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BIOGENIC CHARACTERISTICS OF SACROSANTOL® BY LIQUID SCINTILLATION COUNTER

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Abstract

In today's world of perfumery, natural ingredients play a very important role in the creation of perfumes. Consumer interest in 'naturals' is increasing exponentially, however supply issues of naturals, create obstacles in their easy adoption, thus challenging perfumers to find new sources of natural ingredients. These concerns have led to an increase in the usage of reconstituted essential oils. Reconstituted natural oils are blends created with natural and biotechnology-derived ingredients that mimic the composition of the original essential oil. However, classifying the product made with a reconstituted essential oil as natural has been challenging. Few methods are currently available to establish the naturalness of materials.

In this study, we have attempted to assess the naturalness of Sacrosantol– a reconstituted sandalwood oil formulated using natural and biotechnology-derived ingredients through radiocarbon (Carbon-14) testing method based on liquid scintillation counting. The standardized ASTM method as well as an improved method based on thermal oxidation and LSC was used for Carbon-14 specific activity determination. The percent modern carbon (pMC) for Sacrosantol was found to be 100 - 105% in both methods, clearly demonstrating the biogenic origin of Sacrosantol.

Keywords: *Santalol, sandalwood oil, Naturals, Liquid Scintillation counter, radiocarbon dating.*

1. Introduction

Consumers today are increasingly oriented towards the use of cosmetics and fragrances with characteristics of naturalness and minimal processing [1]. These features are perceived by consumers as synonymous with health and the environment [2]. While synthetic materials make the bulk of palette of a perfumer, many semi-synthetic and natural materials are increasingly getting introduced. Essential oils are one among the several raw materials used extensively in both perfumery and cosmetics.

Essential oils (EOs) are aromatic oily liquids extracted from different parts of plants, for instance, leaves, barks, seeds, flowers, peels and roots of the plant [3,4]. They are obtained by expression, fermentation, effleurage or extraction but among all the methods, steam distillation and hydro distillation are widely used for commercial production of EO's [5]. However, the demand for essential oils far outstrips the supply, leading to adulteration and usage of synthetic replacers in reconstituted essential oils [6]. These issues are noticeable mainly in high value-added oils like sandalwood oil which are difficult to obtain due to overexploitation of natural resources. Further, the addition of synthetics leads to an inability to claim support as natural or natural derived ingredients.

Therefore, the current market focus is shifting toward biotechnology-derived components being used to formulate reconstituted essential oils [7]. This aids in natural product substantiation as well. High-value oils like sandalwood oil have been reconstituted using biotechnology-derived and other natural ingredients. Sacrosantol is one such reconstituted sandalwood oil. This reconstituted oil has been compared to Natural sandalwood oil for its chemical and olfactive similarity, safety and skin benefits, using various in vitro models [8]

Natural verification of essential oils or reconstituted essential oils is difficult [9]. Since they are complex mixtures, it is often difficult to analytically identify a single component that could be checked for naturality. Among the many methods, which have been used to study the naturalness of a product, are chiral chromatography, molecular resonance rotational spectroscopy (MRRS) and isotopic determination of Carbon-14. However, chiral chromatography and MRRS are complex and expensive methods to implement and analyze as compared with isotopic determination of Carbon-14. The determination of Carbon-14 is performed with either accelerator mass spectrometer (AMS) or liquid scintillation counting (LSC) methods. AMS involves rigorous sample preparation and again is very expensive, but very precise and accurate. On the other hand, the direct method based on LSC is less expensive but uncertainty involved is higher when compared to the AMS method.

Carbon-14 determination by LSC method has been previously used to study samples like biodiesel for its biogenic origin [10]. We have extended this application of LSC for the study of essential oils. LSC works on the principle of excitation of the aromatic solvent molecules through the energy released from radioactive decay, the energy is next transferred to the scintillator (also sometimes referred to as the "phosphor" or "fluor"). This energy absorbed through the scintillators produces excited states of the electrons, which decay to the ground state and produce a light pulse characteristic of the scintillator. The light is detected by the photomultiplier tube of the liquid scintillation counter [11].

In the first method [12] which follows the ASTM D6866 procedure, the sample is directly analyzed in LSC without any combustion. However, one of the problems associated with direct method is that issue of chemical compatibility of the liquid scintillator with the oil sample being analysed. Hence, an alternate method based on the oxidation of the sample using thermal sample oxidizer is adopted. The oxidizer converts carbon species into the CO_2, which is trapped in a suitable absorber and measured using LSC

2. Experimental

Instrument: Quantulus 1220 Liquid Scintillation Counter

Samples and reagents: Two samples of Sacrosantol were taken up for analysis. One sample was subjected to ASTM method and the other was subjected to combustion and LSC.

3. Methodology

The sacrosantol sample was subjected to the standard ASTM D6866 method and the ^{14}C concentration is represented in terms of percent modern carbon values (pMC).

Thermal oxidation and Liquid Scintillation Counting:

The combustion of the sample and subsequent analysis with LSC is performed irrespective of the type and color of the sample taken for analysis. In this method, the sample is combusted in a conventional tube furnace system (Pyrolyser-Trio

Furnace System, Raddec International, UK) with a temperature profile suitable for the type of sample being combusted [13]. The CO_2 thus produced is trapped in an absorber as discussed by Bharath et al [14]. The sample is mixed with a suitable scintillator and analysed using Quantulus 1220 liquid scintillation counter.

4. Results And Discussion

The analytical measurement is cited as "percent modern carbon (pMC)". This is the percentage of Carbon-14 measured in the sample relative to a modern reference standard (NIST 4990C). The result is reported as "% Bio-based Carbon". This indicates the percentage carbon from "natural" (plant or animal by-product) sources versus "synthetic" (petrochemical) sources. Table 1 summarizes the results of the sample via the two methods.

Sr. No.	Sample Sent	Method	Value (pMC %)
1	Sacrosantol	Thermal oxidation & Liquid Scintillation Counter	100.1 ± 1.7
2	Sacrosantol	Direct analysis of the sample (without combustion) by LSC - ASTM	105

Table 1 Results of the sample via the two methods.

5. Conclusion

Percent Modern Carbon (pMC) value between 95 and 105% indicates no addition or dilution with fossil fuel-derived organic carbon. A pMC value greater than 105% indicates the likely addition or dilution with ^{14}C- labeled material or a carbon source derived from decades-old growth. A pMC value of less than 95% indicates the likely addition or dilution with the fossil fuel-derived material. A pMC value of 0% indicates that all organic carbon is from fossil fuel/synthetic derived material.

The results from both tests firmly establish that Sacrosantol is from 100% natural source and corroborates its application as an alternative to natural sandalwood oil in perfumery and cosmetic applications. This method can further be applied to study other reconstituted oils and prove their biogenic nature.

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