Detecting traces of methyl eugenol in essential oils

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Introduction

Regulatory authorities are becoming more concerned about even low levels of suspected toxins in flavour, fragrance and medicinal applications. For example, allyl alkoxybenzenes (e.g. safrole, estragole (methyl chavicol), methyl eugenol), common constituents of many essential oils, are of concern because high doses have caused cancer in rodents.

This concern is often exaggerated considering that:

- safety threshold doses some 1000 times less than the offending concentration recommended (e.g. IFRA Standards);
- carcinogenesis is not always considered a threshold phenomenon;
- the validity of dose extrapolation from rodents to humans is uncertain;
- many complete essential oils also contain anti-carcinogenic congeners.

Hence reliable methods for the determination of trace quantities of allyl alkoxybenzenes in essential oils and formulated products are essential.

Determinations

Gas Chromatography using FID and MS detection illustrated the complexity of these essential oils and the difficulties encountered in locating trace constituents.

Using tea tree oil, “Oil of Melaleuca, terpinen-4-ol type” as a model, careful column selection for GC-FID (DB 1701 Length 60 m x 0.25 mm Dia x 0.25 μm film (mid polarity) ) facilitated the separation and determination of methyl eugenol to the ppm level (Figure 1).

Because of the absence of competing mass spectral ions from Melaleuca oil components, GCMS in SIM mode using the m/z 178 ion (Varian Factor-Four VF-5ms, 30m x 0.25mm id, 0.25 μm film) facilitated the determination of methyl eugenol (Figure 2).

Such SIM traces, although excellent for determining analytes like methyl eugenol, give a false impression of the complexity of such oils.

The FID analysis of 100 randomly selected commercial tea tree oils gave methyl eugenol determinations ranging from 0 to 554 ppm (mean 209 ppm). Comparison of four exhaustively, laboratory-distilled tea tree oils determined by both FID and GCMS-SIM gave close agreement (Table) using two different methods in two different laboratories.

Discussion

Both GC-FID and GCMS-SIM methods were found suitable for determining trace quantities of methyl eugenol in essential oils.

These GC methods confirmed that, in Melaleuca alternifolia essential oil, methyl eugenol does not exceed 600 ppm (mean 209 ppm) for commercial distillations and 700ppm for exhaustive laboratory distillations. This result quantifies previous reports of trace amounts and is 20 times less than reports in a recently published SCCP opinion which must have used non-commercial Melaleuca data from other species. When plotted against published results, the dangers presented by methyl eugenol in tea tree oil are negligible because calculated absorption from recommended use is 10^6 times less than the level known to cause tumours in rodents

*100 mg neat oil applied per day = 1.25 mg/kg for 80 kg user = 1.250 μg/kg = 0.25 μg/kg methyl eugenol (0.2mg/ml in oil) = 0.0014 μmol/kg per mg = 1.4 x 10^-9 mol/kg, mg = 1.4 x 10^-9 x 6.023 x 10^23 molecules mol/kg = 8.4 x 10^14 molecules methyl eugenol/kg applied = 4.2 x 10^14 molecules methyl eugenol/kg absorbed (assuming 50% absorption).

*Hepatocellular Carcinomas (f = 0.909983)
(Pesto Eaters)
(Present in Food)

Methyl eugenol dosage for 100mg/day application of tea tree oil compared to flavour/food and pesto use of methyl eugenol (based on Fig.8 in Waddell 2002).

The authors would like to thank Claude Cassegrain from Cassegrain Tea Tree Oil for funding much of the methyl eugenol determination work and the Rural Industries Research and Development Corporation for funding participation in this symposium and Warwick Press, Industry & Investment NSW for preparing this poster.