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Review

Preparative supercritical fluid chromatography

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CONTENTS

1. PREPARATIVE CHROMATOGRAPHIC PROCESSES

Elution chromatography was first discovered as a preparative process by Tswett' for the fractionation of chlorophyll. It is therefore not surprising that preparative supercritical fluid chromatography (SFC) was proposed at the very beginning of the development of SFC by Klesper *et al.*[2], who stated that "the porphyrins could be recovered at the outlet valve". In fact, the unique physico-chemical properties of supercritical fluids, leading to the easy separation of fractionated compounds from the eluent, have convinced many workers that preparative SFC might be a very useful and relative easy tool in comparison with preparative gas chromatography, which is unsuitable for heavy and thermolabile compounds and preparative liquid chromatography, in which fraction–eluent separation can be problematic.

As shown in Fig. 1, preparative elution chromatographic processes are based on the same concept, whatever the eluent, with the following steps: periodic injection of the feed into a continuous flow of eluent; chromatographic separation due to selective interactions of the feed with both the eluent and the stationary phase; detection at the column outlet and fraction collection; separation of the fractionated compounds from the eluent; and purification and recycling of the eluent when economical (e.g., large-scale production).

Obviously, as long as small amounts of pure products (10^{-3}–1 g) are required, e.g., for structure analysis, bench-scale equipment derived from common analytical apparatus can be used with adoption of non-destructive detection, fraction collection and eluent removal. However, for industrial-scale production (1 g/h-l kg/h),

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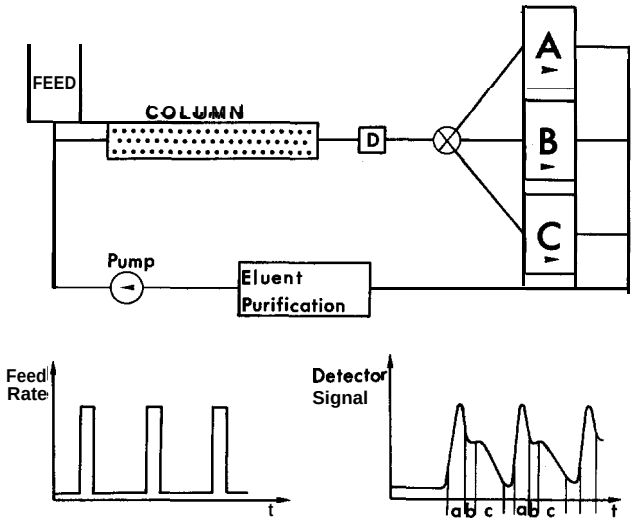

Fig. 1. Schematic diagram of the concept of preparative elution chromatography.

large-scale equipment will have to be designed, posing very different problems, even if the same concept is applied. In preparative SFC, most studies have been devoted to the former aspect, but very promising results are just starting to appear concerning the latter.

2. SMALL-SCALE PREPARATIVE SFC

As the general design of an analytical packed-column SFC instrument (Fig. 2) is always the same whatever the source [commercial or laboratory made from high-performance liquid chromatographic (HPLC) components], the equipment described in the literature is similar and differs only in the way fraction collection is performed: at atmospheric pressure; at high pressure; by adsorption on a solid followed by elution with a liquid solvent; or by dissolution in a liquid solvent.

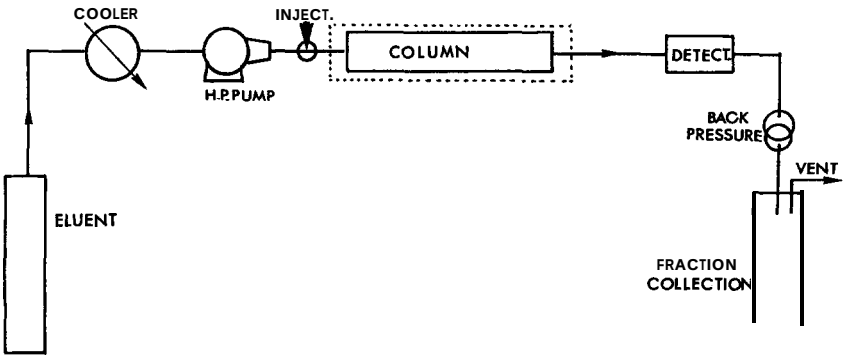

Fig. 2. General scheme of small-scale preparative SFC. H.P. = High-pressure; DETECT. = detection.

2.1. Collection at atmospheric pressure

This was first been used with mobile phases that are liquid at room temperature. As the pressure is reduced to atmospheric pressure by means of a capillary restrictor or a micrometric valve, liquid fractions can be collected at the outlet with a conventional LC fraction collector[3]. However, with mobile phases that are gaseous at room temperature and atmospheric pressure, fractions can also be collected directly at the outlet of a capillary restrictor or a back-pressure regulator, provided that the flow-rate remains relatively low and that the pressure reducer is sufficiently heated[4,5]. In most instances and particularly for volatile compounds, a special design of traps will be required to ensure efficient condensation of the products. An example was given by Flament and Keller'.

2.2. *Collection at high pressure*

The first attempts at collection at high pressures were made by Gouw and Jentoft[3] for collecting solutes dissolved in gaseous eluents, such as carbon dioxide or nitrous oxide. They developed an original system, consisting essentially of a rotating LC fraction collector mounted in a high-pressure container pressurized with nitrogen. The pressure in the container was adjusted so that the emerging mobile phase was still a liquid at the detector outlet. This liquid could then be evaporated, whereas the solutes were left as dry residues in test vials placed in the fraction collector. However, this type of device has rapidly been replaced by a more convenient system, consisting of a series of high-pressure vessels, selected by a switching valve. The vessels used by Campbell and Lee[6] were pressurized with nitrogen and cooled to about 3°C, so that solutes could be collected by slowly reducing the pressure using a micrometric valve.

Other workers have used the knowledge acquired with small-scale supercritical fluid extraction for trapping pure components in high-pressure separators after pressure reduction and for recycling the eluent after cooling and recompression[7,8]. This approach will be useful for larger-scale preparative SFC.

Alternative methods described include adsorption of the solutes on a solid followed by elution with a liquid solvent[9,10] and dissolution in a liquid solvent in which the solutes dissolve preferentially' [1].

2.3. *Applications*

Like other preparative chromatographic methods, preparative SFC is useful for the production of pure fractions when verification of the separation, identification (by mass, NMR or IR spectrometry) or thermodynamic studies are required.

Most of the early preparative studies, such as those of Gouw and Jentoft[3], were carried out to demonstrate the feasibility of SFC by proving first that the observed peaks were not artefacts and second that no degradation was occurring in the column. The collected fractions were either recombined and run again or identified by mass spectrometry[11–15].

To check the quality of flavour and essential oil (black pepper and clove extract) fractionations, Flament and Keller' developed an original two-dimensional chromatographic method by coupling SFC and thin-layer chromatography (TLC) (Fig. 3).

Preparative SFC can also be considered as a final enrichment step for trace analysis (off-line quantification or identification), as reported in Table 1[3,5,6]. Moreover, coupling with supercritical extraction or solute recycling can be performed to concentrate the solute and to increase the resolution of the separation[4,16].

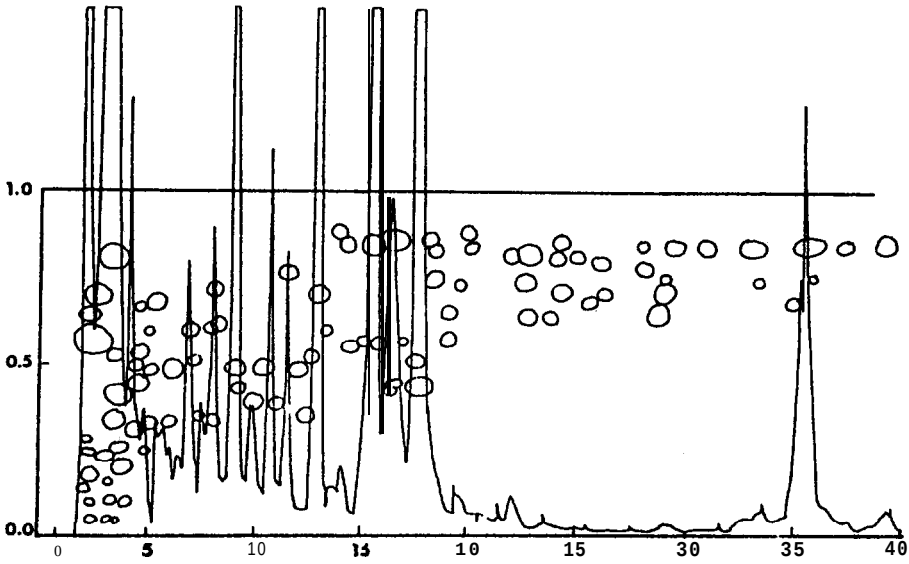

Fig. 3. Fractionation of clove essential oil by combined SFC-TLC. SFC conditions: column, C_{18}, 25 x 1 cm I.D.; CO_2 flow-rate, 4 ml min^{-1} at 32 MPa, 40°C; modifier, methanol (5%); laser detection at 150°C. TLC conditions: silica gel plate; Eluent, hexane–ethyl acetate (3:2). Horizontal axis: time in min; vertical axis: laser detector signal and TLC chromatogram. Reproduced from ref. 5, with permission.

3. LARGE-SCALE PREPARATIVE SFC

Large-scale preparative SFC seems to be a promising method for producing fractions free from solvent and, at first sight, it is surprising that there have been so few reports on semi-industrial preparative SFC separations. In practice, it turns out to be very difficult to cope with many of the problems, such as eluent recycling, eluent-product separation, periodic injection of the feed, fraction collection and column technology, all of which are much more crucial than on a smaller scale. However, we can mention here the work done by Khosah[18] on the laboratory scale on elution with a supercritical fluid of compounds adsorbed on a porous material (natural or not, mineral or polymeric). Khosah discussed the technical and economic feasibility of the process on the pilot scale. Alkio *et al.'* more recently published some results obtained with a preparative SFC unit built by conversion of a supercritical fluid extractor. Columns of 0.3-2 1 could be mounted in this unit, which were swept at flow-rates of up to 8 kg/h of liquid carbon dioxide. However, both devices lack experience and automation and industrial development in the near future is unlikely.

Promising results have been obtained in our laboratory since 1982. We have built a fully automated pilot unit, which can accommodate columns of I.D. up to 6 cm and lengths up to 1 m. The eluent can be totally recycled and its flow-rate through the column can reach 50 kg/h[19]. A general scheme of the process is shown in Fig. 4. The first studies carried out with a synthetic mixture of naphthalene derivatives showed the feasibility of the process, its good reliability and good stability of the hydrodynamics. Some technological difficulties regarding eluent-product separation and column

TABLE 1

EXAMPLES OF SMALL-SCALE PREPARATIVE SFC FRACTIONATIONS

Solute	Eluent	Column	Recovery	Ref.
Benzo[*a*]pyrene, benz[*a*]anthracene in automobile exhaust or refinery solid waste	CO_2	Alumina	Concentrate for off-line quantification	3
Polyaromatic hydrocarbons from mineral oil	CO_2	Alumina	Concentrate for chromatographic analysis	3
di-n- and di-isobutyl-phthalate (1 mg)	CO_2	Bare silica (5 μm), 1 cm I.D.		
Black pepper extract	CO_2–methanol	C_{18}, 1 cm I.D.	TLC coupling	
Clove extract	CO_2–ethanol	C_{18}, 1 cm I.D.	TLC coupling	
Coal tar (10–20 g)	CO_2	Bare silica (40–63 μm) or NH_2-bonded silica (30–70 μm), 4.6 mm I.D.		
Mineral oil distillate (35 g)	CO_2	Molecular sieve 5 Å	1.7 g isoalkanes, 1.65 g n-alkanes	8
Styrene oligomers (100 mg)	*n*-Pentane–methanol	Porasil A, 5 mm I.D.	20 mg of 15 pure oligomers	9,15
Styrene oligomers (35 mg)	CO_2	Porasil C, 2.6 mm I.D.		12-14
	CO_2	Reversed-phase, 4.6 mm I.D.	100 mg	17

efficiency were encountered and led to the design of high-performance separators" and to the adaptation of axial compression" to preparative SFC. We also investigated the possibility of adding a modifier, which would open the field of preparative SFC to more polar molecules[22].

The real potential of the process has been shown to be in the area of industrial separations. The purification of a vitamin intermediate and the fractionation of polyunsaturated fatty acid esters that may be useful in the treatment of cardiovascular and heart diseases [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) esters], were chosen as illustrative examples. The purification of a vitamin intermediate has been carried out on a 60 x 6 cm I.D. silica column using a carbon dioxide–methanol mixture as the eluent. Very high purities (> 99%) could be achieved, but the yields were relatively low[23,24].

Regarding fatty acid ester fractionation, the first attempts at using an axial compression column led to a pure EPA fraction (> 90%) with a relatively high yield

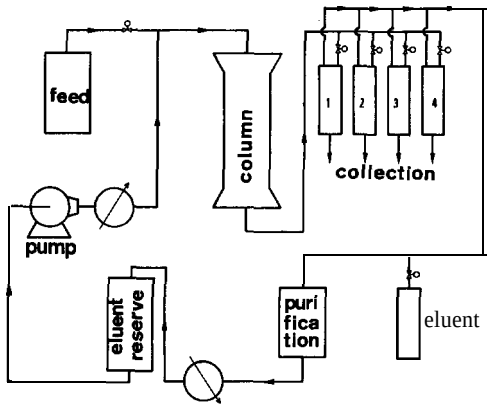

Fig. 4. General scheme of a large-scale preparative SFC unit.

(cu. 15 g/h) and to a DHA fraction (80%) with a yield of up to 20 g/h. These results were obtained on a 22 x 6 cm I.D. silica column with pure carbon dioxide as the eluent; a comparison with results obtained by HPLC indicated that preparative SFC will be a competitor to HPLC in the near future for this type of separation[24].

4. FUTURE DEVELOPMENTS

Small-scale preparative SFC can be very useful for purifying small amounts of key products and for the identification of the main impurities. It has a wider range of applicability than gas chromatography (GC) (extended to heavy compounds) and it is much faster than HPLC. However, although several collection device are discussed in this paper preparative, no SFC apparatus has reached the stage of commercial availability, apart from that from JASCO[4,16], which does not seem to be reliable and quantitative. Special attention must be paid to pressure reduction, eluent-product separation and fraction recovery for preparative SFC to become a quantitative method of fractionation.

Sample preparation by preparative SFC seems to be of a great interest for many studies, but it does not seem to be the most convenient method for trace analysis, for reasons given in this survey. Direct coupling of supercritical extraction with capillary SFC and with mass or infrared spectrometry would be preferable and would give a better performances for this kind of study.

Large-scale preparative SFC, on the other hand, is just reaching commercial development and seems to be very promising for the final purification or fractionation of fine chemicals or natural compounds. Unlike preparative GC, heavy, thermally labile compounds can be treated, and unlike preparative HPLC, the products can be recovered free from solvent and thus be directly usable for tests, reactions or final uses. Moreover, the coupling of supercritical fluid extraction and preparative SFC makes it possible to produce highly purified substances without any contact with organic solvents, which is of great interest for the pharmaceurical industry. However, the necessary use of a modifier (co-solvent) for the elution of polar compounds reduces the interest in preparative SFC for application to the fractionation of such compounds although, even in this instance, solvent removal is often easier than it is in HPLC.

5. SUMMARY

Preparative SFC has been investigated since the first development of SFC in order to collect pure components for identification (1962) and new devices for sample collection have been investigated. During the 1980s, SFC has come of age as an analytical tool complementary to GC and HPLC. Similarly, preparative SFC is being developed as a large-scale production process in parallel with recent developments in preparative GC and HPLC.

The various versions of preparative SFC that have been proposed, differing essentially in fraction collection and eluent recycling, have been surveyed. Future developments of both small- and large-scale preparative SFC are discussed in comparison with competitive GC and HPLC processes.

REFERENCES

1 M. S. Tswett, ***Ber. Dtsch. Bot. Ges.,*** 24 (1906) 315-323 and 384393.
2 E. Klesper, A. H. Corwin and D. A. Turner, ***J.*** Org. ***Chem.,*** 27 (1962) 700–701.
3 T. H. Gouw and R. E. Jentoft, ***J. Chromatogr. Sci.,*** 11 (1979) 313–327.
4 M. Saito, Y. Yamauchi, T. Hondo and M. Senda, in M. Perrut (Editor), ***Proceedings of the 1st International Symposium on Supercritical Fluids, Nice, October 1988,*** Institut National Polytechnique de Lorraine, Nancy, 1988, pp. 381-388.
5 Y. Flamant and U. Keller, in M. Perrut (Editor), ***Proceedings of the 1st International Symposium on Supercritical Fluids, Nice, October 1988,*** Institut National Polytechnique de Lorraine, Nancy, 1988, pp. 381-388.
6 R. M. Campbell and M. L. Lee, ***Am. Chem. Soc. Div. Fuel Chem.*** Prepr., 30 (1985) 189-194.
7 M. Alkio, T. Harvala and V. Kompa, in M. Perrut (Editor), ***Proceedings of the 1st Infernational Symposium on Supercritical Fluids, Nice, October 1988,*** Institut National Polytechnique de Lorraine, Nancy, 1988, pp. 389-396.
8 W. Ecknig and M. J. Polster, ***Sep. Sci. Technol., 21 (1986)*** 139–156.
9 N. M. Karayannis and A. H. Corwin, ***Anal. Biochem., 26 (1968)*** 34–50.
10 N. M. Karayannis, A. H. Corwin, E. W. Baker, E. Klesper and J. A. Walter, ***Anal.*** Chem., 40 (1968) 17361739.
11 E. Klesper and W. Hartmann, ***Eur.*** Polym. J., 14 (1978) 77-88.
12 R. E. Jentoft and T. H. Gouw, ***J. Chromatogr. Sci., 8 (1970)*** 138–142.
13 T. H. Gouw and R. E. Jentoft, ***J. Chromatogr., 68 (1972) 303-323.***
14 R. E. Jentoft and T. H. Gouw, ***Anal.*** Chem., 44 (1972) 681-686.
15 W. Hartmann and E. Klesper, ***J. Polym. Sci., Polym. Lett.*** Ed., 15 (1977) 713–719.
16 J. L. Millet, ***Analusis***, 15, No. 4 (1987) 3842.
17 M. Rawdon and T. Norris, Science, 222 (1983) 253-259.
18 R. P. Khosah, presented at the ***Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, 1988.***
19 M. Perrut, ***Fr. Pat.. 8*** 209 649 (1982); ***Eur. Pat., 0 099 765 (1984); U.S. Pat., 4 478 720 (1983).***
20 M. Perrut, ***Fr. Put., 8*** 510 468 (1985); ***Eur. Par., 0 212 999 (1986).***
21 C. Berger, L. Doguet and M. Perrut, ***Fr. Pat. Request, 1989.***
22 P. Jusforgues and M. Perrut, ***Fr. Put., 8*** 610 809 (1986).
23 C. Berger and M. Perrut, ***Technoscope Biofutur, 75 (1989) 3-8.***
24 C. Berger, ***Ph.D. Thesis,*** Institut National Polytechnique de Lorraine, Nancy, 1989.