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Original Article

Evolution and Confirmation of UV Spectrophotometric Approach for Simultaneously Estimating Allicin and *Azadirachta indica* Extracts in Niosomal Formulation

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ABSTRACT

Background: Generally, it is quite difficult to precisely tell the exact amount of drug in any formulation after formulating it using the active ingredient and other excipients. **Aim:** Based on the simultaneous equation method in niosome formulation, a straightforward, quick, accurate, error-free and cost-effective spectrophotometric approach for the simultaneous quantification of Allicin and *Azadirachta indica* has been devised. **Materials and Methods:** The method involved estimation using two wavelengths. Since the absorbance of Allicin and *Azadirachta indica* reaches its maximum at 273 and 257 nm, respectively, these wavelengths were used to measure the absorbance and to estimate their relative concentrations. Allicin and *Azadirachta indica* both follow Beer-Lambert's rule at concentrations between 10 and 50 µg/mL. **Results:** The strategy can be applied for the regular estimation of Allicin and *Azadirachta indica* niosome formulation using the simultaneous equations as the concentration in the formulation and recovery were quite good (>95%) and reliable. It was designed and verified in accordance with ICH criteria. Methanol was used as a solvent for the process. **Conclusion:** The method proved to be useful, simple and precise for quantifying the extracts.

Keywords: Absorbance, Extract, Herbal, Precision, Wavelengths.

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INTRODUCTION

Allicin (diallyl thiosulfinate) having molecular mass 162.273 g/mol and molecular formula $C_6H_{10}OS_2$ is not stable to a considerable extant and quickly transformed to mono, di, trisulfides and other complexes. Allicin is an organosulfur chemical derived from garlic from family Amaryllidaceae. When fresh garlic is diced or crushed, the enzyme alliinase transforms alliin into allicin, which is responsible for fresh garlic's odour. Garlic and other Allium species contain this significant organosulfur molecule, which has a variety of biological actions including anti-viral, anti-fungal, anti-bacterial, and anti-parasitic effects.[1]

Originally isolated and characterized in 1944, allicin is sulfenic acid's thioester and is now the predominant and most physiologically active organosulfur component in garlic. The chemical allicin has a characteristic smell that is weakly dissolved in water and is reminiscent of freshly crushed garlic.[2] Alliin (S-allyl cysteine sulfoxide) which is a non-proteinogenic amino acid can be changed to allicin (Figure 1) when tissue damage occurs in response to the enzyme alliinase.[3] Due to its low molecular weight and beneficial lipophilic properties, allicin has a high permeability. Because of this, allicin can pass across blood-brain barriers and phospholipid bilayers with ease.[4] *Azadirachta indica (A. indica)* or Neem is also referred to as the margosa tree or Indian neem. Since ancient times, it has been widely utilized in homoeopathic, unani, and ayurvedic treatment. *A. indica* trees belong to the family Meliaceae, which is sometimes known as the mahogany family. They grow quickly and are known for their ability to withstand droughts. The *A. indica* tree spring up to be 150-200 years old, becoming a huge shade tree with a broad, rounded canopy.[5]

Concentrates of *Azadirachta indica's* leaves and bark have been studied for their antioxidant potential; the results of the investigation clearly demonstrated the strong antioxidant qualities of *A. indica* leaf and bark extracts/fractions cultivated in the lower regions.[6] According to the results of an examination, *A. indica* leaf concentrate administered orally at a concentration of 200 mg/kg showed significant anti-inflammatory efficacy in rodents' cotton pellet granuloma test.[7] Previous research has shown that oil seeds have antipyretic and anti-inflammatory properties, as well as immune modulating and anti-inflammatory effects when combined with concentrations of bark and leaves.[8,9]

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Other than these neem also shows various other medicinal effects like antiviral, antibacterial, hepatoprotective, wound healing, etc.

As allicin and *A. indica* have medicinal and therapeutic effects a method must be developed for the determination of the concentrations of both in a mixed formulation. Various methods using HPLC[10] and HPTLC[11] have been devised for determining the concentration of allicin in a formulation. The current paper throws light on a simple UV spectrophotometry-based estimation method for determining the concentrations of *A. indica* and allicin in a formulation.

Equipment and Procedure

Equipment

A two beamed UV spectrophotometer attached to a computer software UV probe 2.0, pair of quartz cuvettes, volumetric flasks, pipettes of borosilicate glass, ultrasonicator digital balance and lyophilizer.

MATERIALS AND METHODS

A. indica leaf powder was purchased from Manakarnika Aushadhalaya, Chinchwad, Pune. Allicin extract was purchased from Janani Organics, Hubballi. All chemicals and solvents used were as per analytical standards and were procured from Neeta chemicals, Chinchwad.

Procedures

Preparation of A. indica extract

Sufficient amount of *A. indica* powder was soaked in 500 mL of methanol and left for 3-4 days. Methanol was used due to the good solubility properties of *A. indica* in it. After the said period, the solution was filtered and the filtrate containing the extract was lyophilized to obtain a powder of the extract.

Preparation of caliberation graph

The standard blends of Allicin and *A. indica* extracts were made by mixing 10 mg of each extract separately in methanol. The end volume was made-up with methanol to obtain a blend composing 100 µg/mL of every extract. Functional solution of 10 µg/mL was examined in the wavelength span of 400-200 nm to check the λ_{max} of each extract. Calibaration curves were constructed to examine Beer-Lambert's Law and regression equations for Allicin and *A. indica* (Figures 2 and 3).

Simultaneous estimation of Allicin and *A. indica*

273 nm and 257 nm were selected as the working wavelength (λ_{max}) for Allicin and *A. indica* extract at which there shown no interference among the extracts. The overlay spectra for the same is shown in Figure 4 The absorptivity values of both the herbal extracts was determined at 273 and 257 nm. Using those values, two simultaneous equations were formed wherein equation (1) and equation (2) at the taken wavelengths. The distributions of both the herbal extracts in the niosomal formulation were computed using the pair of two simultaneous equations.

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

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

Cx: Concentration of Allicin in µg/mL,

Cy: Concentration of *A. indica* in µg/mL,

A_1: Absorbance of sample blend at 257 nm,

A_2: Absorbance of sample blend at 273 nm,

ax_1: Absorptivity of allicin at 273 nm,

ax_2: Absorptivity of *A. indica* at 257 nm.

Concentrations of both the extracts in the niosomal formulations can be calculated by decoding equations (1) and (2). Reliability of the equations was verified by using standard solution containing mixture of original samples of both the extracts, determining their absorbance at the taken wavelengths and computing concentration of both the constituents.

Examination of niosomal formulation

1 mL of niosomal solution containing 10 mg of both herbal extracts was added to a 50 mL flask. Then the extract was mixed in methanol. The specimen solution was strained using Whatman filter paper. The blend was diluted to a concentration of approximately 20 µg/mL. The absorbance of *A. indica* and allicin sample solutions was measured against a blank at 257 nm and 273 nm wavelengths, respectively.

Confirmation of the Method Established[12-16]

Linearity

For each extract, sufficient dilutions of standard stock solutions were tested using the proposed procedures. Beer-Lambert's concentration span for allicin and *A. indica* extracts was 2-10 µg/mL. The linearity data for the approach is shown in Table 1.

Accuracy

To validate the suggested approach, recovery tests were conducted at 80, 100, and 120% of the trial concentration following ICH recommendations. The retrieval research was conducted thrice at every stage. The recovery trials' outcomes are described in Table 2.

Distinctness

Interday and within the day distinctness

To measure interday and within the day distinctness, the sample solution was tested on the same and other days at varied time breaks (six repetitions). The results are shown in Table 3.

Ruggedness

It expresses the precision of laboratory variances, such as various analysts. To test the method's robustness, the standard was spiked three times by different analysts using the same equipment. The results have been provided in Table 4.

Capping of Detection

The quantitation capping is established by analyzing samples with familiar analyte concentrations and determining the minimal amount for accurate and precise quantification.

$$\text{Capping of detection} = \frac{3.3\sigma}{S}$$

σ=the standard error of the result,

S=the slope of the calibration graph.

Limit of calculation

The quantitation limit is often obtained by analyzing samples with familiar analyte concentrations and determining the minimal amount for accurate and precise quantification.

$$\text{Limit of calculation} = \frac{10\sigma}{S}$$

σ=the standard error of the result,

S= the slope of the calibration graph.

Formulation analysis

The analysis results of both the formulations were done using the simultaneous equations. The results are as shown in Table 5.

RESULTS

The linearity ranges for *A. indica* and allicin are 2-10 µg/mL at specific wavelengths. The coefficients of correlation for *A. indica* at 257 nm and allicin at 273 nm are 0.995 and 0.992, respectively. Both extracts have good regression readings at their specific wavelengths, and the retrieval study responses demonstrate that even tiny variation in drug concentration in the blend may be reliably identified by the suggested methodology. The percent analysis of *A. indica* and allicin in niosomal formulation was 13.47±1.274 and 15.25±1.534 standard deviation, respectively, with a standard deviation of less than 2.

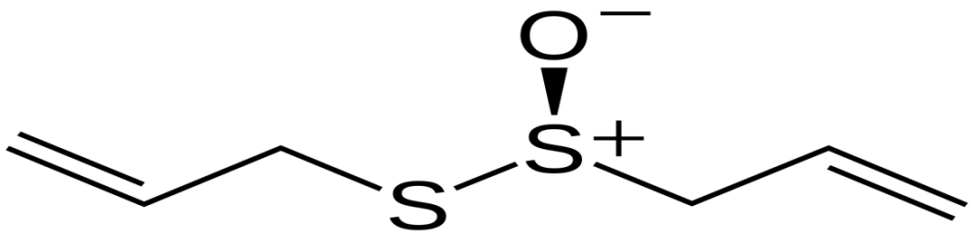

Figure 1: Chemical arrangement of Allicin.

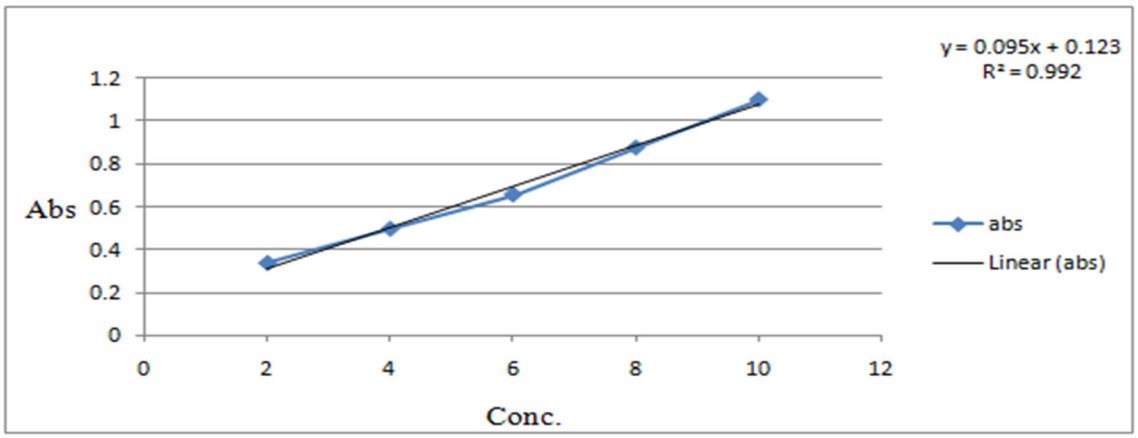

Figure 2: Caliberation curve of Allicin.

Table 1: Result of validation studies.

Parameters	*A.indica*	Allicin
Λ_{max}	257	273
Linearity range	2-10 µg/mL	2-10 µg/mL
Linearity equation	y = 0.056x+0.140	y = 0.095x+0.123
R^2	0.995	0.992
Slope	0.056	0.095
Capping of Detection	0.141 µg/mL	0.459 µg/mL
Limit of Calculation	0.257 µg/mL	0.673 µg/mL

Table 2: Retrieval studies.

Concentration of the extract put to the preparation	*A.indica* % Retrieval ±Standard Error*	% Comparative Standard Error	Allicin % Retrieval±Standard Error *	% Comparative Standard Error
80%	99.26±0.0055	0.24	99.68±0.0025	0.04
100%	99.57±0.0043	0.56	99.87±0.0052	0.126
120%	102.39±0.002	0.013	101.75±0.0026	0.47

*Mean of three calculations.

Table 3: Interday and within the day distinctness.

	Interday distinctness		Within the day distinctness	
Extract	% Quantity obtained ±SD*	% Comparative Standard Error	% Quantity obtained ±SD*	% Comparative Standard Error
A. indica	98.74±0.0048	0.67	96.76±0.0033	0.17
Allicin	97.01±0.0031	0.44	99.81±0.0037	0.67

* Mean of six calculations.

Table 4: Ruggedness analysis.

Niosome preparation	Extract	% Quantity obtained ± Standard Error*
Analyst 1	*A. indica*	99.67±0.0025
	Allicin	101.63±0.0032
Analyst 2	A. indica	99.17±0.002
	Allicin	102.79±0.0042

Table 5: Analysis of Formulation.

Formulation	Extract	Amount Found µg/mL ± S.D*
Niosome formulation	*A. indica*	13.47±1.274
	Allicin	15.25±1.534

*Mean of three calculations.

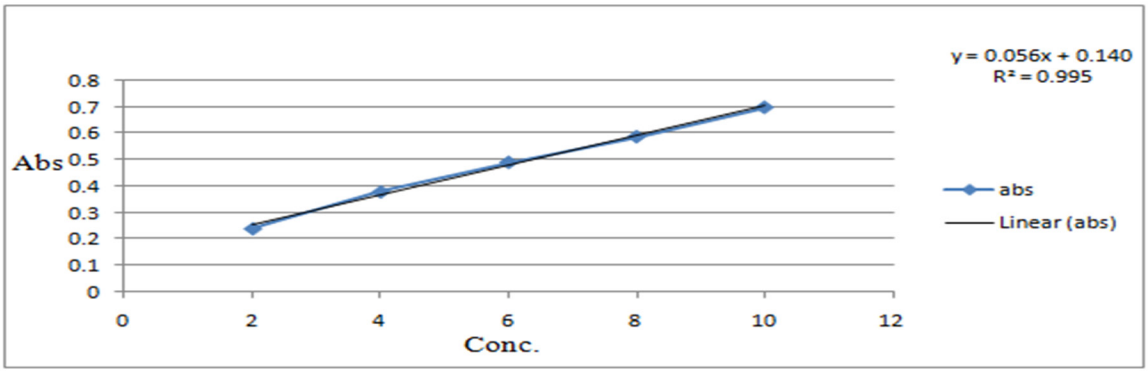

Figure 3: Caliberation curve of *A. indica.*

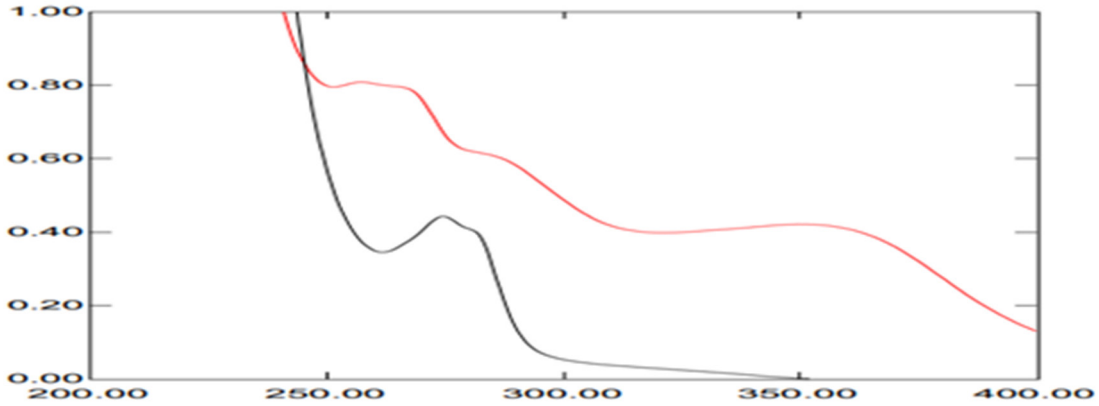

Figure 4: Overlay spectra of max absorption of Allicin and *A. indica.*

Precision is measured by examining both interday and intraday precision. In both intra and inter-day distinctness studies, % RSD of less than 2.0% shows good repeatability and intermediate accuracy.

DISCUSSION

The suggested spectrophotometric approach was found to be simple, fast, accurate, error-free, and cost-effective. It has been validated for linearity, accuracy, precision, specificity, and repeatability. This approach can accurately estimate Allicin and *A. indica* extract levels in niosome formulations. The study pointed out that the simultaneous equation method is quite useful for quantifying the drug in a formulation. The findings obtained in the study were quite precise.

CONCLUSION

The UV Spectrophotometric method for the simultaneous estimation of the herbal extracts in the formulation is easy, precise, accurate and can be used for other different kinds of drug estimations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***A. indica*:** *Azadirachta indica*.

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