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Computerized capillary gas chromatographic identification and determination of Siberian fir oil constituents

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Abstract

A method for the computer-assisted identification of peaks on a chromatogram on the basis of their retention indices on two capillary columns of different polarity was developed and applied to the identification and determination of Siberian fir oil components

1. Introduction

High-resolution capillary gas chromatography is still the most suitable technique available for the separation and identification of complex mixtures of volatile aroma compounds. This work is a part of our efforts to develop a rapid and simple, but reliable, method for a food and perfumery control system in Estonia. The purpose of this particular study was to develop a computerized capillary GC method with two columns of different polarity for analysing fir oil samples for terpenoid compounds.

The volatile constituents of conifer needles and oleoresins have been studied by different GC methods by several groups [1–3]. Siberian fir oil is produced by steam distillation from needles, twigs and sprouts of the plants *Abies sibirica* Ledeb. Average yields of the oil are 1.2–3%.

2. Experimental

Experiments were performed on a Chrom-5 gas chromatograph (Laboratorní Přístroje, Prague, Czech Republic) equipped with a flame ionization detector. Helium was used as the carrier gas with a splitting ratio of about 1:150. A Hewlett-Packard Model 3390A integrator and IBM 286 personal computer were applied for data processing.

Table 1 specifies the columns used and the conditions of analysis.

Characteristic properties of industrial Siberian fir oil samples were as follows: origin, Krasnoyarsk Region, Siberia; colour, light-yellow, clear liquid; odour, specific coniferous odour; density (ρ^{20}), 0.902–0.903 g/cm³; refractive index (n_D^{20}), 1.469–1.470; and acid value, 0.102–0.123 mg KOH/g. Five samples of the oil (0.2–0.4 μ l) were analysed by GC without any preliminary separations. All analyses were performed in triplicate.

The individual terpenoid compounds in the fir oil were identified by comparison of their retention indices (*I*) determined on the two capil-

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Table 1
Capillary columns used and operating conditions

Parameter	Bonded stationary phase	
	Polydimethyl siloxane OV-101	Polyethylene glycol 20M PEG 20M
Column length (m)	50	60
Column I.D. (mm)	0.20	0.32
Stationary phase film thickness (μm)	0.50	0.25
Plate number for <i>n</i> -decane at 90°C	145 000	300 000
Retention factor for <i>n</i> -decane at 90°C	1.45	0.32
Injector temperature (°C)	250	250
Helium flow-rate, (ml/min)	0.25-0.28	0.87-1.00
Column temperature (°C)	70-180	7 min at 70, then 70-180
Programming rate (°C/min)	1	2

lary columns with authentic data either determined in our laboratory or obtained from the literature [4,5]. The temperature-programmed *I* was calculated with the following equation [6]:

$$I = 100 \cdot \frac{t_{R(x)} - t_{R(z)}}{t_{R(z+1)} - t_{R(z)}} + 100z$$

where t_R = retention time, x = substance of interest and z and $z + 1 = n$ -alkanes with z and $z + 1$ carbon atoms emerging before and after substance x , respectively. The reproducibility of *I* expressed in terms of the standard deviation was 0.1-2.5 index units.

A program written in BASIC was used to calculate the *I* value for each chromatographic peak on both columns. Then the *I* on PEG 20M of each component present in the sample was compared with data obtained for standards on PEG 20M existing in the library, and possible identifications were made. In the next step the program looked up in the library the data for the same standard compound on OV-101 and made a comparison with the *I* values for each chromatographic peak obtained on OV-101. When a match was obtained, the component contents determined with the two columns were compared. The possibility of peak overlapping on OV-101 was also taken into account. A tolerance of 0.2% was selected for each *I* and 8% for the component concentration in a sample.

A flow diagram of the computer program is

shown in Fig. 1; a listing of the program is available from the authors on request.

3. Results and discussion

The retention indices of terpenoid compounds on OV-101 and PEG 20M identified in Siberian fir oil are reported in Table 2. Typical gas chromatograms of a fir oil sample analysed on the two columns are presented in Figs. 2 and 3. All terpenoid compounds investigated were completely separated on the OV-101 and PEG 20M columns except *p*-cumene, β -phellandrene and limonene on OV-101.

The quantitative composition of fir oil was determined by using the internal normalization method and checked by the internal standard method. *n*-Dodecane for OV-101 and *n*-tetradecane for PEG 20M column were used as standard markers. The purity of the standards was higher than 98%. The relative calibration coefficients for oxygen compounds were obtained from the literature [7].

The concentrations of the individual terpenoid compounds of a typical fir oil sample determined with the internal normalization and the internal standard methods are given in Table 3. Comparison of the two methods showed good agreement in component composition. The relative deviation between the results of two methods did not exceed 5%.

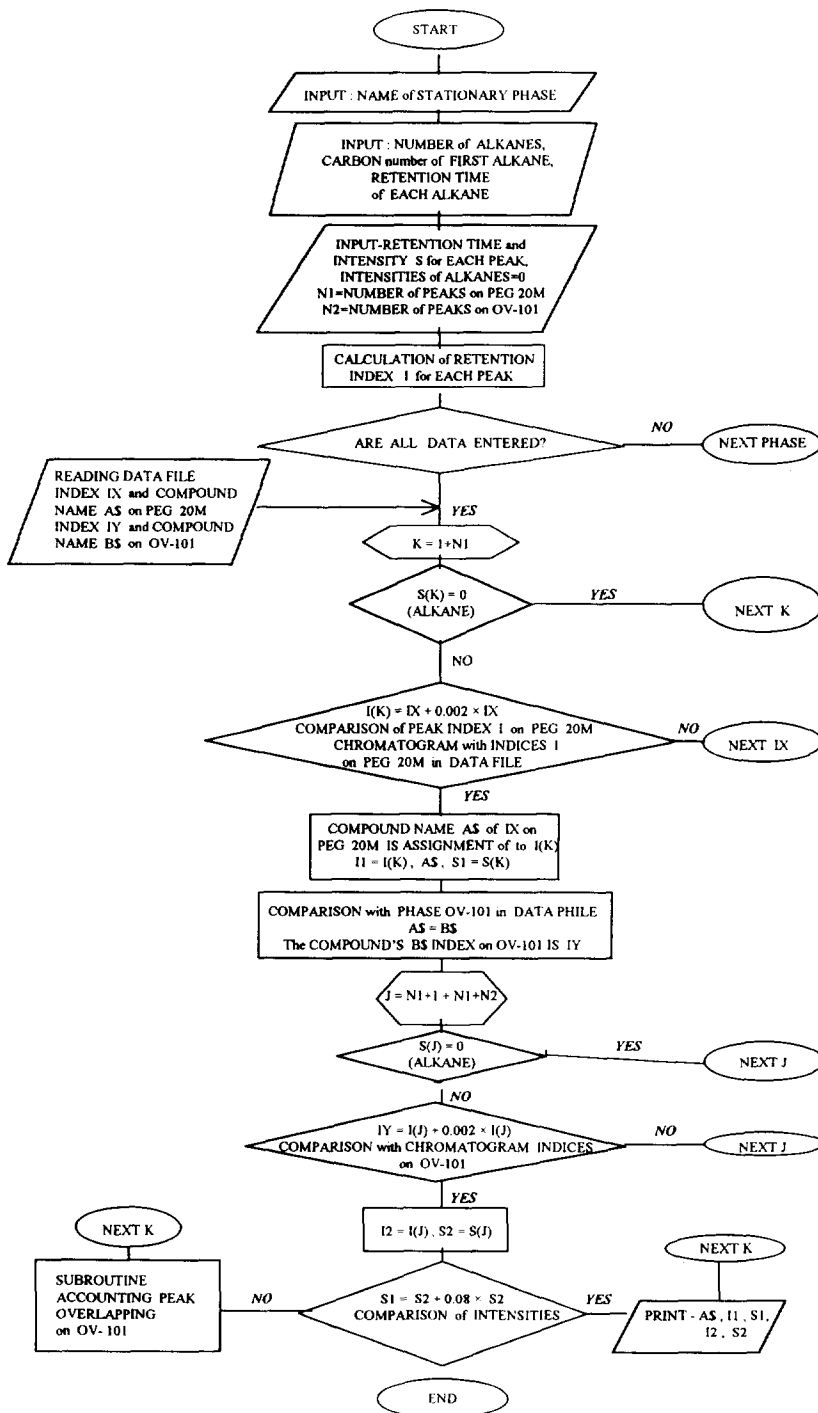


Fig. 1. Flow diagram of computer program.

Table 2
Retention indices of terpenic compounds on OV-101 and PEG 20M columns with temperature programming

Peak no.	Compound	I	
		OV-101	PEG 20M
1	Santene	880	1000
2	Tricyclene	919	1020
3	α -Pinene	930	1033
4	Camphene	943	1083
5	β -Pinene	967	1118
6	Myrcene	979	1167
7	α -Phellandrene	995	1172
8	Δ^3 -Carene	1005	1158
9	α -Terpinene	1009	1186
10	<i>p</i> -Cumene	1016	1277
11	β -Phellandrene	1018	1218
12	Limonene	1020	1209
13	γ -Terpinene	1045	1253
14	Terpinolene	1076	1290
15	Terpinen-4-ol	1120	1634
16	Camphor	1130	1524
17	Borneol	1152	1705
18	Bornyl acetate	1268	1591
19	Geranyl acetate	1361	1765
20	β -Caryophyllene	1406	1604
21	α -Humulene	1438	1673

The reproducibility of GC analysis determined as the standard deviation with a 95% probability was 0.001–1.1. The relative standard deviation (R.S.D.) was 0.5–4% for compounds with oil contents more than 0.2% and up to 8% for the minor components (<0.2%). As can be seen from Table 3, the reproducibility of the results

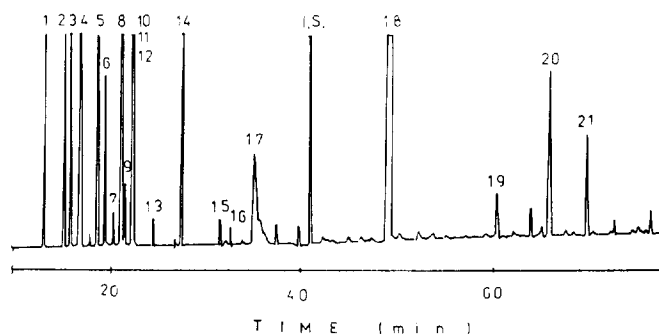


Fig. 2. Chromatogram of Siberian fir oil obtained on OV-101 column. Peak numbers refer to the compound listed in Table 2. I.S. = internal standard (*n*-dodecane).

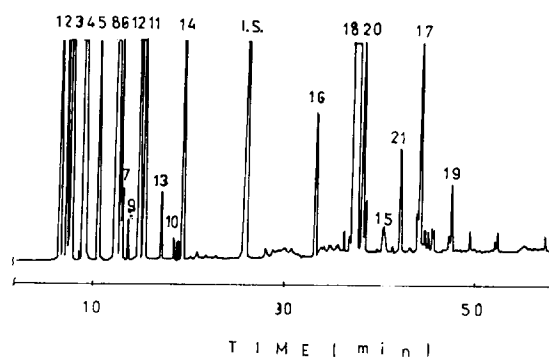


Fig. 3. Chromatogram of Siberian fir oil obtained on PEG 20M column. Peak numbers refer to the compounds listed in Table 2. I.S. = internal standard (*n*-tetradecane).

was better using the internal normalization method (<4%).

The results obtained demonstrated the possibility of using the simpler and rapid internal normalization method for the GC analysis of fir oil.

In the five Siberian fir oil samples studied, bornyl acetate (30–34%), camphene (22–24%), α -pinene (10–12%) and Δ^3 -carene (11–13%) were identified as major components, constituting about 80% of oil composition. The total content of santene, tricyclene, β -pinene, limonene, β -phellandrene, terpinolene, β -caryophyllene and borneol amounted for over 16% of oil composition.

In comparison with fir oils of other *Abies* species (European *A. alba* and *A. balsamifera* Mich.) [8], bornyl acetate occurred in higher

Table 3
Composition of the fir oil

Peak no.	Compound	Internal standard method		Internal normalization method	
		Content (% , w/w)	R.S.D. (%)	Content (% , w/w)	R.S.D. (%)
1	Santene	2.51 ± 0.11	1.8	2.52 ± 0.05	0.8
2	Tricyclene	2.37 ± 0.07	1.2	2.38 ± 0.04	0.7
3	α-Pinene	13.67 ± 0.42	1.2	13.74 ± 0.56	1.6
4	Camphene	24.16 ± 0.72	1.2	24.27 ± 0.62	1.0
5	β-Pinene	1.57 ± 0.08	2.1	1.57 ± 0.08	2.1
6	Myrcene	0.68 ± 0.02	1.2	0.69 ± 0.02	1.2
7	α-Phellandrene	0.12 ± 0.01	3.4	0.12 ± 0.005	1.7
8	Δ ³ -Carene	12.15 ± 0.49	1.6	12.23 ± 0.16	0.5
9	α-Terpinene	0.10 ± 0.01	4.0	0.10 ± 0.005	2.0
10	p-Cumene	0.14 ± 0.02	6.2	0.15 ± 0.01	2.7
11	β-Phellandrene	2.36 ± 0.15	2.6	2.47 ± 0.09	1.5
12	Limonene	3.98 ± 0.15	1.5	4.08 ± 0.08	0.8
13	γ-Terpinene	0.14 ± 0.02	5.8	0.14 ± 0.01	2.9
14	Terpinolene	1.10 ± 0.05	1.8	1.12 ± 0.02	0.7
15	Terpinen-4-ol	0.15 ± 0.02	5.4	0.14 ± 0.01	2.9
16	Camphor	0.05 ± 0.01	8.0	0.05 ± 0.005	4.0
17	Borneol	1.56 ± 0.20	5.2	1.66 ± 0.08	2.0
18	Bornyl acetate	30.81 ± 1.10	1.4	30.96 ± 0.45	0.6
19	Geranyl acetate	0.18 ± 0.01	2.2	0.17 ± 0.01	2.2
20	β-Caryophyllene	0.58 ± 0.03	2.0	0.59 ± 0.03	2.0
21	α-Humulene	0.33 ± 0.02	2.4	0.33 ± 0.02	2.4
Total		98.71		99.42	

concentrations in the Siberian fir oil samples investigated.

4. Conclusions

The computerized capillary GC method described here is simple and convenient for the identification and determination of terpenoid compounds in fir oils. This method may be applied to the quality control of commercial fir oils and also other essential oils, whereas other analytical procedures, e.g., spectroscopy and refractometry, do not allow the determination of the composition of individual components.

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