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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF *NIGELLA SATIVA* SEED OIL AND GINGER EXTRACT IN THE SAME DOSAGE FORM

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ABSTRACT: The combination of *Nigella sativa* seed oil and ginger extract is used as antiulcer agent and to treat abdominal pain, diarrhoea and flatulence. The paper describes validated simultaneous equation method for the simultaneous estimation of *Nigella sativa* seed oil and Ginger extract in a combined dosage form. *Nigella sativa* seed oil and Ginger extract were found to have absorption maxima at 256 and 282 nm respectively in 5% SLS solution. Both these drugs obeyed Beer's law in the concentration range of 100-500µg/mL. The high values of correlation coefficients (r^2) indicated good linearity of calibration curve for both the drugs. The results of analysis have been validated statistically and by recovery study the value of standard deviation ranging from 97-99.21 % for *Nigella sativa* seed oil and 98-101.6 % for ginger extract were indicative of the accuracy and precision of the proposed method. The proposed method was successfully applied for the determination of *nigella sativa* seed oil and Ginger extract in the pharmaceutical formulation. This method was found to be simple, sensitive, accurate, precise and economical and applicable for the simultaneous determination of *Nigella sativa* seed oil and Ginger extract in combined dosage form.

INTRODUCTION: Nigella sativa is an annual flowering plant, native to south and southwest Asia. Nigella sativa oil contains an abundance of conjugated linoleic (18:2) acid, thymoquinone, dithymoquinone, melanthin, nigilline, damascenine, and tannins. Melanthin is toxic in large doses and nigelline is paralytic, so this spice must be used in moderation. Thymoquinone, found in the seed oil extract of N. sativa, has been shown to have anti-neoplastic effects in rats and mice and in cultured human cells from several types of cancer, including pancreatic ductal adenocarcinoma.

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It has protective antioxidant and anti-inflammatory [1-2] effects, and promotes apoptosis (cell death) of the cancer cells.

Ginger - the "root," or actually the rhizome, of the plant *Zingiber officinale* has been a popular spice and herbal medicine for thousands of years. It has a long history of being used as medicine in Asian, Indian, and Arabic herbal traditions. In China, for example, ginger has been used to help digestion and treat stomach upset, diarrhea, and nausea for more than 2,000 years.

Ginger is native to Asia where it has been used as a cooking spice for at least 4,400 years. It has been used to help treat the common cold, flu-like symptoms, headaches, and painful menstrual periods [3]. Ginger has been used to treat diseases related to gastrointestinal tract such as flatulence, indigestion, nausea and vomiting [4-6].

The ginger extract composes of oleoresin containing volatile oils of gingerols, shogoals, α-zingiberene, β- bisabolene, β- sesquiphellandrene and *ar*-curcumene [7].

Literature survey revealed that various analytical methods such as UV spectroscopy, HPLC, pulse polarography can be used for determination of two drugs. The UV spectrophotometric analysis[8] is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use.

The present study involves developing and validating spectrophotometric method for simultaneous determination of floating beads containing *Zingiber officinale* extract and *Nigella sativa* seed oil as a drug candidate, which remain in stomach or upper part of GIT for prolonged period of time, therefore the maximum drug release is maintained at desired site.

MATERIALS AND METHODS:

Instrument & Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1800, Japan), attached to a computer software UV probe 3.2, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Analytical balance (Sartorius Balance), volumetric flasks, pipettes of borosilicate glass.

Reagents and Materials

Nigella sativa seed oil was extracted with ether in laboratory, Ginger extract was received as a gift sample from Konark Herbals and Health Care, Nani Daman, Sodium Lauryl Sulphate, Distilled water, Whatman filter paper

Preparation of diluents

Sodium Lauryl Sulphate solution (5%). [Dissolve 5 gm of Sodiul Lauryl Sulphate (SLS) in 100 mL of water]

Preparation of Standard Stock Solutions

Accurately weighed *Nigella sativa* seed oil (100 mg) and Ginger extract (100 mg) was transferred to a separate 10 mL volumetric flask and dissolved and diluted to the mark with SLS solution to obtain a standard solutions having concentration *Nigella sativa* seed oil (10 mg/mL) and Ginger extract (10 mg/mL).

Method:

In simultaneous equation method, five working standard solutions having concentration 100, 200, 300, 400, 500 µg/mL for both *Nigella sativa* seed oil and Ginger extract (100 mg) were prepared in SLS solution and λmax of *Nigella sativa* seed oil and Ginger extract (100 mg) were calculated, absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations;

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2} \quad \ldots\ldots\ldots\ldots (1)$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2} \quad \ldots\ldots\ldots\ldots (2)$$

Where, Cx and Cy= Concentration of *Nigella sativa* seed oil and Ginger extract respectively.

A_1 = Absorbance of mixture at 256 nm

A_2 = Absorbance of mixture at 282 nm

ax_1 = Absorptivity of *Nigella sativa* seed oil at 256 nm

ax_2 = Absorptivity of *Nigella sativa* seed oil at 282 nm

ay_1 = Absorptivity of Ginger extract at 256 nm

ay_2 = Absorptivity of Ginger extract at 282 nm

Method Validation:

Linearity

Calibration curves were plotted over a concentration range of 100 – 500 µg/mL for both *Nigella sativa* seed oil and Ginger extract. Accurately measured standard working solutions of *Nigella sativa* seed oil (0.1, 0.2, 0.3, 0.4 and 0.5 mL) and Ginger extract (0.1, 0.2, 0.3, 0.4 and 0.5 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with SLS solution, and the absorbance was measured at 256 nm and at 282 nm for both drug. The calibration curves were constructed by plotting absorbance vs. concentration and the regression equations were calculated.

Precision:

Intraday Precision

Mixed solutions containing 100-500 µg/mL of *Nigella sativa* seed oil and 100-500 µg/mL of Ginger extract were analyzed 3 times on the same day and % RSD was calculated.

Interday Precision

Mixed solutions containing 100-500 µg/mL *Nigella sativa* seed oil and 100-500 µg/mL of Ginger extract were analyzed on 3 different days and % RSD was calculated.

Accuracy

The accuracy of the method was determined by calculating recoveries of *Nigella sativa* seed soil and Ginger extract in mixture by the standard addition method. Known amounts of standard amount of Ginger extract was added at 50, 100 and 150 % levels to pre-quantified sample solutions of 1000 µg/mL *Nigella sativa* seed oil + 1000 µg/mL Ginger extract mixture. The absorbance of *Nigella sativa* seed oil and Ginger extract were recorded at λ_1 and λ_2. The percentage recovery was calculated by measuring the absorbance of both drug at their absorbance maxima and fitting these values into simultaneous equation. Each response was average of three determinations.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response, S = Slope of calibration curve.

Analysis of Ginger extract and *Nigella sativa* seed oil in Combined Dosage Form

Beads equivalent to 0.3 gm of *Nigella sativa* seed oil and 0.1 gm of Ginger extract were weighed and transferred into 10 mL volumetric flask. Using this solution, dilution equivalent to 200µg/mL was prepared. Absorbance of the resulting solution was measured at 256 nm and 282 nm against SLS solution, relative concentration of two drugs in the sample was calculated using above equations (1) and (2).

RESULTS AND DISCUSSION:

In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all wavelengths, which was fulfilled in case of both these drugs. The two wavelengths used for the analysis of the drugs were 256 nm (λmax of *Nigella sativa* seed oil) and 282 nm (λmax of Ginger extract) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of *Nigella sativa* seed oil (256 nm) and Ginger extract (282 nm) in SLS solution is shown in **(Figure 1)**.

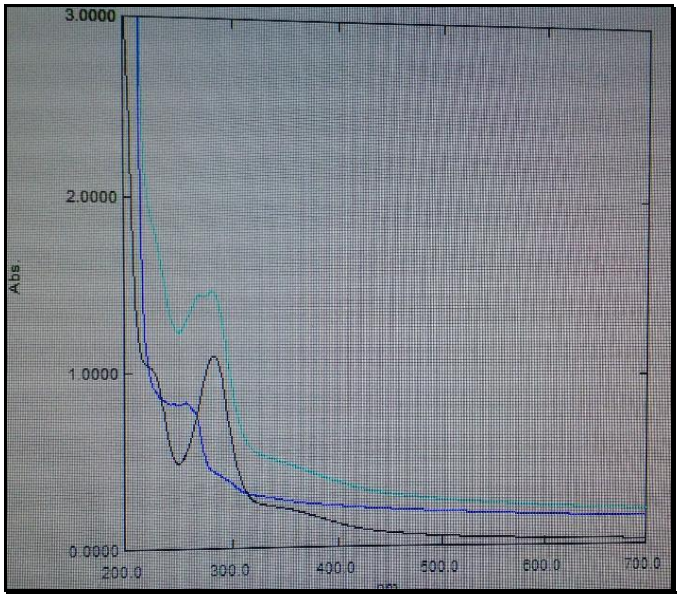

FIGURE 1: OVERLAIN ABSORPTION SPECTRA OF *NIGELLA SATIVA* SEED OIL (256 NM) AND GINGER EXTRACT (282 NM) IN SLS SOLUTION

Validation of the Proposed Method

Calibration curve

Linear correlation was obtained between absorbance versus concentrations of *Nigella sativa* seed oil and Ginger extract in the ranges of 100 – 500 µg/mL. Regression parameters are mentioned in table 1 and the calibration curves of these two drugs at 256 nm and 282 nm were validated by the high value of correlation coefficients of regression **(Table 1).**

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER FOR THE PROPOSED METHOD

Parameters	*Nigella sativa* seed oil	Ginger extract
Wavelength range (nm)	256	282
Beer's law limit (µg/mL)	100-500	100-500
Regression equation (y = mx + c)	y=0.0011x+0.0161	y=0.0011x+0.0118
Slope	0.0011	0.0011
Intercept	0.0161	0.0118
Correlation coefficient (r)	0.997	0.998
System Precision (%RSD)		
1. Intraday Precision (n=3)	0.55-0.86%	0.49-1.21%
2. Interday Precision (n=3)	0.84-1.38%	1.32-1.82%
Accuracy (% Recovery) (n=3)	97-103	98-104
LOD(µg/mL)	2.63	2.5
LOQ(µg/mL)	8.33	8.4
Assay (±SD) (n=3)	95.66±0.9	98±1.2

% RSD = Percent relative standard deviation
LOD = Limit of detection
LOQ = Limit of quantitation
SD = Standard deviation
n = number of replicates

The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined **(Table 2)**. The method was successfully used to determine the amounts of *Nigella sativa* seed oil and Ginger extract present in the beads **(Table 3 and Table 4)**.

TABLE 2: RECOVERY DATA FOR THE PROPOSED METHOD

Drug	Level	Amount of sample taken (µg/mL)	Amount added (µg/mL)	Amount added (%)	% Mean Recovery ± SD*(n=3)
Nigella sativa seed oil	I	200	0	0	97±1.2
	II	200	100	50	98±1.74
	III	200	200	100	99.25±1.23
	IV	200	300	150	99.21±1.25
Ginger extract	I	200	0	0	98±0.58
	II	200	100	50	99.33±0.96
	III	200	200	100	102±0.25
	IV	200	300	150	101.6±0.21

*SD is Standard deviation and n is number of replicates.

TABLE 3: FORMULATION COMPOSITION

Sr. no.	Ingredients	% w/v
1	Sodium alginate	3
2	Calcium Chloride	10
3	Ginger extract	1
4	*Nigella sativa* seed oil	3
5	Tween 80	1

TABLE 4: ANALYSIS OF *NIGELLA SATIVA* SEED OIL AND GINGER EXTRACT BEADS

Formulation	Amount added (%w/v)		Amount found (%w/v)		%Amount found	
Nigella sativa seed oil and Ginger extract Beads	*Nigella sativa* seed oil	Ginger extract	*Nigella sativa* seed oil	Ginger extract	*Nigella sativa* seed oil	Ginger extract
	3	1	2.87	0.98	95.66±0.9	98±1.2

CONCLUSIONS: The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of *Nigella sativa* seed oil and Ginger extract. The developed method was validated as per ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis of *Nigella sativa* seed oil and Ginger extract in their combined pharmaceutical formulations.

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