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A validated HPTLC method for determination of tea tree oil from cosmeceutical formulations

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Abstract

A HPTLC method has been developed and validated for the determination of tea tree oil from cosmeceutical formulations. Tea tree oil concentration was estimated by analyzing the terpinen-4-ol content. The method employed TLC aluminium plates precoated with silica gel 60F-254. The solvent system consisted of toluene and ethyl acetate in the ratio 85:15. The calibration curve of terpinen-4-ol was linear in the range of 100–900 ng. The polynomial regression data for the calibration plots showed a good linear relationship with $r^2 = 0.9949$. The Rf value of terpinen-4-ol was found to be 0.62 ± 0.05. The method was validated for precision and accuracy. The minimum detectable amount was found to be 60 ng. The limit of quantitation was found to be 100 ng. The drug content was within the limits (±5% of the labeled content of the formulations). The recovery of tea tree oil was greater than 99%. The method was found to be simple, sensitive, precise, accurate and specific for estimation of tea tree oil from formulations.

Keywords: HPTLC; Tea tree oil; Terpinen-4-ol; *Melaleuca alternifolia*

1. Introduction

The use of essential oils in complementary medicine, particularly aromatherapy and also in the cosmetic and perfumery industry is becoming increasingly popular. The essential oil distilled from the leaves of *Melaleuca alternifolia*, commonly called tea tree is well known for its antimicrobial activity and has enjoyed increased medicinal uses in recent years [1–3]. Detailed GC and GC–MS analyses of oil of *M. alternifolia* have identified several constituents including terpinen-4-ol (Fig. 1), which is of primary importance in deciding the suitability of tea tree oil for medicinal use. Oil of *Melaleuca* (terpinen-4-ol type) (AS 2782-1985) is the current standard oil. It sets a terpenin-4-ol content of not less than 30% and 1,8-cineole content of not more than 15% [4–8].

The estimation of essential oil in formulations is often time-consuming, expensive and cumbersome. The advantage of HPTLC is that a large number of samples can be simultaneously analyzed in a short period of time. The method utilizes less quantity of solvents, thus lowering the cost of analysis. The method of detection does not place any restriction on the choice of the mobile phase.

The objective of this study was to develop a simple, sensitive and specific HPTLC method for the estimation of tea tree oil. In this study the quantitative HPTLC method was developed for the estimation of tea tree oil in formulations. Tea tree oil concentration was determined by estimating the terpinen-4-ol content, which is the major component of the oil.

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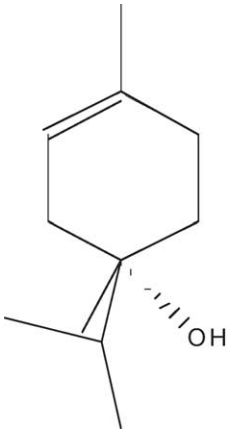

Fig. 1. Chemical structure of terpinen-4-ol the active component of tea tree oil.

2. Experimental

2.1. Chemicals and reagents

Tea tree oil was purchased from Blossom Kochhar Beauty Products, New Delhi, India. Dabur Research Foundation, India, gifted authentic sample of terpinen-4-ol (99.5%). Isopropyl myristate, triethanolamine, Tween-80, toluene, ethyl acetate, methanol, acetic acid and sulphuric acid were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. Glycerol monostearate, calamine, kaolin, bentonite, methyl paraben and glycerol were purchased from E. Merck, Mumbai, India.

2.2. Instrumentation

The HPTLC system consisted of a CAMAG Linomet IV-automatic spotting device, a CAMAG twin-trough chamber (20 cm × 10 cm), CAMAG Cats-4 software and a 100 μl HPTLC syringe (SGE, Australia). Sample application was done on precoated silica gel aluminium Plate 60-254 (SG 60 F254, layer thickness 0.02 mm, Merck, Germany). Densitometric analysis was carried out utilizing CAMAG TLC scanner-3. Integration was performed using CAMAG TLC scanner/Integrator system LCI-100.

2.3. Tea tree oil formulations

Microemulsion was prepared using 23% isopropyl myristate, 5% tea tree oil, 42% Tween 80, 6% glycerol and 24% water. Microemulsion was prepared by slow trituration of the aqueous glycerol solution in isopropyl myristate, tea tree oil–Tween 80 mixture. Trituration was carried out until the mixture became hazy or turbid to establish the region of clear isotropic mixtures [9]. Phase studies showed existence of microemulsion region in which a glycerol-to-water mass ratio of 2:8 at 30% total concentration of glycerol and water and isopropyl myristate–tea tree oil to Tween-80 mass ratio of 2:3 produced the largest microemulsion region. This ratio was selected for the preparation of microemulsion for further experiments. The particle size of the formulation was determined using a Malvern particle size analyzer. Microemulsion had a mean particle size of 30 ± 14.6 nm (95% confidence interval).

Colloidal bed formulations are pastes or creams, sometimes described as mudpacks. They are prepared using kaolin, calamine and bentonite, which are termed as 'clays' [10]. The formulation was prepared using 34% kaolin, 10% calamine, 6% bentonite, 3.5% glyceryl monostearate, 0.25% methyl paraben, 10% glycerine, 5% tea tree oil and water to make 100% composition. The clays were presterilised at 150 °C in a dry heat oven for 1 h to remove microbial contamination if any. Water and glycerin were added to the clays along with glyceryl monostearate and methyl paraben and mixed well. Tea tree oil was dispersed with Tween 80 and mixed until a fine paste was obtained. The particle size as determined by Malvern particle size analyzer was 50 ± 16.2 μm.

2.4. HPTLC method

In the proposed HPTLC method the reference standard (terpinen-4-ol) was spotted on to the precoated TLC plates. The chromatographic development was performed using a mixture of toluene and ethyl acetate (85:15) as mobile phase with two-dimensional development under the following conditions: chamber saturation time, 45 min; temperature, 25 ± 2 °C; migration distance, 80 mm. After development, the TLC plates were dried completely. To visualize the zones, plates were sprayed with an anisaldehyde reagent and heated for 15 min at 105 °C. Quantification of terpinen-4-ol was achieved by scanning with CAMAG TLC scanner 3 (slit dimension, 4 mm × 0.1 mm; scanning speed 1 mm/s; wavelength of determination, 366 nm at absorbance mode) and the automated software produced the chromatogram by plotting absorbance against Rf values.

2.5. Calibration plots

A series of standard curves were prepared over a concentration range of 100–900 ng for terpinen-4-ol ($n = 3$; nine standards). The data of area under the peak versus drug concentration was treated by linear least square regression analysis.

2.6. Analysis of tea tree oil

The tea tree oil was run on precoated TLC plates as described in HPTLC method above. Tea tree oil concentration was determined by quantifying the terpinen-4-ol content. The chromatogram was developed by scanning with CAMAG TLC scanner 3 (slit dimension, 4 mm × 0.1 mm; scanning speed 1 mm/s; wavelength of determination, 366 nm at absorbance mode) and the automated software produced the chromatogram by plotting absorbance against Rf values.

2.7. Analysis of formulations

The formulations were analyzed by the use of the same mobile phase. Formulations equivalent to 2 g were accurately weighed and dissolved in 100 ml of ethanol. To ensure complete extraction of the terpinen-4-ol it was sonicated for 15 min. The resulting solution was centrifuged at 3000 rpm for 5 min and the supernatant was analyzed for terpinen-4-ol content. One milliliter of the supernatant was dissolved in 10 ml of ethanol. Ten microliters of each of these solutions were spotted on plates and analyzed for terpinen-4-ol in the same way as described by the HPTLC method.

2.8. Quantitation

In order to estimate the limit of detection and limit of quantification, terpinen-4-ol was spotted in the range of 25–2250 ng. The limit of detection was calculated to be 3 times the standard deviation and 10 times the standard deviation value gave the limit of quantification [11–13].

2.9. Accuracy

Recovery studies were carried out to check the accuracy of the method. Recovery experiments were performed by adding three different amounts of terpinen-4-ol i.e., 25, 50 and 75% of the amount of terpinen-4-ol analyzed from the tea tree oil formulations and resultant was reanalyzed ($n = 6$).

2.10. Precision

The intra-day precision was evaluated by analyzing terpinen-4-ol repeatedly at concentration range of 100–900 ng ($n = 5$). The inter-day precision was evaluated by analyzing terpinen-4-ol at concentration range of 100–900 ng over a period of 10 days ($n = 5$).

3. Results and discussion

3.1. Chromatography

In this study the quantitative HPTLC method was developed for the estimation of tea tree oil in formulations. The solvent system comprising of toluene and ethyl acetate (85:15) was found to give a good separation and resolution of terpinen-4-ol with out interference from the other materials. The peak area on the chromatogram was used for quantitative determination. During the development of the HPTLC method it was observed that a pre-saturation of the TLC chamber with mobile phase for at least 45 min was required to obtain a good separation with reproducible Rf values. The Rf value of terpinen-4-ol was found to be 0.62 ± 0.05. The terpinen-4-ol content of tea tree oil was found to be $40 \pm 1.2\%$.

3.2. Specificity

Derivatization of chromatogram with anisaldehyde reagent provided pink coloured spot at Rf value 0.62 corresponding to pure terpinen-4-ol, which differentiates closely related structures, indicating the method, is specific.

3.3. Calibration curves

The polynomial regression data (Table 1) for the calibration plots ($n = 3$) showed a good linear relationship over concentrations range of 100–900 ng. No significant difference was observed in the slopes of standard curves (ANOVA, $P > 0.05$).

3.4. Quantitation

The minimum detectable amount with a signal to noise ratio of 3:1 was found to be 60 ng. The limit of quantitation with a signal to noise ratio of 10:1 was 100 ng.

3.5. Drug content analysis

The results of the terpinen-4-ol content of the developed formulations by HPTLC are depicted in Table 2 and that for recovery studies are depicted in Table 3. The terpinen-4-ol content of the developed formulations was found to be well within the limits (±5% of the theoretical value). The drug content analysis and recovery studies along with the fact that tea tree oil is soluble in ethanol proved that the efficiency of the extraction recovery of terpinen-4-ol was complete.

Table 2
Drug content ($n = 6$)

Formulation	Amount of drug spotted (ng) (terpinen-4-ol equivalent)	Mean concentration of drug found (ng ± S.D.) (terpinen-4-ol equivalent)	Coefficient of variation (%)
Microemulsion	400	400.66 ± 1.86	0.46
Colloidal bed	400	400.0 ± 2.36	0.59

Table 1
Polynomial regression data for standard curves ($n = 3$)

	$r^2 \pm$ S.D.	Slope ± S.D.	Intercepts ± S.D.
Terpinen-4-ol, 100–900 ng	0.9949 ± 0.0019	85.91 ± 0.3465	166.63 ± 10.513

Table 3
Recovery studies ($n = 6$)

Formulation	Amount drug analyzed (terpinen-4-ol equivalent) (ng)	Amount of drug added (terpinen-4-ol) (ng)	Theoretical concentration (ng)	Total amount analyzed (ng)	% Recovery ± S.D.
Microemulsion	400	100	500	499	99.00 ± 0.71
	400	200	600	599	99.50 ± 0.65
	400	300	700	698.83	99.61 ± 0.72
Colloidal bed	400	100	500	501.83	101.83 ± 1.34
	400	200	600	601.73	100.86 ± 0.94
	400	300	700	701.5	100.50 ± 0.62

Table 4
Intra and inter-day reproducibility studies ($n = 5$)

Amount of terpinen-4-ol (ng)	%CV
Intra-day reproducibility	
100	5.5
300	6.2
500	6.4
700	6.8
900	5.9
Mean	6.16
Inter-day reproducibility	
100	7.1
300	7.8
500	7.5
700	8.1
900	7.4
Mean	7.58

3.6. Accuracy

Table 3 depicts the accuracy of the quantitation of terpinen-4-ol. The recovery of terpinen-4-ol was found to be more than 99%.

3.7. Precision

Table 4 shows the intra-day and inter-day precision studies of terpinen-4-ol at different levels. It was observed that, the percentage coefficient of variation of intra-day precision and inter-day precision was comparable and within the limits.

4. Conclusion

A new HPTLC method has been developed for the identification and quantification of tea tree oil in formulations. The method was found to be simple, sensitive, precise, accurate and specific for estimation and can be conveniently employed for studies on tea tree oil formulations.

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