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Journal of Chromatography A, 799 (1998) 265–273

JOURNAL OF CHROMATOGRAPHY A

Capillary supercritical fluid chromatography of pyrethrins and pyrethroids with positive pressure and negative temperature gradients[1]

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Received 23 July 1997; received in revised form 29 October 1997; accepted 3 December 1997

Abstract

Thermolabile molecules, such as pyrethrins and pyrethroids, are separated by supercritical fluid chromatography (SFC). Chromatography on a fused-silica capillary column is optimized by measuring the retention factor, peak resolution, and plate height. The separation of pyrethrins and pyrethroids by SFC was accomplished by the use of a positive pressure gradient and negative temperature gradient. The density of both gradients was comparable. Efficient separation of these pesticides was obtained with a linear isothermal pressure gradient of 0.2 MPa/min from 11.1 up to 22.3 MPa at a temperature of 90°C. Using a negative isobaric temperature gradient with a rate of −1.2°C/min from 130 down to 80°C, and holding 80°C for 10 min at 16.2 MPa, good results were obtained. The retention times of the analytes were constant. © 1998 Elsevier Science B.V.

Keywords: Pressure gradients; Temperature gradients; Pyrethrins; Pyrethroids; Pesticides

1. Introduction

The quantity of pesticides released into the global environment averages about 640×10^6 kg per year [1]. The use of chlorinated hydrocarbons (lindane, DDT, aldrin) is limited or prohibited in many countries because of their accumulation and persistence in fat [2]. Depending on their molecular structure there are various groups of pesticides: organophosphoroacid esters (dichlorvos, DDVP) [3], carbaminacid esters [1,3] and pyrethroids. The latter are technical derivatives of natural pyrethrins which can be extracted from chrysanthemum flowers. They are applied to sprays, or are released from electrically heated small paper strips, or they are often applied in high concentrations to carpets. The commercial pyrethrum extract made of dried blossoms consists of six pesticides, optically active esters of (+)-*trans* configured chrysanthemic acid and pyrethric acid with cyclic keto alcohols (+)-cinerolon, (+)-jasmolon and (+)-pyrethrolon. The substance names, cinerin, jasmolin and pyrethrin, are based on the keto alcohols. Because of the two acids there are two different groups: chrysanthemates and pyrethrates. They show high insect toxicity combined with low human toxicity and good plant compatibility [4,5]. In Fig. 1 structures, names and molecular masses of pyrethrins and common pyrethroids are shown.

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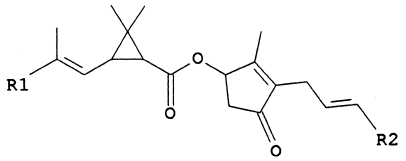
[1]Presented at the 1st SFE/SFC/XSE Symposium, Siegen, 1–2 October 1996.

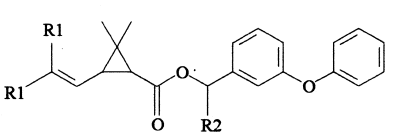
0021-9673/98/$19.00
PII S0021-9673(97)01236-3

a.

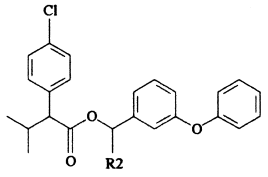


substance	R_1	R_2	total formula	MG [g/mol]
Cinerin I	$-CH_3$	$-CH_3$	$C_{20}H_{28}O_3$	316.44
Jasmolin I	$-CH_3$	$-CH_2-CH_3$	$C_{21}H_{30}O_3$	330.47
Pyrethrin I	$-CH_3$	$-CH=CH_2$	$C_{21}H_{28}O_3$	328.46
Cinerin II	$-COOCH_3$	$-CH_3$	$C_{21}H_{28}O_5$	360.46
Jasmolin II	$-COOCH_3$	$-CH_2-CH_3$	$C_{22}H_{30}O_5$	374.48
Pyrethrin II	$-COOCH_3$	$-CH=CH_2$	$C_{22}H_{28}O_5$	372.47
Allethrin	$-CH_3$	-H	$C_{19}H_{26}O_3$	302.42

b.



b1



b2

substance	formula	R_1	R_2	total formula	MG [g/mol]	Isomers
Phenothrin	b1	$-CH_3$	-H	$C_{23}H_{26}O_3$	350.46	4
Permethrin	b1	-Cl	-H	$C_{21}H_{20}Cl_2O_3$	391.29	4
Cypermethrin	b1	-Cl	-CN	$C_{22}H_{19}Cl_2NO_3$	416.30	8
Fenvalerate	b2	—	-CN	$C_{25}H_{22}ClNO_3$	419.91	4
Deltamethrin	b1	-Br	-CN	$C_{22}H_{19}Br_2NO_3$	505.20	8

Fig. 1. (a) Names, structures and molecular masses (MG) of pyrethrins and allethrin; (b) names, structures, molecular masses and number of isomers of the studied pyrethroids.

Various investigations of the chromatographic separation of pyrethrins and pyrethroids have been carried out in recent years.

For GC, elution temperatures above 200°C and hot injection ports allow pyrethin I+II to tautomerize, which results in very poor peak shapes [6,7]. Class and Böhner [8] successfully separated the pyrethrins with GC by applying temperatures below 200°C and programmed temperature injection. They used a very short capillary column. The disadvantage is an insufficient resolution of other compounds in a natural sample.

The use of thin-layer chromatography (TLC) requires derivatization of the pyrethrins to get co-

lored molecules, and the separation of all six pesticides is not sufficient [9]. In HPLC a solvent gradient is mainly used. A baseline separation of pyrethrins in HPLC cannot be achieved [10–14]. In supercritical fluid chromatography (SFC) many parameters influence the chromatographic separation. Various gradients of pressure, density or temperature can be applied. In addition, the solvent or flow-rate can be varied. A combination of different gradients enlarges the number of choices further, making it even more difficult for a beginner [15–20]. Using a pressure gradient in SFC it is possible to separate pyrethrins and pyrethroids [21–23]. Positive pressure and negative temperature gradients are optimized by systematic examination of chromatographic values in this paper.

2. Experimental

2.1. Instrumentation

In this study, an MPS/225 SFC-system (Suprex, Pittsburgh, PA, USA) with flame ionization detection (FID) and a timed-split, pneumatically driven four-port injection valve (Valco Instruments, Houston, TX, USA) was used. A volume of 100 nl of an internal loop rotator (Valco-CSSA C 14W.1) (GAT, Bremerhaven, Germany) was flushed with SFE-grade carbon dioxide as mobile phase (Air Products, Hattingen/Ruhr, Germany) onto a fused-silica capillary column (DB 5) (J&W Scientific, Folsom, CA, USA). The column was 20 m×0.1 mm I.D. A self-made Guthry-Restrictor [24,25], which was prepared in less than 30 min, was placed 15 mm under the detector jet of the FID system. The detector temperature was kept at 325°C. The flow-rates of the flame gases were 30 ml/min hydrogen (5.0, Messer Griesheim Düsseldorf, Germany) and 280 ml/min synthetic air (Messer-Griesheim). The detector signal was recorded either with a Shimadzu Integrator C-R6A (Kyoto, Japan) or a personal computer equipped with GC-star software (Varian, Darmstadt, Germany).

2.2. Chemicals

Dichloromethane (CH_2Cl_2) was Nanograde quality (Promochem, Wesel, Germany). The pyrethroids (allethrin, phenothrin, permethrin, cypermethrin, fenvalerate and deltamethrin, each 97%) were obtained from Labor Dr. Ehrensdorfer (Augsburg, Germany). Three different standard solutions, A, B and C, were prepared: solution A contained 2.41 mg allethrin, 2.48 mg phenothrin and 2.22 mg permethrin per ml CH_2Cl_2, and was used to optimize the chromatography. Solution B contained 4.44 mg allethrin, 3.74 mg phenothrin, 13.75 mg permethrin, 14.11 mg cypermethrin, 5.60 mg fenvalerate and 21.45 mg deltamethrin per ml CH_2Cl_2. Solution C was prepared by diluting 10 μl of a pyrethrum extract (~25%) (Fluka, Neu Ulm, Germany) in 1 ml CH_2Cl_2.

3. Results and discussion

3.1. Positive pressure gradient

Solution A was used to optimize SFC for static conditions, i.e. constant temperature and constant pressure. Peak resolution (R_s) combines retention factor (k), selectivity (α) and theoretical plate height (H). We have calculated all three from the chromatographic data. H or better N (plate number) is a measure of the separation efficiency.

The parameters k, α, H, N and R_s were determined according to following equations [26,27]:

$$\text{retention factor } k = (t_R - t_M)/t_M \quad (1)$$

$$\text{selectivity } \alpha = k_2/k_1 \quad (2)$$

$$\text{theoretical plate height } H = \frac{L}{8 \times \ln 2} \cdot \left(\frac{w_h}{t_R}\right)^2 \quad (3)$$

$$\text{plate number } N = L/H \quad (4)$$

$$\text{peak resolution } R_s = \frac{1}{4} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k}{1+k}\right) \cdot \sqrt{N} \quad (5)$$

where t_M is the hold-up time, t_R is retention time, L is column length and w_h is half peak width. The parameters were calculated from chromatograms ($n=3$) after 4 h equilibration under isobaric and isothermal conditions. The temperature was varied between 60 and 120°C in steps of 20°C. The pressure

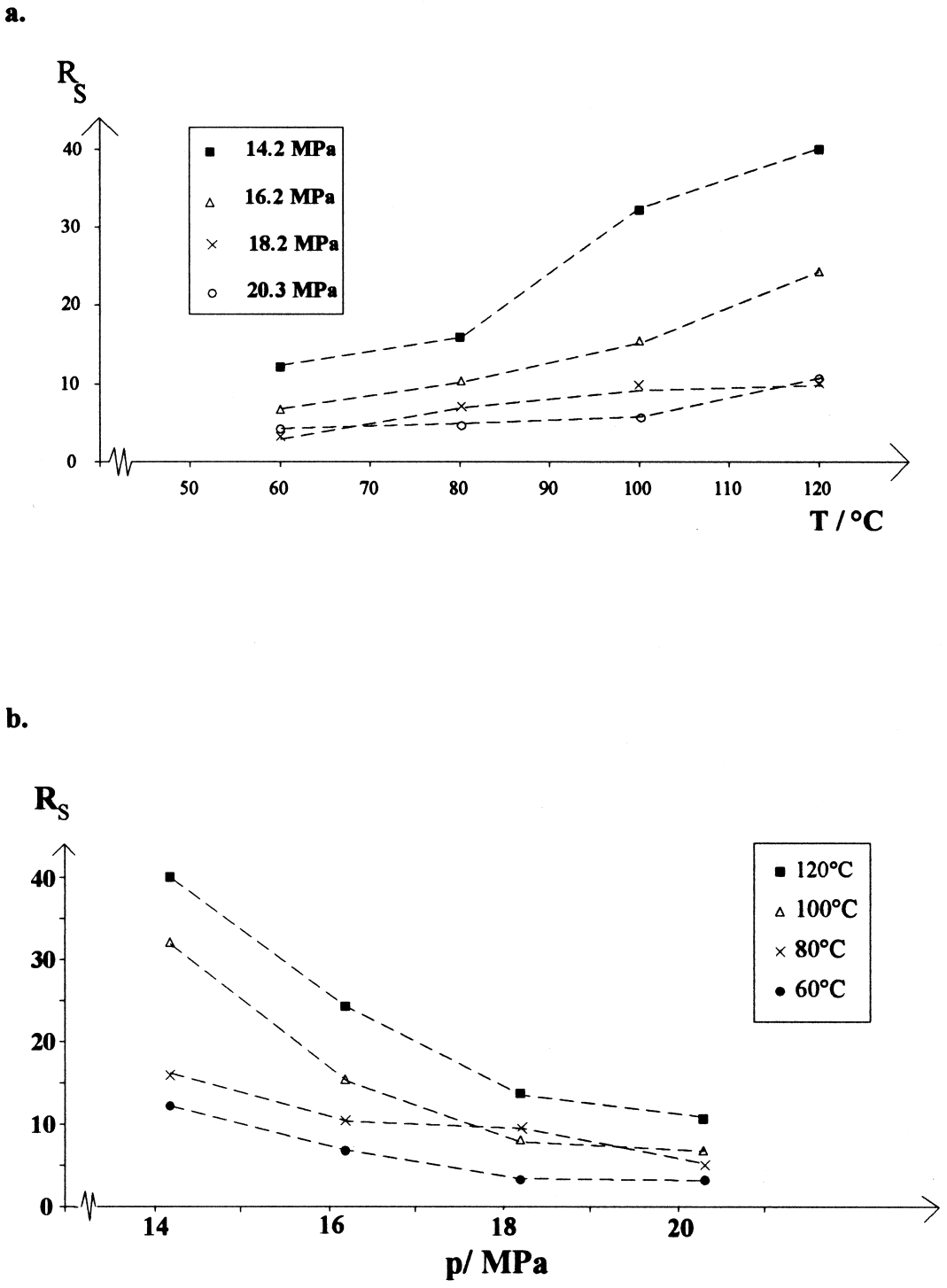


Fig. 2. (a) Resolution R_s (phenothrin–allethrin) versus temperature at different pressures; (b) resolution R_s (phenothrin–allethrin) versus pressure at different temperatures.

steps of 2.0 MPa varied between 14.2 and 22.2 MPa. Fig. 2a illustrates the resolution R_s (calculated according to Eq. (5)) between phenothrin and allethrin versus temperature using pressures from 14.2 up to 20.3 MPa. The resolution at a constant pressure increases with the temperature from 60 to 120°C. A suitable separation temperature is 120°C.

Solution B was used to evaluate chromatographic performance under dynamic viz. pressure conditions. The same linear pressure program starting at 11.1 MPa with 0.2 MPa/min up to 22.3 MPa was applied at four different temperatures: 80, 90, 100 and 120°C. The most efficient separation was achieved at 90°C.

Fig. 3a shows a chromatogram of solution B. The dark areas in the chromatogram show permethrin and isomers of fenvalerate.

In Fig. 3b a chromatogram of pyrethrins in CH_2Cl_2 (solution C) is illustrated.

3.2. Negative temperature gradient

A change of density is also possible by using a negative temperature gradient under isobaric conditions. Fields and Lee applied a positive temperature program parallel to a pressure gradient [28]. Wenclawiak chose a negative temperature gradient as a substitute for pressure programming [29,30]. The above-described pressure gradient was equivalent to an increase of density from 0.24 to 0.59 g/ml CO_2. It is possible under appropriate working conditions to cover the same density range by applying a negative temperature gradient.

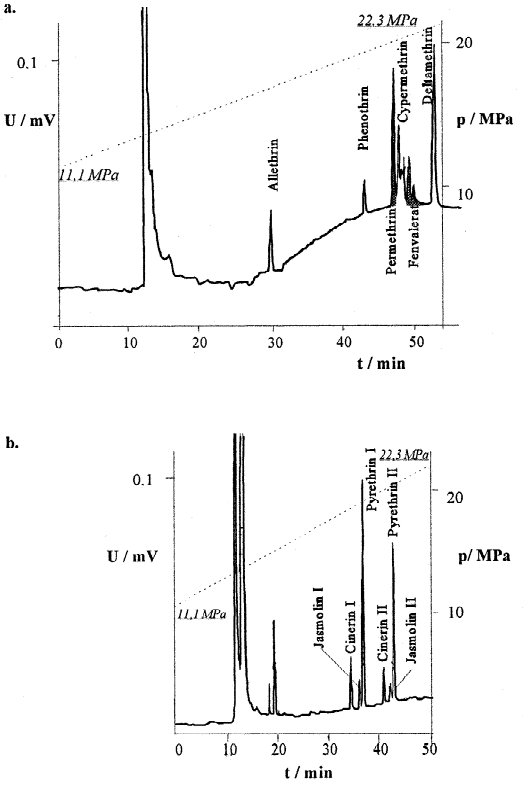


Fig. 3. (a) Chromatogram of pyrethroids with a positive pressure gradient; (b) chromatogram of pyrethrins with a positive pressure gradient. Pressure program: from 11.1 MPa at 0.2 MPa/min to 22.3 MPa; temperature 90°C; detection (FID) temperature, 325°C.

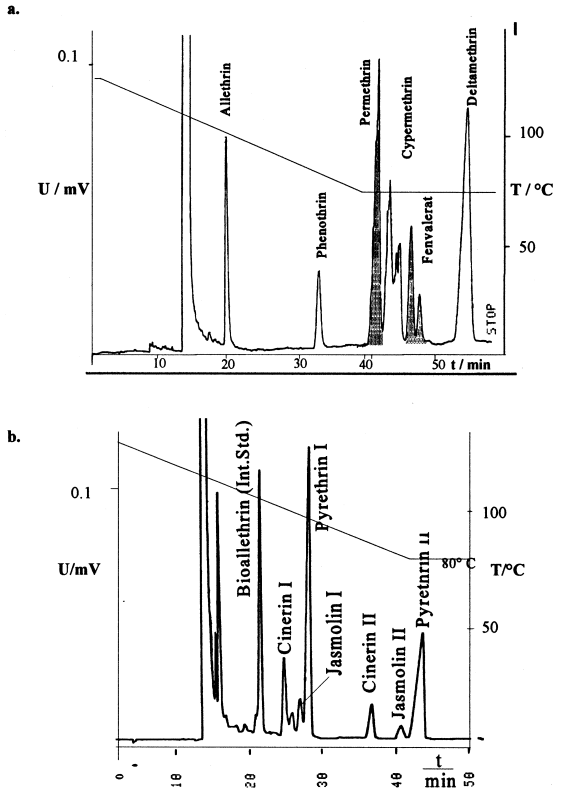


Fig. 4. (a) Chromatogram of pyrethroids with a negative temperature gradient; (b) chromatogram of pyrethrins with a negative temperature gradient. Temperature program: 130°C at 1.2°C/min; holding at 80°C for 10 min; pressure, 16.2 MPa; detection (FID) temperature, 325°C.

Chromatograms were recorded with isobaric and isothermal conditions. It was measured in the same temperature and pressure ranges as mentioned before. Fig. 2b shows the change in resolution (phenothrin–allethrin) depending on pressure at different temperatures. A strong decrease of R_s values along with a pressure increase from 14.2 to 20.3 MPa is noticed. The highest R_s values are found at a temperature of 120°C and the lowest at 60°C. An acceptable separation could be expected at a pressure range of 14.2–17 MPa.

A temperature program starting at 130°C down to 80°C, with a rate of −1.2°C/min holding at 80°C for 10 min with various constant pressures were tested. At a pressure of 16.2 MPa the pyrethroids contained in solution B (Fig. 4a) and the pyrethrins contained in solution C (Fig. 4b) could be separated.

The change of density during the chromatographic process for both gradients (0.240–0.590 g/ml) is illustrated in Fig. 5.

The density increases during an isotherm positive pressure gradient, whereby the solubility of the analyte in the mobile phase is also increasing. In contrast, the volatility of the substances is unchanged. Using an isobaric negative temperature gradient, two contrary substance properties compete with each other during chromatography. At a high temperature the volatility prevails. Decreasing the temperature increases the density and the solvating power of the mobile phase.

According to Gere et al. [31] flow-rates of supercritical fluid and expanded gas were calculated. The flow-rate of the fluid (u_F) using the pressure gradient is nearly constant (Fig. 6a). It decreases slightly from 11.5 (12.1 MPa) to 10.5 μl/min (22.3 MPa). In contrast to this the flow-rate of the expanded gas increases (u_G) from 2.1 to 4.2 ml/min. The pump action – control and adjust – is not perfectly reproducible so that retention times of the analyte differ from run to run.

The flow-rate of the fluid (u_F) using the negative temperature gradient decreases from 13.0 μl/min at 120°C to 7.9 μl/min at 80°C (Fig. 6b). The flow-rate of the gas (u_G) is nearly constant. It increases from 2.95 to 3.05 ml/min. Due to the constant pressure, the gas flow behind the restrictor is also constant, which does not generate a baseline drift in a chromatogram. This is shown by the horizontal baseline in Fig. 4a,b. On the contrary, the chromatograms in Fig. 3a,b exhibit a baseline drift because of the increase of the gas flow. Another phenomenon is the difference of the retention times. Using the temperature gradient the retention times are very reproducible. The relative standard deviation of the retention

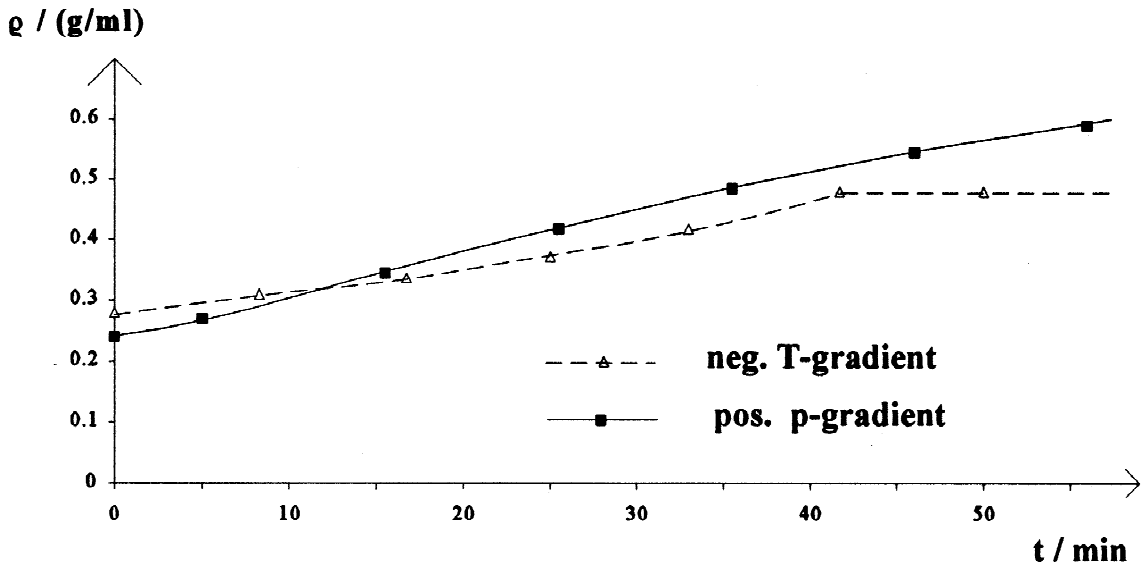


Fig. 5. Change of density for negative temperature gradient and positive pressure gradient used in this study.

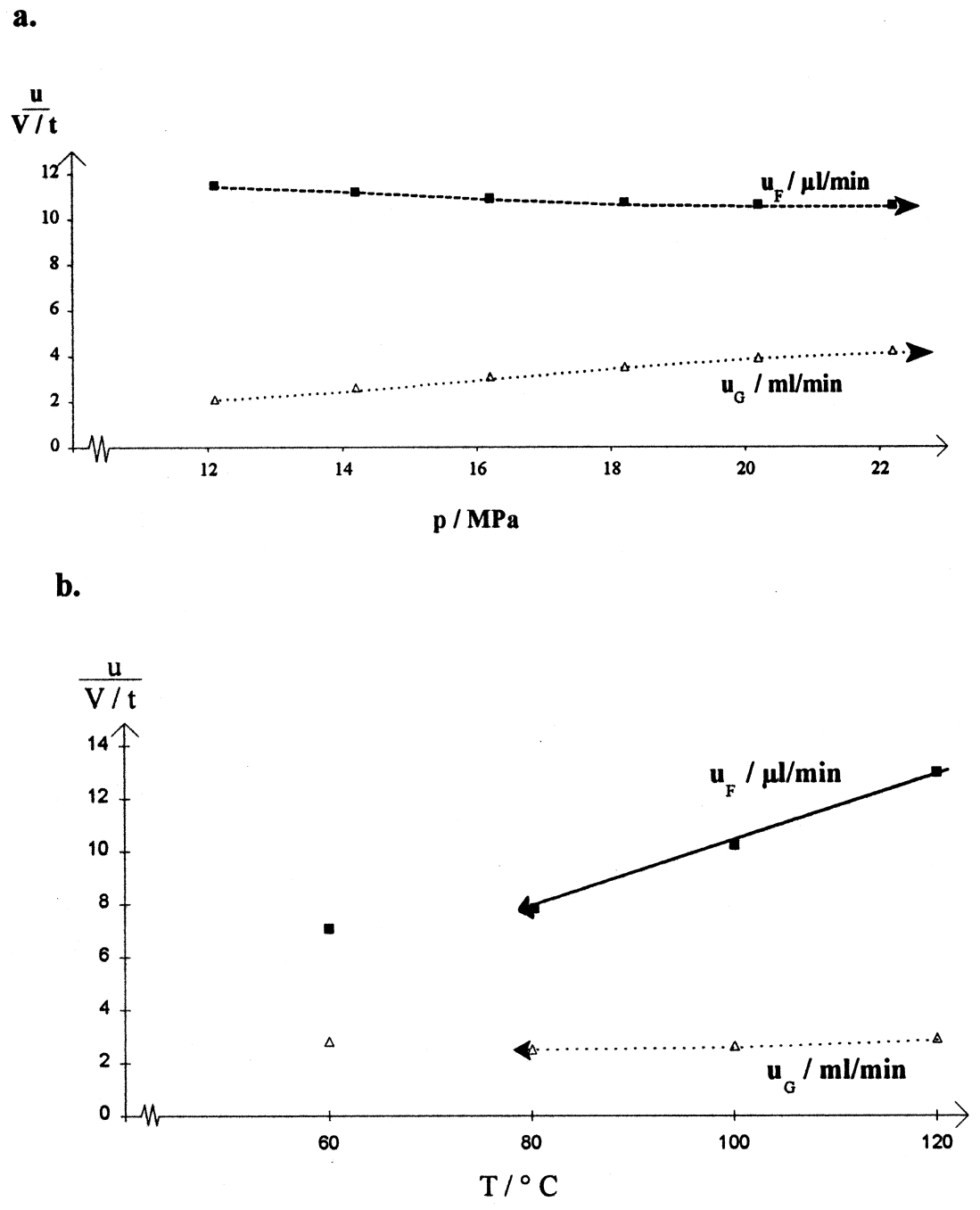


Fig. 6. (a) Flow-rates with the positive pressure gradient, $T=90°C$; (b) flow-rates with the negative temperature gradient, $p=16.2$ MPa; u_F, flow-rate of the fluid; u_G, flow-rate of the expanded gas.

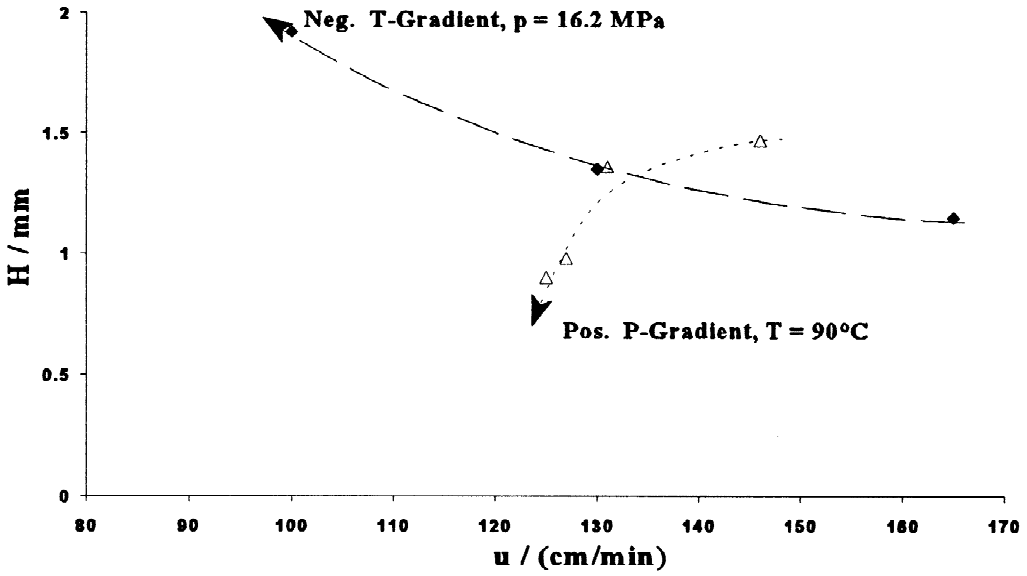


Fig. 7. Linear velocity (u) versus theoretical plate hight (H) with positive pressure gradient and negative temperature gradient.

time for allethrin with $n=15$ is less than 0.2%. The reason for the very good reproducibility is the fast column equilibration between runs, because of the temperature which has a uniform effect on the entire surface of the column. Whereas using the pressure gradient, the equilibration happened only from the inlet of the column.

Fig. 7 illustrates the theoretical plate height dependence on the linear velocity (u) during the chromatography with both gradients. The arrows indicate the change of u during the gradient in the chromatographic process. It is seen that the linear velocity decreases in both gradients. Using a negative temperature gradient u decreases from 165 to 100 cm/min, but H increases from 1.2 to 1.9 mm. This has a bad consequence on the chromatography, which is represented by the broad peaks in Fig. 4. During a pressure program a smaller decrease of u (145–125 cm/min) is obtained: H decreases from 1.7 to 0.9 mm. The chromatography of the pesticides is positively influenced by pressure gradients. This is shown by the narrow peaks in Fig. 3.

4. Conclusion

The separation of pyrethrins and pyrethroids in SFC was optimized by measuring retention factor, peak resolution, selectivity and plate height. With the pressure gradient, R_s values increase with an increase of temperature. Correspondingly, during negative pressure gradient R_s values decrease with an increase of pressure. An isothermal (90°C), linear pressure gradient of 0.2 MPa/min from 11.1 to 22.2 MPa and an isobaric (16.2 MPa) negative temperature gradient of −1.2°C/min from 130 to 80°C holding for 10 min is used. The retention times are reproducible. The density using both gradients is in a range of 0.25–0.59 g/ml. With the optimized SFC method it is possible to analyse samples of soil or waste water containing pyrethroids or pyrethrins. In the future packed columns will be investigated.

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