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# DEVELOPMENT A VALIDATED HPTLC METHOD FOR QUANTIFICATION OF LINALOOL IN THE OILS AND EXTRACTS OF SAUDI ARABIAN *OCIMUM BASILICUM* AND *LAVANDULA ANGUSTIFOLIA*

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**Abstract:**

*Linalool is an important constituents found in the many Lamiaceae herbs. Ocimum basilicum and Lavandula angustifolia are parts of the several Arabs foods, traditional medicine and homemade formulations. Though, the lack of operative quality control methods for the selection of quality raw materials and formulations having linalool. A validated high performance thin layer chromatography (HPTLC) method was developed and applied for the quantification of linalool in the essential oils and alcoholic extracts of O. basilicum and L. angustifolia from Saudi Arab local markets. Silica gel 60F-254 pre-coated plates (10 × 10 cm²) were selected for the separation and analysis of linalool compound. The mobile phase, n-hexane: ethyl acetate (8: 2, v/v), wavelength at 460 nm, and $R_f$ (0.31) value were used for the separation, detection and quantification of linalool respectively. The developed method was validated for linearity ($R^2$ = 0.9988), limit of detection (LOD) (6.99 ng), limit of quantification (LOQ) (14.05 ng), accuracy (% recovery =99.58 ± 0.38), and precision (RSD <1%). The amount of Linalool was found to be 170 µg/g and 2900 µg/g in the alcoholic extract and essential oil of O. basilicum and 120 µg/g and 1400 µg/g of alcoholic extract and essential oil of L. angustifolia respectively. The developed HPTLC method was found to be accurate, sensitive, precise, and suitable for the quantification of linalool in the herbal formulations containing these herbs.*

***Keywords:*** *Extracts, Essential oils, Linalool, O. basilicum, L. angustifolia, HPTLC, quantification*

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## INTRODUCTION:

The extract of plants provides several bioactive compounds and it is used in the preparation of dietary supplements, nutraceuticals, functional food ingredients or cosmeceuticals [1]. *Ocimum basilicum* L is commonly known as basil (Arabic: Al-Rehan) and *Lavandula angustifolia* Mill is commonly known as true lavender (Arabic: Al-khuzaama) and both of these are belong to the Lamiaceae family but grow in the different parts of Saudi Arabia [2-4]. In Al-Khobah village of Saudi Arabia, decoction of *O. basilicum* is traditionally used to treat spasm and gastric ulcer [5]. In Makkah region of Saudi Arabia, the infusion of leaves and flowers of *O. basilicum* is traditionally used to treat general and unspecified respiratory ailments [6]. The extracts of *O. basilicum* having strong antioxidant and antibacterial properties and are commonly used for the treatments of coughs, diarrhea and flatulence, worm infestations, stomachic and antispasmodic in different parts of the world [7]. The methanolic extract of *O. basilicum* has been evaluated for various pharmacological effects such as strong inhibitory effects on HIV-1 transcriptase, platelets aggregations, enhancing spermatogenesis, antimicrobials, antioxidants [8]. Lavender is commonly used for the carminative, diuretic, antiepileptic, anti-rheumatic, and headache and migraine. The extracts, fractions, and essential oil of *L. angustifolia* demonstrated for the therapeutic effects such as CNS-depressant, anticonvulsive, and antibacterial effects [9]. The methanolic extract of *L. angustifolia* has strong antioxidant and protects myocardium against isoproterenol-induced myocardial infarction (MI) [10]. Recently, *O. basilicum* obtained from Asir Region of Saudi Arabia has been reported that L-Linalool (60.97%) is the main metabolites in the essential oil [11]. GC-MS analysis of Lavender oil shows the major contents linalyl acetate (25-55%) and linalool (20-30%), and it is used in food manufacturing as an additive flavor in beverages, ice-cream, candy, baked goods and chewing gum [12]. Recently, fingerprints based on chromatographic progression especially high performance thin layer chromatography (HPTLC) play an important role in the quality control of the extracts, oils, herbal formulations, food products, cosmetics and several other products [13]. Nowadays, due to its reliability, use of micro or nanogram analytes, specificity, and reducing time of analysis and per analysis cost, the method of HPTLC has become a routine analytical technique [14-15]**.** The additional advantage of this method is minimal exposure risks, less pollution risk, repetitive detection facility of the chromatogram with same or changed parameters and assay of numerous compositions in nutraceutical and pharmaceutical preparation, herbal formulations, crude extracts and essential oils simultaneously [16-17]. GC-MS and HPLC method generally used for the detection of the lavender and basil, extracts and essential oil samples [18-20]. HPTLC quantification of Myristicin and Linalool simultaneously for the Parsley, Dill, and Celery leaves extract has been developed but not explore for the essential oils [21]. Literature survey revealed that no method has been reported for the quantitation of linalool from *O. basilicum* and *L. angustifolia* leaves extract and essential oils. So, in the present study, a HPTLC method for the quantification of linalool in both extract and essential oils has been developed.

## MATERIAL AND METHODS:

### Plants materials

The leaves of Basil and lavender were purchased from the local markets of Riyadh, Kingdom of Saudi Arabia and were authenticated from Department of Pharmacy, Prince Sattam Bin Abdulaziz University (PSAU), Al-Kharj (KSA) by Dr. Osman A. Elmakki. The voucher specimens for the Basil (PSAU-10-CPH-2017) and Lavender (PSAU-09-CPH-2017) were deposited at the herbarium, Department of Pharmacognosy at PSAU. The plants material were powdered and stored in airtight glass bottle at room temperature for further studies.

### Chemicals and standard

All solvents used for the extraction and stocks solution preparation were of analytical grade and purchased from E-Merck (Darmstadt, Germany). Standard Linalool (Purity: 97% w/w) was also obtained from E-Merck.

### Preparation of methanolic extracts

Exactly, 10 g of stored powdered samples of *O. basilicum* and *L. angustifolia* were sonicated on the sonicator with 100 ml methanol (at room temperature) separately for 2h. The extracts were filter using vacuum Buchner funnel (Sintered glass, 125ml). The filtrates were concentrate and dry at 40°C using a rotary evaporator (Buchi, Switzerland, R-124). The dried methanol extracts were stored in airtight glass bottle at room temperature for the chromatographic analysis.

### Preparation of essential oils

200 g of stored powdered samples of *O. basilicum* and *L. angustifolia* were separately subjected to hydrodistillation at 100 °C using a clevenger type apparatus until no more essential oil was present. The

obtained essential oils were dried over sodium sulfate (anhydrous) powder and stored at 4 °C in a sealed glass vial for the chromatographic analysis.

**Preparation of Standard and samples**

Standard solution of Linalool was prepared by transferring of accurately 5 mg of linalool into 100 ml flask (Volumetric), add 30 ml of methanol. Then, it was sonicate for 5 min and final volume up to 100 ml was made with methanol to achieved 10 µg/ml concentration. Accurately 10 mg of extracts and essential oils extracted from *O. basilicum* and *L. angustifolia* were separately dissolved in 100 ml volumetric flask, add 30 ml methanol. Then, it was sonicate for 5 min and final volume up to 100 ml was made with methanol to achieved 100 µg/ml.

**Method of development**

**Chromatography conditions**

Chromatography analysis was performed on aluminium-backed 10 cm×20 cm, 0.2mm thick coated with HPTLC Silica gel 60 F254 (Merck, Darmstadt, Germany). Aliquots of standard and each samples were applied to the plates as 6 mm bands using an automatic TLC Sampler Linomat-V (CAMAG, Switzerland) fitted with a microliter syringe with nitrogen flow, at the distance 8mm from the bottom. A constant rate of application (150 nlxs-1) was used. Linear ascending development of the plates were carried out in the horizontal developing chamber (CAMAG, 10 × 10 cm) which was previously saturated for 30 min at 22 °C with hexane: ethyl acetate 8:2 (10 ml, %, *v/v*) as mobile phase. After development, scanning of plates were performed in the absorbance mode using TLC densitometric scanner III (CAMAG) furnished with win-CATS-V 1.2.3 software (CAMAG), at 460 nm, using the deuterium lamp. The slit dimensions was kept at 4 × 0.45 mm and 20 mm/s scanning speed were employed for scanning of chromatogram. The derivatization of the chromatogram was did by spraying with anisaldehyde-sulfuric acid reagent, follow by heating at 105 °C for 5 min. After 30min, all the plate was observed under UV cabinet (CAMAG).

**Calibration curve of Linalool**

The content of linalool was determined by using the calibration curve established with a standard concentration range from 200 to 700 ng/spot. The different volumes of stock (10 µg/ml) solution 20, 30, 40, 50, 60 and 70 µl were spotted on HPTLC plate to achieved the concentration 200, 300, 400, 500, 600 and 700 ng/spot, respectively (band width 6 mm, distance between tracks 12 mm) using automatic sample spotter. Each concentration peak area was plotted against the concentration of injected linalool. The linearity of present HPTLC technique for linalool was documented between 200 and 700 ng/spot. The concentration peak area was plotted against the concentration linalool.

**Specificity**

Specificity was established by spotting the samples of extracts and essential oils of *O. basilicum* and *L. angustifolia* and linalool (Standard) together. The band for linalool from the solutions of extract and essential oils were confirmed by comparing the Rf of standard linalool and the spectra of the bands of samples to the standard. The peak purity of linalool was studied by comparing the spectra at start, middle, and end positions of the bands.

**Validation of the method**

Guideline, International Conference on Harmonization (ICH, 2010) [22] were followed for the validation of the HPTLC developed method for sensitivity, accuracy, precision, and robustness.

**Sensitivity**

In order to assessment the LOD (limit of detection) and LOQ (limit of quantification) different concentration of linalool were spotted on TLC plate along with methanol as blank, succeeding the similar to above method. The slope of calibration curve (S) and standard deviation (SD) of blank were used to LOQ (3.3×SD/S) and LOQ (10×SD/S) determination.

**Accuracy**

The accuracy the present HPTLC densitometric method was determined by the recovery of standard (Standard) after several dilutions. The Pre-analyzed samples (200 ng/spot) were added by spiking with the standard linalool (0, 50, 100, and 150%) and assessed. The values of % recovery and percentage relative standard deviation (% RSD) for each concentration were calculated.

**Precision**

The precision the present HPTLC densitometric method was calculated by Inter-day and intra-day variation study. These were studied by analyzing standard solution of linalool (300, 400, 500 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % RSD.

**Robustness**

Robustness of the present HPTLC densitometric method was assessed to evaluate the effect of small changes in the condition of chromatographic whereas identification of linalool. It was determined by altering

the mobile phase polarity. The results were expressed as %RSD.

**Estimation of Linalool in the extracts and essential oils of *O. basilicum* and *L. angustifolia***

To determine the content of linalool in the extracts and essential oils of *O. basilicum* and *L. angustifolia* from local markets of KSA. The sample and oil solution (100 µg/ml) applied on the TLC plate followed by development and scanning following the present method.

**Statistical Analysis**

Excel 2013 Microsoft was used for the statistical analysis.

## RESULTS AND DISCUSSION:

**Method of developments**

In the present study, mixtures of the mobile phase was adjusted for the development of appropriate and accurate method of densitometric HPTLC for the identification and quantification of linalool. The mobile phase *n*-hexane: ethyl acetate (8:2 %, *v/v*) resulted a sharp, symmetrical, and well resolved peak at *Rf* value of (0.31) (Figure 1).

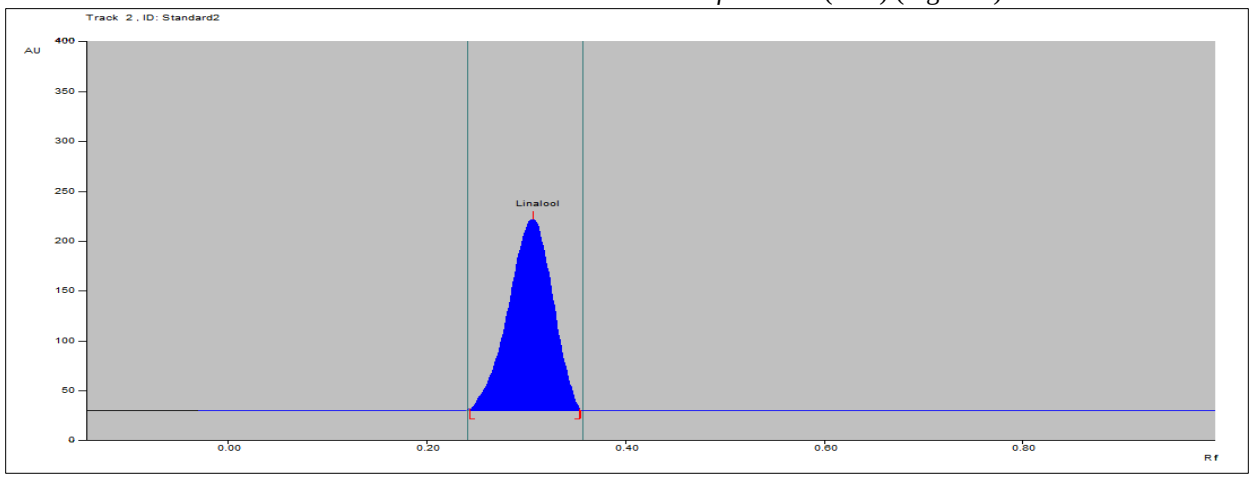


**Figure 1:** HPTLC chromatogram of Linalool.

**Table 1**: Linear regression data for the calibration curve of linalool

| | |
|---|---|
| Linearity (range ng x spot$^{-1}$) | $y^2$ = 25.128x + 6019.5 |
| Correlation coefficient ($r^2$) | $r^2$ =0.9988 |
| Slope ± SD | 25.128 ± 0.1614 |
| Intercept ± SD | 6019.5 ± 67.71 |
| Standard error of slope | 0.06635 |
| Standard error of intercept | 3.22 |
| Confidence interval (95%) of slope | 4.342 – 5.023 |
| Confidence interval (95%) of intercept | 790- 897.2 |
| LOD (ng.spot$^{-1}$) | 6.99 |
| LOQ (ng.spot$^{-1}$) | 14.05 |

Average of six times repeated determinations

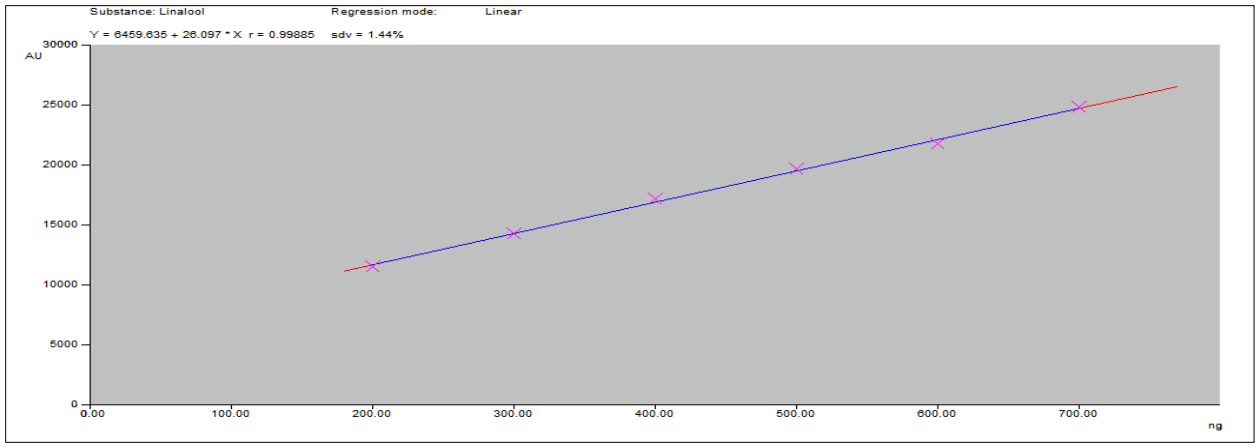


**Figure 2:** Calibration curve for standard linalool (*n* = 6).

**Table 2:** Accuracy of the developed method

| Samples added to analyte (%) | Theoretical content (ng) | Conc. found (ng) ± SD | % Recovery | % RSD |
|---|---|---|---|---|
| 0 | 200 | 199.17 ± 0.75 | 99.58 | 0.38 |
| 50 | 300 | 294.00 ± 2.76 | 98.00 | 0.94 |
| 100 | 400 | 395.83 ± 1.72 | 98.96 | 0.44 |
| 150 | 500 | 490.17 ± 5.23 | 98.03 | 1.07 |

Average of three times repeated determinations

**Table 3:** Precision of the developed HPTLC method

| Conc. | Repeatability (Intraday precision) (n=6) | | | Intermediate precision (Interday) (n=6) | | |
|---|---|---|---|---|---|---|
| 300[a] | 13734.83 ± 68.39[b] | 27.92[c] | 0.50[d] | 13712.17 ± 104.12[b] | 0.21[c] | 0.76[d] |
| 400[a] | 16144.50 ± 84.89[b] | 34.66[c] | 0.53[d] | 6333.20 ± 112.57$_b$ | 0.23[c] | 0.70[d] |
| 500[a] | 18731.00 ± 89.70[b] | 36.63[c] | 0.48[d] | 9242.80 ± 33.83[b] | 0.24[c] | 0.63[d] |

(Where: a, b, c, and d ng/spot, Area ± SD, SE, and % RSD respectively)

**Table 4:** Robustness of the proposed HPTLC method

| Conc. | (Hexane: Ethyl acetate) | | | Results (n = 6) | | |
|---|---|---|---|---|---|---|
| | Original | Changes | Ethyl acetate | Area ± SD | % RSD | Rf |
| 300 (ng/spot) | 8:2 | 8:1.9 | +0.1 | 13729.20 ± 86.60 | 0.63 | 0.28 |
| | | 8:2 | 0.0 | 13789.20 ± 81.49 | 0.59 | 0.31 |
| | | 8:2.1 | -0.1 | 13709.20 ± 33.15 | 0.69 | 0.32 |

**Table 5**: Amount (µg/g) of Linalool found in extract and essential from *O. basilicum* and *L. angustifolia*

| Plants | Extracts | Essential oils |
|---|---|---|
| *O. basilicum* | 170µg/g | 2900 µg/g |
| *L. angustifolia* | 120µg/g | 1400 µg/g |

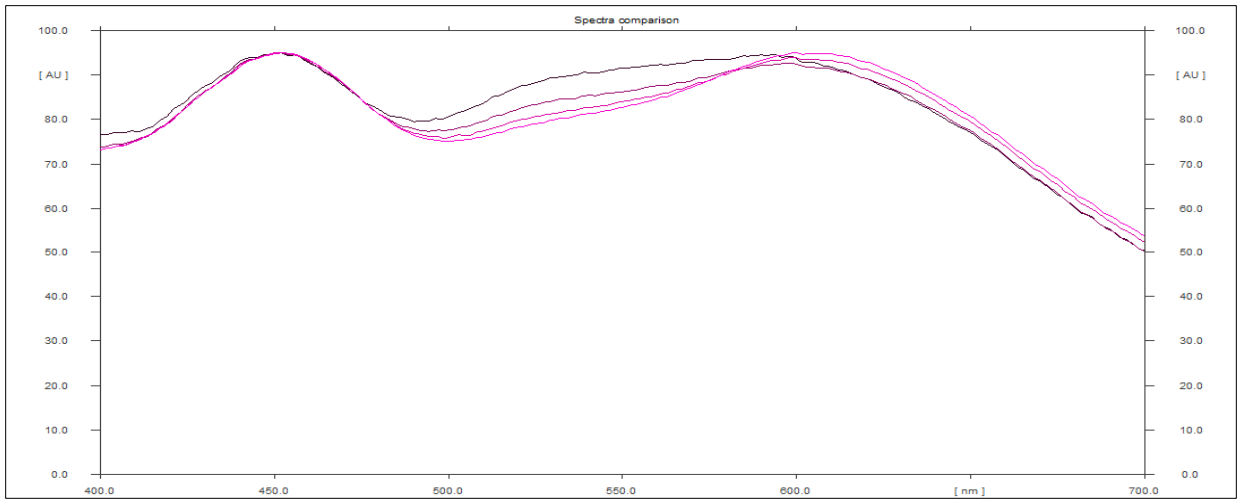


**Figure 3**: superimposing the UV spectra found in extracts and essential oils of *O. basilicum* and *L. angustifolia* with that of the standards in all tracks.

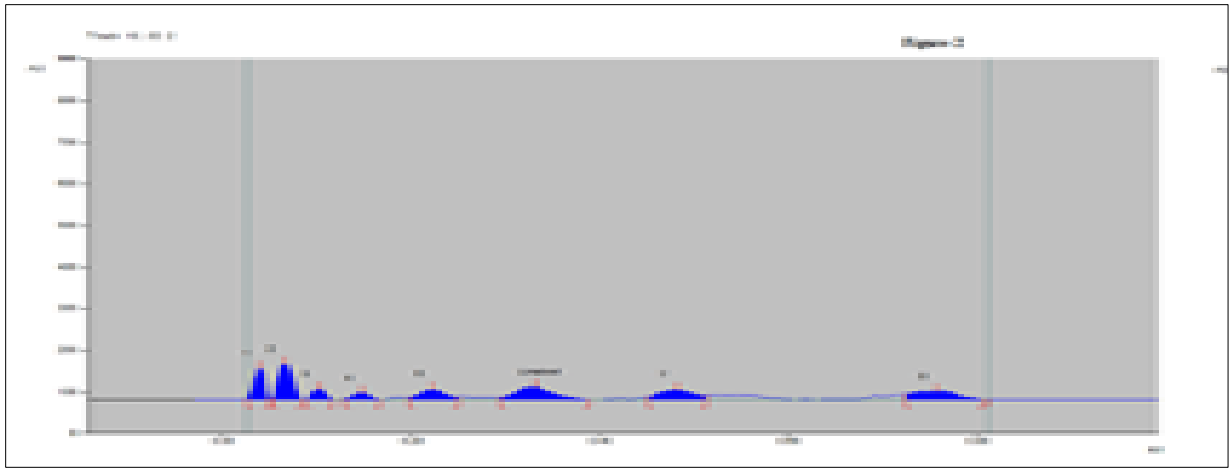

**Figure 4:** Densitometric chromatogram of methanol extracts of leaf of L. angustifolia after derivatization at 460 nm

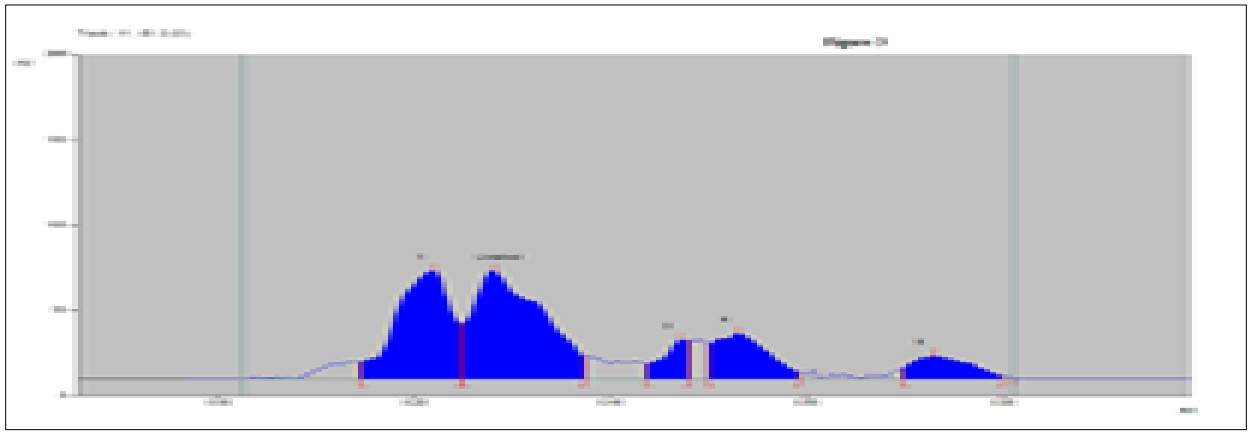

**Figure 5:** Densitometric chromatogram of essential oil from leaves of L. angustifolia after derivatization at 460 nm.

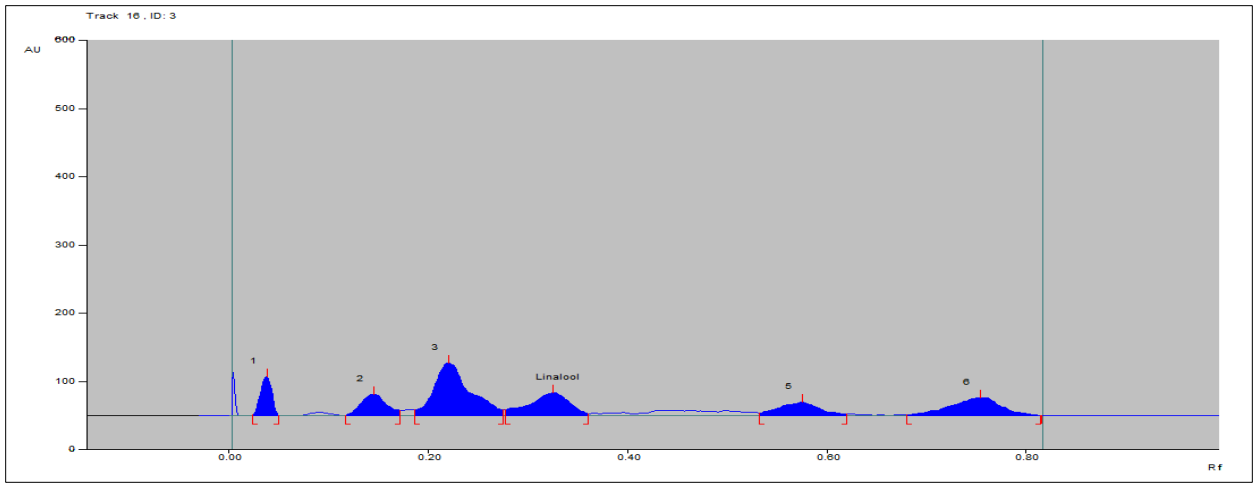


**Figure 6:** Densitometric chromatogram of methanol extracts of leaves of O. basilicum after derivatization at 460 nm.

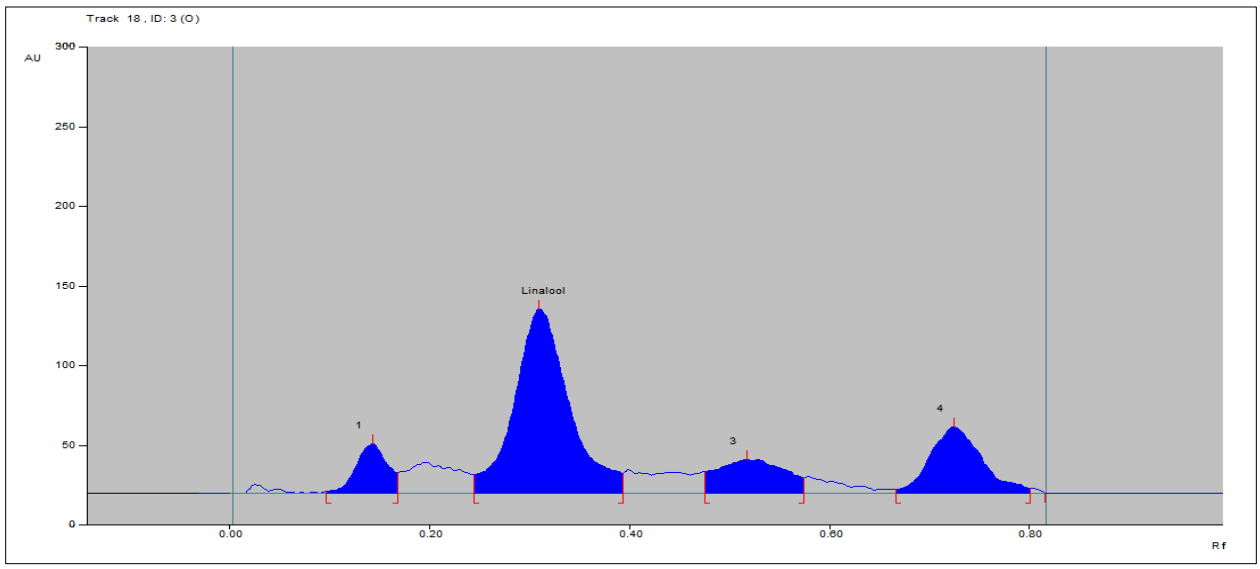


**Figure 7.** Densitometric chromatogram of methanol essential oil from leaves of *O. basilicum* after derivatization at 460 nm.

**Linearity**

The calibration plot of peak area against concentration of linalool was linear in the range 200–700 ng/spot. The linear regression equation is Y = 25.128x + 6019.5 (Figure 2). The data's have shown a good linear relationship over the concentration range of 200–700 ng/spot (Table 1). The correlation coefficient (r2) of standard curve was highly significant (0.9988). The linearity of calibration graphs and adherence of the system to Beer's law are validated by high value of correlation coefficient and the standard deviation (SD). The intercept value is noticed to be less than 2% (RSD 0.28%). No significant difference is observed in the slopes of standard curves ($p < 0.05$).

**Specificity**

The specificity of the present method was determined by comparing the sample and standard peak for its Rf (0.31). The identification of standard (Linalool) was confirmed by superimposing the UV spectra of the samples and standards (Figure 1). Superimposing the UV spectra of *O. basilicum* and *L. angustifolia* with that of the standards in all tracks indicating specificity of the method [23].

**Method of Validation**

**Sensitivity**

The LOD and LOQ were 6.99 and 14.05 ng respectively which indicate the adequate sensitivity of the method (Table 1). The results of LOD and LOQ

indicated that the developed method can be apply in the wide range for quantification of Linalool in the oils and extracts [24].

**Accuracy**

The samples (pre-analyzed) were spiked with standard linalool at four different concentration (0, 50, 100, and 150%) levels and these were re-analyzed by the proposed method. The recovery of the experiment was shown in triplicate, which validated a good recovery of 98.00–99.58% (%RSD), indicated the accuracy of the present HPTLC method [25].

**Precision**

The results of Inter-day and intra-day variation, articulated as SD (%) and were shown in (Table 3). The range of % RSD (0.48–0.53 for repeatability (Inter-day) and 0.63–0.76 for intermediate (intra-day) precision. These low values of % RSD (<1%) indicated that the developed method was precise [26].

**Robustness**

The Results of robustness was shown in (Table 4). The range % RSD (0.46%–1.29%) were obtained on the basis of introducing small change in the TLC procedure, hence, it represents the robustness of the proposed method [27].

**Estimation of linalool in the extracts and essential oils of *O. basilicum* and *L. angustifolia***

The band at Rf (0.31) corresponding to linalool was detected in the chromatogram of the extracts and oils of *O. basilicum* and *L. angustifolia* with other components. In the chromatogram, there were no intervention from the other compositions present in the samples were detected. Figures 4 & 5 were shown the chromatogram of extract and oil of *L. angustifolia* respectively and Figure 6 & 7 were shown the chromatogram of extract and oil of *O. basilicum* respectively. The amount of linalool in the extracts and oils of *O. basilicum* and *L. angustifolia* were quantified using calibration curve plotted with linalool. The content of linalool in the extract and essential oils of *O. basilicum* were found to 170 µg/g and 2900 µg/g respectively and in the extract and essential oils of *L. angustifolia* were found to 120 µg/g and 1400 µg/g respectively (Table 5). The previous study has been reported the quantitative analysis of linalool in *O. basilicum* and *L. angustifolia* extracts and essential oils by the Gas chromatographic method [28-29]. In previous report, Linalool and Myristicin were simultaneously estimated quantitatively from Parsley, Dill, and Celery leaves extracts using HPTLC [30] but our present finding focus on both extracts and essential oils and may be an additional method to be use in quality control of both extracts and essential oils products.

## CONCLUSION:

The developed densitometric HPTLC method was found to be sensitive, simple, rapid, and precise. The recovery of linalool from the extracts and essential oils of *O. basilicum* (96.67%–102.92 % and 97.72%–99.29%) and *L. angustifolia*. 96.67%–102.92% and 97.72%–99.29 %,) respectively, showing the accuracy of the developed densitometric method, hence can be applicable for routine analysis and quality control of linalool containing oils and extracts in any type of formulations, food products, cosmetic products and flavoring agents.

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## REFERENCES:

1. Peschel W, Sanchez-Rabaneda F, Diekmann W, Plescher A, Gartzia L, et al. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem, 2006; 97: 137-150.
2. Sodany YM, Bazaid SA, Mosallam HA. Medicinal Plants in Saudi Arabia: I. Sarrwat Mountains at Taif, KSA. Acad. J. Plant Sci, 2013; 6(4): 134-145.
3. Al-Ali KH, El-Beshbishy HA, El-Badry AA, Alkhalaf M. Cytotoxic activity of methanolic extract of *Mentha longifolia* and *Ocimum basilicum* against human breast cancer. Pak. J. Biol. Sci, 2013;16(23): 1744-50.
4. Khayyat S, Al-Kattan M, Basudan N. Phytochemical Screening and Antidermatophytic Activity of Lavender Essential Oil from Saudi Arabia. Int. J. Pharmacol, 2018; 14: 802-810
5. Abdel-Kader MS, Hazazi MA, Elmakki OA, Alqasoumi SI. A survey on traditional plants used in Al Khobah village. Saudi Pharmaceutical Journal, 2018; 26(6):817-821.
6. Alqethami A, Hawkins JA, Teixidor-Toneu I. Medicinal plants used by women in Mecca: urban, Muslim and gendered knowledge [published correction appears in J. Ethnobiol. Ethnomed.

2017 Dec 15;13(1):71]. J. Ethnobiol. Ethnomed, 2017; 13(1): 62.
7. Demir H, Kalayc S. Chemical Composition and Antimicrobial Activity of Essential Oils of *Ocimum basilicum* var. album (L.) Benth, *Lavandula angustifolia* subsp. *angustifolia*, *Melissa Officinalis* belonging to Lamiaceae Family. J. Food Sci.Eng, 2017; 7: 461-471.
8. Khalil AB. Antimicrobial Activity of Ethanolic Extracts of *Ocimum basilicum* leaf from Saudi Arabia. Biotechnology, 2013; 12(1): 1-4
9. Hajhashemi V, Ghannadi A, Sharif B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. J. Ethnopharmacol, 2003; 89: 67–71.
10. Ziaee M, Khorrami A, Ebrahimi M, et al. Cardioprotective Effects of Essential Oil of *Lavandula angustifolia* on Isoproterenol-induced Acute Myocardial Infarction in Rat. Iran J. Pharm. Res, 2015; 14(1): 279–289.
11. Helal IM, Ashraf El-Bessoumy, Erwa Al-Bataineh, et al., "Antimicrobial Efficiency of Essential Oils from Traditional Medicinal Plants of Asir Region, Saudi Arabia, over Drug Resistant Isolates," BioMed Research International, vol. 2019, Article ID 8928306, 9 pages, 2019.
12. Hassiotis CN, Lazari DM, Vlachonasios KE. The effects of habitat type and diurnal harvest on essential oil yield and composition of *Lavandula angustifolia* Mill. Fresenius Environ. Bul, 2010; 19: 1491–1498.
13. Kamboj A, Saluja AK. Development of validated HPTLC method for quantification of stigmasterol from leaf and stem of *Bryophyllum pinnatum*. Arab. J. Chem, 2017; 10(2): S2644-S2650
14. Alam P, Yusufoglu H, Alam A. HPTLC densitometric method for analysis of thymoquinone in Nigella sativa extracts and marketed formulations. Asian Pac. J.Trop. Dis, 2013; 3(6): 467–471.
15. Singhvi G, Shukla VK, Ukawala R, Gampa G, Saha RN. Development of a new, rapid and sensitive HPTLC method for estimation of Milnacipran in bulk, formulation and compatibility study. Arab. J. Chem., 2017; 10(2): S2417-S2423
16. Panchal H, Amin A, Shah M. Development of Validated High-performance Thin-layer Chromatography Method for Simultaneous Determination of Quercetin and Kaempferol in *Thespesia populnea*. Pharmacognosy Res. 2017; 9(3): 277–281.
17. Firke SD, Patil AS, Patil RR, Patil AS. Stability-Indicating HPTLC Method for Estimation of Benazepril in Bulk and Tablet Dosage Form. Anal. Chem. Lett, 2018; 8:4, 552-564.
18. Beale DJ, Morrison PD, Karpe AV, Dunn MS. Chemometric analysis of lavender essential oils using targeted and untargeted GC-MS acquired data for the rapid identification and characterization of oil quality. Molecules, 2017; 22: 1339
19. Rout KK, Singh RK, Barik DP, Mishra SK. Thin-Layer Chromatographic Separation and Validated HPTLC Method for Quantification of Ursolic Acid in Various Ocimum Species. J. Food Drug Anal, 2012; 20(4): 865-871
20. Avetisyan A, Markosian A, Petrosyan M, et al. Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. BMC Complement. Altern. Med, 2017; 17(1): 60.
21. Stan M, Opris O, Lung I, Soran ML. High-Performance Thin-Layer Chromatographic Quantification of Myristicin and Linalool from Leaf Extracts of Microwave-Irradiated Parsley, Dill, and Celery. JPC – J. Planar Chromat. Modern TLC, 2014; 27(2): 97-101.
22. International Conference on Harmonisation (ICH) ICH harmonised triplicate guideline on validation of analytical procedures. In: International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use; Switzerland: ICH. 2010.
23. Fahim M, Singh M, Kamal YT, Mukhtar HM, Ahmad S. A high performance thin layer chromatographic method for the estimation of colchicine in different formulations. J. Pharm. Bioallied Sci, 2015; 7(4): 260–263.
24. Ashraf K, Mujeeb M, Ahmad A, Amir M, Mallick MN, Sharma D. Validated HPTLC analysis method for quantification of variability in content of curcumin in *Curcuma longa* L (turmeric) collected from different geographical region of India. Asian Pac. J. Trop. Biomed, 2012; 2(2): 584–588.
25. Chandel S, Barhate CR, Srivastava AR, Kulkarni SR, Kapadia CJ. Development and Validation of HPTLC Method for Estimation of Tenoxicam and its Formulations [published correction appears in Indian J. Pharm. Sci; 2012;74(2): 188. Kulkarni, S K [corrected to Kulkarni, S R]]. Indian J. Pharm. Sci, 2012; 74(1): 36–40.
26. Rathee D, Rathee S, Rathee P, Deep A, Anandjiwala S, Rathee D. HPTLC densitometric quantification of stigmasterol and lupeol from *Ficus religiosa*. Arab. J.chem, 2015; 8(3): 366-371

27. Alam P, Alam A, Anwer MK, Alqasoumi SI. Quantitative estimation of hesperidin by HPTLC in different varieties of citrus peels. Asian Pac. J. Trop. Biomed. 2014; 4(4): 262–266.
28. Farouk A, Fikry R, Mohsen M. Chemical Composition and Antioxidant Activity of *Ocimum basilicum* L. Essential Oil Cultivated in Madinah Monawara, Saudi Arabia and its Comparison to the Egyptian Chemotype. J. Ess. Oil Bearing Plants, 2016; 19 (5):1119-1128.
29. Stanojevic LP, Marjanovic-Balaban ZR, Kalaba VD, Stanojevic JS, Cvetkovic DJ, Cakic MD. Chemical Composition, Antioxidant and Antimicrobial Activity of Basil (*Ocimum basilicum* L.) Essential Oil. J. Ess.Oil Bearing Plants, 2017; 20(6): 1557-1569
30. Alexa E, Danciu C, Radulov I, Diana Obistioiu D, Sumalan RM, Adriana Morar A, Dehelean CA. Phytochemical Screening and Biological Activity of *Mentha piperita* L. and *Lavandula angustifolia* Mill. Extracts. Anal. Cell. Pathol, 2018; 2018: 2678924.