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Short communication

Method development and validation for the GC–FID assay of *p*-cymene in tea tree oil formulation

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Abstract

This paper describes the development and validation of an isothermal gas chromatography–flame ionisation detection (GC–FID) method for the assay of pure tea tree oil. The chromatographic conditions of the method employ a 5% carbowax packed column (20 m × 0.25 mm), isothermal elution with hydrogen at a column flow of 36 ml/min, injector and detector temperature at 220 °C and oven temperature at 100 °C, and a 1.5 μl injection volume. Samples and standard were diluted in hexane. The calibration curve for *p*-cymene was linear ($r^2 = 0.9995$) from 20 to 120% range of the analytical concentration of 100 μg/ml. The precision of this method was calculated as the relative standard deviation (R.S.D.) was 0.66% ($n = 6$). The R.S.D. for intermediate precision study was 0.13 and recovery of the *p*-cymene ranged between 93.39 and 97.86%. The limits of detection and quantitation were determined to be 2.08 and 10.39 ng/ml, respectively.
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Keywords: Tea tree oil; *p*-Cymene; Gas chromatography; Assay; Validation; Quality assurance

1. 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 myrtle 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 is pale yellow-green or colourless in appearance, and has a fresh, spicy, agreeable odour and therapeutic grade. Tea tree oil turns out to be one of the most useful of all essential oils, especially as an antiseptic, antibiotic, antibacterial, anti-fungal, anti-inflammatory, antiviral, and anti-infectious properties [1]. Tea tree oil has also been used successfully in the treatment of many other conditions and is now increasingly employed by herbal practitioners in many countries. Cuts, wounds, ulcers, sores, boils, burns, throat, bronchial and sinus infections, mouth ulcers, infected gums and many other conditions have all responded remarkably well to treatment with this essential oil. Its chemical content is not different from eucalyptus or rosemary, except that it has an unusually high content of terpinen-4-ol, an alcohol, which constitutes some 35% of the best quality oils. It is also worth noting that a thorough analysis of the oil revealed the presence of four constituents which have not been found anywhere else in nature: viridiflorene, present at 1%, B-terpineol (0.24%) 1-terpineol (trace) and allyl hexanoate (trace) [2]. Chemically *p*-cymene is a 1-methyl-4-(1-methylethyl) benzene [3] occurs in a number of essential oils (Fig. 1).

Quality assurance (QA) of the essential oil obtained from *M. alternifolia* is now clearly defined by the draft ISO 4730 "Oil of Melaleuca-Terpinen-4-ol Type". This standard identifies a set of chemical parameters (Table 1) that stipulates the range of 14 key terpenes. It should be stressed that this standard is primarily a QA tool for trade in pure tea

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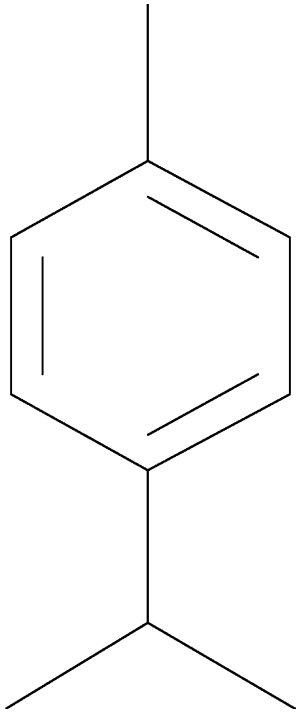

Fig. 1. Chemical structure of *p*-cymene.

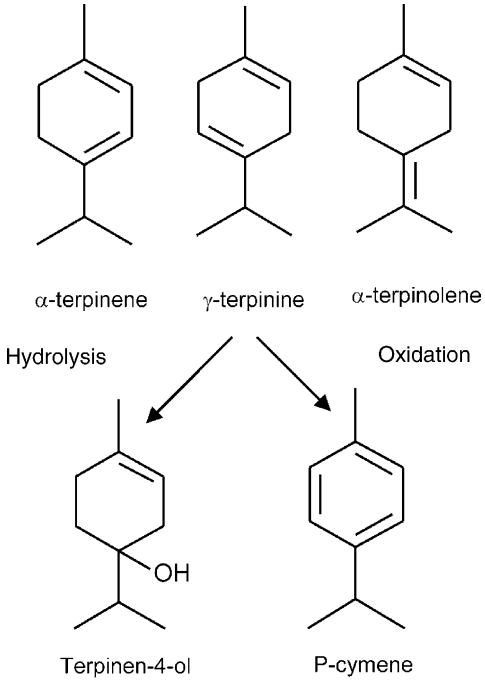

Fig. 2. Degradation pathways of some monoterpene hydrocarbons.

tree oil and does not provide a definitive composition for optimum concentrations of principal bioactive compounds. The issue of optimal bioactivity of tea tree oil has been addressed [4,5] and must be linked ultimately with QA. As therapeutic uses are identified and formulated products developed, significantly narrower ranges for tea tree oil constituents may be required to satisfy the more stringent demands placed on therapeutic and pharmaceutical raw materials. *p*-Cymene concentration can rise to levels approaching its upper limit (Table 1). Two pathways are operating here (Fig. 2), one involving hydrolysis of the pi bond at C-4 to produce terpinen-4-ol, the other involving oxidation of the *p*-menthane skeleton to its benzene analogue, *p*-cymene.

Table 1
Normative values of tea tree oil constituents

Constituent	Maximum (%)	Minimum (%)
1,8-Cineole	15	–
α-Terpinene	13	5
γ-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
Aromadendrene	7	Trace
δ-Cadinene	8	Trace
Globulol	3	Trace
Viridiflorol	1.5	Trace

The first pathway must involve water, naturally present in the oil through the steam distillation extraction process and possibly, trace volatile organic acids that may catalyse the reaction. The second pathway is well known in terpene chemistry however the various oxidation agent and catalysts that are involved in tea tree oil degradation require further investigation.

GC and GC–MS methods for tea tree oil have been reported [6,7]. GC methods are very useful in the determination of essential oils, and offers a significant improvement in sensitivity over previous reports. I 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. Owing to the widespread use of GC in routine essential oils analysis, it is necessary that good GC methods are developed and that these are thoroughly validated [8]. 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. This method can also be used to identify product degradation of *p*-cymene in stability studies.

2. Experimental

2.1. Materials

All chemicals and reagents were of the highest purity. The *p*-cymene reference material used was purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA) and hexane was purchased from Merck (Darmstadt, Germany).

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