Development And Evaluation of Herbal Formulation Containing Tulsi and Tabasheer Along with A Novel Method for Simultaneous Estimation of Selected Active Chemical Constituents of Tulsi and Tabasheer

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Abstract: Ayurvedic medicine play a major roles in treatment or cure of variety of diseases with the additional advantage of negligible side effects. Looking at the tremendous benefits of Ayurvedic formulation, a successful attempt has been made to develop a novel herbal Ayurvedic formulation using Tulsi and Tabasheer in the form of Churna. In the present study, we aimed to develop a churna containing Tulsi and Tabasheer as focused herbs for Ayurvedic formulation development. The developed churna is evaluated successfully along with the development of the novel analytical method for simultaneous estimation of selected active constituents Tulsi and Tabasheer. Based on the above findings, it can be concluded that the novel Ayurvedic formulation of Churna containing Tulsi and Tabasheer was successfully developed and evaluated. The novel analytical technique was also advanced or validated for the determinations of Carvacrol or Lignin as selected active chemical constituents of developed Churna formulation of Tulsi and Tabasheer. Result: The 3:2 ratio was discovered to be the best for preparing Tulsi and Tabasheer. For both herbs, the assessment parameters are within conventional limits. Carvacrol or lignin was discovered to have ranges of 10 to 50 g mL-1 or 10 to 40 g mL-1, correspondingly. For carvacrol or lignin, the LOD value was 1.65 g mL-1 but also 1.182 g mL-1, accordingly, while the LOQ values were 5.445 g mL-1 as well as 3.90 g mL. Tulsi or Tabasheer have 3.0% w/w carvacrol but also 1.7 percent w/w lignin in mixed Methanolic extraction of the 2 plants, according to this approach.

Keywords: Carvacrol, Derivative Spectroscopy, Evaluation, Herbs, Lignin, Tabasheer, Tulsi.

1. INTRODUCTION

According to World Health Organization (WHO), herbs as fresh or dried, powdered plants material that can be used raw or further processed and prepared to become finished herbal products/ Ayurvedic Products [1]–[3]. Herbs and various formulations containing herbs are currently used for a variety of purposes in trade and commerce. Tulsi is indeed an oral disinfectant as well as a natural mouth freshener. Mouth ulcers may also be treated with Ocimum Sanctum. While also safeguarding the teeth, holy basil kills the microorganisms that cause dental plaque, cavities, tartar, or foul breath. Various Ayurvedic formulas are used to cure a variety of ailments, including Arishta, Asava, Rasa Rasayan, Lauha, Vati, Churna, Avaleha, Ghrita, Parpati, Tailaa, and Guggulu. Tulsi's anti-inflammatory effects protect the eyes against viral, bacterial, and fungal diseases. It also decreases stress or calms eye irritation. Tulsi or Tabasheer are two of the most often utilized herbs for treating a broad range of ailments, particularly respiratory ailments. Carvacrol is just a polyphenol found in Tulsi, a herbal remedy. Tulsi or Tabasheer was chosen for innovative Ayurvedic formulation or development due to their vast application. No formulations have yet been established employing these two commonly utilized herbs.

There is a variety of chemical constituents present in Tulsi like Eugenol, Carvacrol, Oleanolic acid, Ursolic acid, and Rosmarinic acid. Similarly, various active chemical constituents of Tabasheer are cellulose, partisans, lignin, ash, silica, starch. Carvacrol or Lignin was chosen as markers for further analytic technique development or validation procedures from Tulsi and Tabasheer. Carvacrol (2-methyl-5 (1-methyl ethyl) phenols) is monoterpenes phenol found in Lamiaceae species such as Origanum, Satureja, Thymus, Thymbra, etc Coridothymus essential

oils. Origanum vulgar has the greatest naturally occurring concentration of carvacrol. CVC possesses a broad spectrum of biological activity, including antibacterial, insecticidal, antifungal, antioxidant, or anticancer properties [4]–[8]. The chemical structure of Carvacrol is represented in the following Figure 1.

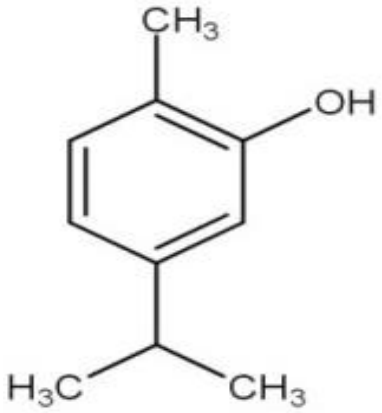

Figure 1: Illustrating the Chemical 2-Dimensional Structure of carvacrol

An alkyl-aromatic polymers found in terrestrial plants cell walls. Lignin gives plants structure but also stiffness, as well as acting as a natural, extremely effective microbial barrier or allowing water or nutrient movement throughout plant tissues. Lignin is chemically (1-[4-[1-[4-[2-[4-[5-(1,2-dihydroxypropyl)-3-(hydroxymethyl)-7-methoxy-2,3-dihydro-1-benzofuran-2-yl]-2-methoxyphenoxy]-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propoxy]-3-hydroxy-5-methoxyphenyl]-3-hydroxy-2-[4-[4-(4-hydroxy-3,5-dimethoxyphenyl)-1,3,3*a*,4,6,6*a*-hexahydrofuro[3,4-c]furan-6-yl]-2-methoxyphenoxy]propoxy]-3-methoxyphenyl]-2-[4-(3-hydroxyprop-1-enyl)-2-methoxyphenoxy]propane-1,3-diol. The chemical structure of Lignin is represented in following Figure 2.

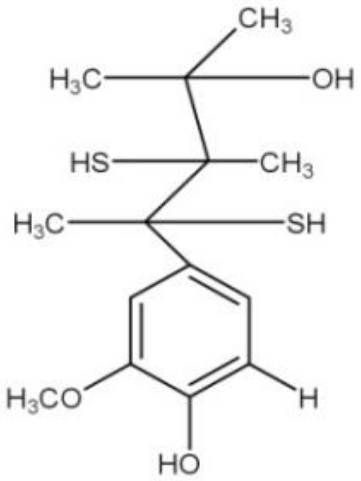

Figure 2: Illustrating the Chemical 2-Dimensional Structure of lignin

Some approaches for determining carvacrol and lignin from the raw material have indeed been disclosed. The measurement of carvacrol in several plant species has been reported using HPLC, spectrophotometric, and TLC techniques. For the measurement of lignin in diverse plant species, TLC, HPLC, spectroscopic, UV Spectroscopic, HPTLC, and spectrofluorimetric techniques have been published. Even though many analytical techniques for quantifying carvacrol or lignin from respective source plants are available in the literature, there is no way for determining both at the same time. Because both carvacrol and lignin absorb UV light strongly, it was assumed that developing a spectrophotometric approach for simultaneous measurement of both chemicals from extract mixes of the newly produced unique Churna would be useful. When compared to chromatographic or electrophoresis, spectrophotometric techniques of analysis are less expensive and easier to use [9], [10]. The goal of this study is to create and test a new Ayurvedic formula using Tulsi or Tabasheer. The chemical ingredients of the new ayurvedic formulation, namely Carvacrol or Lignin, would be determined concurrently using the extraction and spectroscopic approach. The recently developed spectroscopic approach for measuring carvacrol or lignin simultaneously will be evaluated according to the ICH requirements for analytical method validation.

2. LITERATURE REVIEW

P Prakash and colleagues looked into the medicinal properties of the Ocimum sanctums Linn the (Tulsi), focusing on the eugenol or its pharmacological effect. Various parts (seeds, leaves, stems, root, bloom, or, surprisingly, the entire plants) of the Ocimum sanctums Linn a small spice found through India, have indeed been suggested in traditional medical frameworks for the treatment of bronchitis, the runs, intestinal illness, constant fever, skin infections, joint inflammation, looseness of the bowels, agonizing eyes illnesses, bug chomps, and other ailments. Antifertility, antidiabetic, anticancer, antifungal, antibacterial, hepatoprotective, adaptogenic antiemetic, antispasmodic, cardioprotective, analgesic, or diaphoretic effects have also been claimed for Ocimum sanctums L. Eugenol Tulsi's medicinal activities are yet unknown because of a functional fixing identified in Ocimum sanctum L. Regardless of the facts that experts in traditional medicine have used Ocimum sanctum L. to treat a variety of illnesses due to its incredible restorative properties or limitless event in India, rational method to combining this traditional clinical practice with the modern medical frameworks isn't readily available [11].

Priya Panchal and her colleagues investigated the phytochemical study of therapeutic herbs (Ocimum sanctum). It's a fragrant plant. Plants have been used as sources of therapeutic substances by humans from the dawn of time. All things considered, everyday objects used to be the key wellspring. Tulsi's vitally compound constituents, such as oleanolic corrosive, ursolic acids, rosmarinic acids, linalool, carvacrol, eugenol, or caryophyllene, have been widely used in food, perfumery, as well as dental or oral items for just a long time, or plant separate keeps going to go the various searching inquiries for more viable plant beginning medications that are less harmful or accessible to low financial populaces Tulsi, like other current medications, may be a COX-2 inhibitor due to its high eugenol content, according to recent research. The goal of this research was to see how well aqueous extracts of Ocimum leaves screened for phytochemicals. This medicinal plant may be employed as pharmaceutical adjuvants in the creation of different dosage forms, according to research [12].

3. METHODOLOGY

3.1. Design:

All of the chemicals were of analytical quality, and pure water was utilized throughout the experiment. Benzchem Pharmacy pvt. ltd., Vadodara, India, provided pure carvacrol but also lignin. Tulsi or Tabasheer methanolic extracts were made in the lab. Commercially available Tulsi or Tabasheer herbs were used. Analytical but also pharmaceutical-grade chemicals were employed throughout. Throughout the investigation, only distilled water was utilized.

3.2. Sample:

According to the conventional technique of churna production by Ayurvedic formulary, a unique churna was produced utilizing Tulsi but also Tabasheer in 3:2 ratios for Tulsi or Tabasheer, accordingly. Tulsi and Tabasheer were identified to be best prepared in a 3:2 ratio. The evaluation parameters for both herbs are within standard limits. The concentrations of carvacrol but also lignin was found to be 10 to 50 g mL-1 or 10.01 to 40 g mL-1, correspondingly. The LOD values for carvacrol and lignin were 1.65 g mL-1 as well as 1.182 g mL-1, respectively, whereas the LOQ value was 5.445 g mL-1 but also 3.90 g mL. As per this method, Tulsi and Tabasheer have 3.0 percent w/w carvacrol but only 1.7 % w/w lignin in a combined Methanolic extract of the two plants.

3.3. Data Collections:

3.3.1. Preparations of the Formulation:

The churn was prepared using the basic procedure outlined in the Indian Ayurvedic Formulary, with the medications well washed and dried. Tulsi was finely ground and sieved to yield 6 grams. In the same way, 4 grams of Tabasheer powder was precisely weighed but also sieved. Even before actual forming preparation, both powders, as well as the moisture content, were verified or dried appropriately. Both powders were sieved at 85# and then combined with the specified percentage for full formulation production.

3.4. Data Analysis:

3.4.1. Standardization of Formulation:

- pH Value: A precise weight of 1.0g of the sample was transferred to a beaker with a capacity of 100mL. The pH meter electrodes were placed in the beaker and the reading was obtained after adding 100mL of water and shaking vigorously for around 1.0 minutes.

- Tapped Density: By accurately hitting a graded chamber to measure containing the examples so that no volume change is observable, the tapped not totally settled.
- Bulk Density: The mass to the volume ratio of the untapped powders samples is bulk density of material (including inter particulate voids volumes).
- Hausner's Ratio: Hausner's ratio is a statistic associated with the flow strength of powder or granular materials. It's the powder's tapped bulk density. Hausner's ratio is not an absolute attribute of a material; its value changes depending on the method used compute it.

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

- Carr's Index: The Carr index is a measure of how compressible a powder is. In pharmaceutical science, the Carr index is widely used to determine a powder's flow capacity.

$$\text{Carr's Index} = \frac{\text{Bulk Density} - \text{Tapped Density}}{\text{Tapped Density}}$$

- The angle of the Reposes: A granular material angle of repose, also known as the critical angles of the repose, is sharpest angle of the fall or dip compared to the horizontal planes to which it may be piled without slumping. This material on the sloping surface is on the edge of slipping at this angle. The angle of repose may be anything between 0 and 90 degrees.

$$\text{Tan } \theta = h/r$$

3.4.2. Preparation for the extracts of Tulsi and Tabasheer:

One gram of the aforementioned powder was extracted three times using three ten-milliliters of methanol, every time for one hour. For each medication, the mixed extracts were independently concentrated as well as the final volumes were adjusted to 25 ml using methanol.

3.4.3. Solutions:

a readymade solution Pure carvacrol and lignin sample of 10 mg mL-1 each were newly produced in methanol. In methanol, standard solution of Tulsi or Tabasheer extracts were produced.

All reagents were evaluated in solution as well as in the analysis for stability. The analytes' behavior remained unaltered up to 80 hours after preparation when maintained in the refrigerator or 72 hours when stored at room temperature. During every kind of experiment results, carvacrol or lignin were determined to be stable. At room temperature, every measurement was taken.

3.4.4. Development of an analytical technique for simultaneous detection of Tulsi or Tabasheer chemical components, namely carvacrol or lignin:

3.4.4.1. First derivative zero crossing method:

Between 200 and 800 nm, the absorptions spectra of the pure medication and its extract were obtained. While recording the main subsidiary spectra, the scaling factor was set to 1 and the delta Lamba was set to 10. The subsidiary proportion amplitudes were assessed against increasing groupings of unadulterated carvacrol and lignin to create adjustment diagrams. Inside the presence of the lignin, a wavelength of 244 nm was used for determining carvacrol since there is no interference from lignin. Similarly, in the presence of carvacrol, a 263 nm wavelength was used for lignin determination since there is no interference from carvacrol (Zero absorbance of carvacrol).

3.4.4.2. Validations Parameter:

- *Accuracy*

Recovery tests were used to assess the accuracy of the suggested approach as well as interference from the extract. The conventional addition technique was used to conduct the recovery studies. The investigation was carried out by adding known concentrations of carvacrol or lignin to samples that had already been examined. The standard recovery numbers were calculated using the mean recovery with percent relative standard deviation upper or lower limits.

- *Precision*

The developed method's intraday or interday accuracy was quantified in terms of percent RSD. For intraday precision, the trials were repeated five times a day, and for interday precision, they were performed five times on five distinct days. The % relative standard deviation was computed five times for both intraday and interday precision. Lastly, the mean percent RSD was determined (percent RSD = [S/X] 100, in which S is the standard deviations of the X is the mean of samples tested).

- *Limit of the detections (LOD) and limit of quantitation (LOQ):*

LOD and LOQ were determined using the 3 s/m or 10 s/m criteria, correspondingly, thus where is standard deviations of samples absorbance (n=10) and m seems to be the slope of suitable calibrations curve.

- *Robustness:*

The technique's robustness was created by summing a little alteration to the maximum wavelengths, as well as a slight modification to the extraction step by adding an extra 2ml of solvent. With these changes, the findings were tested for robustness, as well as values for both carvacrol and lignin were reported.

4. RESULTS AND DISCUSSION

The churn was prepared as per the general method described in the Ayurvedic Formulary of India. The original picture of the prepared Churna is represented in Figure 3.

Figure 3: The Original Picture of the Prepared Churna is Represented

The newly developed churna was tested for basic organoleptic properties which were represented in Table 1.

Table 1: Illustrating the Data of Organoleptic Parameters.

PARAMETER	OBSERVATION
Appearance/ form	Powder
Color	Greenish Brown
Taste	Bitter
Odour	Characteristic

The newly developed Ayurvedic formulation was standardized for various parameters and a summary of parameters is mentioned in Table 2, represented below.

Table 2: Data of Standardization Parameter of Newly Develop Ayurvedic Formulation.

Sr. No	Formulation	Parameter	Standard value	Observed value*
1.		pH Value	5.5 – 8	6.5 ± 1.024
2.		Bulk Density	0.18 – 1.25	0.49 ± 0.55

3.	TULSI + TABASHEER (FORMULATION)	Tapped Density	0.6 – 0.9	0.90 ± 1.24
4.		Hausner's ratio	1.4 – 2.9	1.83 ± 0.974
5.		Carr's index	0.30 – 0.60	0.45 ± 0.691
6.		Angle of Repose	25 - 50	42.33 ± 0.022

Chemical elements of Tulsi or Tabasheer, namely Carvacrol or Lignin, were chosen for further technological development or evaluation for simultaneous identification of both markers without previous separations. Between 10 g mL-1 as well as 5.00 g mL-1, and 10.00 g mL to 50 g mL, respectively, carvacrol or lignin conformed to Beer's rule. As seen in Figure 4, the absorption spectra of the two chemicals, Carvacrol and Lignin, were quite similar.

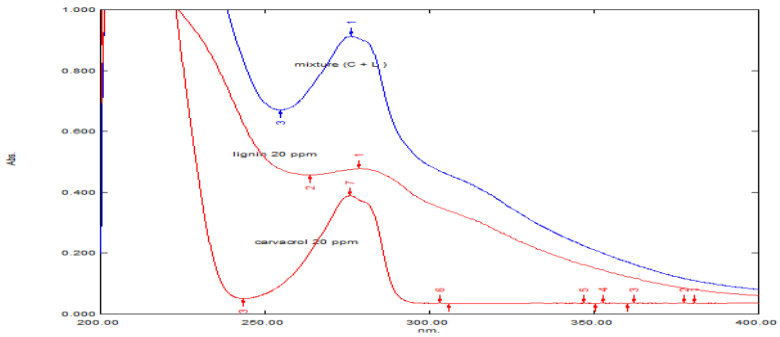

Figure 4: Zero-order Overlain Spectra of Carvacrol, Lignin, and Mixture of Carvacrol and Lignin.

Measurement results of absorption in zero-order spectra made simultaneous detection of the aforesaid substances impossible. As a result, first derivative spectroscopy was used to estimate all of the chemicals at the same time. Derivative spectroscopy has a higher resolution but also allows for the analysis of many drugs simultaneously without the need for pre-treatment or physical separation. Figure 5 shows the first derivative overlaying spectra of carvacrol or lignin.

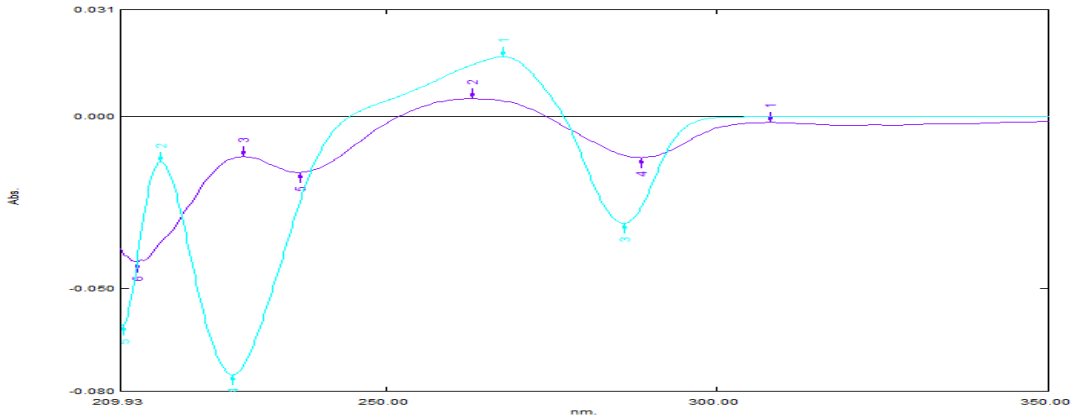

Figure 5: Overlay First Derivative Spectra of the Carvacrol and Lignin.

The derivatives ratio amplitudes were measured with increasing concentrations of pure Carvacrol but also pure Lignin to produce calibration graphs. Carvacrol in the presence of Lignin was determined at 244nm where there is no interference of Lignin. The spectra representing a zero-crossing point of Lignin were represented in Figure 6.

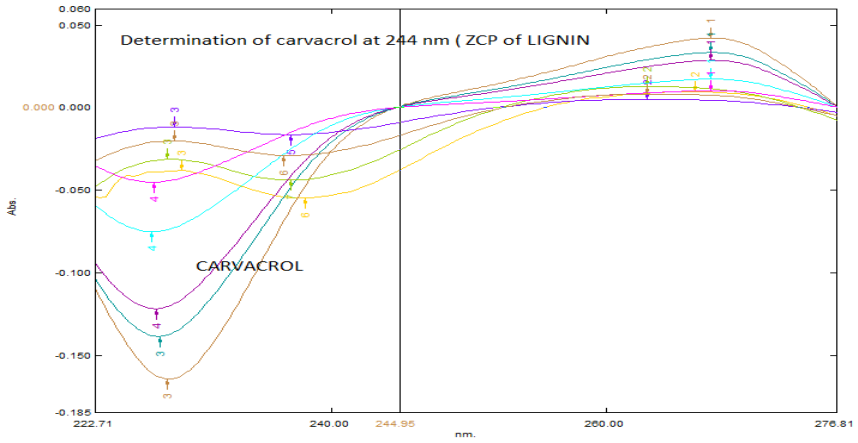

Figure 6: Determination of the Carvacrol Zero Crossing Point (ZCP) of Lignin at 244nm

Lignin, in the presence of Carvacrol, was determined at 263 nm where there is no interference of Carvacrol. The spectra representing a zero-crossing point of Carvacrol were represented in Figure 7.

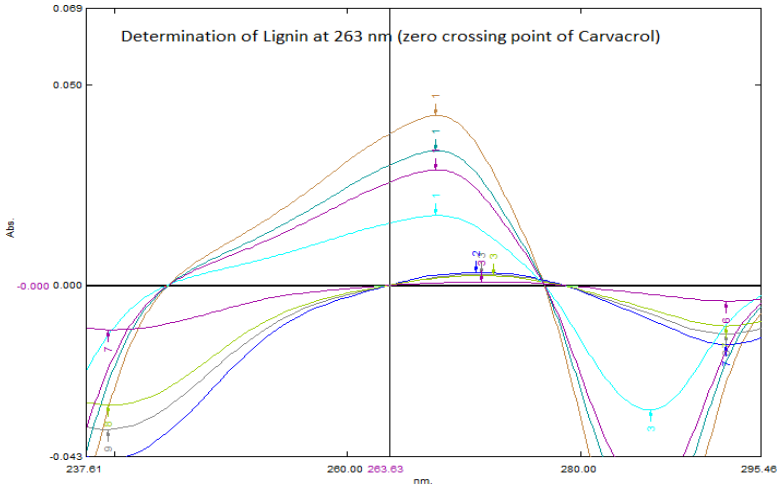

Figure 7: Determination of the Lignin at zero crossing point of Carvacrol

Between the concentrations of 10 - 50 g/ml but also 10 - 40 g/ml, carvacrol, as well as lignin, follow Beer's law. Tulsi but also Tabasheer extracts were combined and evaluated for carvacrol and lignin, respectively, using the suggested first-order derivative spectroscopic method. Tulsi methanolic extracts had 3.1 0.259 percent carvacrol, whereas Tabasheer methanolic extracts included 1.71+0.314 percent lignin. Table 3 shows the results of determining Carvacrol or Lignin using methanol extraction of Carvacrol or Lignin, both separately as well as concurrently.

Table 3: Determination of Carvacrol and Lignin Fro, Tulsi and Tabasheer Extracts

Sr. No	**Samples**	**% W/W of the markers compound*** ± SD	**Method of the determinations**
1.	Tulsi – Methanolic extracts (carvacrol)	3.1 ± 0.259	Zero orders UV-visibles spectroscopy
2.	Tabasheer – Methanolic extracts (lignin)	1.71 ± 0.314	Zero orders UV-visible spectroscopy

3.	Extracts of Tulsi or Tabasheer (mixture)	3.0 ± 0.512*[a] 1.7 ± 0.221 *[b]	First derivatives spectroscopy

*Mean value of three determinations

*a Markers compound of the samples of Tulsi

*b Markers compound of the samples of Tabasheer

The devised first orders derivative approach was successfully verified for simultaneous Lignin or Carvacrol determination. Table 4 shows the findings of recovery investigations, whereas Table 5 shows the results of different validation criteria.

Table 4: Result of Recovery Studies of Carvacrol or Lignin by the Developed Methods.

Sr. no	**The number of markers generated in extracts**		**(g mL-1) amount of pure markers added)**		**Percent Regaining**	
	carvacrol	**lignin**	**carvacrol**	**lignin**	**Carvacrol**	**lignin**
1.	3.0 ± 0.512%	1.7 ± 0.221%	1.4	1.4	97.66	99.66
2.			2	2	97.68	98.22
3.			2.6	2.6	98.68	98.25
Mean					97.66	98.22
Standard deviation					0.58	0.82

Table 5. Results of the Validation Parameter Obtained by Developed Methods for Carvacrol and Lignin

Validation parameter	**Results**	
	Carvacrol	**Lignin**
Analytical λ_{max}	244 nm	263 nm
Beer's law range (µg mL^{-1})	10 - 50 µg mL^{-1}	10 - 40 µg mL^{-1}
Slopes	0.001	0.0003
Intercepts	0.000	-0.0019
Correlations coefficients	0.998	0.998
Accurateness	97.66 ± 0.58	98.22 ± 0.82
Precision (%RSD)	1.1 07	0.973
LOD (µg mL^{-1})	1.65	1.182
LOQ (µg mL^{-1})	5.445	3.900
Robustness (%RSD)	1.142	1.579

The results of all the validation parameters are within the acceptance criteria for analytical method development and validation.

4. CONCLUSION

From overall investigation and experiments, it is concluded that the novel Ayurvedic formulation containing Tulsi and Tabasheer is developed and evaluated successfully for all the evaluation parameters of Ayurvedic formulation evaluation. The unique approach for simultaneous estimation of chosen markers of novel formulations has been effectively developed or verified. The newly devised derivative spectrophotometric approach for simultaneous quantification of carvacrol or lignin is simples, specific, accurates, precise, fast, and inexpensive, indicating its suitability for routine pharmaceuticals analysis.

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