Retention of some components in supercritical fluid chromatography and application to bergamot peel oil fractionation

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Abstract

Supercritical fluid chromatography was used in order to investigate the possibility of detoxification of an essential oil. Bergamot peel oil contains phototoxic compounds, the psoralens, which must be removed prior to its use. The retention of the major constituents of the oil was determined under various levels of pressure (from 75 to 160 bar) and temperature (37 to 57°C) of pure carbon dioxide. The highest selectivity against psoralens, and specifically, against bergapten was obtained at low pressure and high temperature. Bergapten elimination was then successfully achieved by adsorption from a supercritical feed.

Keywords: Essential oils; Psoralens; Bergapten

1. Introduction

Citrus peel oils are widely used in the perfume and cosmetic industries. Among them, bergamot peel oil is the most valuable oil due to its unique fragrance and freshness. The essence is also used in the pharmaceutical industry because of its antiseptic and antibacterial properties. However, strong limitations have been imposed on its use, since bergamot oil contains several coumarins and psoralens which may be deleterious. Citropten, for example, tends to precipitate inducing turbidity of the product. Another compound, bergapten, is a strong phototoxic agent responsible for allergenic effects on human skin [1]. More recently, Morlière et al. [2] note that the major component of the bergamot oil, bergamottin (5-geranoxypsoralen) may also contribute to the photo-

biological properties of the bergamot oil-containing materials. For these reasons, the bergamot peel oil content in perfume formulations is considerably reduced. The IFRA (International Fragrance Association) recommends a maximum content of 0.4% cold-pressed bergamot oil in products that are applied on skin areas exposed to sunshine against 2% for a lemon peel oil [3]. Psoralen elimination can be realized by first removing the volatile species by vacuum distillation and treating the residue by selective elution from a solid adsorbent [4]. However, the technique suffers the disadvantage of thermal degradation induced by the distillation. Therefore, there is considerable interest in developing milder processes that can produce a bergamot oil without or at low psoralen content and without degradation of the volatile fractions.

The peculiar characteristics of supercritical fluids make them very attractive for use in selective

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fractionation. Supercritical carbon dioxide (SC-CO$_2$) has been widely used as a selective solvent and has found many applications in extraction processes [5–7]. However, to improve the selectivity within the extractable materials, an adsorbent may be introduced into the supercritical extraction system. Several applications using silica gel as an adsorbent have been reported. Lim et al. [8] and Shishikura et al. [9] investigated cholesterol reduction, respectively from anhydrous milk fat and from butter oil by continuous carbon dioxide extraction in conjunction with on-line adsorption on silica based sorbents. With a specific reference to *Citrus* peel oil fractionation, some authors proposed to fractionate the oil during desorption. The selective desorption of lemon, bergamot peel oil and bigarade from silica gel was obtained using supercritical carbon dioxide at increasing pressures [10–12]. The use of increasing pressure allows the modulation of the solvating ability of the supercritical solvent, in the same way that liquid solvents of different eluting strength yield to recover selectively compounds in liquid displacement techniques [13–15]. The supercritical desorption was started at a low pressure of 75 bar in order to remove the hydrocarbon terpenes first. The pressure was then increased to 85 bar to desorb the oxygenated compounds which exhibited a stronger affinity towards the silica based sorbent. The same principle was used by Yamauchi and Saito [16] who fractionated 0.5 ml of the lemon peel oil by supercritical fluid chromatography. Hydrocarbons and their oxygenated derivatives were successively eluted by carbon dioxide using a pressure gradient of 100 to 200 bar. At the end of the process, ethanol was added to the fluid phase to recover the non-volatile substances.

The previously described processes are performed in a semi-batch manner since it is necessary to batchwise charge the oil onto the desorption column [10–12]. About 20 g of oil is charged onto 100 g of the adsorbent, with a waiting period of 12 h before starting the desorption procedure. To overcome these limitations, the oil can be mixed with a supercritical fluid in order to continuously feed the sorbent column. As the adsorption proceeds, effluent fractions of various compositions will be recovered and the column will be selectively enriched, according to the relative affinity of the solutes for the sorbent.

The objective of this work was to use the fixed-bed adsorption technique with a supercritical fluid in order to fractionate the oil and eliminate the psoralens. To achieve these results, we first studied the influence of the pressure and the temperature on the retention of major components of the oil by supercritical fluid chromatography. Once the conditions which yielded highest selectivity were determined, we performed the fractionation of the bergamot oil by continuously flowing the oil, diluted in supercritical carbon dioxide, through the adsorbent column.

2. Experimental

2.1. Apparatus

Supercritical fluid chromatography (SFC) was performed with laboratory equipment, which consisted of a dual piston pump for supercritical fluid delivery (CP3000 Model) and a UV spectrophotometer equipped with a high pressure cell (Spectro-Monitor SM3100). Both are manufactured by LDC (Thermo Instruments, LDC Division, France). Carbon dioxide (N45 grade, Alphagaz) was previously cooled to $-2°C$ by circulation through an ethylene glycol bath, which also cooled the jacketed pump heads. A Tescom valve (Model 26-1722-24-084, 6000 p.s.i., Automatismes Appliqués; 1 p.s.i.= 6894.76 Pa) at the outlet of the adsorption column regulated the upstream pressure. The column was a stainless-steel tube (15 cm×4.6 mm I.D.) filled with silanized silica (Merck No. 7719) of particle diameter of 63–200 μm. The sorbent was chosen as a compromise between potentiality and cost since it may be further used in an industrial scale. The tubing and column were thermostated by an oven (Shimadzu CTO-6A Model, Touzard and Matignon; accuracy 1°C). The detection wavelength was set to 200 nm for detection of the terpenes and to 313 nm for the psoralens. The injections were performed by a 6-way VALCO valve through an external injection loop of 20 μl. Components were diluted in heptane for terpenes and in chloroform for non-volatile species (Carlo Erba, HPLC grade) at a concentration range of 1 g/l. The mobile phase flow-rate was 2 ml/min.

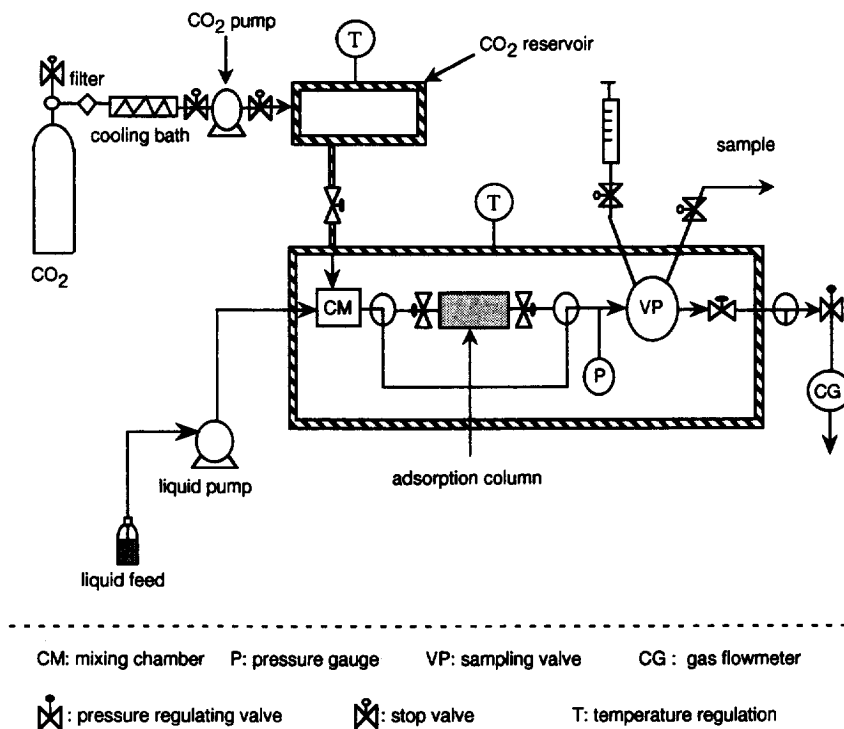The adsorption experiment for the oil was per-

Fig. 1. Schematic diagram of the system used for the adsorption process.

formed using different apparatus (Fig. 1). An additional pump (CM 3200, Thermo Instruments) delivered the oil that was then mixed with the carbon dioxide in a dynamic mixing chamber (Thermo Instruments). The SC-CO_2 flow-rate and the bergamot oil flow-rate were 1.35 ml/min and 30 µl/min, respectively. Pressure was monitored using a transducer (Top Industrie) and was regulated by a Tescom valve (Model 26-1722-24-084, 6000 p.s.i.). The column was held in an air chamber (WTB Binder, Series ED, Touzard and Matignon) whose temperature was controlled within ±1°C. The column was a stainless-steel tube (15.5 cm×15 mm I.D.) provided with a stop valve (Autoclave Engineers) at each end. The overall mixture was driven through a sampling valve (Valco, external loop of 250 µl) and then flashed to atmospheric pressure through the Tescom valve. The CO_2 stream flowed through a liquid trap to remove the precipitated solutes, and then to a volumetric flow meter (Magnol, Model G4).

Sampling was performed through the 6-way Valco valve. Two exits of the valve were equipped with a low volume Whitey valve (Swagelock), in order to gently depressurize the sampled supercritical solution into a flask. The complete recovery of solutes was performed by flushing the valve loop with 4 ml of heptane.

2.2. Materials

α-Pinene, β-pinene, myrcene, limonene, γ-terpinene, β-caryophyllene, linalool, citronellal, citral, linalyl acetate, citronellyl acetate, geranyl acetate, coumarin, bergamottin, citropten and bergapten were purchased from Extrasynthese (research grade) and were stored at −18°C. Carbon dioxide (N45 grade, Alphagaz) was purified by flowing through packed beds of silica and octadecyl-bonded silica (Merck). The sorbent was a silanized silica gel (Kieselgel 60, Merck No. 7719), which was dried prior its use for 12 h in a vacuum oven at 100°C. Some characteristics of the sorbent and the bed are given in Table 1. The sorbent was chosen from literature results on

Table 1
Properties of the silica Kieselgel 60 and of the sorbent bed

Property		Value
Surface area (N_2 BET) (m^2/g)		502
Particle size (μm)		63–200
Mean particle diameter (μm)		105
Porous volume (ml/g)		0.8
Mean pore diameter (Å)		60
Carbon content, (%, w/w)		4.47
Bed porosity		0.65
Bed length and diameter (mm)	analytical scale	150×4.6
	semi preparative scale	155×15

desorption of lemon peel oil by supercritical carbon dioxide [8–10]. The bergamot peel oil was a gift from C.A.L. (Grasse, France).

2.3. Analytical methods

Adsorption experiments required analysis of both the feed and the effluent composition. Samples were analysed by gas chromatography according to the volatile character of the compounds. The chromatograph (GC 6000, Carlo Erba) was equipped with an on-column injector (injection volume of 2.0 μl), a capillary column (Hewlett-Packard HP-5, 25 m×0.2 mm I.D., 0.5 μm film thickness) and a flame ionization detector. The carrier gas was helium. A temperature program of 70°C for 5 min followed by an increase of 3°C/min to 163°C allowed a good resolution of the compounds, except for linalyl acetate and geraniol. Peak areas were integrated by a LDC integrator (Thermo Instruments). Identification of compounds was based on the comparison of retention times with standards. Peak areas were converted into concentration by calibration curves which were fitted satisfactorily with a linear regression ($R^2=0.999$). A gas chromatogram of the bergamot crude oil is given in Fig. 2a. Since citral is a mixture of neral and geranial, two peaks can be observed on the chromatogram.

The coumarins and psoralens were analysed by liquid chromatography. The compounds were separated on a LiChrosorb Si column (250×4.6 mm I.D., 5 μm, Touzard and Matignon) with a mixture of 20% (v/v) of chloroform in heptane at a 1.5 ml/min flow-rate. The column was thermostated to 30°C (Shimadzu oven, Touzard and Matignon). The UV

detection wavelength was set at 313 nm (Spectro-Monitor SM3100, Thermo Instruments). The injection volume was 20 μl (Rheodyne). Peak areas were recorded by a LDC integrator (Thermo Instruments). The calibration curves, provided by analysis of mixtures of known concentration, were satisfactorily regressed ($R^2=0.999$). A liquid chromatogram of the bergamot crude oil is given in Fig. 2b.

3. Results and discussion

Bergamot peel oil is mainly composed of a volatile fraction consisting of terpene hydrocarbons, their oxygenated derivatives and aliphatic oxygenated compounds, and of a non-volatile residue, including coumarins, psoralens, waxes, carotenoids, sterol, tocopherols and paraffins. The aim of this study was first to examine the retention behavior of some representative compounds in order to obtain optimum conditions which will achieve a good selectivity between the terpenes and the psoralen derivatives. We selected as model compounds some major constituents of each group, e.g., limonene, linalool, citral, linalyl acetate, bergamottin, bergapten and citropten. Coumarin was also studied despite the absence of this compound in the peel oil, since its simple structure compared to the others made it a border component between the volatile and the high-molecular-mass compounds.

3.1. Determination of retention factor by supercritical fluid chromatography

Retention of volatile terpenes was studied for pressures ranging from 75 to 125 bar, whereas experiments were performed up to 160 bar for the non-volatiles. The influence of temperature was also investigated. Results are expressed in terms of the retention factor, k', defined as $(t_r-t_0)/t_0$, where t_r is the residence time of the solute and t_0 the residence time of an unretained species. Since the temperature and the pressure of both column and surroundings were controlled, t_0 was calculated for every set of experimental conditions.

The evolution of the retention factor k' against pressure at 47°C is given in Fig. 3 for both the volatile and the non-volatile compounds. The re-
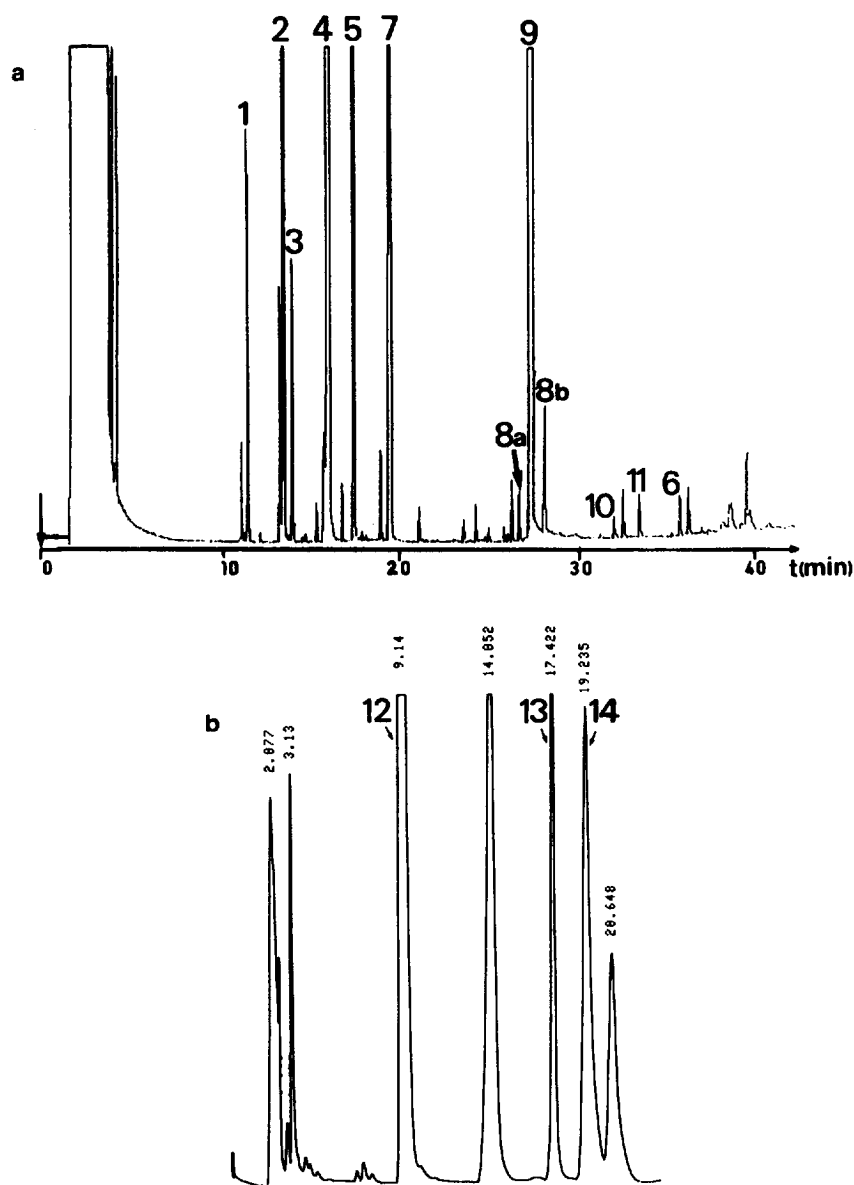
Fig. 2. Analysis of the bergamot peel oil. (a) Gas chromatogram; (b) liquid chromatogram. 1 = α-pinene, 2 = β-pinene, 3 = myrcene, 4 = limonene, 5 = γ-terpinene, 6 = β-caryophyllene, 7 = linalool, 8 = citral, 9 = linayl acetate/geraniol, 10 = citronellyl acetate, 11 = geranyl acetate, 12 = bergamottin, 13 = bergapten, 14 = citropten.

tention was found to increase from the hydrocarbon limonene to the polar volatiles and to the non-volatile compounds, with the highest k' value for citropten. When the pressure is increased, retention decreases sharply, so that the retention factors of the non-volatiles range from 10 to 30 instead of 45 to 110 at low pressure.

The relative retention order of the various components could be rationalized on the basis of the relative strength of the solute interactions with both the sorbent and the fluid. Solute–sorbent interactions can be described by the reduced adsorption energy parameter defined by Snyder [17] to explain relative retention of components in liquid chromatography,
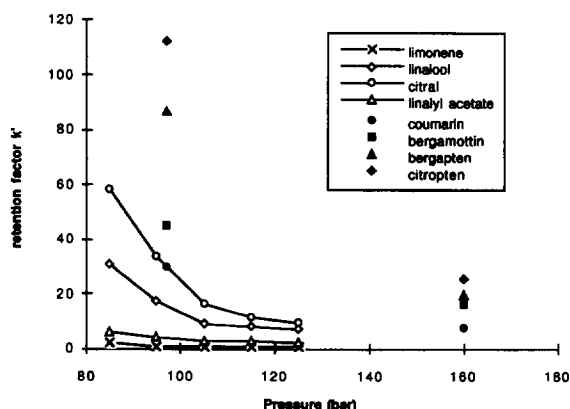
Fig. 3. Retention factors of the model solutes as a function of pressure at 47°C.

whereas, the strength of the interactions between the solute and the fluid can be qualified by the solubility. Estimation of the energy parameter by group contribution provides three groups: limonene whose adsorption energy is 0.9, the oxygenated terpenes, whose energies lie between 6.1 and 6.7 and the non-volatile compounds, whose energies are between 9.5 and 11.0. Therefore, compounds that have oxygenated functionalities show higher affinity for the sorbent. The solubility reflects the solute–solvent interactions. According to Stahl et al. [18] mono-terpene hydrocarbons such as limonene are more soluble than the oxygenated terpenes in supercritical carbon dioxide. Furthermore, high-molecular-mass and low vapor pressure are properties known to be detrimental to solubility [19,20]. Thus, coumarin and psoralen derivatives are less soluble than the volatile compounds. As a result of interaction balance, limonene shows the weakest interaction for the sorbent and the strongest interaction for the fluid, so it is a poorly retained component. In contrast, non-volatiles show strong interaction with the sorbent, associated with poor solubility. The oxygenated terpenes lie between these two groups with a medium affinity for both the fluid and the sorbent.

Fig. 4 focuses on the influence of pressure and temperature on the retention of the terpenes. The capacity factors decrease sharply as the pressure increases up to 105 bar, and do not change extensively beyond that pressure. The influence of temperature depends on the pressure. For pressure below

105 bar, an increase in temperature improves the retention, whereas at pressure above 105 bar, improvement is minor. The temperature effect depends also on the compound. The more retained the solute, the more influential the parameter. As an example, for a pressure set to 85 bar, the retention factors of limonene, linalool and citral decrease by two, three and over five, respectively when the temperature decreases from 47°C to 37°C.

The observed behaviour with regard to pressure and temperature are quite usual in supercritical fluid chromatography [21,22]. The decrease of the retention factor with an increasing pressure is due to the enhancement of the solvation ability of the fluid. The influence of temperature is more complex since the temperature has opposing effects on the fluid density, thus on the solvating ability of the fluid, on the vapor pressure of the solute, and also affects interaction with the adsorbent via the adsorption constant. Within the range of pressure investigated, the influence of temperature on the fluid density overcompensates the effect on the solute vapor pressure. Therefore, by decreasing the solvating ability of the fluid, the rising temperature leads to an increasing retention factor. Similar behavior was observed with the non-volatile species (Fig. 5). However, the retention factor decreased only by 40% when temperature decreased from 57°C to 47°C, a much lower decrease than that observed for the volatile species.

3.2. Adsorption experiment

SFC provided the relative retention values of the major compounds of bergamot peel oil as a function of pressure and temperature. Results showed that bergapten was strongly retained on the sorbent compared to the other volatile terpenes and thus, its removal by adsorptive process could be considered. Conditions leading to higher selectivity would be at lower pressure, ca. 85 bar. However, experimental difficulties of maintaining a constant flow-rate and a constant concentration of the oil in the supercritical medium occured when the adsorption experiment was performed in such conditions. Therefore, the pressure was set to 105 bar. The temperature was 47°C.
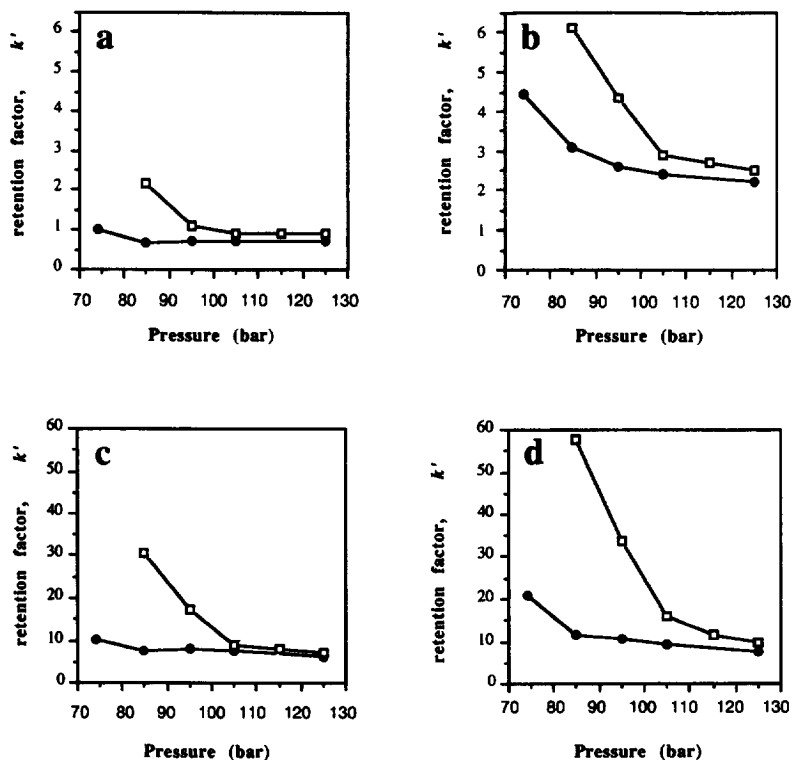
Fig. 4. Evolution of the retention factor of the volatile compounds as a function of pressure and temperature: (●) 37°C; (□) 47°C. a = Limonene, b = linayl acetate, c = linalool, d = citral.
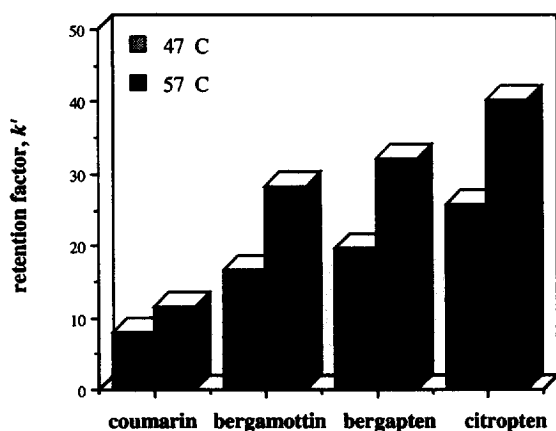

Fig. 5. Influence of temperature on the retention factors of the non-volatile components. $P = 100$ bar.

The adsorption procedure started by pressurizing the system including the column with pure CO_2. The column was then isolated and the mixture of bergamot peel oil and supercritical CO_2 was prepared by continuously mixing the flows coming from each pump. The column was by-passed as long as the concentration of the feed varied. When a constant feed composition was achieved, the column was placed in-line and the feed flew continuously through the adsorbent. Regular sampling of the effluent were performed, in order to trace the breakthrough curves of each solute of the feed with accuracy. The breakthrough curves of volatile compounds were obtained from gas chromatographic analysis, whereas the breakthrough curves for coumarins and psoralens were obtained from liquid chromatographic analysis.

Fig. 6 shows the breakthrough curves of some components of the bergamot peel oil. The ratio of the effluent concentration to the feed concentration is
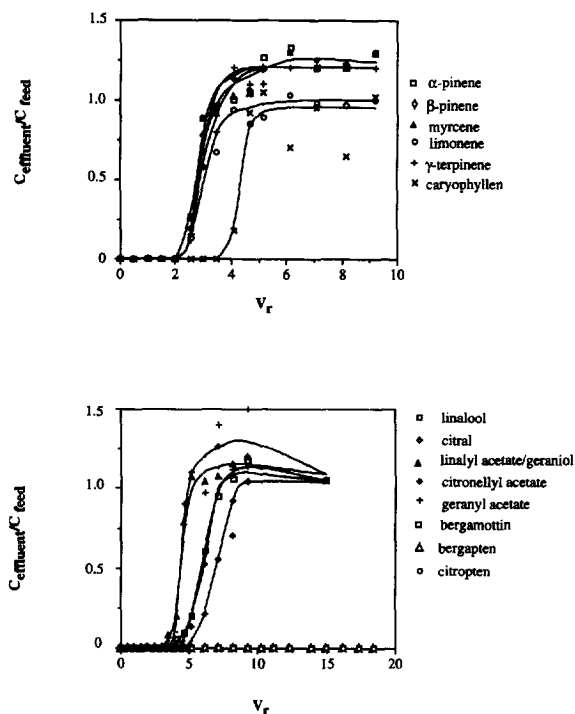
Fig. 6. Breakthrough curves of the major components of the bergamot peel oil diluted in carbon dioxide. $P = 105$ bar. $T = 47°C$. CO_2 flow-rate = 1.35 ml/min, oil flow-rate = 30 μl/min.

plotted against the reduced volume (V_r) which is the ratio of the effluent volume to the void volume (V_v) of the column. The reduced volume has the same meaning than the retention factor, k'. Indeed, the loaded volume (V_L) is the sum of the effluent volume (V_{eff}) collected at the outlet of the column and of the void volume:

$$V_L = V_v + V_{eff}$$

With a reduced volume expressed as $V_r = V_{eff}/V_v$, we obtain

$$V_L = V_v(1 + V_r)$$

If the column was used in the elution chromatographic mode, the response to a pulse injection would have been a peak, whose location is the retention volume. Provided that the column is not overloaded, it corresponds to the reduced volume measured at the half height of the breakthrough curve, $V_{r0.5}$. Since the retention factor is related to the

retention time or the retention volume by the expression

$$V_{retention} = V_v(1 + k')$$

$V_{r0.5}$ and k' are analogous. Therefore, the breakthrough curve position was expected to be in the same order as the retention factors evaluated from the chromatographic measurements. Indeed, the three groups of compounds showed different breakthrough volumes. Hydrocarbon terpene breakthrough curves were observed first, with a similar behavior whatever the terpenes. Only the sesquiterpene β-caryophyllene was slightly more retained. The oxygenated derivatives breakthrough curves were observed later, with discrepancies relative to their molecular structure. Also, according to the retention order provided by the chromatographic studies, the non-volatile compounds were expected to give high breakthrough volumes. Indeed, no traces of coumarin nor psoralens were detected in the effluent.

In order to quantify the fractionation efficiency, we focused on the major compounds labelled on the chromatograms given in Figs. 2a and b and reported in Table 2. Percentages were expressed on a mass basis and dealt with the factor response of each identified solute. Table 2 also reports the composition of the effluent when fractionated in three fractions, from 0 to 3 V_r, 3 to 5 V_r and 5 to 18 V_r, or when only one whole fraction (F_T) is collected from 0 to 18 V_r. Fractionation of the effluent leads to oil of various composition. According to the breakthrough curves of the solutes, the first fraction contains only hydrocarbon terpenes whose percentages are different from the crude oil. The fraction is highly enriched with α- and β-pinene, myrcene and γ-terpinene. Oxygenated terpenes begin to appear in the second fraction, and the discrepancies in the breakthrough volumes correspond to various percentages of the oxygenated derivatives. The oxygenated content of this fraction is mainly due to the presence of linalyl acetate and geraniol, since citral and linalool show higher breakthrough volumes. On the other hand, the breakthrough curves of most of the hydrocarbons have reached their plateau. Their percentages are still higher than in the crude oil but, due to the presence of the oxygenated terpenes, they decrease compared to the content of the first fraction. As the percolation of the oil continues, the break-

Table 2
Composition in percentage of the crude bergamot peel oil, of the effluent fractions (F_1, F_2, F_3), of the total effuent (F_T) and of the adsorbed content

Compound	Crude oil	F_1 (0–3 V_r)	F_2 (3–5 V_r)	F_3 (5–18 V_r)	F_T (0–18 V_r)	Adsorbed content
Hydrocarbons						
α-Pinene	1.06	8.1	1.9	0.91	1.00	0.39
β-Pinene	4.64	30.7	9.0	4.54	4.90	1.83
Myrcene	0.77	7.2	1.7	0.89	0.96	0.31
Limonene	26.40	21.5	45.8	37.24	37.80	28.25
γ-Terpinene	5.00	32.4	10.7	5.51	5.96	2.43
β-Caryophyllene	0.18	0	0.2	0.23	0.23	0.33
Total %	38	100	69.4	49.3	50.9	34.14
Oxygenated compounds						
Linalool	15.8	0	2.0	14.2	13.3	22.84
Citral	1.04	0	0.03	0.77	0.72	1.61
Linalyl acetate/geraniol	43.3	0	28.4	35.3	34.70	35.91
Citronellyl acetate	0.16	0	0	0.12	0.11	0.21
Geranyl acetate	0.28	0	0.16	0.23	0.22	0.17
Total %	60.6	0	30.6	50.7	49.1	60.74
Non-volatiles						
Bergamottin	1.04	0	0	0	0	4.16
Bergapten	0.14	0	0	0	0	0.34
Citropten	0.17	0	0	0	0	0.62
Total %	1.4	0	0	0	0	5.12

$T = 47°C$. $P = 105$ bar. CO_2 flow-rate = 1.35 ml/min. Oil flow-rate = 30 μl/min.

through volumes of most of the oxygenated terpenes are reached, increasing their content in the effluent. The third fraction is thus characterized by a similar content of hydrocarbons and oxygenated terpenes compared to the ratio 38%:61% of the crude bergamot oil. The ratio of 49%:51% comes mainly from the higher content in limonene (37% against 26% in the crude oil) and from the lower content in linalyl acetate and geraniol (35% against 43%).

The main result of detoxification of the bergamot peel oil, is that none of the fraction contains bergapten, citropten or bergamottin since these compounds are strongly retained by the sorbent. This purification may however be detrimental to the flavor of the oil, since the processed oil will have a lower content of the oxygenated terpenes which are responsible for the olfactory characteristics. Other processes of psoralens elimination have also to deal with disadvantages such as partial decomposition or degradation of some of the components.

Furthermore, continuously flowing the oil through an adsorption column could be considered as a first step prior to selective desorption. With regard to the composition of adsorbed species at the end of the adsorption step (last column in Table 2), one may remark that the column contains less hydrocarbons than the crude oil and is also enriched, mainly with linalool and citral. Therefore, if a selective desorption with supercritical carbon dioxide is further performed as proposed by several authors, a highly flavored fraction enriched with linalool and citral would be expected. The adsorption and desorption cycles in series would also yield a continuous process of oil purification, which is economically attractive.

4. Conclusion

SFC was used to compare the retention of the major compounds of the bergamot peel oil as a function of pressure and temperature. Results

showed that the toxic bergapten exhibited a much higher retention than the hydrocarbons or oxygenated terpenes, with a retention factor in the range of 80 compared to 35 for citral. Thus, its removal from a supercritical feed of the oil by a continuous adsorptive process was considered with conditions of pressure and temperature of 105 bar and 47°C, respectively. Depending on the volume —or the time— of the effluent collection, the process yields individual fractions of various composition of hydrocarbons and oxygenated terpenes or only one fraction (from 0 to 18 V_r), free of toxic compounds. Therefore, bergapten elimination from bergamot peel oil was successfully performed on the fixed-bed column packed with a silanized silica gel. Although the oil has to be highly diluted to obtain a good selectivity (flow-rates of oil and carbon dioxide were 0.03 and 1.32 ml/min, respectively) the adsorption allows to process about 10 g of the oil for 19 g of the sorbent. Furthermore, if this step is considered as a preliminary step prior to selective desorption, the purification of the oil and its fractionation could be realized by a continuous cyclic adsorption–desorption process.

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