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Note

Quantitative determination of minor components in essential oils: determination of pulegone in peppermint oils

CARLO BICCHI and CARLOTTA FRATTINI

Laboratorio Risonanza Magnetica Nucleare e Spettroscopie Applicate alla Tossicologia, Facoltà di Farmacia, University of Turin, Corso Raffaello 31, 10125 Turin (Italy)

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Peppermint oils (*Mentha piperita* L.) are widely used as flavouring agents in the pharmaceutical, confectionery and food industries. The identification of the geographical origins of peppermint oils is important, as oils from different sources, with small differences in chemical composition, differ widely in taste, aroma and price. Therefore, the identification, determination and toxicological properties of minor components are important. The toxicity of pulegone is interesting[1–3], and maximum allowable concentrations in mint beverage and confectionery have been proposed in Europe (EEC and Conseil de l'Europe). Recently, Grundschober[3] reviewed the toxicological properties and use of pulegone. Based on this review, the International Organization of the Flavour Industry Committee of Experts has recommended that the presence of pulegone in finished food products be restricted as follows: maximum in mint-flavoured candies, 250 ppm; maximum in all other foods and beverages, 20 ppm.

The quantitative determination of pulegone by gas–liquid chromatography (GLC) has been studied previously[4–7]. In this paper we report an improved GLC method applied to *M. piperita* L. var. *rubescens Italo-Mitcham* (commercial name, menta piemonte). A problem is that pulegone (minor component) has a retention time, according to the columns employed, that is either very near to that of menthol (main component), with consequent overlap (Fig. 1), or very similar to those of isomenthol and some sesquiterpene hydrocarbons (*e.g.*, γ-cadinene and caryophyllene)[8,9].

Our study was then oriented in two directions: (1) the separation of carbonyl compounds (pulegone) from peppermint oil constituents by extraction with Girard reagent[10] and GLC determination, and (2) silylation of hydroxy compounds (menthol) in order to change their retention times and to achieve a GLC separation.

EXPERIMENTAL

GLC analyses

GLC analyses were performed with a Perkin-Elmer Model Sigma 1 gas chromatograph equipped with a flame-ionization detector (FID). A 2 m × 0.125 in. I.D. 5% NPGS and a 3 m × 0.125 in. I.D. 20% Carbowax 20M nitroterephthalic

acid (TPA) stainless-steel column were used. The carrier gas (nitrogen) flow-rate was 30 ml/min. Temperatures were programmed from 80° to 180° at 4°/min. Methyl cinnamate (Merck, Darmstadt, G.F.R.) was used as the internal standard.

Extraction with Girard D reagent[10]

Peppermint oil (1 g) is dissolved in 15 ml of methanol and 3 g of Girard D reagent are added. The slurry is heated under reflux for 2 h, poured into 250 ml of distilled water, subjected to continuous extraction for 2 h with diethyl ether to remove the products that have not reacted, and acidified to pH 3 with dilute hydrochloric acid. After standing for 12 h the mixture is extracted three times with light petroleum (b.p. 40–70°). The extracts are concentrated by distillation at room temperature under reduced pressure, to give the carbonyl fraction of mint oil. It is then possible to identify the pulegone peak in the gas chromatogram of the extracted fraction (Fig. 1). Pulegone extraction is complete, as shown by comparison with a reference sample of menthol, menthone, isomenthone and pulegone in approximately the same proportions as in peppermint oil. The only problem with this method is to ensure complete extraction of menthol with diethyl ether, after reaction with Girard D reagent, because of the large amount of menthol present and its solubility in water. The best solution is to extract continuously with diethyl ether; although time consuming, the extraction becomes almost complete.

Silylation of menthol

Menthol can easily be silylated. The silyl derivative has a different volatility and, consequently, a different retention time to the parent compound, with improved GLC separation.

Peppermint oil (1 g) is added to 0.2 ml of pyridine, 0.1 ml of N,O-bis trimethylsilylacetamide and 0.1 ml of trimethylchlorosilane. After standing for 1 min, an exactly weighed amount of methyl cinnamate internal standard is added and 0.2 μl is injected into the gas chromatographic column. No detectable amounts of trimethylsilyl derivatives of the enolic form of pulegone were found. Fig. 1 shows the chromatogram of the silylated oil: the retention time of menthol is much lower and the pulegone peak is clearly isolated, as was demonstrated by the addition of pure pulegone to total oil.

Mass spectrometry

The mass spectrum of silylated menthol indicates the expected structure: menthyl–O–Si$(CH_3)_3$, *m/e* 228 (molecular peak), 143 (base peak), 75, 73, 138, 95, 213, 123, 157, 171.

Mass spectra were obtained by use of a modified Varian Aerograph 1200 gas chromatograph interfaced by means of a variable-slit separator with a Varian CH7A spectrometer. A 2 m × 0.125 in. I.D. stainless-steel column packed with 5% NPGS on Chromosorb W (80–100 mesh) was used. The helium flow-rate was 9 ml/min in the output of the fore-vacuum pumps. Mass spectra were recorded at 70 eV with a line-of-sight inlet system temperature of 200°, ion source temperature 220° and ion source pressure $2 \cdot 10^{-6}$ torr.

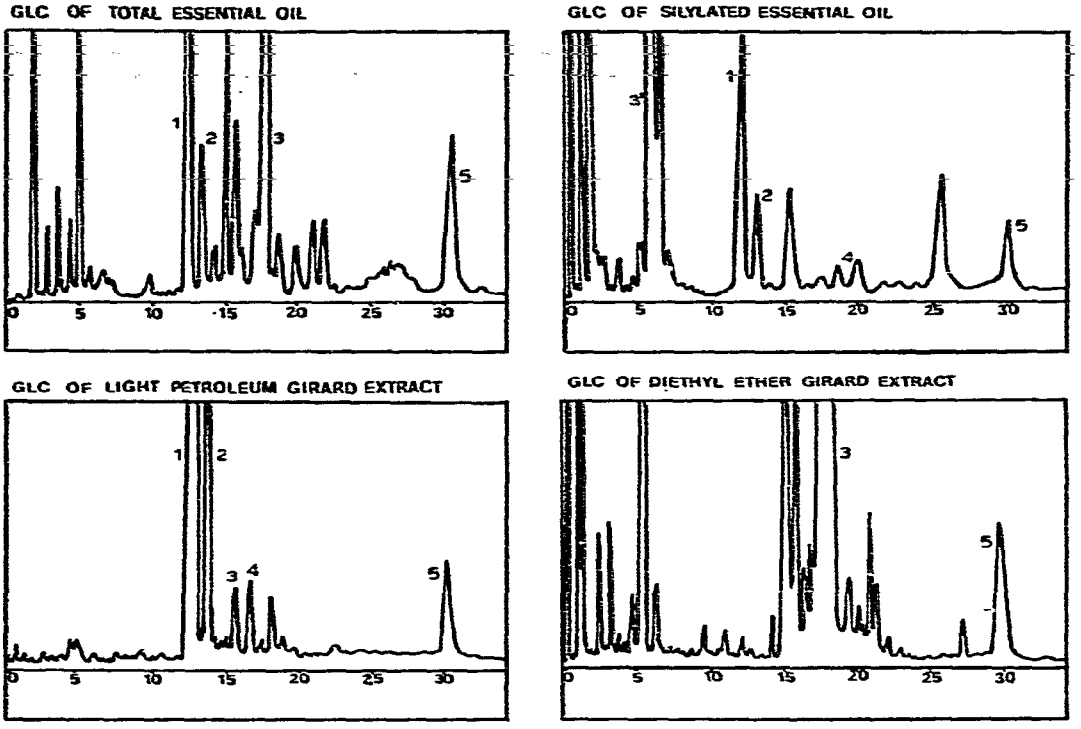

Fig. 1. Gas–liquid chromatograms of essential oils before and after extration or silylation. Peaks: 1 = menthone; 2 = isomenthone; 3 = menthol; 3′ = silylated menthol; 4 = pulegone; 5 = methyl cinnamate.

RESULTS AND DISCUSSION

The methods described were applied repeatedly (3–5 times) to five standard solutions, containing variable amounts (exactly weighed) of menthol, menthone, and isomenthone. The method involving extraction with Girard D reagent gave a recovery of pulegone of 85 ± 2%. With the silylating method it is possible to determine 95 ± 1% of pulegone present.

The results lead to the following conclusions. The silylating method does not require intermediate operations which may cause losses or chemical alterations, and is suitable for routine analysis, not only of pulegone, but also of all minor components that do not undergo silylation and, because of the presence of hydroxy compounds with similar retention times, would not be identified and quantitatively determined.

The method involving extraction with Girard D reagent gives less reproducible results and is slower, but is not destructive. Moreover, this method does not alter the components, but only splits the oils into two fractions (carbonyl and non-carbonyl compounds), allowing a reconstruction of the total GLC pattern. This is useful because a very fine characterization of peppermint oil allows effective quality control, with obvious commercial implications.

The two combined methods not only are useful in investigating the geographical origins and quality of peppermint oils, allowing detailed analyses of non-alcoholic minor components, but have also proved helpful in other research (*e.g.*, chemotaxonomy).

Finally, the methods described were applied to samples of *M.piperita* L. var. *rubescens Italo-Mitcham* from ten geographically different Piedmontese cultivations, and the results are reported in Table I.

TABLE I

GLC RESULTS FOR SAMPLES FROM TEN GEOGRAPHICAL ORIGINS

Sample	*Concentration of pulegone in oil (%)*	
	Extraction method	*Silylation method*
1	0.30	0.34
2	1.94	2.08
3	0.85	0.90
4	1.65	1.81
5	0.45	0.50
6	2.21	2.43
7	1.23	1.42
8	1.86	2.06
9	0.70	0.83
10	0.72	0.93

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