Journal of Chromatography, 247 (1982) 356–358
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,072

Note

Analysis of essential oil mixtures in ointments

KARL-ARTUR KOVAR* and ALBRECHT SAKMANN
Pharmazeutisches Institut der Universität Tübingen, Auf der Morgenstelle 8, D-7400 Tübingen (G.F.R.)
(First received April 28th, 1982; revised manuscript received May 28th, 1982)

Essential oils are useful medicaments for the treatment of various diseases and are applied as antirheumatics, expectorants, carminatives and anti-inflammatory agents. Several essential oils are frequently mixed together in ointments, liniments and liquors. In order to establish the quality of such commercial preparations, it is necessary to determine the mixing ratio of particular essential oils. Previous quantitative analyses of essential oil mixtures by high-performance liquid chromatography (HPLC) in comparison with gas chromatography (GC)[1] showed that both methods are suitable but should be applied in different situations. For essential oil mixtures that contain strong UV-absorbing constituents, HPLC is preferred as it produces more complete resolution and is faster. On the other hand, GC is to be preferred for those mixtures which contain non-specifically detectable compounds.

In this work, these elementary findings were applied to the analysis of essential oil mixtures in ointments. A complex composite unguent containing both oxygenated and oxygen-free terpenes, *e.g.* pine needle, eucalyptus, mint and melissa oil, in addition to camphor and menthol was selected as a model. The principle of the proposed method is based on the separation of the terpene compounds from the ointment base by steam distillation using *n*-hexane to take up the volatile compounds. The diluted *n*-hexane solution was investigated by GC and the composition and mixing ratio of the essential oils were determined via their terpene contents using fenchone[2] as an internal standard.

EXPERIMENTAL

Steam distillation

Steam distillation was carried out in a special apparatus for the determination of essential oils in vegetable drugs as described in the European Pharmacopoeia (Ph.Eur.III)[3]. An accurately weighed 5.0-g sample of unguent was introduced into the distillation flask and 50.0 mg of fenchone and 300 ml of water were added. Then the condenser apparatus was attached and the distillation rate was adjusted to 2–3 ml/min. The distillate was collected in the graduated tube, using 1.00 ml of *n*-hexane to take up the volatile compounds. After 2 h the volume of the *n*-hexane solution in the graduated tube was read and the volume of a blank carried out under the same conditions using 5.0 g of ointment base was substracted (see the Ph.Eur.III[3]). The difference represents the amount of essential oil mixture and terpenes in the weight of ointment taken. The result was calculated as millilitres of oil per 100.0 g of ointment.

0021-9673/82/0000–0000/$02.75

Gas chromatography

A Hewlett-Packard Model 5711A gas chromatograph equipped with hydrogen flame-ionization detector (FID) and a Model 5671 A, automatic sample injector, was used. GC columns (4.2 m × 0.3 cm I.D.) were packed with 10% Carbowax 20M on Chromosorb W AW DMCS (60–80 mesh). The temperature of the injector and detector was 250°C and the column oven was heated to 80°C. Four minutes after injection the temperature was programmed at a rate of 4°C/min to 150°C, at which temperature it was maintained for 8 min. The carrier gas (nitrogen) flow-rate was 25 ml/min. A 2.0-μl volume of the collected distillate, diluted with *n*-hexane (1:10) and dried over anhydrous sodium sulphate, was injected. Peak areas were determined with a Hewlett-Packard Model 3370 integrator. Terpene contents were calculated using fenchone as an internal standard. Melissa oil was determined via citronellal and limonene, mint oil via menthone, eucalyptus oil via cineol and pine needle oil via peak 1 (a mixture of several monoterpene hydrocarbons).

RESULTS AND DISCUSSION

GC was preferred to HPLC because stability studies had to be carried out under constant conditions over a long period, and because HPLC columns decrease in efficiency much faster than GC columns. Isolation of the essential oils including camphor and menthol from the ointment was essential in order to avoid carbonization of the dissolved ointment base in the injector, contamination of the column and interference with peaks of the oxygen-free terpenes in the chromatogram.

Steam distillation carried out in the special apparatus described in the Ph.Eur.III[3] was effective. Under these conditions the volatile constituents of the ointment base (0.23 ml per 100.0 g ≈ 1.86% of the total mixture) did not interfere in the analysis of the terpenes as they were eluted later. Contrary to the method of the Ph.Eur.III[3], *n*-hexane was used instead of xylene to collect the volatile compounds. otherwise the determination of the percentage content of pine needle oil would have been difficult. Loss of *n*-hexane was not disadvantageous as the internal standard fenchone had been added to the distillation flask before steam distillation took place.

On analysis of an ointment sample containing 11.5 g of essential oils and terpenes per 100.0 g (≈ 12.36 ml per 100.0 g; $d_{20}^{20} = 0.9022$), an average value of $\bar{x} =$ 12.02 ml per 100.0 g ($n = 10$) using ointment base as a blank was obtained; this corresponds to a recovery of 97.2%.

The GC conditions proposed by Kovar and Friess[1] were slightly modified by using columns loaded with more Carbowax (10%); thus a higher effective plate number (>4200) was achieved in order to improve the resolution of the critical pair limonene–cineol and to facilitate the quantification of pine needle oil. The content and recovery of each essential oil were determined via characteristic components (Fig. 1, Table I), which had been selected before preparing the unguent. Each of the related terpenes in the distillate was identified by mass spectrometry. The peaks of camphor (7), cineol (3), menthol (8) and pine neddle oil (1) were well separated and quantified. The determination of mint oil via menthone (5), however, was complicated by incomplete separation from citronellal (6).

Quantification of melissa oil based on measuring geraniol (9) did not give satisfactory results and other constituents, *e.g.*, citronellal (6) and limonene (2), were therefore employed. Citronellal showed a better recovery but also a higher standard deviation than limonene. Accordingly, citronellal was used to measure the amount of melissa oil in in the mixture, whereas limonene served in stability studies in order to

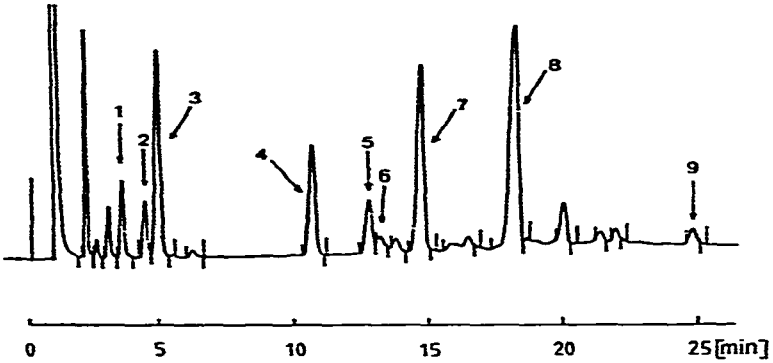

Fig. 1. GC separation of a mixture of essential oils and terpenes in ointment after steam distillation. Peaks: 1 = pine needle oil; 2 = limonene; 3 = cineol; 4 = fenchone (internal standard); 5 = menthone; 6 = citronellal; 7 = camphor; 8 = menthol; 9 = geraniol.

register any small loss of its content. The recoveries of the individual essential oils and of camphor and menthol in mixtures were found to be between 98.9 and 101.9%, the relative standard deviations ranging from 0.8 to 2.16% (Table I). Thus, the proposed method proved to be highly satisfactory for the analysis of mixtures of essential oils and terpenes. In principle, it can also be applied to other mixtures of different ratios and compositions.

TABLE I

RECOVERY OF ESSENTIAL OILS AND TERPENES FROM THE PREPARED OINTMENT (n = 10)

Essential oils and terpenes	*Peak No. in Fig. 1 for determination*	*Content (g per 100.0 g of ointment)*		*Standard deviation (g per 100.0 g of ointment)*	*Relative standard deviation (%)*	*Recovery (%)*
		Calculated	*Found*			
Pine needle oil	1	1.100	1.103	0.017	1.58	100.3
Eucalyptus oil	3	2.210	2.184	0.043	1.99	98.8
Mint oil	5	1.970	1.973	0.028	1.43	100.1
Melissa oil	6	1.870	1.905	0.041	2.16	101.9
Limonene	2	1.870	2.271	0.021	0.94	121.5
Camphor	7	2.000	2.006	0.016	0.80	100.3
Menthol	8	2.808	2.800	0.051	1.82	99.7

Stress tests at 41, 51 and 61 C over a period of 12 weeks were carried out under the conditions proposed by Grimm and Schepky[4]. Significant deviations from the original contents of each of the essential oils were not observed; this result agrees with those of Neuwald and Scheel[5]. It is concluded that the essential oils and terpenes used in the present work are stable towards autoxidation for more than 5 years.

REFERENCES

1 K.-A. Kovar and D. Friess, *Arch. Pharm. (Weinheim)*, 313 (1980) 416.
2 H. Glasl and H. Wagner, *Deutsch. Apoth.-Ztg.*, 114 (1974) 146 and 363.
3 *European Pharmacopoeia*, Vol. III, Maisonneuve S.A., 57 Sainte-Ruffine, France, 1975, p. 68.
4 W. Grimm and G. Schepky, *Stabilitätsprüfungen in der Pharmazie*, Editio Cantor, Aulendorf, 1980.
5 F. Neuwald and D. Scheel, *Pharm. Ind.*, 31 (1969) 879.