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Gas chromatographic determination of monoterpenes in essential oil medicinal plants

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ABSTRACT

Gas chromatographic (GC) techniques for the determination of the major biologically active monoterpenoids in peppermint, fennel, garden sage and creeping thyme plant material and water infusions were developed. The analysis requires 1–2 g of plant material or 100–200 g of infusion and takes no more than 1.5 h, including distillation and GC. The techniques allow the analysis of the herbs and infusions without the determination of the total essential oil content and weighing of isolated oils for chromatography. It was found that the essential oil component ratios were changed on dissolution in water during the preparation of infusions. The average values of the extraction factors for monoterpene alcohols, ketones, phenols and peroxides extracted from plant material are ten times those for related hydrocarbons and ethers extracted under the same conditions.

INTRODUCTION

Essential oil medicinal herbs are widely used in the U.S.S.R.in traditional and scientific medicine for treating many diseases [1,2]. The following components of essential oils are considered to be the most important biologically active substances in different medicinal herbs: menthol, menthyl acetate and methone for peppermint (*Mentha piperita* L.), fenchone and anethole for fennel (*Foeniculum vulgare* Mill.), cineole and camphor for garden sage (*Salvia officinalis* L.) and thymol and carvacrol for creeping thyme (*Thymus serpyllum* L.) [1].

Infusions are most often used for phytotherapy. According to the U.S.S.R. Pharmacopoeia [2], they represent aqueous extracts of plant materials and contain complex mixtures of water-soluble low- and high-molecular-weight compounds of various classes. It may be expected that infusions of the plant material are likely to contain only small amounts of essential oil constituents.

The aim of this work was to develop simple and rapid methods for the determination of the main biologically active terpenoids in plant material and infusions of peppermint, fennel, garden sage and creeping thyme, in order to allow us to determine objectively the quality of plant raw material and related medicines, to optimize their methods of production and to solve some other problems.

Terpenoids are usually determined by gas chromatography (GC) of essential

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oils isolated from samples by various methods. For essential oil isolation different methods are used, *e.g.*, extraction with organic solvents [3,4], hydrodistillation [5,6], the headspace method [7], retention by sorbents [8] or cooling traps [9], evaporation in a modified injector [10] and combinations of these methods.

For the isolation of essential oil components from plant materials and infusions we selected the hydrodistillation method, owing to its simplicity and rapidity, the relative stability of the analytes and the possibility of their quantitative isolation during distillation. Hydrodistillation can also be combined with GC using the internal standard method. As a result, we developed methods that are simpler and faster than those described in the literature for the determination of terpenoids in plant materials and water infusions.

EXPERIMENTAL

Distillation apparatus and distillation

The apparatus used is a modified version of that applied to for the determination of essential oils in plant materials according to the U.S.S.R. Pharmacopoeia [11] and the British Pharmacopoeia [12] methods. The modified apparatus is approximately half the size and has a more effective inverse condenser and other constructional improvements. The apparatus is shown in Fig. 1 (dimensions are given in millimetres). Condenser A is connected with chamber C, which is the oil and water condensate receiver. The receiver has orifice B closed with a stopper during distillation. The level of liquid in the receiver is maintained by arm D, which returns condensed water to flask.

Accurately weighed plant material (1–2 g) and 100 ml of water or 100–200 g of infusion, 20–40 g of sodium chloride (salting-out agent) and a few small pieces of porous earthenware were placed in a 500-ml flask, which was then joined to the apparatus. Before distillation, water was poured into the apparatus up to maximum level and 0.2–0.5 ml of a 4.95% (w/v) solution of methyl salicylate (internal standard) in hexane was accurately measured and introduced into receiver C through orifice B.

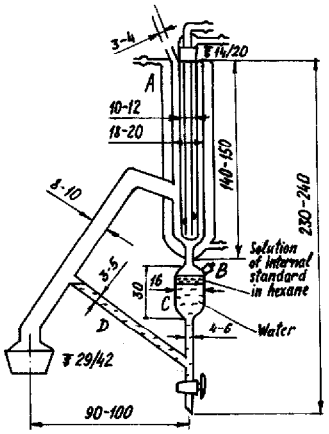

Fig. 1. Apparatus for essential oil isolation.

The internal standard solution was used as the collecting solvent for the hydrodistilled oil constituents.

During distillation, the essential oils and water vapour condensed in the condenser and the condensate dropped into the reciver. The terpenoids were dissolved in a mixture of hexane and internal standard but water was automatically returned through arm D into the flask with plant material or infusions. Because of the high partition coefficient between hexane and water, virtually full retention of oil components by hexane was achieved during distillation.

A distillation time of 45 min was estimated to be adequate for complete isolation of the analytes from all the herbs and infusions studied. After the end of distillation, 0.2–0.5 μl of the obtained mixture of isolated oils and internal standard were injected into the gas chromatograph.

To establish possible losses of analytes, the major components in peppermint, fennel, garden sage and creeping thyme oils were determined before and after distillation. Essential oils (0.2 g) with known composition were accuratley weighed and placed in a flask containing 100 ml of 20% sodium chloride solution when distilled for 45 min. A mixtgure of distilled essential oil with internal standard in hexane was chromatographed and the distillation recovery for the analytes was calculated. The results obtained (from 93.2 to 100.0%) indicated that the losses were insignificant.

The reproducibility of the isolation method was determined by distillation of five peppermint leaf samples with the same mass and composition. These samples were selected from well milled and mixed peppermint leaves. The essential oil from each sample was isolated and the determination of menthol, menthyl acetate and menthone was carried out. The results of statistical treatment [relative standard deviation (n = 5) for menthol = 1.98%, for menthyl acetate = 2.66% and for menthone = 3.57%)] confirm the high reproducibility of both isolation and analysis.

Gas chromatography

An LHM-80 (the 6th model) gas chromatograph equipped with a flame ionization detector was used. Thymol and carvacrol determinations were carried out on a 2 m × 3 mm I.D. stainless-steel column packed with 5% Superox 20M on Chromosorb W-HW (100–120 mesh) (Alltech) with temperature programming from 100 to 220°C at 4°C/min. In other instances a 3 m × 3 mm I.D. column with the same stationary phase was used and the temperature was programmed from 100 to 190°C at 3°C/min. The carrier gas (helium) and hydrogen flow-rates were 25 ml/min and the air flow-rate was 250 ml/min. The injector and detector were thermostated at 190 and 220°C, respectively.

The chromatography takes 30 min and full analyses, including hydrodistillation, not more than 1.5 h.

The determination of analytes was carried out by the internal standard method with methyl salicylate [4.95% (w/v) solution in hexane] as the internal standard. Mixtures of internal standard and essential oils isolated from each sample were chromatographed five times. The content was calculated by comparing the areas under the peaks of the analytes and internal standard and taking into account the response factors.

Menthol, camphor and thymol which corresponded to the U.S.S.R. Pharmacopoeia requirements, were used as standard substances. Menthone was obtained by

oxidation and menthyl acetate by acetylation of menthol. Other reference substances were isolated from essential oils: cineole from eucalyptus oil by *o*-cresol extraction, anethole by cooling from an ethanolic solution of fennel oil, fenchone from fennel oil by preparative column chromatography on silica gel using gradient elution with hexane–diethyl ether and carvacrol by alkaline extraction from thyme oils which are rich in carvacrol. Their authenticity was confirmed by IR spectrometry and their purity was determined by GC.

The response factors of analytes measured with respect to methyl salicylate under the conditions mentioned above were menthol 0.529, menthyl acetate 0.644, menthone 0.503, fenchone 0.498, anethole 0.531, cineole 0.497, camphor 0.504, thymol 0.513 and carvacrol 0.502.

Plant material and infusions

All the medicinal plant raw material samples investigated corresponded to the U.S.S.R. Pharmacopoeia [2] requirements and were bought in pharmacies. Fennel fruits were milled using a coffee grinder before analysis and preparation as infusions.

The infusions were prepared by the U.S.S.R. Pharmacopoeia [11] method: the plant material and water, taken in ratios appointed above, were heated in a special eathenware beaker for 15 min on a water-bath, then cooled by air to room temperature for 45 min and filtered. The ratios of plant material to water were taken according to the literature [13]: for leaves of peppermint and garden sage 1:40, for fennel fruits and for creeping thyme herb 1:20.

RESULTS AND DISCUSSION

It was found that complete distillation of analytes from all the species of plant materials and infusions investigated took 45 min. The dependences of the amounts of menthol, menthone and menthyl acetate isolated from peppermint leaves on distillation time are shown in Fig. 2.

Limonene has a retention time near to that of cineole using Superox 20M as stationary phase and would interfere in the determination of cineole. However, using

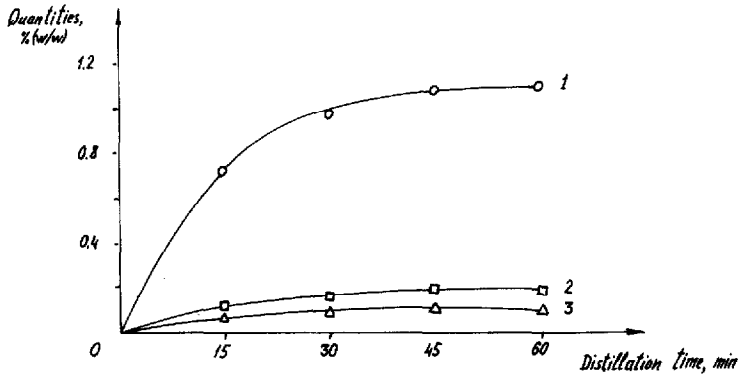

Fig. 2. Dependences of amounts of (1) menthol, (2) menthone and (3) methyl acetate isolated from peppermint leaves on duration of distillation.

a column with 3% OV-17 on Chromosorb W-HP (100–120 mesh) it was found that garden sage oils isolated from the investigated plant material samples contain only traces of limonene and cineole could be reliably determined.

The contents of major monoterpenoids in a set of peppermint leaves, fennel fruits, garden sage leaves and creeping thyme herb were determined by the developed methods. It can be seen from Table I that samples of one herb may have wideley differing contents and ratios of oil constituents. It is evident that medicinal preparations from these samples can vary in their medicinal efficacy.

The results of the determination of monoterpenoids in infusions (Table II) indicate that the contents of these compounds in infusions also differ significantly.

These methods allow the quantitative evaluation of the efficiency of the U.S.S.R. Pharmacopoeia method for infusion preparation. For this purpose we cal-

TABLE I

RESULTS OF MONOTERPENOID DETERMINATION IN PLANT MATERIAL

Plant material sample	Compound determined					
	Content (%, w/w)	R.S.D.[a] (%)	Content (%, w/w)	R.S.D.[a] (%)	Content (%, w/w)	R.S.D.[a] (%)
Peppermint leaf	*Menthol*		*Menthone*		*Menthyl acetate*	
20985	1.095	1.81	0.184	2.68	0.097	2.62
20986	0.277	2.64	0.149	3.53	0.137	2.82
1460987	0.843	1.65	0.098	1.30	0.175	2.18
81287	0.689	1.98	0.400	3.57	0.223	2.66
10188	1.351	1.71	0.188	1.45	0.192	2.59
Fennel fruits	*Anethole*		*Fenchone*			
80685	2.05	1.08	0.41	2.32		
10586	1.94	3.29	0.48	1.73		
80288	2.52	2.53	0.46	2.43		
90288	2.68	1.84	0.67	2.08		
100288	2.44	3.24	0.58	2.57		
240387	2.11	4.52	0.42	2.75		
Garden sage leaf	*1,8-Cineole*		*Camphor*			
50383	0.064	2.62	0.099	2.15		
31285	0.085	3.36	0.109	1.64		
70687	0.133	1.81	0.362	1.87		
181287	0.130	2.72	0.193	3.19		
161287	0.117	1.81	0.170	2.38		
20388	0.146	1.74	0.216	2.06		
Thyme herb	*Thymol*		*Carvacrol*			
10684	0.414	1.63	0.547	1.34		
20684	0.349	3.06	1.054	2.01		
50684	0.211	2.12	1.403	2.41		
130684	1.001	1.46	0.089	5.53		
150684	0.491	1.24	0.516	1.94		
470684	0.288	1.56	0.086	2.76		

[a] Relative standard deviation.

culated the extraction factor, which is the ratio between the amounts of a substance in the initial plant material and dissolved in water.

It was found that aqueous infusions of individual samples of herbs prepared under standard conditions show differing extraction efficiencies for the main constituents of the essential oils. Table II shows that the extraction factors for various substances are from only 1.0 to 45.2% at best. This illustrates the low efficiency of the infusion preparation method because the majority of the biologically active compounds is not used.

The extraction factors for a particular substance from different plant material samples are similar under the same conditions, indicating that the monoterpenoid content in infusions is approximately proportional to their content in the initial plant material and that infusions with higher concentrations of monoterpenoids can be preparated from herbs with high contents of these compounds.

Comparing the chromatograms of essential oils isolated from plant material and related infusions (Fig. 3), it can be noted that the ratio of the main components of

TABLE II

RESULTS OF MONOTERPENOID DETERMINATION IN INFUSIONS PREPARED BY U.S.S.R. PHARMACOPOEIA METHOD [2]

Plant material sample taken for infusion preparation	Compound determined								
	Content $\times 10^{-3}$ (%, w/w)	R.S.D.[a] (%)	E.F.[b] (%)	Content $\times 10^{-3}$ (%, w/w)	R.S.D.[a] (%)	E.F.[b] (%)	Content $\times 10^{-5}$ (%, w/w)	R.S.D.[a] (%)	E.F.[b] (%)
Infusions of peppermint (1:40)	*Menthol*			*Menthone*			*Menthyl acetate*		
20985	8.25	1.91	30.1	1.21	1.87	26.3	3.64	5.84	1.5
20986	2.62	2.34	37.7	1.02	1.44	27.3	4.11	6.78	1.2
1460787	7.82	1.02	37.1	0.69	3.52	28.4	4.37	7.12	1.0
Infusions of fennel (1:20)	*Anethole*			*Fenchone*					
80288	2.38	2.41	1.9	5.13	2.18	21.1			
90288	2.43	2.03	1.8	4.75	2.24	14.3			
100288	1.89	2.19	1.5	3.99	3.42	13.7			
Infusions of garden sage (1:40)	*Cineole*			*Camphor*					
50283	0.44	3.99	27.8	0.99	1.15	40.2			
70687	0.75	3.55	22.6	3.98	1.83	45.2			
161287	0.24	2.39	31.9	1.62	1.92	38.1			
181287	0.78	2.44	24.1	1.73	1.24	35.8			
20388	1.31	1.25	35.7	2.37	1.52	43.9			
Infusions of creeping thyme (1:20)	*Thymol*			*Carvacrol*					
10684	3.72	3.51	17.9	5.02	3.35	18.4			
20684	3.49	2.69	20.2	12.33	3.47	23.4			
150684	3.61	2.77	14.7	3.99	2.88	15.5			

[a] Relative standard deviation.
[b] Extraction factor.

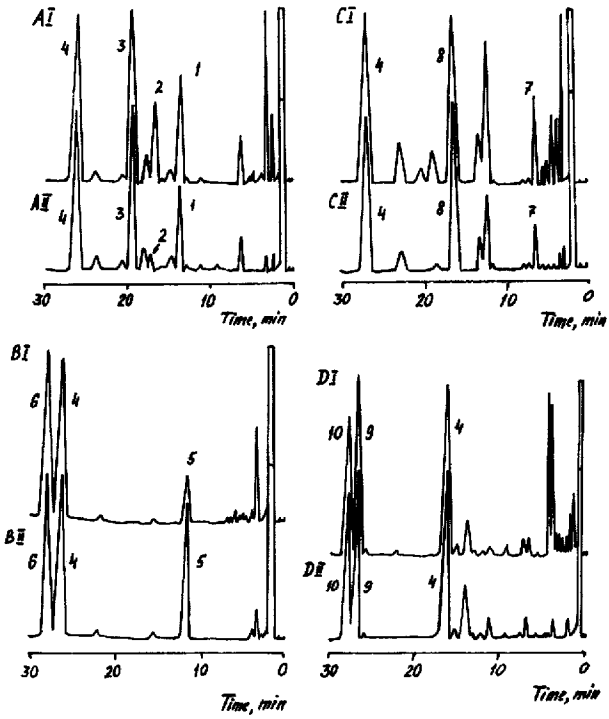

Fig. 3. Chromatograms of essential oils isolated from (A) peppermint, (B) fennel, (C) garden sage and (D) creeping thyme plant material samples (I) and infusions (II) prepared from them. Peaks: 1 = menthone; 2 = menthyl acetate; 3 = menthol; 4 = methyl salicylate (internal standard); 5 = fenchone; 6 = anethole; 7 = cineole; 8 = camphor; 9 = thymol; 10 = carvacrol.

essential oils is significantly changed when dissolved in water during the preparation of infusions. Fig. 3 and Tables I and II show that the preparation of infusions significantly lowers the proportion of menthyl acetate, menthone, etc., in peppermint oil, of anethole in fennel oil, of cineole and other constituents in garden sage oil and of monoterpene hydrocarbons and other constituents in creeping thyme oil.

The substances with an "open" oxygen-containing group such as the alcohols menthol, thymol and carvacrol and the ketones camphor, menthone and fenchone are more readily extracted by infusion than compounds where oxygen is "blocked" such as the ether anethole or the ester menthyl acetate. The epoxide cineole gives an anomalously high extraction factor.

To obtain comparable results we also studied the fennel and creeping thyme infusions made at a plant material-to-water ratio of 1:40. The extraction factors under these conditions were as follows: for fenchone 19.8–26.6%, for anethole 2.0–4.8%, for thymol 27.7–34.8% and for carvacrol 26.8–38.1%. The extraction factors of monoterpene hydrocarbons from all herbs were 0.7–2.1%.

Under these conditions the monoterpene alcohols, phenols, ketones and epoxides have extraction factors of more than 20%, on average ten times those of related hydrocarbons, ethers and esters.

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