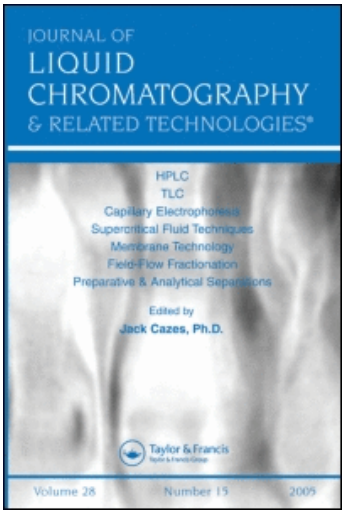

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Simple HPLC Method for the Determination of Thymoquinone in Black Seed Oil (Nigella Sativa Linn)

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SIMPLE HPLC METHOD FOR THE DETERMINATION OF THYMOQUINONE IN BLACK SEED OIL (*NIGELLA SATIVA LINN*)

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ABSTRACT

A simple and reliable isocratic normal phase HPLC method for the determination of thymoquinone in black seed oil (*Nigella Sativa Linn*, Ranculaceae) is described. After oil extraction with methanol, thymoquinone is analyzed using Econosphere CN column. The mobile phase consists of hexane:2 propanol (99:1 v/v), thymoquinone is monitored by UV detection at 295 nm. This method is quite specific and sufficiently sensitive with a lower limit of 5 nmoles/ml, within day and between-day assays showed variation coefficient below 5%.

INTRODUCTION

Nigella Sativa Linn (Ranculaceae), grows in Mediterranean countries and is cultivated in others. The black seed oil has a long history of folklore medicine in Arabian and other countries for the treatment of various diseases (1,2) such as; asthma, respiratory appression, cough, headache, diuretic, lactagogue and others. The main constituents of black seed oil are fixed oil, volatile oil and alkaloids.

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Investigators attributed the pharmacological activities of black seed oil to its thymoquinone content (the main constituent of the volatile oil) which represents 18.4 - 24% w/w of the volatile oil (3,4). Other constituents detected in black seed oil were thymol, dithymoquinone (thymoquinone dimer), monoterpenes, phenols and some ester (5,6).

El-Tayeb (7) described his own experience in folk medicine and the success he had achieved in treatment of various systemic and dermatological diseases following treatment of patients which extract of the black seed oil or seeds powder either alone or mixed with some other natural products. Abou-Basha et al (8) recently reported a thin layer chromatography quantitative assay of thymoquinone in black seed oil with a limit of detection of 100 nmoles/ml.

Accordingly, we describe here a simple, reliable, more sensitive, and rapid HPLC assay for thymoquinone in black seed oil that could be routinely performed in most laboratories.

MATERIALS AND METHOD

Chemicals

Thymoquinone authentic with 99.9% purity was purchased from Aldrich Chemical Co., Milwaukee, WI, USA. Hexane and 2 propanol were HPLC grade (Springfield, New Jersey, USA). The two black seed oil analysed were obtained from the local market.

Chromatography

The HPLC system consisted of a Bio-Rad 1350 solvent delivery pump, a Rheodyne model 7125 injector, a Waters Lambda Max 481 variable wavelength detector set at 295 nm and a Hewlett-Packard 3394 A integrator. The column used was CN normal phase (250 mm x 4.6 mm I.D., Econosphere™ CN, particle size 10 μ) purchased from Alltech Associates, Inc, Deerfield, IL.,USA.

Sample preparation

1 ml of methanol was added to 1 ml of oil (commercial black seed oil) in a glass centrifuge tube with cover. Vortex mix for 2 mins. The methanol top layer was transferred to a glass

tube. The methanol was evaporated under nitrogen stream. The residue was reconstituted with 1 ml of mobile phase and 20 μl injected into HPLC system.

RESULT AND DISCUSSION

Chromatograms

A typical chromatogram of thymoquinone was presented in Figure 1, Chromatogram of thymoquinone in oil was shown in Figure 2.

Thymoquinone has retention time of R_t 4.48 min at 295 nm. At this wave length there is no interference neither from dithymoquinone (thymoquinone dimer) nor from thymol. Dithymoquinone and thymol were detected at 260 and 275 nm respectively.

Linearity

The calibration curve of thymoquinone were constructed over the range of 0.1-30 nmoles with a correlation coefficient 0.999 (n=6). Each determination (n=6) of the thymoquinone content of black seed oil consisting of calibration curve and oil extracts of interest, were done on the same setting. The lower limit of detection was 5 nmoles/ml.

Variability and percentage recovery

High and low valued quality control sample (30 and 0.5 nmoles) were assayed six times a day on the same day and several days during a two-weeks period, to evaluate the precision of the assay. The within day variability (coefficient of variation) were 2.0 and 3.0 respectively for 30 and 0.5 nmoles. The day to day variation 3.5 for both values. The black seed oil sample 3 (1 ml) spiked with 500 nmol internal thymoquinone standard. The spiked sample extracted as in sample preparation and 20 μl injected into HPLC system. The spiked sample assayed 6 times during two weeks period. The recovery of thymoquinone from spiked sample 3 was 100% with a coefficient of variation 2.5%.

Table 1 summarizes the results for the quantitative assay of thymoquinone as the main active constituent of the volatile oil in black seed oil.

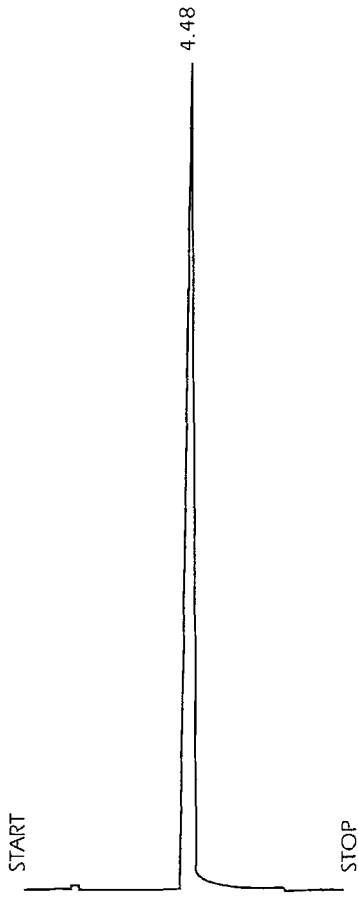

Figure 1. Chromatogram of thymoquinone authentic sample. Column CN (25cm x 4.6mm i.d., Econosphere CN, particle size 10μ); mobile phase: hexane:2-propanol (99:1 v/v); flow rate: 1ml/min; chart speed; 0.5cm/min; temperature: 23° C; detector; UV 295nm; sensitivity: 0.01aufs; sample quantity: 10 nmol.

It has been shown that the volatile oil revealed some pharmacological activities such as bronchodilators (9, 10, 11), increases bile flow and concentration of bile salts (12) and decreases blood pressure in dogs (13) and in rats (14). Indeed, Marozzi *et al* (15) claimed that the pharmacological activities of black seed oil is due to its thymoquinone contents which varies according to the method of manufacturing the oil. This is verified by the results shown

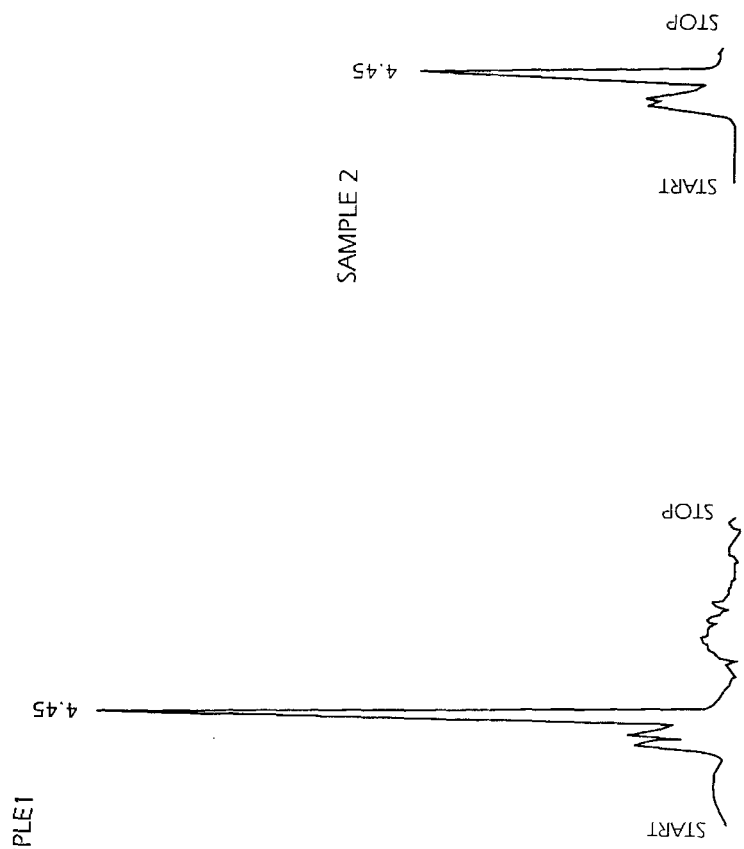

Figure 2. Chromatogram of oil sample 1 and 2. Column CN (25cm x 4.6mm i.d., Econosphere CN, particle sifze 10µ); mobile phase: hexane : 2-propanol (99:1 v/v); flow rate: 1ml/min; chart speed: 0.5cm/min; temperature 23°C; detector UV 295nm; sensitivity: 0.01aufs.

Table 1. Analysis of thymoquinone content in commercial black seed oils and sample 3 spiked with thymoquinone internal standard

Black seed oil	Mean* ± S.D. nmoles/20 µl	CV %	Thymoquinone nmoles/ml
Sample # 1	55.5 ± 1.78	3.2	8.3×10^3
Sample # 2	16.1 ± 0.58	3.6	2.4×10^3
Sample # 3	undetected	-	<5
Sample # 3 spiked	10.0 ± 0.25	2.5	0.1×10^2

* Mean of 6 determinations

in Table 1. Oil sample #1 has a higher thymoquinone content than oil sample #2 while thymoquinone is not detected in oil sample # 3. This requires the drug regulatory authorities to set up a standard limit for this active constituent (thymoquinone) in black seed oil, in order to set up a quality control criteria for this preparation.

CONCLUSION

A simple and reliable method for rapid determination of thymoquinone in black seed oil have been developed.

The HPLC procedure is sensitive with a lower limit of 5 nmoles/ml and a coefficient of variation less than 5% The method is suitable for routine analysis or thymoquinone in black seed oil.

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