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Note

Estimation of menthone, menthofuran, menthyl acetate and menthol in peppermint oil by capillary gas chromatography

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An assessment of the quality of oils from plants such as lemon, spearmint, peppermint and eucalyptus can usually be performed by gas chromatography using conventional packed columns[1,2]. However, there is often a need to use columns with differing polarities to determine all the constituents which affect the quality of the oil.

The specificity and sensitivity of capillary column gas chromatography has enabled the separation and measurement of compounds in complex mixtures which hitherto has been impossible[3]. Capillary columns are eminently suitable for the evaluation of such mixtures as petroleum oils and essential oils where the pattern of compounds can be used either qualitatively for "fingerprinting" or for quantitative information[4]. Peppermint oil (from *Mentha piperita* L.) contains over thirty known compounds with many minor compounds as yet unidentified. Peppermint oil is used in confectionary, liquor, dental paste and pharmaceutical preparations.

Commercial quantities of peppermint oil are produced in Tasmania and feasibility studies for peppermint and spearmint oil production are currently being made in Victoria. Quantitative data are therefore required to evaluate the effect of factors such as varying photoperiod, temperature, plant density and time of harvest on the levels of menthone, menthofuran, menthyl acetate and menthol in the growing plant[5,6]. Clark and Menary[7] reported the use of a capillary column for the analysis of peppermint oil. However, for quantitation they assumed equal detector response for all compounds in the oil.

The purpose of this paper is to describe the use of an SP-1000 capillary column to obtain quantitative measurements of peppermint oil compounds using calibration factors and to estimate the repeatability of the procedure.

EXPERIMENTAL

Reagents

Menthyl acetate menthone and menthol were obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.), menthofuran from Eastman Kodak (Rochester, NY, U.S.A.), 2-methylnaphthalene from Aldrich (Milwaukee, WI, U.S.A.) and hexane (low in aromatic hydrocarbons) from Ajax Chemicals (Auburn, Australia).

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Capillary column installation

A support-coated open-tubular (SCOT) glass capillary column (43 m × 0.5 mm I.D.) from Chromalytic Technology (Melbourne, Australia) coated with SP-1000 was fitted into an aluminium support cage which was then fastened to the oven roof of the gas chromatograph. One end of the capillary column was connected via a T piece union to the detector by glass lined stainless-steel tubing (0.4 mm I.D.). The other end of the capillary column was connected to a splitless injector (SGE, Melbourne, Australia) by nickel tubing (0.25 mm I.D.). Make-up gas was piped to the detector by connecting a length of coiled stainless-steel tubing to the T piece union from the other injector port inside the oven.

Swagelok® fittings were used to modify the inlet/outlet carrier gas lines at the rear of the chromatograph to enable helium and nitrogen to be used as carrier gas and make-up gas, respectively. Helium was passed through moisture and oxygen traps and the gas flow to the column was regulated by a Porter model VCD 1000 variable flow controller.

Gas chromatography

A Packard-Becker 419 gas chromatograph equipped with dual flame ionization detectors and dual injectors was used. The injection port temperature was 190°C and detector temperature 190°C. The multilinear temperature programmer was used as follows. Initial temperature of 64°C was held for 3 min, then the temperature was raised at 0.5°/min to 80.5°C, then at 5°/min to the final temperature of 155°C, with an isothermal hold of 12 min at 155°C. The carrier gas was helium at a flow-rate of *ca.* 2 ml/min with nitrogen (18 ml/min) as make-up gas. Air flow was 300 ml/min and hydrogen flow 30 ml/min. The velocity of the carrier gas was about 21.5 cm/sec whilst the capacity ratio (k) of the column was 6.5 using docosane at 185°C. A Spectra-Physics (System 1) computing integrator with post-run calculation accessory was used to measure peak areas and compute the concentrations of the relevant compounds.

Preparation of standards and samples

The purities of menthol, menthone, menthyl acetate, menthofuran and 2-methylnaphthalene were determined by gas chromatography.

A standard calibration mixture was made by weighing approximately 100 mg of menthone, 200 mg of menthofuran, 100 mg of menthyl acetate and 200 mg of menthol into a 10-ml volumetric flask then adding 2 ml of the internal standard solution (5% (w/v) 2-methylnaphthalene in hexane) and making to 10 ml with hexane. The individual weights of compounds and internal standard concentration with their respective retention times were then entered into the computing integrator. Calibration factors for each compound were obtained by injection of the prepared calibration mixture and were automatically applied by the integrator to subsequent samples.

Peppermint oil samples were prepared by weighing 0.2 g of oil into a 10-ml volumetric flask, adding 2 ml of internal standard solution and making to 10 ml with hexane. If the solution was cloudy, anhydrous sodium sulphate was added to remove traces of moisture. Recoveries were determined by the addition of known weights of each of the four compounds to peppermint oil.

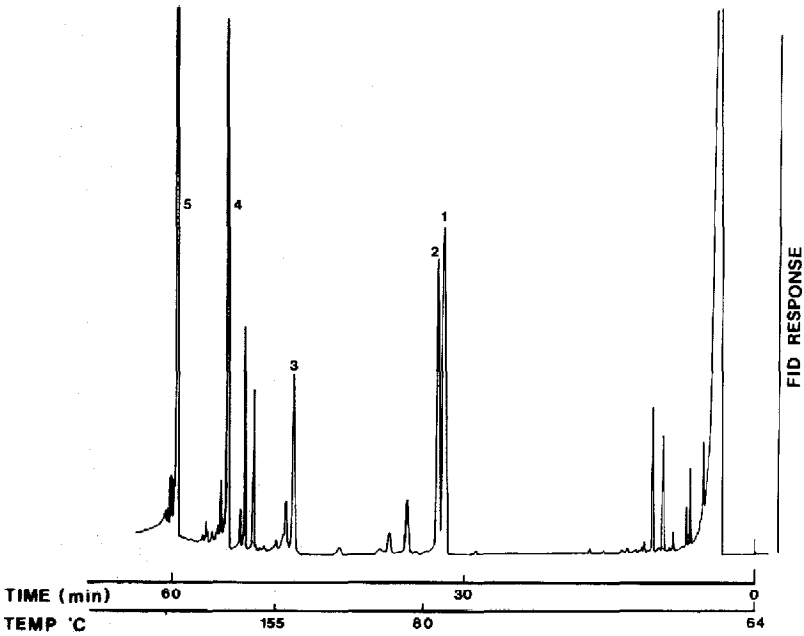

Fig. 1. Gas chromatogram of peppermint oil with conditions as described in the text. Peaks: 1 = menthone; 2 = menthofuran; 3 = menthyl acetate; 4 = menthol; 5 = 2-methylnaphthalene (internal standard).

RESULTS AND DISCUSSION

A typical chromatogram of peppermint oil which was obtained using the conditions and procedures as described is shown in Fig. 1. The total time for analysis was approximately 60 min.

The multilinear temperature programmer allowed adjustment of the temperature programme to optimise the separation of menthone and menthofuran. Earlier studies with a SCOT FFAP column (50,000 effective plates) failed to separate these two compounds; however, the present column (60,500 effective plates) provides satisfactory resolution as shown by the separation of peaks 1 and 2 in Fig. 1. As both the

TABLE I

RECOVERIES OF MENTHONE, MENTHOFURAN, MENTHYL ACETATE AND MENTHOL FROM PEPPERMINT OIL

Number of determinations: 5.

Additive	*Amount added (mg)*	*Mean recovery (%)*	*Standard deviation*
Menthone	19.5	89	2.4
Menthofuran	35.3	97	4.0
Menthyl acetate	15.1	97	0.9
Menthol	118.0	98	1.1

TABLE II

AMOUNTS (%, w/w) OF MENTHONE, MENTHOFURAN, MENTHYL ACETATE AND MENTHOL IN PEPPERMINT OIL FOUND IN REPEAT DETERMINATIONS

Sample	*Menthone*	*Menthofuran*	*Menthyl acetate*	*Menthol*
1	23.27	10.91	3.90	32.16
2	24.02	11.34	3.97	32.82
3	23.92	10.78	3.99	33.87
4	23.80	10.45	3.93	33.05
5	23.27	10.35	4.12	32.67
6	22.98	10.92	4.14	32.97
Average	23.54	10.79	4.01	32.92
Standard deviation	0.42	0.36	0.10	0.56
Coefficient of variation (%)	1.78	3.34	2.49	1.70

SP-1000 and FFAP phases are susceptible to oxidation, both carrier gas and make-up lines should contain oxygen and moisture traps. The column has been used intermittently for three years and over three hundred injections have been made with little loss in resolution.

The modifications to make-up gas and carrier gas connections allow the cheaper nitrogen to be used as make-up gas whilst allowing helium with its advantages of decreased time of analysis and better separation at higher temperatures to be used as carrier gas.

Calibration factors may vary due to differing flame conditions, but typical values for menthone, menthofuran, mentyl acetate and menthol were 1.44, 1.75, 1.43 and 1.25, respectively. Recoveries of menthone, menthofuran, menthyl acetate and menthol from peppermint oil are shown in Table I.

Repeatability data were not given in a paper describing a similar procedure for peppermint oil analysis[7]; however, the data shown in Table II confirm that the repeatability expressed as a coefficient of variation was better than 4% for the four relevant compounds.

The separation of compounds in peppermint oil by this method using SCOT columns should enable quantitative measurement of isomers[8] of compounds such as menthol to be made and allow evaluation of the influence of some of the minor compounds on oil quality.

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