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METHOD DEVELOPMENT AND VALIDATION FOR THE GC ASSAY OF α-PINENE IN TEA TREE OIL FORMULATION

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Abstract

The present study was oriented towards the development and validation of Gas chromatography-flame ionization detection (GC-FID) method for pure Tea tree oil (TTO). Separation was achieved by Stationary phase ECTM- WAX, coated with polyimide that gives notorious flexibility of the capillary column, Film thickness (0.25µm), length 30m x0.25mm i.d and the Injector and detector temperature, 230 and 240°C. The data was acquired by data processing system, Nuchrom GC Software. The samples and standard were prepared with dichloromethane. The calibration curve for α- pinenewas linear (r^2= 0.998) from 0.1% to 0.5% range of the analytical concentration of pureα- pinene oil. The optimal separation was achieved at 9.69 minute.% R.S.D for the intra-day and inter-day precisions was found to be 0.949 and 0.781 respectively. The limits of detection and quantitation were determined to be 0.0003 % and 0.001 % respectively. Analytical performance demonstrated that the proposed method is highly sensitive, precise and accurate and hence was successfully applied for the quantification ofα- pinenein formulation of TTO.

Keywords: *Tea Tree oil, GC, Validation.*

Introduction

Tea tree oil *(Melaleuca alternifolia)* is distilled from the fronds of a tree native to New South Wales, Australia, and parts of New Zealand. This tree, M. alternifolia, is a member of the myrtaceae family, and is extremely hardy and disease resistant. The leaves have been employed medicinally for centuries by New South Wales Aborigines, and the name is said to stem from a visit by Captain Cook, whose crew made a tea from the leaves. Tea tree oil has been proven to be a powerful yet natural antiviral, antibacterial, antifungal medicine [1]. It is being used as a very effective first aid remedy and against countless skin ailments, infections, cuts, scrapes, burns, insect bites and skin spots etc.

Tea tree oil is not just soothing and disinfecting, it is capable of penetrating into the lower skin layers with its anti-inflammatory, disinfectant, analgesic (pain-killing) and cicatrizant (wound-healing) qualities. It is also effective against gram positive bacteria like *Staphyloccusaureus, Staphyloccusepidermidis, Staphyloccuspneumoniae, Staphyloccusfaecalisetc*, gram negative bacteria like *Escherichia coli, Pseudomonas aeruginosaetc* and Fungi like *Trichophytonmentagrophytes, Aspergillusniger, Aspergillusflavus, Candida albicans, Microsporumcanis, Thermoactinomycetes vulgaris* [2].

The standard identifies a set of chemical parameters (Table 1) that stipulates the range of 1

The tea tree oil is widely used in the cosmetic industry due to its wide therapeutic uses. From its 14 constituent the α-pinene is one of the major constituent, it has anti-viral [4]. Antiseptic and antibacterial properties, so we believe that the availability of this method, with its increased sensitivity and selectivity, will be very useful for the determination of p-cymene in therapeutic and pharmaceutical preparations.

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

α–pinene

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Table 1. Values of tea tree oil constituents

Constituent	Maximum (%)	Minimum (%)
1,8 Cineole	15	-
α-Terpinene	13	5
λ-Terpinene	28	10
p-cymene	12	0.5
Terpinen-4-ol	-	30
α-terpineol	8	1.5
α-pinene	6	1
Terpinolene	5	1.5
Limonene	4	0.5
Sabinene	3.5	Trace
Aromodendrene	7	Trace
δ-cadinene	8	Trace
Globulol	3	Trace
Viridifloral	1.5	Trace

The aim of this study was to develop an assay, rapid and accurate GC–FID method for the determination of p-cymene content in pure tea tree oil formulation.

2. Experimental

2.1 Chemicals and reagents

All chemicals and reagents used were of Analytical Grade. Working standard of α-pinene was purchased from Sud pines Pvt ltd. Jammu, India, Tea tree oil was gifted by Katyani oils, Delhi.

2.2 Instrument

A NUCON model (5765) gas chromatograph (GC) equipped with a flame ionisation detector (FID) was used to analyze the samples. Separation was achieved by Stationary phase ECTM- WAX, coated with polyimide, Film thickness(0.25μm), length 30m x0.25mm i.d and the Injector and detector temperature, 230 and 240°C. The data was acquired by using data processing system, Nuchrom software.

2.3 Preparation of the standard and sample solutions

A standard solution of α-pinene (0.1%) was prepared by dissolving 0.1ml in 100 ml of dichloromethane. A 10 % solution of tea tree oil sample was prepared by dissolving of 10 ml in 100 ml of dichloromethane.

2.4 Column Temperature Programming

Column temperature programming was set as shown in Table 2, Initial temperature was 40℃ and 10℃/min rising is set upto 80℃ then change in the rising temperature was drop to 2℃/min from 10℃/min upto 90℃, temperature was kept on Hold for 10 minute at 90℃. Then again rising at the rate of 2℃/min up to 100℃ and then after 100℃/min rise was kept 10℃/min upto 240℃.

Table 2 Column Temperature Programming

Time(mins)	Temperature(^{o}C)	Rate(^{o}C/min)	Hold Time(min)
0	40	10	-
4	80	2	-
9	90	-	10
19	90	2	-
24	100	10	-
38	240	-	-

Results and Discussion

The chromatographic conditions were optimized in order to provide a simple, accurate, and economical analytical method, which can be employed for routine quality control of TTO in pharmaceutical dosage forms. The injection port and detector temperature were set to 230 and 240°C respectively and oven temperature program was set as shown in table 2. The solvent, column and acquisition parameters were chosen to be a starting point for the method development. However, the separation produced using these parameters were excellent. The retention time of α-pinene was approximately 9.69 min (Fig - 2) with good peak shape and USP tailing was approximately 1.0. A five point calibration curve was constructed with working standards and was found linear (r^2 =0.998) (Fig-2).The developed GC method with FID detector was accurate, precise, reproducible and sensitive. All the validation parameters of the method were shown to be good results. Accuracy and precision were determined by elaboration of standard calibration curve, i.e. (intra-day) and (interday). The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be 0.949 and 0.781 respectively.The α-pinene showed 96% - 98% recoveries from the formulation prepared in the lab. Moreover the %R.S.D. (less variation) shows good precision of the developed method. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods was specific as none of the excipients interfered with the analytes of interest. Hence, the method was suitably employed for assaying of TTO in the commercial formulations.

Fig. 2 Chromatogram showing the elution of α-pinene at their respective retention times

3.1 Validation of the Proposed Method

3.1.1 method validation

The methods were validated according to International Conference on Harmonization (ICH) guidelines(ICH Guidelines 1996, 1994) for validation of analytical procedures in order to determine the linearity, precision and recovery, limit of detection, limit of Quantification [7] [8]

3.1.2 calibration curve (linearity)

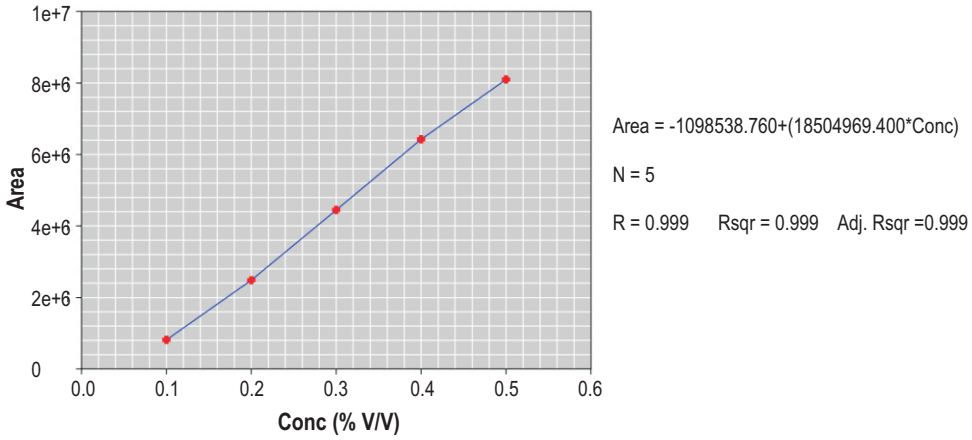
Calibration curve was constructed by spiking five different concentrations of α-pinene were studied from approximately 0.1 to 0.5 %. The chromatograms were found to be linear over an analytical range of 0.1, 0.2, 0.3, 0.4 and 0.5% of pure α-pinene oil and reproducible with time. The linear regression equation was calculated by the least squares method. The coefficient of determination was found to be 0.998 as shown in Fig 3.

3.1.3 precision (repeatability and reproducibility)

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as %R.S.D for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on 3 different days and the results documented as standard deviation and %R.S.D. Both repeatability (within a day precision) and reproducibility (between days

precision) were determined as follows. Solutions containing three different concentrations of the calibration curve i.e. 0.2, 0.3 and 0.4% were prepared. Nine injections at each of the specified concentration levels were injected within the same day for repeatability, and over a period of 3 days i.e. 3 injections/day for reproducibility. Mean and relative standard deviation was calculated. Inter-day as well as intra-day, gave an R.S.D. value 0.949 and 0.781 respectively.

Fig. 3 Calibration curve at the range of 0.1% to 0.5%



3.1.4 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for α-pinene were found to be 0.0003% and 0.0011% respectively.

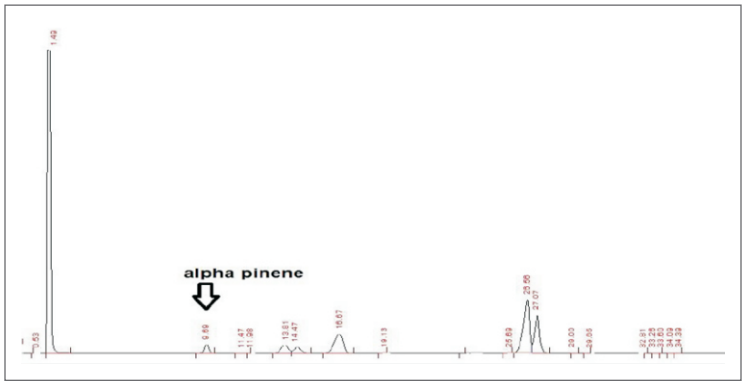
3.2 Recovery studies

The accuracy of the method was determined by sample (cream)with known amounts of the α-pinene reference substance. Mean recoveries for sample was found to be 96.29%.

3.3 α-pinene in Tea tree Oil

The α-pinenein Tea tree oil was confirmed by comparing the chromatogram obtained from Tea tree oil and Reference α-pinene retention time for α- pinene in both cases are found to be same i.e. about 9.69 minutes (Fig. 4).

Fig. 4 Chromatogram showing the elution of Tea tree oil, in this chromatograph the elution time of α-pinene was same as in the reference.



Conclusion

This method for assaying α-pinene from a tea tree oil sample is simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification.

Moreover the shorter duration of analysis for α-pinene make this method suitable for routine quantitative analysis in pharmaceutical dosage forms.

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