Reversed Phase High Performance Liquid Chromatography of Essential Oils

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Z. Naturforsch. **35 c,** 675–681 (1980); received June 20, 1980

High Performance Liquid Chromatography (HPLC), Essential Oils, Terpenes, Terpenoids, *Cistus*, Cistaceae

A reversed phase high performance liquid chromatographic procedure for the separation of essential oils is described. Applying water-acetonitrile elution systems on octyl and octadecylsilane-bonded silica, complex mixtures of sequiterpenes and oxygenated volatile constituents can be resolved, comparably to the quality of gas chromatographic analysis. Photometrical detection at different wavelengths can be an important parameter in optimizing the separation of essential oil constituents. The application of the described method is demonstrated with naturally occurring mixtures of terpenes from leaves of *Cistus ladanifer*.

Introduction

Existing studies on high performance liquid chromatography (HPLC) of essential oils lack the possibility of a broad application, and still the method of choice for saturated constituents is gas chromatography using capillary columns [1].

The apparent major problem in using the technique of HPLC for the analysis of essential oil constituents arises from the applied solvent systems which limit photometrical detection. Because of the lack of chromophoric groups in most constituents either refractive index (RI) or low UV monitoring are necessary. The use of RI detection goes to the account of sensibility and dependability and it turned out that some types of compounds escape detection [2]. The alternative detection with UV light limits the selection of solvent systems, which most of them – *e.g.* the widely applied methanol – show high absorptions at lower wavelengths. This is a common problem if gradient elution is necessary to resolve highly complex mixtures. Therefore most communications report on HPLC of compounds which are UV-detectable above 240 nm [2–6].

Schwanbeck and Kubeczka [7] demonstrated an excellent separation of terpenehydrocarbons using *n*-pentane on a silicagel column with UV detection at 220 nm, at which no applied compound escaped detection. However, this procedure makes it necessary to operate at very low temperatures (–15 °C).

This present communication reports results on reversed phase HPLC of essential oils using water-acetonitrile mixtures and UV detections at 200, 220, and 254 nm and we demonstrate different selectivities of octyl and octadecylsilane-bonded silica which complement each other.

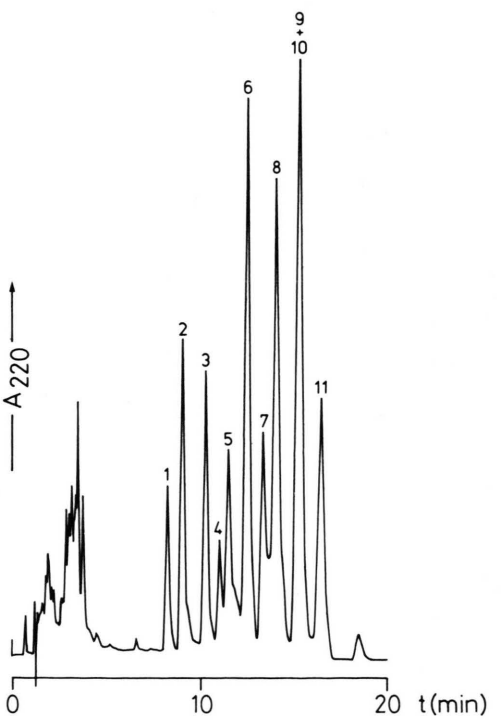

Fig. 1. HPLC resolution of a standard mixture of sesquiterpenes on LiChrosorb RP-18 using 85% acetonitrile in water with a flow-rate of 2 ml/min. For peak identification see No. in Table I.

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0341-0382/80/0900-0675 $ 01.00/0

Materials and Methods

Extraction of essential oils from *Cistus* plants, which were grown in the field of the Botanical Institute of the University of Cologne, was carried out by steamdistillation of the leaves. Fractionation of the oil according to functional groups was achieved on SiO_2-columns as described previously [8–10]. Standard samples of monoterpenes and monoterpenoids were purchased from Fluka, Buchs (Switzerland), Roth, Karlsruhe, Schuchardt, München, and EGA-Chemie, Steinheim. Sesquiterpenes were a generous gift of Dr. E. Klein, Dragoco, Holzminden.

The liquid chromatograph used was Spectra-Physics (Santa Clara, Calif., USA) and included two Model 740B pumps with 740B pump control units, a 714 pressure monitor, a 744 solvent programmer, and injection was done via a Rheodyne rotary valve with a 20 µl loop. Detection was achieved with a Schoeffel SF 770 UV-VIS spectroflow monitor and a Spectra-Physics SP 8200 dual-beam UV-VIS detector (interference filter). Retention times were obtained with an Autolab System I computing integrator (Spectra-Physics). The chromatographic columns (250 × 4 mm) were prepacked with LiChrosorb RP-8 or RP-18 (both 5 µm), E. Merck, Darmstadt. Elution systems are described in the legends.

Gas chromatographic analysis was carried out with a Hewlett-Packard (Model 5830 A) chromatograph with a 18850 A GC terminal. The liquid phase in the used capillary columns was 10 m Sp 2100. The temperature program was 45–170 °C (4 °/min) with 30 min upper limit for terpenes and 10 min upper limit for terpenoids.

Results and Discussion

Essential oils represent a complex mixture of almost any type of organic, mostly volatile compounds. In general, these mixtures are preferably analyzed by gas chromatography using highly selec-

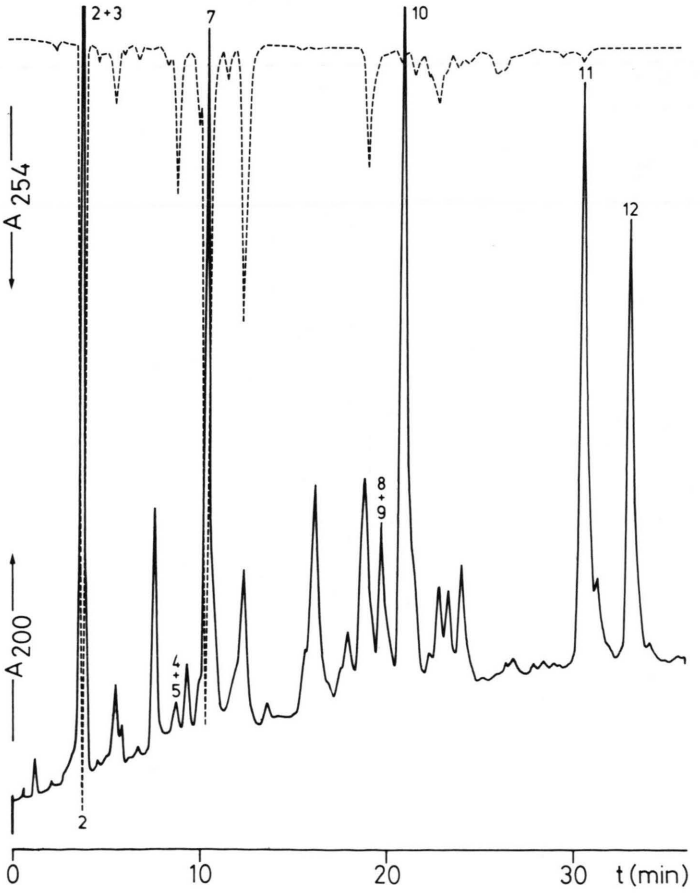


Fig. 2. HPLC separation of constituents of the 2-chloropropane fraction [8–10] from the essential oil of *Cistus ladanifer* on LiChrosorb RP-8 using a linear gradient from 40–70% acetonitrile in water within 40 min (2 ml/min). For peak identification see No. in Table II.

tive capillary columns [1] with an universal detection by flame ionization.

In the last few years there is accumulating knowledge about the application of high performance liquid chromatography to analyses of essential oils. Most literature centers mainly on those constituents, which are photometrically detectable at wavelengths higher than 240 nm [2–6] and Ross [5] stated, that many volatile constituents could not be analyzed by HPLC with UV detection because of the lack of chromophoric groups of many terpenes and terpenoids. The alternative widespread RI detection is not very useful because of its low sensitivity, deficient dependability and as shown by Komae and Hayashi [2] exhibits selective detection. The latter which also concerns UV detection can be of an advantage because impurities can be filtered out. This feature surpasses gas chromatography. By selective detection one can acquire information relative to the degree of unsaturation in individual compounds analyzed by HPLC. Latz and Ernes [11] demonstrated a selective fluorescence detection of *citrus* oil components.

Schwanbeck and Kubeczka [7] and the results in the present study demonstrate the possibility that any desired constituent of essential oils can be analyzed by HPLC with UV detection at 200 and 220 nm. This requires the use of solvents, which do not exhibit a background absorption at these wavelenghts. Schwanbeck and Kubeczka [7] published an excellent separation of terpenehydrocarbons with *n*-pentane as solvent on a silicagel column, requiring, however, the operation at low temperatures (−15 °C). Our results show that similar quality HPLC of these compounds can be achieved with water-acetonitrile mixtures on reversed phase columns and no applied compound escaped detection at 200 or 220 nm. Tables I and II list the compounds which were analyzed. On LiChrosorb RP-18 a standard mixture of 11 sesquiterpenes was resolved within 17 min. Only β-maaliene and α-gurjunene elute simultaneously with a retention time of 15.33 min (refer to Fig. 1). On LiChrosorb RP-8 this mixture eluted with only 5 peaks, however, β-maaliene and α-gurjunene were separated. These different selectivities of RP-8 and RP-18 for terpenes are most striking when applying oxygenated monoterpenes as to be seen in Table II. These two phases complement each other in separations of these compounds. Some compounds which are un-

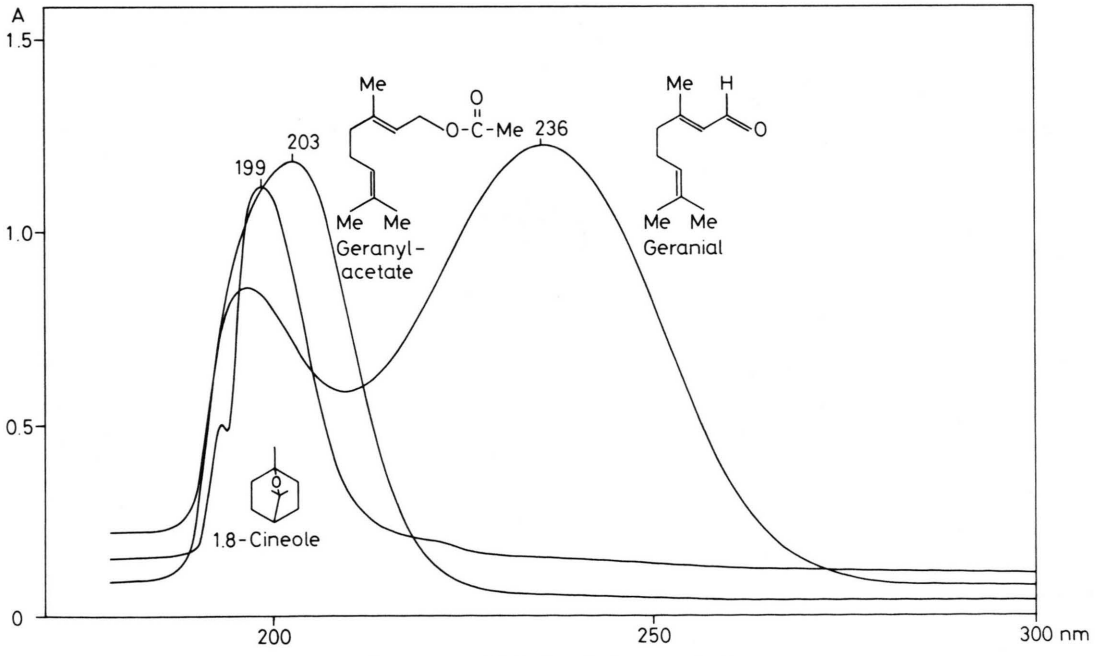


Fig. 3. UV spectra of geranial, geranylacetate, and 1,8-cineole in acetonitrile.

Table I. Structures [12] and retention times of sesquiterpenes which were applied to HPLC on LiChrosorb RP-18 using 85% acetonitrile in water (2 ml/min). Fig. 1 shows the chromatogram.

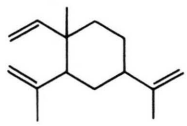
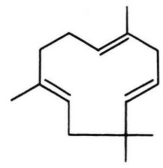
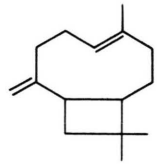
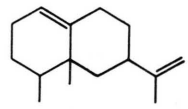
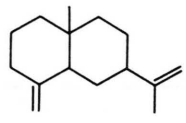
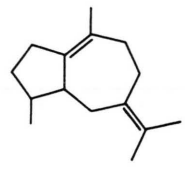
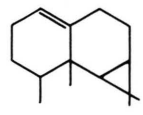
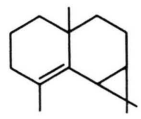
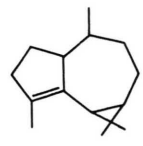
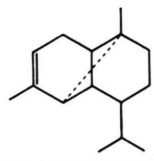
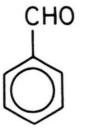
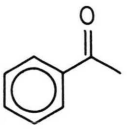
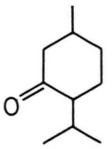
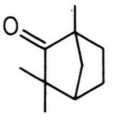
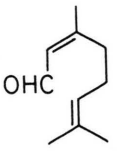
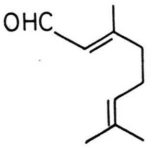
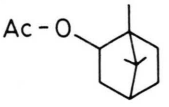
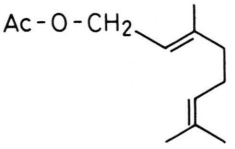
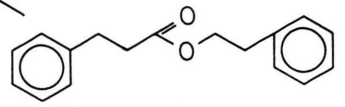
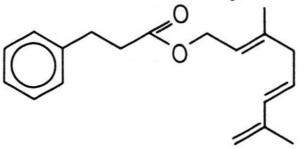
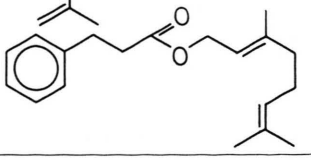
No.	Compound	Structure	t_R [min]
1	β-Elemene		8.32
2	Humulene		9.10
3	Caryophyllene		10.36
4	Valencene		11.08
5	β-Selinene		11.53
6	δ-Guaiene		12.53
7	Longifolene		13.42
8	Calarene		14.08
9	β-Maaliene		15.33
10	α-Gurjunene		15.33
11	Copaene		16.53

Table II. Structures and retention times of oxygenated volatile essential oils constituents which were applied to HPLC on LiChrosorb RP-8 and RP-18 using a water-acetonitrile gradient as described in Fig. 2.

No.	Compound	Structure	t_R [min]	
			RP-8	RP-18
1	Benzaldehyde		3.80	2.78
2	Acetophenone		4.02	3.02
3	Menthone		4.02	3.02
4	1,8-Cineole		8.58	7.78
5	Fenchone		8.58	7.20
6	Neral		9.27	7.20
7	Geranial		10.07	7.78
8	Bornylacetate		18.88	14.72
9	Geranylacetate		18.88	14.72
10	Phenylethylphenylpropanoate		19.92	14.72
11	Dehydrogeranyl-phenylpropanoate		29.37	23.07
12	Geranylphenylpropanoate		31.85	25.93

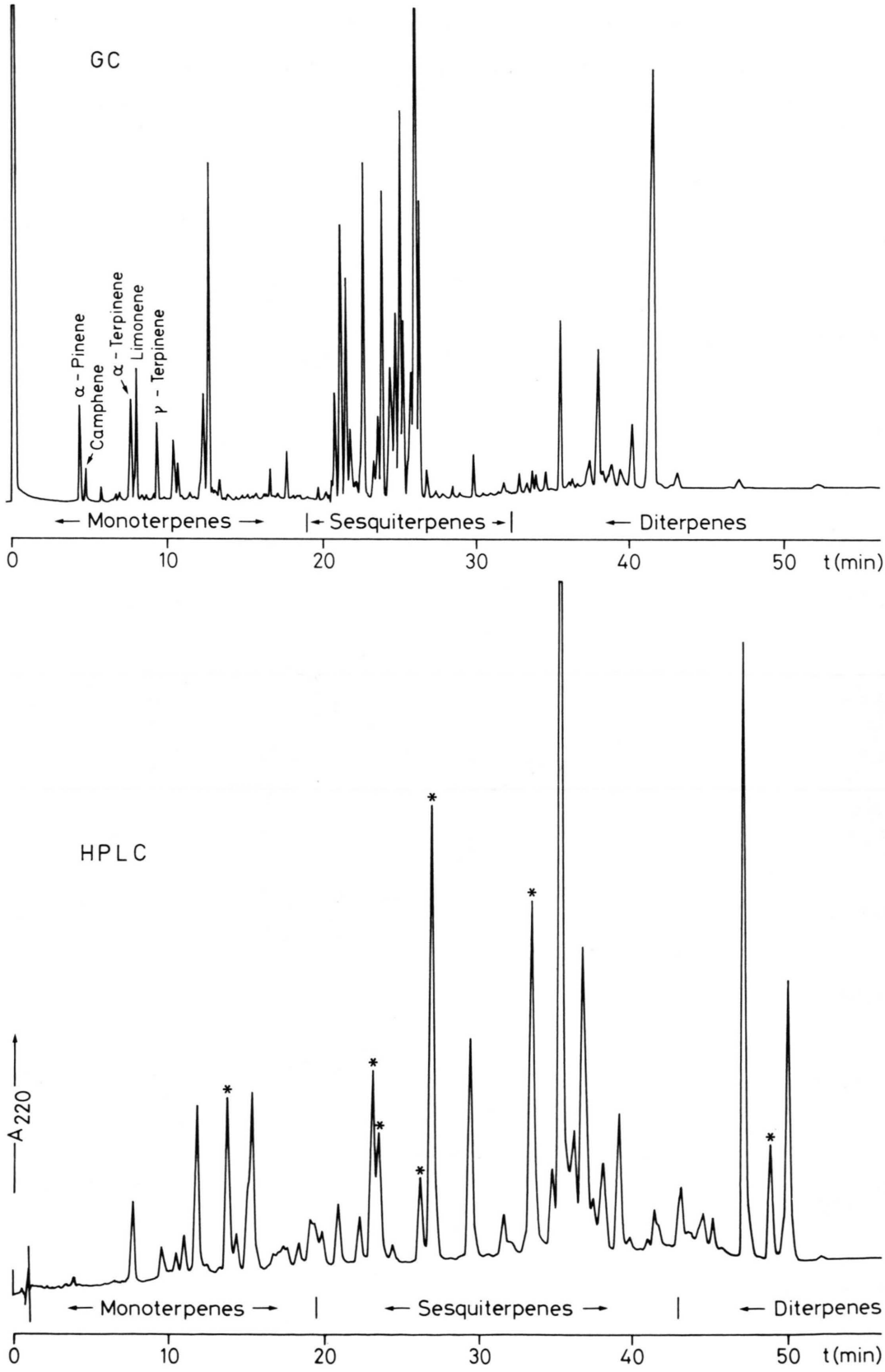


Fig. 4. GC and HPLC resolution of constituents of the *n*-pentane fraction [8–10] from the essential oil of *Cistus ladanifer*. HPLC was done on LiChrosorb RP-18 using a linear gradient from 50% acetonitrile in water to acetonitrile within 45 min (2 ml/min). Asterisked peaks are selectively detectable at 240 nm, which might be due to a certain degree of unsaturation.

separable on both columns can be analyzed by using different wavelengths. This is demonstrated in the application to the oxygenated monoterpenes in the essential oil of *Cistus ladanifer* (Fig. 2). The simultaneous detection at 200 and 254 nm enables the analysis of acetophenone and menthone, the latter filtered out at 254 nm. The same stands for the analysis of 1,8-cineole and geranial, which are unseparable on RP-18. Fig. 3 shows UV spectra of 1,8-cineole, geranial, and geranylacetate. The distinct absorption behaviour of this type of compounds opens the possibility to optimize a separation problem by means of the detection wavelength.

Another application of the described method is shown in Fig. 4. The *n*-pentane fraction [8–10] of the essential oil from *Cistus ladanifer* was resolved on RP-18 using a water-acetonitrile gradient. Comparison with GC demonstrates the high selectivity of this HPLC for sesqui- and diterpenes. Monoterpenes, identified in the gas chromatogram, could not be satisfactorally analyzed by HPLC.

Conclusion

HPLC on C_8 and C_{18} bonded silica using water-acetonitrile mixtures and a UV detection at 200 and 220 nm offers an efficient and dependable method for the separation of sesquiterpenes and oxygenated monoterpenes, resembling the quality of GC analysis. The possibility to expand this technique to a preparative scale and selective UV detection might lead HPLC to be well established in investigations on essential oil constituents. In preliminary experiments we received good results in applying the above described methods to preparative separations of sesqui- and diterpenes on LiChroprep columns.

Acknowledgements

The support by the Deutsche Forschungsgemeinschaft to one of the authors (D. S.) is gratefully acknowledged. We thank Claudia Kisselstein for assistance in GC analysis and Josef Calvis for drawing the figures.

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