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Short communication

Feasibility study of using refrigerant 1,1,1,2-tetrafluoroethane as the mobile phase in high-speed counter-current chromatography

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Abstract

Newly synthesized environmentally benign refrigerant 1,1,1,2-tetrafluoroethane (R134a) was used as the mobile phase in high-speed counter-current chromatography (HSCCC) in this study. R134a remains as a liquid under mild pressure and becomes a gas at atmospheric pressure. Accordingly, operation of HSCCC equipped with regular polytetrafluoroethylene tubing was satisfactory. Since R134a turned into gas at ambient temperature and pressure, further concentration for the collected fractions after elution was not required. A mixture of four standard components of *Angelicae radix* was separated to demonstrate the feasibility of using this new mobile phase in HSCCC. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Counter-current chromatography (CCC) is a form of liquid chromatography. The retention of the liquid stationary phase in the column requires no solid support; therefore permanent adsorption of analytes does not occur. Separation is entirely attributable to partitioning of analytes between the stationary and mobile phases. Conventionally, the solvent systems are made by mixing two immiscible liquids. After thorough saturation of each other, one of the two portions is used as the mobile phase and the other as the stationary phase. Without support from the solid packing material, liquid stationary phases are retained in the separation tubing by either gravitational or centrifugal force. Due to the large capacity of the stationary phase allowed in the separation tubing,

CCC has been recognized as a useful preparative separation technique. In the last few years, two new techniques named pH-peak-focusing and pH-zone-refining CCC have been developed and proved to be highly efficient preparative methods [1–4]. Refer to the literature [5–8] for thorough description of theory and application for CCC and high-speed CCC (HSCCC). Recently we modified a solar coaxial CCC apparatus suitable for using supercritical fluid carbon dioxide as the mobile phase [9]. Separations can be accomplished in a more versatile way through fine-tuning the solvating power of supercritical fluid carbon dioxide by changing the temperature and pressure [10]. In addition, the supercritical fluid carbon dioxide becomes a gas when leaving the column, which spares further concentration of the collected fractions. The advantages are, however, overshadowed by the fact that pressure-resistant tubing and valves are required for the operation. Furthermore, polyether ether ketone (PEEK) tubing

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which worked smoothly for the anti-twist mechanism in the slow-speed solar coaxial apparatus failed to remain intact in HSCCC [11].

Alternatively 1,1,1,2-tetrafluoroethane (R134a) is employed as the mobile phase to replace common liquids in this work. R134a is one of the hydro-fluorocarbons that were synthesized to substitute the ozone-depleting chlorofluorocarbon refrigerants. Cantrell and Blackwell demonstrated that R134a possessed different selectivities and solvation power on retention than carbon dioxide and methanol-modified carbon dioxide when used as the mobile phase in chromatography [12]. In addition, Blackwell and Stringham [13] examined the mixture of carbon dioxide and R134a as the mobile phase of near-critical chromatography. This mobile phase showed strong temperature and compositional dependence on both retention and selectivity. The same research group did several other works to demonstrate the promise of using R134a as mobile phase or modifiers in super- and sub-critical fluid chromatography in analytical applications [14–17]. Jackson and Fulton [18] examined the possibility of using R134a and some hydrochlorofluorocarbons as extraction solvents for polar components in the presence of surfactants. Roth investigated the possibility to employ these alternative refrigerants as super-critical extraction solvents from their thermodynamic prospect [19].

R134a remains as a liquid under relatively low pressure; thus the operation using a CCC apparatus with polytetrafluoroethylene (PTFE) tubing is adequate. The liquid evaporates into a gas at room temperature and pressure, and separates from analytes directly after coming out of the column. Thus, it also possesses the advantage of using supercritical fluid carbon dioxide as the mobile phase in CCC. The operational pressure for the fluid to have an adequate solvent strength, however, would be much lower than supercritical fluid CO₂. We examined the feasibility of using R134a as the mobile phase in the separation of a mixture of four standard components of *Angelicae radix* using HSCCC in this work. Although components separated in this study are of relatively low polarity, R134a may potentially also be used to elute more polar compounds due to its higher dipole moment (2.06 D) than that of CO₂ (0 D) [18]. In fact, it has been reported that even more

polar analytes were more readily eluted using R134a as the mobile phase than methanol-modified carbon dioxide [12,15].

2. Experimental

2.1. CCC instrumentation and procedures

The instrumental layout is shown in Fig. 1. A Pharma-Tech (Baltimore, MA, USA) Model CCC-1000 HSCCC was housed in an oven with a $\pm 1^\circ\text{C}$ precision. Three column modules were wound from a 29.5-m long PTFE tube (2.54 mm O.D. \times 1.59 mm I.D.), that gave a total volume of 177 ml. The oven was adjusted to the experimental temperature. The stationary phase was then delivered to fill the column by an Isco 260D syringe pump (Lincoln, NE, USA). After the excessive stationary phase was discarded, R134a was drawn from the liquid tank (~ 6.4 bar) to the pump and pressurized to the desired pressure. While the CCC rotor was revolving at 800 rpm, the mobile phase was pumped into the column. The stationary phase was gradually replaced by the R134a at a flow-rate of about 2 ml/min and collected in a graduated cylinder via Path 1. When the mobile phase flow reached the outlet, a burst of R134a vapor was observed and the flow-rate indicated on the pump head also increased drastically. The flow-rate was then gradually stabilized. The stationary phase was collected a further 10 min and the collected volume was used to roughly estimate the phase retention ratio. The experiment was finished if only the stationary phase retention was to be measured.

If a chromatographic run was followed, the mobile phase was allowed to flow one more hour after stationary phase displacement to reach hydrodynamic equilibrium, because the two phases were not pre-saturated like in the conventional operation. Then the R134a flow was switched using the six-port switch to pass through a Bio-rad (Hercules, CA, USA) 1801 UV-Vis HPLC detector then to a tube, via Path 2. The flow-rate was fine-adjusted using a metering valve (Valve V2). It usually took several minutes to attain a steady baseline signal after path switch to allow a sample injection. The attached outlet stainless steel tubing (1.59 mm O.D. \times 0.254 mm I.D.) immersed in ~ 2 ml methanol was used for collecting

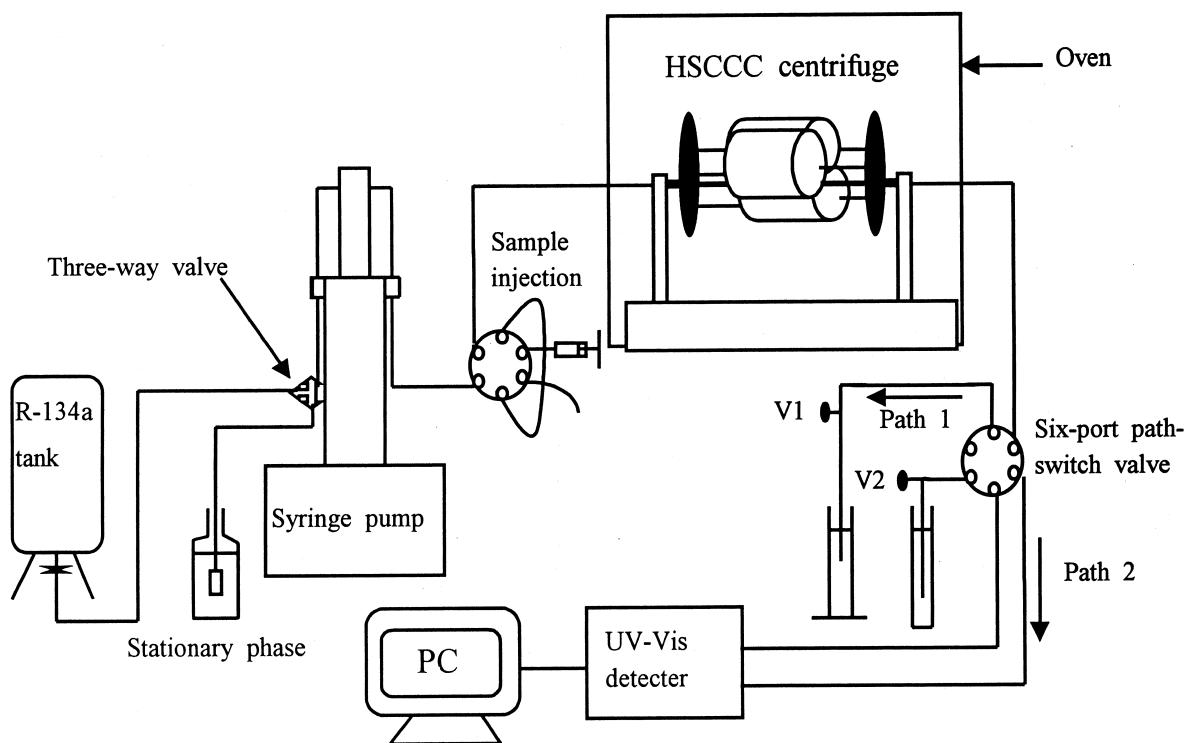


Fig. 1. Schematic diagram of the instrument layout.

components of interest. A 100- μ l sample solution was injected and the effluent was monitored at 212 nm. The sample collecting was operated under a hood.

2.2. Chemicals

Five standard components of *Angelicae radix* were examined in this study. Their structures are shown in Fig. 2. *p*-Cymene (>95%) was obtained from TCI (Tokyo, Japan); *trans*-ferulic acid (99%), bergapten (99%) and safrole (97%) from Aldrich (Milwaukee, WI, USA); HPLC-grade isopimpinellin from Extrasynthese (Genay Cedex, France). The sample solutions were prepared in methanol. HPLC-grade methanol and ethyl acetate were purchased from Mallinckrodt (Paris, KY, USA). Refrigerant 1,1,1,2-tetrafluoroethane (99.9%) was purchased from Ausimont (Milan, Italy).

3. Results and discussion

Because a PTFE tubing was used as the separation column, we tried to keep the pressure as low as possible. After careful testing we found steady flow could be obtained when the applied pressure was above 10 bar. Since the upper pressure limit for the PTFE tubing was rated 20.7 bar (300 p.s.i.), the operational pressure range was therefore about 10–20 bar for this system. Because R134a is a gas at ambient temperatures and pressures, it is not possible to measure the partition coefficient (K) in the solvent system in the conventional manner. We therefore assumed the polarity of R134a was similar to that of chloroform. Since the target compounds were of medium polarity, a R134a–methanol–water two-phase system was considered a reasonable selection. Both the head-to-tail and tail-to-head elution modes [5–7] were tested. No significant phase retention was observed for the tail-to-head flow direction. Phase

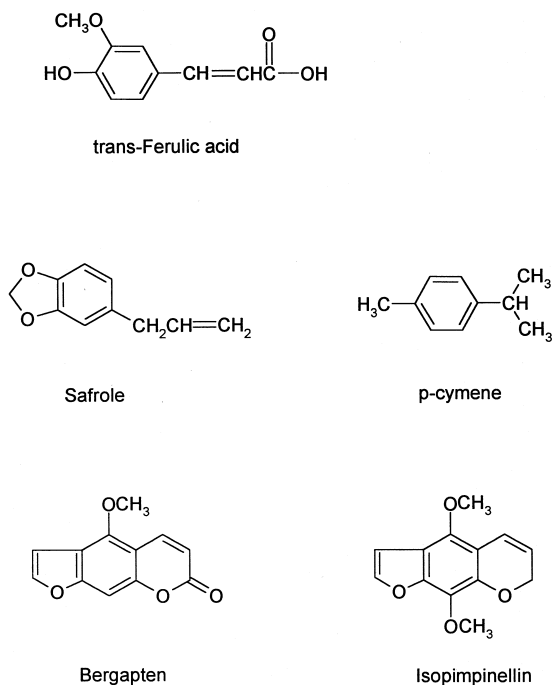


Fig. 2. Structures of bergapten, *trans*-ferulic acid, isopimpinellin, *p*-cymene and safrole.

retention ratios (S_F) were examined under different compositions using the head-to-tail mode, and the estimated results are shown in Table 1.

3.1. Elution of *trans*-ferulic acid

Extraction or chromatography of polar analytes suffers from the low solvating power using supercritical fluid carbon dioxide. A polar modifier usually is added to enhance the analyte solubility in the fluid. The relatively high dipole moment of R134a might act as a successful eluent for the polar *trans*-ferulic

acid. However, this component was not eluted out of the column after 3 h using the aqueous stationary phases of three different ratios of methanol (10, 40 and 60%, v/v). In order to reduce the dissociation of this acid, the pH of the aqueous solution was thus adjusted to acidic. Under pH of 2.3, 3.5, 4.6 and 5.9 the mobile phase was still unable to elute this component. Since the property of R134a was assumed to be close to that of chloroform, the partition coefficient of *trans*-ferulic acid was measured using a methanol–water–chloroform (6:4:4) system. The K value found was 0.74, which suggested a reasonable elution time for *trans*-ferulic acid. The result contradicted the assumption of the similarity between R134a and chloroform.

3.2. Separation for a mixture of bergapten, *p*-cymene, isopimpinellin and safrole

With a low ratio of methanol in water, it was not possible to resolve the *p*-cymene and safrole pair, and the bergapten and isopimpinellin pair well. The capacity factors of all the components were found to increase with increasing methanol content in the stationary phase. Two chromatograms using stationary phases of slightly different methanol portions are shown in Fig. 3. As can be seen, the resolution of the two pairs using the stationary phase of methanol–water (70:30) edged that of using methanol–water (65:35) at the expense of longer elution time. In order to obtain better resolution, an even higher methanol content was apparently required. However, higher methanol proportion in the aqueous solution lowered the retention volume drastically (see Table 1), and thus was prohibited. In fact, the baseline signal of using methanol–water (70:30) exhibited higher noise level than that of using methanol–water (65:35). The hydrodynamic stability became difficult to reach with higher methanol content. The small peak shown in Fig. 3a, becoming a shoulder of *p*-cymene in Fig. 3b, could be impurities in the *p*-cymene sample. It usually appeared as an independent peak in most chromatographic runs. The dip appearing just before the component *p*-cymene was due to the sample solvent and was used to calculate the phase retention ratio. Since the mobile and stationary phases were not pre-saturated, the stationary phase depletion was expected throughout

Table 1
Phase retention ratios under different stationary compositions

Methanol–water	S_F (%)
10:90	81
20:80	81
50:50	81
60:40	80
70:30	76
80:20	Negligible
90:10	Negligible

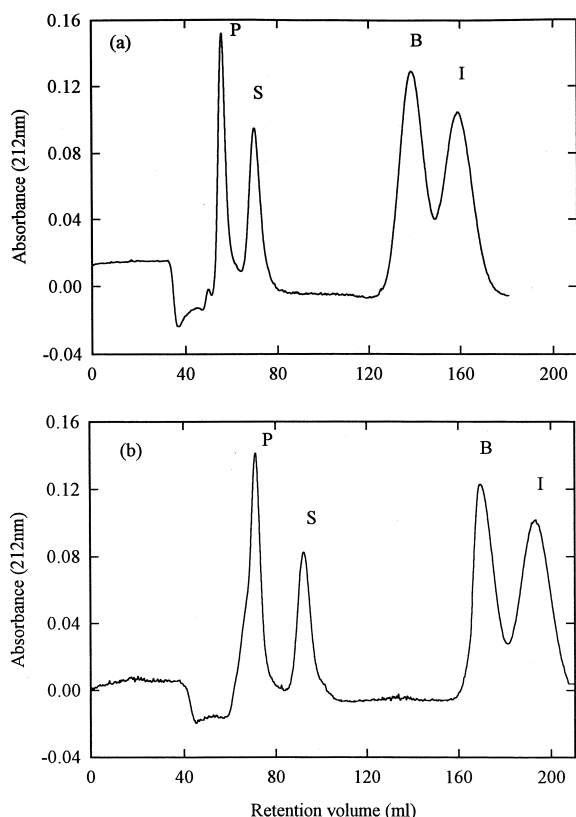


Fig. 3. Chromatograms of a mixture of bergapten (B) (500 $\mu\text{g/ml}$), isopimpinellin (I) (500 $\mu\text{g/ml}$), *p*-cymene (P) (250 $\mu\text{g/ml}$) and safrole (S) (500 $\mu\text{g/ml}$). (a) Stationary phase, methanol–water (65:35), $S_F=81\%$; (b) stationary phase, methanol–water (70:30), $S_F=77\%$. The effluent was monitored on-line by a UV detector at 212 nm. Experimental conditions: apparatus, HSCCC centrifuge; column, semipreparative multilayer coil, 1.59 mm I.D., 177-ml coil volume; flow-rate, 2.0 ml/min; injection volume, 100 μl ; under 20 bar and 25°C

the elution. Accordingly, the phase-retention ratio at the end of the elution should be different from the calculated value using the solvent front. It is also noted that the composition of the stationary phase at the completion of the elution would be also different from that at the beginning due to the same reason.

Although both separations, shown in Fig. 3, were performed under 20 bar and 25°C, experiments at 10 bar and 25°C, 15 bar and 25°C, and 20 bar and 20°C, were actually also performed using the stationary phase of methanol–water (65:35). The results (not shown) displayed no significant difference from the chromatogram in Fig. 3a. Since the operational

temperatures and pressures in this study were far below the critical temperature (101.5°C) and pressure (40.4 bar) [20,21], very small density changes were expected according to the equation of state derived by Sorner and Strom [22], and thus the solvation power of R134a. Accordingly, it is not surprising to observe the negligible effects on varying temperature and pressure.

In conclusion, we successfully demonstrated the prospect of using R134a as the mobile phase for separating samples of relatively low polarity without modifying the commercialized instrument. However, one gives up many advantages or introduces complications for spontaneous evaporation related to: partition coefficient measurement, mobile phase pre-equilibration, pressure and temperature conditions, choice of mobile phase, and potential air pollution.

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References

- [1] A. Weisz, A.L. Scher, K. Shinomiya, H.M. Fales, Y. Ito, *J. Am. Chem. Soc.* 116 (1994) 704.
- [2] A. Weisz, A.L. Scher, Y. Ito, *J. Chromatogr. A* 732 (1996) 283.
- [3] Y. Ma, Y. Ito, *Anal. Chem.* 68 (1996) 1207.
- [4] Y. Ma, Y. Ito, *J. Chromatogr. A* 771 (1997) 81.
- [5] N.B. Mandava, Y. Ito (Eds.), *Countercurrent Chromatography: Theory and Practice*, Marcel Dekker, New York, 1988.
- [6] W.D. Conway, *Countercurrent Chromatography: Apparatus, Theory and Applications*, VCH, New York, 1990.
- [7] Y. Ito, W.D. Conway (Eds.), *High-Speed Countercurrent Chromatography*, Wiley-Interscience, New York, 1996.
- [8] Y. Ito, *CRC Crit. Rev. Anal. Chem.* 17 (1986) 65.
- [9] T. Yu, S.-E. Li, Y.-H. Chen, H.P. Wang, *J. Chromatogr. A* 724 (1996) 91.
- [10] T. Yu, Y.-H. Chen, *J. Chromatogr. A* 790 (1997) 31.
- [11] Y.-L. Tsai, Master Thesis, National Chiao Tung University, Taiwan, 1998.
- [12] G.O. Cantrell, J.A. Blackwell, *J. Chromatogr. A* 782 (1997) 237.

- [13] J.A. Blackwell, R.W. Stringham, *J. Chromatogr. A* 796 (1998) 355.
- [14] J.A. Blackwell, R.W. Stringham, *Chirality* 9 (1997) 693.
- [15] J.A. Blackwell, L.E. Schallinger, *J. Microcol Sep.* 6 (1994) 551.
- [16] J.A. Blackwell, R.W. Stringham, *J. High Resolut. Chromatogr.* 20 (1997) 631.
- [17] J.A. Blackwell, R.W. Stringham, *Anal. Chem.* 69 (1997) 4608.
- [18] K. Jackson, J.L. Fulton, *Langmuir* 12 (1996) 5289.
- [19] M. Roth, *Anal. Chem.* 68 (1996) 4474.
- [20] K. Fujiwara, S. Nakamura, M. Noguchi, *J. Chem. Eng. Data* 43 (1998) 55.
- [21] H. Aoyama, G. Kishizawa, H. Sato, K. Watanabe, *J. Chem. Eng. Data* 41 (1996) 1046.
- [22] M. Sorner, K.H.U. Strom, *Can. J. Chem. Eng.* 73 (1995) 854.