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QUANTIFICATION OF EUGENOL (FROM CLOVE OIL) IN MARKETED HERBAL TOOTHPASTE PRODUCTS

Ashutosh Jadhav* and Akshay Charegaonkar

Abstract: A straightforward and accurate HPTLC (high-performance thin-layer chromatographic) approach has been created and validated for the quantification of eugenol in commercial toothpaste samples. Using toluene-ethyl acetate (97:3) as the mobile phase, chromatographic separation was performed on pre-coated silica gel F254 HPTLC plates along with standard eugenol. The UV absorbance spectrum of eugenol indicated 281 nm as its lambda max and hence this wavelength was used for quantification. Linearity, precision, selectivity, limit of detection, limit of quantification, and accuracy of the method were all validated as per ICH Q2R1 guidelines. A correlation coefficient of 0.9967 was obtained from linear calibration curve in the 2 to 14 µg/band range. The relative standard deviations for the intra-day (n = 8) and inter-day (n = 8) precision, respectively, ranged from 1.78% to 2.34% and from 1.78% to 2.11%. The limits of detection and quantification were found to be 2.156 g band-1 and 4.581 g band-1, respectively. The accuracy from real life samples was determined to be 97.00% when expressed as a percentage of recovery. The method was specific and did not exhibit matrix interference. This is an affordable, easy to use, reliable method and suitable for use by QC and regulatory labs.

Keywords: Eugenol, Clove oil, herbal toothpaste, High performance thin layer chromatography/ HPTLC, anti-inflammatory, antioxidant, antimicrobial, traditional medicine, oral hygiene.

Introduction*:* Natural clove oil has been used for its medicinal properties for a very long time. It is produced from dried flower buds of a indigenous tree to Indonesia i.e. clove tree. A substance called eugenol found in clove oil has been demonstrated to have analgesic and anti-inflammatory effects. Clove tree is a medium-sized tree that is also known by the names Syzygium aromaticum, Eugenia aromatica, Eugenia caryophyllata, or Eugenia caryophyllus. It is a member of the Myrtaceae family and possesses a variety of biological qualities, including antioxidant and antibacterial capabilities. (D.D. Shete, M.A. Patmas, S.P. Patil, 2020)

Because of this, it has been recommended in traditional medicine as a therapy for a range of conditions linked to inflammation and infection. In traditional medicine, clove oil and bud are used as analgesics to relieve tooth pain. Clove buds contain flavonoids, phenolic acids, and derivatives of hydroxyl-benzoic, hydroxy-cinnamic, and hydroxyl-phenyl propane acids. Caffeine, ferulic, elagic, gallic, and salicylic acids are examples of phenolic acids present. Additionally, clove buds contain about 18% of clove oil, an essential oil that also contains trace levels of farnesol, benzaldehyde, 2-heptanone, -ethyl hexanoate, -caryophyllene, -humulen, -pinene, -limonene, and -eugenyl acetate. The primary phytocompound of cloves and clove oil i.e., eugenol (C10 H12 O2), has a spicy aroma and is a phenolic phenyl propane (2-methoxy-4-(prop-2-en1-yl) phenol) that is yellow and oily.

In addition to clove, eugenol has been found in other biological sources, e.g., flowers of the Tilia species and the Tolu balsams of *Myroxylon balsamum* (Leguminosae). the underground parts of Valeriana officinalis (Valerianaceae), *Pimenta dioica* (Myrtaceae) fruits, *Myristica fragrans* (Myristicaceae) oil from the kernels,etc.

Eugenol has inhibitory effects on *S. mutans* at a variety of levels, including suppressing the production of acid,

***Corresponding author**

Anchrom Customer Support Laboratory, A-101, Shree Aniket Apartments, Navghar Rd, Mulund (E), Mumbai 400081, India

E-mail: ashutoshjadhav0603@gmail.com

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the synthesis of water-insoluble glucans, and the ability of S. mutans to adhere to hydroxyapatite beads. The gram-negative oral pathogens Prevotella intermedia, Porphyromonas gingivalis, and Actinomyces viscosus are particularly susceptible to the anti-microbial effects of eugenol. Eugenol therapy is a simple but potent therapeutic strategy that can prevent dental issues. (Diego Francisco Cortés-Rojas, 2015)

Materials and methods:

Materials: Eugenol standard was purchased from Gala Ayurvedic Bhandar, Mulund, Mumbai, India and five toothpastes (3 samples and 2 blanks) were purchased from Apollo pharmacy, both from, Mulund, Mumbai, India.

Reagents and standards: The analytical-grade chemicals and solvents used were all purchased from E-Merck.

Preparation of solutions:

Standard preparation: Weigh 1 mg of eugenol in 1 mL of chloroform **(A)** and 1 mg of clove oil in 1 mL of chloroform **(B).**

Sample preparation: Weigh 1000mg toothpaste sample in tarson tubes, add 5 mL of water. Vortex it thoroughly until the paste is completely dispersed in water. Add 3 drops of 1- Octanol (used as defoaming agent) and 5mL chloroform. Vortex the solution for 2 minutes and centrifuge it at 2000 RPM for 5 minutes. After centrifuge discard the supernatant and add 5 mL of chloroform, filter, and use the filtrate as the sample.

Instrumentations and chromatographic conditions

The HPTLC procedure follows the USP/EP/IP guidelines i.e., the application of the eugenol was performed in band length of 8 mm with a CAMAG Switzerland 100 µL sample syringe (Hamilton, Switzerland) on the HPTLC Aluminum Silica Gel 60 F254, 20x10cm plates (Merck Catalogue No. 1.05548.0007) with 200 µm thickness [using a (CAMAG Switzerland) Automatic TLC sampler applicator. 1 µL/s for standards and 10 µL/s for samples was the application speed with 13 mm center to center spacing between two bands. Linear ascending development was executed in a 20 cm × 10 cm twin trough glass chamber (CAMAG). An Automatic Development Chamber (CAMAG) with 33% relative humidity was used. It was saturated with the mobile phase vapor (Toluene: Ethyl acetate 97:3 v/v.). The saturation time for the mobile phase in the chamber lined with saturation pad was 20 min at room temperature (25ºC±2ºC). The plate was developed to 70 mm from bottom edge. (CAMAG) TLC Scanner-IV coupled with (CAMAG) visionCATS software was employed for densitometric scanning in the reflectance absorbance mode, keeping the slit dimension 6 mm × 0.45 mm with a scanning speed of 20 mm/s at 281nm. Peak areas with linear regression were utilized for the evaluation.

Results:

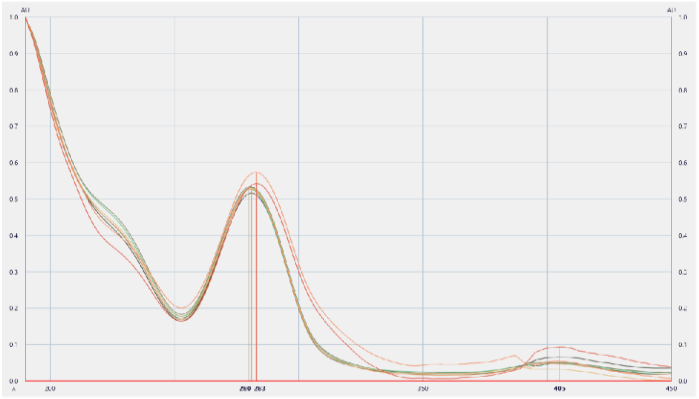


Fig 1: Absorbance spectrum of eugenol indicated a λmax of 281 nm. The spectra of the eugenol at 0.45 Rf matches with corresponding bands at 0.45 Rf from all 3 samples.

Using CAMAG densitometric scanning, multi-level quantification was done, and the amount of eugenol was found to be 0.5857%, 0.6751% and 0.1782% respectively in the toothpaste sample.

Specificity

Toothpaste samples and the internal standard were well separated and were efficiently detected at the Rf of 0.45 (±0.02) The Rf and UV absorbance spectra of the reference standard and the sample matched exactly, indicating the specificity of the method. The blank samples did not generate any peaks at the RF of the analyte, indicating the absence of matrix interferences.

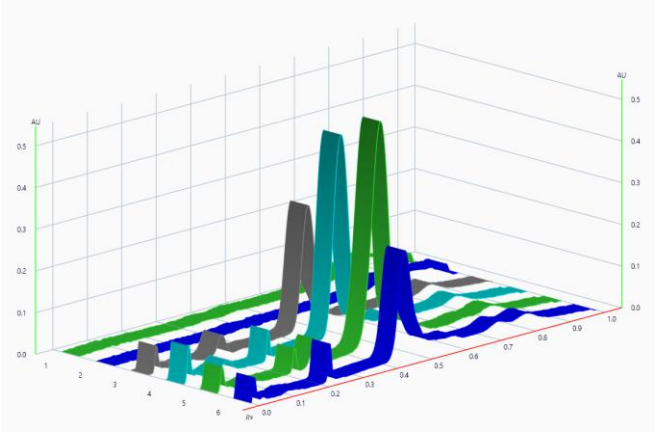


Fig 2: Track 1- diluent, track 2 - mobile phase, track 3 – Eugenol standard, track 4 – sample 1, track 5- sample 2 and track 6 – sample 3.

Precision and accuracy: Precision and accuracy of the HPTLC method were determined by carrying out intra-day and inter-day analyses of the three quality control samples in replicates. The relative standard deviation for precision ranged from 1.78% to 2.34% and from 1.78% to 1.81% for intra- and inter-day analysis, Accuracy was calculated and found out between 97.02%.

Concentration [µg band -1]	RSD intra-day (n = 8)	RSD inter-day (n = 8)
5	1.78	1.48
	1.89	1.81
	2.34	2.11

Table 1: Represents the precision estimated from market samples which is calculated as % RSD.

Calibration curve:

The amount of standard which was applied on the plate was 2, 4, 6, 8, 10, 12, 14 µl, and the sample applied was from 5 µl and 10 µl. The regression mode was kept at linear 2 and linear regression line is y = 2.391 x 10^{-9} χ + 3.431 x 10^{-3}, coefficient of variance is 2.01 % and correlation coefficient is 0.999.

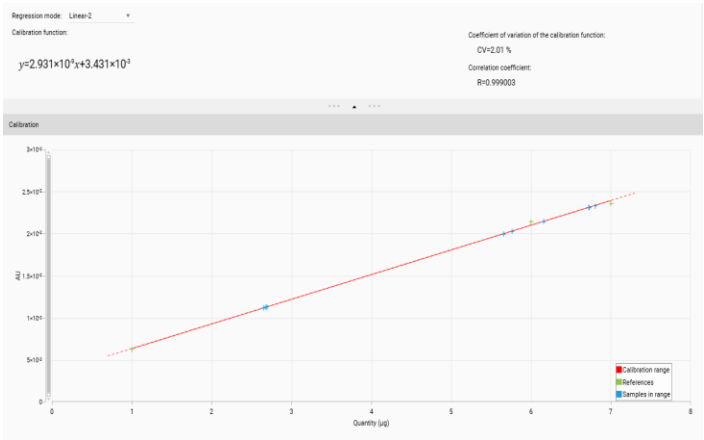


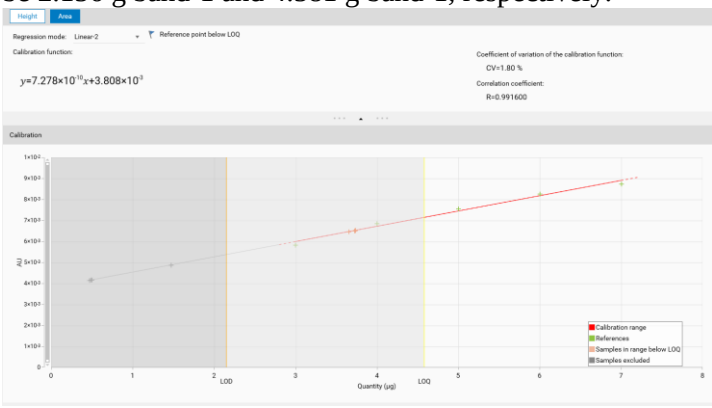
Fig 3: Calibration curve.

Sam,	Amount of Eugenol present in 1 gram toothpaste (in weight percentage)
Market toothpaste sample 1	0.6921%
Market toothpaste sample 2	0.6394%
Market toothpaste sample 3	0.1717%

Table 2: The amount of eugenol (used as marker for clove oil) found in herbal toothpaste samples.

Limit of detection (LOD) and Limit of quantification (LOQ):

The limits of detection and quantification were found to be 2.156 g band-1 and 4.581 g band-1, respectively.



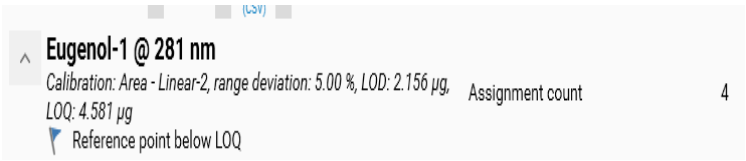


Fig 4: LOD and LOQ.

Ruggedness and robustness:

The method's ruggedness and robustness were evaluated by intentionally introducing minor variations into the optimized chromatographic conditions. Factors modified were saturation time and the volume of band application etc. Additionally, adjustments were made to the development process as below.

Parameters	Eugenol Rf	Observation
Saturation time (20 minutes)	0.4[illegible]	Robust
Saturation time (30 minutes)	0.46	Robust
Volume of application (4 µl)	0.45	Robust
Volume of application (5 µl)	0.45	Robust
Twin trough chamber (standalone)	0.44	Rugged
Automatic development chamber	0.45	Rugged

Table 3: Represents the robustness and ruggedness of the method.

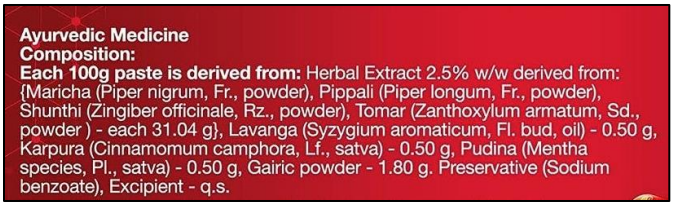


Fig 5: Label claim for toothpaste 1

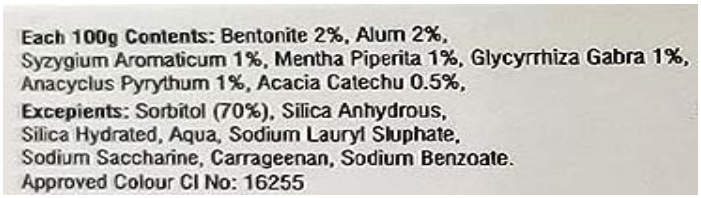


Fig 6: Label claim for toothpaste 2

Fig 7: Label claim for toothpaste 3

Conclusion: Eugenol plays a significant role in toothpaste samples due to its various medicinal properties and benefits. With the help of this method, we can check the amount as well as identify clove oil as eugenol in marketed samples of toothpaste.

The toothpaste matrix is hard to separate but with HPTLC routine a fast and simple method has been developed to extract eugenol from toothpaste. This method is useful for quality control and checking label claims in regulatory labs. This method can also be used to check content from batch to batch.

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Conflict Of Interest: All authors declare that they have no conflicts of interest.

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