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Original Research Article

Estimation of eugenol in different tulsi ayurvedic formulations by RP-HPLC method

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ABSTRACT

Aim: The aim of the present is to develop simple, accurate and precise RP-HPLC method for the quantification of Eugenol content in various tulsi based ayurvedic formulations.
Materials and Methods: HPLC was carried out by reverse –phase technique on a C18 column with a mobile phase composed of Acetonitrile: Methanol in the proportion of 50:50 v/v, at a flow rate of 1.0 mL /min. The detection was carried at 281 nm by UV- Visible detector. The linearity was established in the concentration range of 10-50 mcg/mL with an R^2 value of >0.999. Method and system precision was established with an RSD of < 2%. Accuracy was performed with standard addition spiking method and the results were found to be good. Robustness was established for change in flow rate, mobile phase composition and wavelength of detection. The assay of eugenol was performed in Eugenol drops, tablets, syrup and mother tincture and the results were reported.
Results: The method was found to be specific, precise, accurate and robust. The proposed method can be used for the reliable quantification of Eugenol.

1. Introduction

Eugenol, a phenolic compound (l-hydroxy-2-methoxy-4-allylbenzene) is one of the important bioactive compound that is present in Ocimum sanctum Linn (Tulsi) in the range of 40-70% which varies from one plant to the other.[1] The widely accepted functional properties of eugenol are its free radical scavenging activity, prevention of reactive oxygen species (ROS) & reactive nitrogen species (RNS) generation, DNA and protein damage, and elevation of cellular anti-oxidant potency.[2] Tulsi is one of the most commonly used herb in many of the ayurvedic formulations like tablets, syrups, capsules and drops etc. Most of the analytical methods reported in the literature were used to estimate eugenol quantity in various extracts of tulsi plant and other types of formulations containing clove oils etc., but not for the tulsi ayurvedic formulations.[3–14] The current aim of the work is to develop and validate RP-HPLC method for the estimation of eugenol in selected ayurvedic tulsi formulations as per ICH guidelines.[15]

2. Materials and Methods

2.1. Instruments and software

Shimadzu Prominence Liquid Chromatograph equipped with LC 20AT pump and SPD-20A UV -Vis detector, Phenomenex Kinetex C18 column (4.5x250 mm, 5μ) and Rheodyne injector is used for the estimation of eugenol in the selected formulations.

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2.2. Materials and solvents

Pure eugenol standard was purchased from TCI Pharma Pvt. Ltd. Tulasi Tablets manufactured by Revinto life science Pvt. Ltd., Satayu Tulasi Drops manufactured by Vita health Pvt. Ltd., COF-15 Syrup manufactured by Revinto life science Pvt. Ltd., and Tulsi mother tincture were purchased from local market. Acetonitrile and Methanol of HPLC grade were purchased from Merck life sciences Pvt.Ltd.

2.3. Method development and validation of reverse phase high performance liquid chromatography

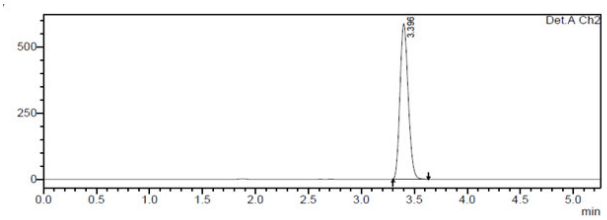
10 mg of Eugenol was weighed and dissolved in few ml of methanol in 10 mL standard flask. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 mg/mL. Further dilutions were made to get required concentrations of 10-50 mcg/mL. Several chromatographic trials were performed to evaluate the parameters like retention time, tailing factor, theoretical plates and capacity factor etc., are within the limits or not. The different assay solutions were prepared to get the required concentration that encompasses the selected linearity range of the standard eugenol solutions.

3. Results and Discussion

After the assessment of the chromatographic parameters obtained from several trial runs, the trial performed with a mobile phase composed of Acetonitrile: Methanol in the proportion of 50:50 v/v, at a flow rate of 1.0 mL /min and detection at 281 nm by UV- Visible detector gave the parameters which are within the limits. A single distinct peak corresponding to eugenol at a retention time of 3.372 min was observed in the chromatogram as shown inFigure 1. So, this condition is optimized for the method validation. Specificity of the method was confirmed by injecting the blank sample which doesn't give any peak at the retention time of the eugenol. Linearity of the method was established by using eugenol standard solutions in the range of 10-50 mcg/mL as shown in Table 1. The correlation coefficient was greater than 0.999 as shown inFigure 2 System precision was established by injecting six standard injections of eugenol in to the LC and the % RSD was found to be less than 2%. Similarly, method precision was established by injecting six assay samples in to the LC and the % RSD was less than 2%. Accuracy was established by using standard spiking method in the levels of 80-120% of the target concentration. % recovery was found to be in the range of 98-102% which was very good. Robustness of the developed method was established for various parameters like change in flow rate, mobile phase composition and wavelength. All the subtle changes doesn't impact the method parameters as all the obtained values were within the acceptance limits as shown in Table 2. Assay of various ayurvedic samples were found to be 11.2 mg/mL for Tulsi drops, 12.6 mg/mL for tablets, 29.1 mg/mL for syrup and 30.4 mg/mL for mother tincture.

Table 1: Linearity of eugenol by RP-HPLC method.

S.no	Concentration (mcg/mL)	Peak area
1	10	268937
2	20	545732
3	30	830437
4	40	1151120
5	50	1456775



Peak at 281 nm	Theoretical plates	Tailing factor	Retention time (Rt)
1.	7629.22	1.230	3.372

Fig. 1: Optimized chromatogram of eugenol standard

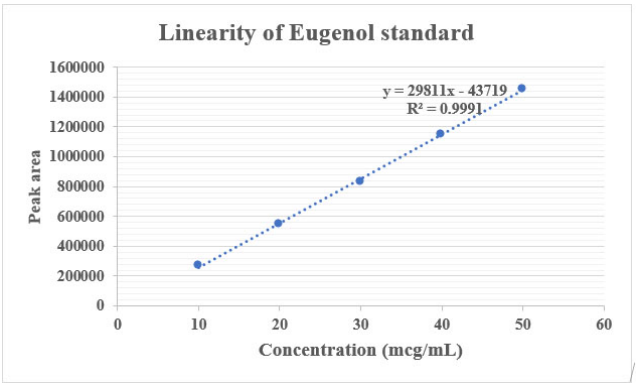


Fig. 2: Calibration curve for eugenol by RP-HPLC method

4. Conclusion

An attempt was made to develop and validate RP-HPLC method for the estimation of eugenol in various tulsi ayurvedic formulations as per ICH guidelines. The developed method was found to be specific, precise, accurate and robust. The proposed method can be used for the reliable quantification of Eugenol.

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Table 2: Robustness parameters for eugenol by RP-HPLC method

Eugenol Parameters	Condition	System Suitability Parameters		
		Theoretical plates	Tailing factor	Retention time
Change in flow rate	0.8mL/min	7873	1.239	3.370
	1.2mL/min	7970	1.230	3.686
Change in detector wavelength	278 nm	7833	1.230	3.369
	284 nm	7759	1.257	3.369
Change in mobile phase ratio	55:45 (Acetonitrile:Methanol)	3919	1.210	3.424
	45:55 (Acetonitrile:Methanol)	4729	1.226	3.430

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7. Conflict of Interest

The author declares that there is no Conflict of interest.

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