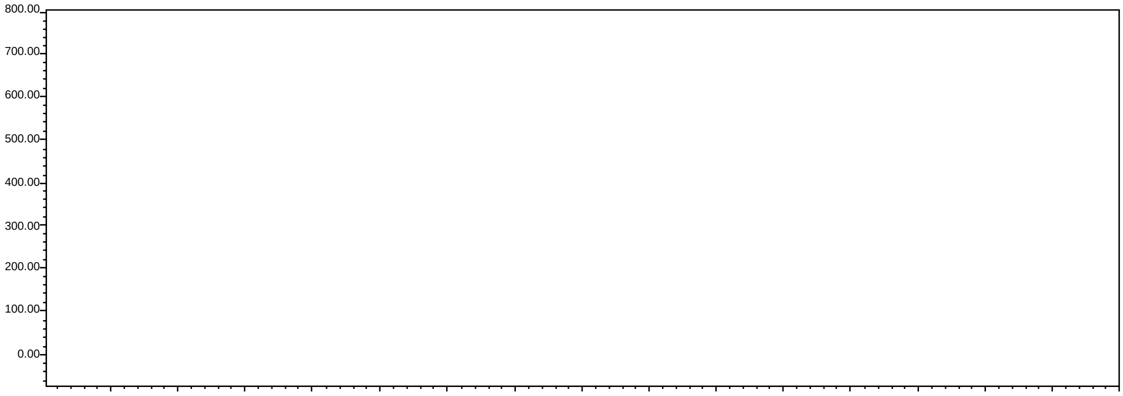
Precision and accuracy

The within-run and between-run accuracy and precision of the assays were determined by assaying calculated four quality control samples in triplicate over a period of three days. The concentrations represented the entire range of the calibration curves. The lowest level was the expected limit of quantitation (LOQ) for each analyte. The second level was within 3 times of LOQ. Calibration curves were prepared and analyzed daily and linear models were used to determine concentrations in the quality control samples. The nine measured concentrations per concentration level (triplicates from three runs) were subjected to estimate the between-run precision. Percent accuracy was determined (using the data from the precision assessment) as the but deliberate variation in the chromatographic conditions for the determination of THQ. Robustness of the method was determined by changing the flow rate (1.1 and 0.9 mL/min) and ratio (58:42 and 62:38) of mobile phase.

Quantification of THQ in extracts and formulations

Different concentrations (25, 50, 100 and 200 µl) of methanolic (5 gm/50 ml), petroleum extracts (5 gm/50 ml) and Baraka capsule (1.62 gm/25 ml) were diluted with methanol to make up the volume 1 ml. Similarly *Nigella sativa* oil (1 mg/ml) was first diluted with methanol to get concentration of 100 µg/ml. Then 10, 20, 40 and 80 µl of it was further diluted with methanol to make up the volume 1 ml. Vortex all

Citation: Iqbal M, Alam P, Anwer MT (2013) High Performance Liquid Chromatographic Method with Fluorescence Detection for the Estimation of Thymoquinone in *Nigella Sativa* Extracts and Marketed Formulations. 2: 655 doi:10.4172/scientificreports.655



Conclusion

In conclusion, a reliable HPLC fluorescence method with high sensitivity, robust, reproducible and short elution time was developed for the determination of THQ in extracts and various formulations. This method was validated for its selectivity, accuracy as recovery, precision and robustness and was successfully applied for the estimation of THQ in various extracts and formulations.

Acknowledgement

Authors are grateful to the Research Center of the College of Pharmacy and the Deanship of the Scientific Research of the King Saud University for their funding and support.

References

1. El-Fatatry HM (1975) Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. Pharmazie 30: 109-111.
2. El-Kadi A, Kandil O (1986) Effect of *Nigella sativa* (the black seed) on immunity. Bulletin of Islamic Medicine. Proceedings of the 4th International Conference on Islamic Medicine, Kuwait pp 344-348.
3. Gali-Muhtasib H, Roessner A, Schneider-Stock R (2006) Thymoquinone: a promising anti-cancer drug from natural sources. Int J Biochem Cell Biol 38: 1249-1253.
4. Khader M, Bresgen N, Eckl PM (2009) *In vitro* toxicological properties of thymoquinone. Food Chem Toxicol 47: 129-133.
5. Gheita TA, Kenawy SA (2012) Effectiveness of *Nigella sativa* oil in the management of rheumatoid arthritis patients: a placebo controlled study. Phytother Res 26: 1246-1248.

Citation: Iqbal M, Alam P, Anwer MT (2013) High Performance Liquid Chromatographic Method with Fluorescence Detection for the Estimation of Thymoquinone in *Nigella Sativa* Extracts and Marketed Formulations. 2: 655 doi:10.4172/scientifcreports.