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CAPILLARY GAS CHROMATOGRAPHIC DETERMINATION OF VOLATILES IN SOLID MATRICES BY DIRECT INTRODUCTION USING A PROGRAMMABLE-TEMPERATURE VAPORIZER

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SUMMARY

A simple method for the rapid and reliable analysis of solid matrices by direct introduction in the glass liner of a programmed-temperature vaporizer is proposed. The procedure requires only very small samples sizes (about 0.1 mg), does not require pretreatment or concentration steps and can be carried out in less than 40 min. A comparison between composition data obtained from *Rosmarinus officinalis* leaves analysis by both simultaneous distillation extraction and direct introduction of the plant in the programmed-temperature vaporizer is also included. The proposed procedure allows the reproducible determination of the volatile composition of a plant with coefficients of variation of less than 5%.

INTRODUCTION

A large number of plant volatiles have been used as raw materials for flavouring foods and beverages and also in the pharmaceutical and cosmetics industries, but at present the need for a reliable and complete analysis of natural plant components still remains as the first step in essential oil studies and further flavour and fragance research.

Gas chromatography has been widely used in order to establish the identity and origin of essential oils, to detect adulterations or compositional changes and also for production quality control. However, some difficulties derived from the complexity of the sample have also been reported, mainly concerning to the sample preparation.

Most essential oils are obtained by distillation but other methods, such as solvent extraction and mechanical pressing, are also applied commercially[1], although the industrial methods for the preparation of essential oils vary to such an extent that considerable batch-to-batch differences can be encountered[2]. In addition, some of the constituents of essentail oils possess low thermal stabilities and others are reactive and therefore some rearrangement reactions can occasionally alter the composition

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of the sample prior to the beginning of the chromatographic analysis itself. Also, losses of trace volatile compounds and the production of artifacts can occur.

The simultaneous steam distillation–solvent extraction technique (SDE)[3–6] provides an extract that can be analysed by capillary gas chromatography without further enrichment. This method has already proved useful for isolating low-concentration volatiles in different types of sample[7,8], its main advantages being the very effective enrichment achieved with a relatively small amount of extraction solvent (1 ml) and the high recoveries obtained for the compounds to be analysed. However, solvent impurities and degradation products still remain as possible sources of interferences even if the purification of the extraction solvent and the concentration step are carefully performed. As far as gas chromatographic analysis is concerned, injection considerations are especially important. Most essential oils are exceedingly complex and it is probable that some injection modes may give rise to compositional changes due to discrimination against some components. Moreover, reliable chromatographic results not only demand the use of high-resolution columns but also require a very narrow starting band. In this respect, additional thermal focusing by locally cooling a section of the capillary column is obviously helpful in order to enhance the chromatographic resolution of low-boiling substances[9,10]. In addition, several workers have previously reported the use of dynamic headspace techniques for determining the essential oil composition of different plants[11–13].

In the last few years, the programmed-temperature vaporizer has proved useful for sampling introduction[14–17]. This vaporizer allows cold injection in three different modes[18] and consequently it eliminates the problems brought about by selective vaporization from the syringe needle and minimizes the discrimination observed[19,20]. A much broader field of application has already been reported[21] but further research on the use of the programmed-temperature vaporizer for sampling volatile compounds is still needed.

The aim of this work was to develop a method for the determination of the volatile composition of plant materials by direct introduction of plants into the programmed-temperature vaporizer injector of the gas chromatograph and subsequent thermal desorption. Expensive and time-consuming sample preparation techniques and the use of potentially hazardous organic extraction solvents which can produce artifacts should be eliminated in this way. A comparison with the data obtained by using the SDE method as the isolation step was also made.

EXPERIMENTAL

Plant material and extraction

Leaves from plants of *Rosmarinus officinalis* L. from Tarazona (Spain) were collected. Fractionation was carried out by using an SDE apparatus in the low-density solvent configuration[4]. A 100-ml volume of water was added to 2 g of dried and crushed leaves and 1 ml of *n*-pentane was used as the extraction solvent. The enrichment step was carried out for 1 h and further concentration of the extract was not required.

Direct introduction into the programmed-temperature vaporizer

A 0.1-mg amount of dried and crushed leaves was introduced without any

pretreatment into the middle of a Pyrex capillary tube (8 cm × 0.7 mm I.D. × 0.9 mm O.D.) between two small plugs of deactivated glass-wool. This capillary was placed in the empty liner of the vaporizer after having interrupted the carrier gas circulation, then the flow was established again and temperature programming and integration were started.

Gas chromatography

Gas chromatographic analyses were performed on a Perkin-Elmer 8320 gas chromatograph equipped with a programmed-temperature vaporizer injector, flame ionization detector and the required software to integrate peak areas. A fused-silica open tubular column (50 m × 0.22 mm I.D.) of BP-20 (SGE) with its first 2 cm inside the capillary containing the sample was used. The operating conditions are given in the figure captions.

Programmed-temperature vaporizer injector and temperature programme

Injections of the extracts obtained with the SDE technique were carried out in the split mode (splitting ratio 1:10) by maintaining the vaporizer at 30°C on injection. This temperature was increased at 14°C/s to 300°C and held at the final temperature for 5 min.

For direct introduction, injections both without and with additional thermal focusing were compared . For injections without thermal focusing the vaporizer was held at 30°C on injection, then increased at 14°C/s to 200°C. A splitting ratio of 1:200 was established 0.3 min after having started the analysis.

For injections performed with additional focusing, sampling was carried out in the split mode (splitting ratio 1:50) with an initial vaporizer temperature of 30°C, which was raised to 200°C in 0.2 min and maintained there for 1 min.

In order to reconcentrate the sample efficiently, an inexpensive and simple laboratory-made device, which allows the direction and the effect of thermal gradients established during on-column focusing to be optimized, was applied. The coolant circulation was initiated prior to the analysis itself and was maintained for 1 min after having started the chromatographic run. The passage of heated nitrogen was initiated 0.7 min after sample introduction and shut off 2.3 min later; therefore, cooled and heated nitrogen were applied simultaneously for 0.3 min. These experimental conditions were selected in such a way that a 'simultaneous double thermal effect' could be achieved in order to improve significatively the chromatographic resolution of the less retained solutes[22].

In all instances the experimental conditions were carefully established by optimizing separately each set of chromatographic analyses.

Gas chromatography–mass spectrometry (GC–MS)

The identity of the compounds was determined by GC–MS using a Konik 2000 gas chromatograph coupled to a VG 12-250 quadrupole mass spectrometer (VG Masslab) (electron-impact mode, 70 eV). The same chromatographic column and temperature programming as mentioned above were applied.

RESULTS AND DISCUSSION

Table I lists the 30 compounds identified by GC–MS in leaves from *Rosmarinus officinalis*; only those peaks contributing more than 0.09% were taken into account. The compositions of volatile compounds determined in the extract obtained by the SDE method and those resulting from direct programmed-temperature vaporizer injection are included. The essential oil compositions determined with the two methods are very similar, camphor (peak 18), α-pinene (2), 1,8-cineole (10), camphene (4), myrcene (7), borneol (26) and limonene (9) being the main contributors to the essential oil composition.

TABLE I

COMPOSITION (%) OF VOLATILE COMPOUNDS RELEASED FROM *ROSMARINUS OFFICINALIS* L. LEAVES

Comparison of the data obtained by SDE and direct programmed-temperature vaporizer injection.

Peak No.	*Identification*	*SDE*[a]	*Direct injection*[a]	
			Without thermal focusing	*With thermal focusing*
1	Tricyclene	0.30	0.10	0.17
2	α-Pinene	13.39	12.82	11.72
3	α-Fenchene	0.11	0.10	0.10
4	Camphene	8.22	7.41	7.24
5	β-Pinene	2.52	2.32	1.98
6	4-Methyl-3-penten-2-one	0.29	0.35	0.34
7	Myrcene	7.11	8.39	6.33
8	α-Terpinene	0.48	0.42	0.38
9	Limonene	3.52	4.49	3.84
10	1,8-Cineole	12.63	11.53	12.67
11	Δ^3-Carene	0.10	0.10	0.10
12	γ-Terpinene	0.40	0.48	0.34
13	3-Octanone	0.76	0.77	0.65
14	*p*-Cymene	1.02	1.36	1.33
15	Terpinolene	0.39	0.47	0.34
16	6-Methyl-3-heptanol	0.11	0.11	0.11
17	Fenchene	0.10	0.10	0.10
18	Camphor	33.16	31.23	32.29
19	Linalool	1.42	1.78	1.35
20	Pinocarvone	0.15	0.19	0.10
21	Bornyl acetate	1.76	2.57	3.43
22	Caryophyllene	1.14	2.17	3.52
23	Carvone	0.10	0.10	0.10
24	Pulegone	0.10	0.10	0.10
25	α-Terpineol	1.52	1.33	1.43
26	Borneol	4.82	5.07	5.27
27	Verbenone	1.77	1.35	2.04
28	Piperitone	0.10	0.10	0.25
29	*p*-Cymen-8-ol	0.13	0.15	0.10
30	Caryophyllene oxide	0.22	0.10	0.39

[a] Average values of the normalized peak areas ($n = 5$). The total may not be 100% as trace amounts of some compounds are not included.

Table II gives the coefficients of variation (C.V.) of the normalized peak areas ($n = 5$) obtained by the different methods. The compounds included were chosen to represent several chemical classes of compounds and to cover a wide range of solubilities, concentrations and boiling points. As expected, C.V.s obtained by analysing the same SDE extract five times are low whereas those obtained from five different SDE extracts of the same sample range between 2.07 and 17.74.

It is clear from Table II that direct injection of the sample into the gas chromatograph allows the most precise results to be obtained if a suitable cooling technique is used to enhance the chromatographic resolution of the less retained solutes. In this case, C.V.s lower than 5.4% are achieved with the only exception of β-pinene. Values far higher are generally obtained if determinations are accomplished by the other procedures that we have studied.

Figs. 1, 2 and 3 show the chromatograms resulting from an SDE extract and direct introduction without and with additional thermal focusing, respectively. When the two analyses performed by introducing the sample directly into the programmed-temperature vaporizer are compared, it can be seen that the poor resolution obtained for the most volatile solutes in Fig. 2 can make the determination of the less retained components of the sample difficult, whereas Fig. 3 demonstrates the advantage of cryofocusing for obtaining narrow solute bands and consequently for the efficient determination of the different essential oil components. As the method described does not require any sample pretreatment or concentration step, the complete procedure can be carried out in less than 40 min.

TABLE II

COEFFICIENTS OF VARIATION OF NORMALIZED PEAK AREAS ($n = 5$) OBTAINED BY ANALYSING *ROMARINUS OFFICINALIS* L. LEAVES BY SDE AND DIRECT PROGRAMMED-TEMPERATURE VAPORIZER INJECTION

Compound	*SDE*		*Direct injection*	
	A[a]	*B*[b]	*Without thermal focusing*	*With thermal focusing*
Camphene	0.62	3.04	7.40	2.49
β-Pinene	0.21	6.34	10.27	7.71
Myrcene	2.34	17.74	13.05	3.09
Limonene	0.41	4.03	11.43	5.43
1,8-Cineole	0.38	5.04	7.74	1.54
γ-Terpinene	0.95	7.87	12.58	4.33
3-Octanone	0.95	14.23	6.36	3.13
p-Cymene	1.11	14.33	12.87	5.40
Terpinolene	1.78	7.00	15.49	4.16
Camphor	0.32	2.07	2.32	1.22
Linalool	0.53	6.63	7.38	1.97
Bornyl acetate	0.90	11.01	14.94	2.78
Caryophyllene	1.71	13.96	9.79	4.64
Borneol	0.56	10.85	8.55	1.95
Verbenone	1.36	15.01	11.21	3.93

[a] Data obtained by analysing the same SDE extract five times.
[b] Data collected from five SDE extracts obtained from the same sample.

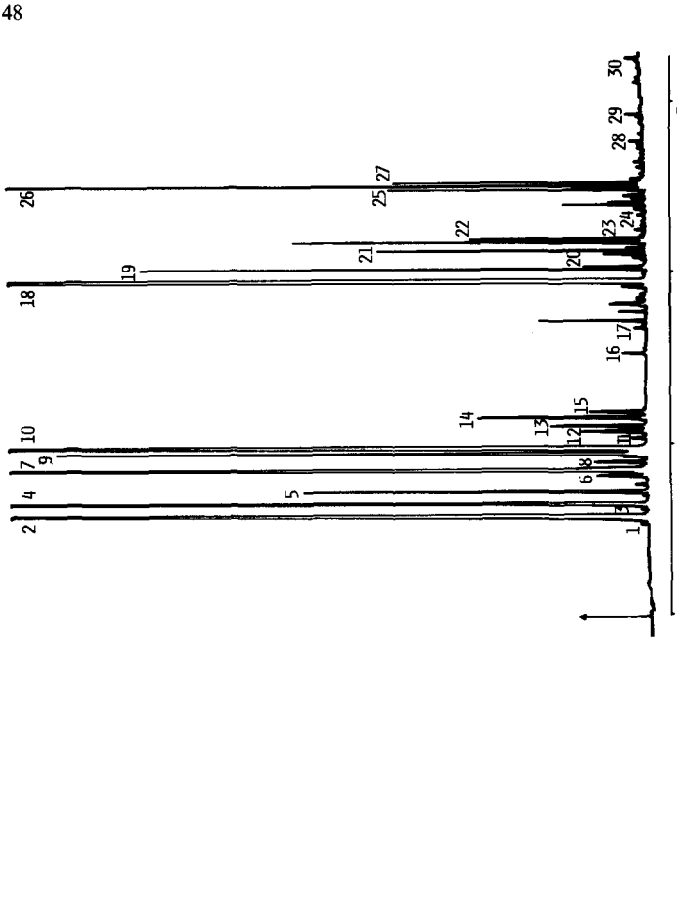

Fig. 1. Chromatogram of 2 μl of an SDE extract from *Rosmarinus officinalis* leaves injected in the split mode. Column: 50 m × 0.22 mm I.D. FSOT coated with a cross-linked film (d_f = 0.25 μm) of Carbowax 20M. Temperature programme: 50°C for 2 min then increased at 5°C/min to 190°C and maintained at this temperature for 5 min. Carrier gas: helium (40 p.s.i.g.). For peak assignments, see Table I.

Fig. 2. Chromatogram obtained by direct introduction into the programmed-temperature vaporizer glass liner of *Rosmarinus officinalis* leaves without additional thermal focusing. Column as in Fig. 1. The column temperature was held initially at 20°C for 0.3 min, then programmed to 60°C at 30°C/min, then to 190°C at 4°C/min and finally held at 190°C for 5 min. Carrier gas: helium (40 p.s.i.g.). For peak assignments, see Table I.

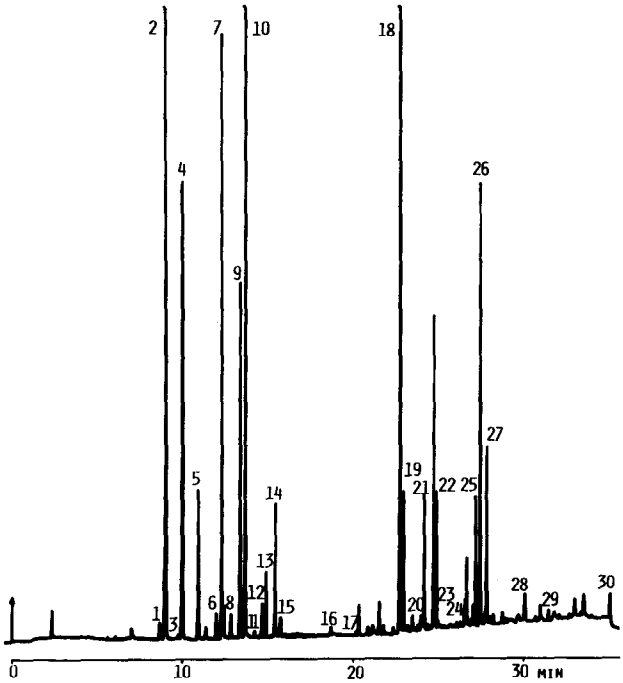

Fig. 3. Chromatogram obtained by direct introduction into the programmed-temperature vaporizer glass liner of *Rosmarinus officinalis* leaves with additional thermal focusing. Operating conditions are outlined under Experimental. Column as in Fig. 1. Temperature programme: 20°C for 1 min then increased at 30°C/min to 50°C and subsequently increased at 5°C/min to 190°C, held for 5 min. Carrier gas: helium (30 p.s.i.g.). For peak assignments, see Table I.

CONCLUSION

Direct introduction of 0.1 mg of plant, without pretreatment, using the glass liner of a programmed-temperature vaporizer allows the reproducible determination of the volatile components. The compositions obtained by using direct sample introduction and SDE fractionation are similar. However, the former method provides a higher precision when thermal focusing is used (C.V.s generally less than 5%). The very small sample sizes required may allow complete chemotaxonomic studies of rare or expensive plants, and also the chromatographic analysis of different parts of individual samples is extremely facile and rapid.

As other isolation procedures are more time consuming and also can cause severe losses of trace volatile components or alter the composition of volatiles, direct sample introduction seems to be an efficient alternative for establishing the composition of volatile components.

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