CHROM. 10,277

COUPLING OF EXTRACTION WITH SUPERCRITICAL GASES AND THIN-LAYER CHROMATOGRAPHY

EGON STAHL

Pharmacognosy and Analytical Chemistry, University of Saarland, Saarbrücken (G.F.R.)

SUMMARY

A procedure is described in which substances are extracted with supercritical gases from small samples and brought directly on to a chromatographic thin layer as a starting band. Carbon dioxide is a particularly suitable gas. Using numerous model substances, the extraction conditions of this coupling technique have been ascertained and general rules formulated. These rules enable forecasts to be made for the industrial application of "fluid extraction".

INTRODUCTION

In the first decade of thin-layer chromatography (TLC) (1957–1967) we were concerned in particular with the standardization, range of application and coupling procedures with the aim of achieving improved identifications of substances[1]. In the second decade we have been interested in the problem of extracting compounds from complex samples (for example, natural drugs) as far as possible without the use of a solvent, and then applying them directly on to the thin layer. It appeared advantageous to use gases for this purpose and two possibilities can be considered: (1) use of the gas simply as a transport medium, and (2) use of the gas in the supercritical state as a genuine solvent.

GASES AS TRANSPORT MEDIA

We used a gas as a transport medium in the so-called carrier gas distillation and sublimation on the micro-scale, which resulted in the TAS procedure[2,3]. In this procedure, the sample is contained in a glass cartridge which is inserted into a heated oven block (Fig. 1), and a carrier gas is passed through slowly at the same time. Vaporizable compounds emerge directly on to a thin layer held in front of the apparatus and can subsequently be chromatographed. Further development of the TAS procedure led to thermofractography[4]. Gas is passed through the sample in this procedure also, but the sample is heated slowly from 50° to 450°. The plate supporting the thin layer is slowly transported at the same time so that the substances are fractionated as a band on the thin layer. A so-called thermofractogram is then obtained after chromatography. The sequence of substances along the abscissa
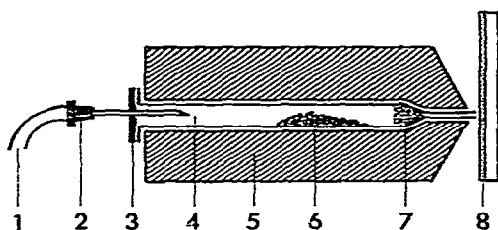
Fig. 1. TAS oven, longitudinal section. 1 = Carrier gas; 2 = injection needle; 3 = seal (silicone membrane); 4 = glass cartridge; 5 = heating block; 6 = sample; 7 = glass-wool; 8 = chromatographic layer.

follows the order of the beginning of their boiling or sublimation, while their sequence along the ordinate follows their chromatographic behaviour. This procedure furnishes more information than the usual two-dimensional chromatographic methods. More than 60 publications have appeared on the TAS procedure and thermofractography[5]. The procedures are well known in Europe and are frequently used, but so far they are less well known in the U.S.A. and other countries.

SUPERCRITICAL GASES FOR MICRO-EXTRACTION

The second possibility depends on the solvent power of compressed gases in the supercritical state. Supercritical or "fluid" gases are those which are at a pressure and temperature above the critical values, and are thus gaseous and compressible. Compression of the gases leads to increases in their density, dielectric constant and solvent-like properties. This phenomenon of supercritical gases was discovered almost 100 years ago by Hannay and Hogarth[6], but only the development of apparatus for high-pressure techniques in the last few decades has made possible the practical application of supercritical gases for extraction.

The gases which can be considered are listed in Table I, together with their critical data, and carbon dioxide is without doubt the most suitable. Its critical temperature is 31.3°, critical pressure 73 bar, critical density 0.45 g/ml and dielectric constant at 1 bar 0.0011.

TABLE I

PHYSICAL DATA FOR GASES USABLE FOR EXTRACTION

Gas	B.p. (°C) at 1 bar	T_c (°C)	P_c (bar)	ϱ_c (g/ml)	Dielectric constant at 1 bar and 0° (ε^{-1})
Carbon dioxide	− 78 (subl.)	31.3	73	0.448	0.0011
Nitrous oxide	− 89	36.5	71	0.457	0.00113
Ammonia	− 33.4	132.3	111	0.24	0.0072
Ethylene	−103.7	9.5	50	0.20	0.0018
Ethane	− 88	32.4	48	0.201	0.0015
Propane	− 44.5	96.8	42	0.220	−
Propene	− 47.4	91.8	45	0.22	−
Trifluorochloromethane	− 81.4	28.8	39	0.58	−
Difluorochloromethane	− 29.8	111.7	39	0.558	−

As can be seen in Fig. 2, both the density and dielectric constant increase with increase in pressure in the supercritical state; the main increase is between 70 and 150 bar. During the last two decades, many patents and patent applications have appeared all over the world, in which new uses of supercritical gases for extraction on an industrial scale are described (Table I)[7]. As far as we know, however, there have been no publications in scientific journals about practical applications.
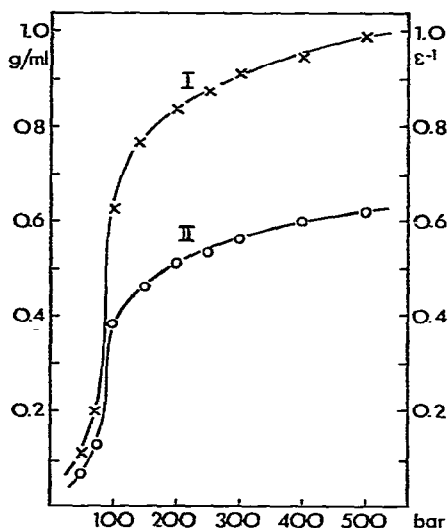

Fig. 2. Relationship between density (I) and dielectric constant (II) of carbon dioxide at increasing pressure and at 40°.

For some time, supercritical gases played a part in the so-called fluid chromatography[8], and attempts were made to utilize their increased solvent power. In my opinion, the failure of the procedure can be attributed to the difficulties of detection and to the development in the meantime of high-performance liquid chromatography (HPLC) as the preferred procedure. We were interested in the simple and direct combination of a high-pressure liquid extraction under mild conditions with TLC. We have constructed such an extraction equipment on the micro-scale and been using it for over 2 years for practical purposes[9]. The chief problem at first was the transition from the high pressures (between 73 and 500 bar) to the thin layer. We solved this problem by using extremely fine capillaries, of I.D. 25–50 μm. Our apparatus and its function are described below with the help of a diagram (Fig. 3).

CONSTRUCTION AND FUNCTION OF THE APPARATUS

The gas, usually pure carbon dioxide or nitrous oxide, is taken from an ordinary commercial cylinder and brought to the desired preliminary pressure of 40–60 bar. It is then compressed to the desired supercritical pressure of, for example, 73–500 bar by means of an electrically driven diaphragm compressor of performance 10–20 Nl/min. The pressure is maintained by using a back-pressure regulator accurate
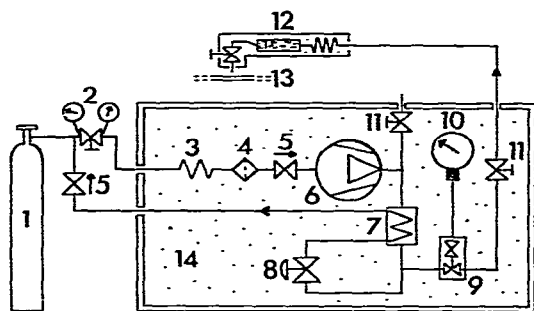
Fig. 3. Schematic diagram of the apparat. ⸲ for fluid extraction coupled directly with TLC. 1 = Steel cylinder; 2 = reducing valve; 3 = pre-heating coil; 4 = filter; 5 = check valves; 6 = diaphragm compressor; 7 = heat-exchange coil; 8 = back-pressure regulator; 9 = damping parts; 10 = precision manometer; 11 = shut-off valves; 12 = micro-autoclave for extraction; 13 = TLC receiving layer; 14 = thermostatically controlled container.

to about 1 bar, and is checked with a precision manometer. The compressor is thermostated at 40°. The supercritical gas then flows into a micro-extraction autoclave, also kept at 40°. Its volume is 2 ml but can be reduced to as low as 50 μl by inserting PTFE cylinders. The exit of the autoclave is sealed with a cut-off valve. When this valve is opened, the supercritical gas loaded with substance flows out through the very fine capillary and thereby expands. The emerging stream of gas strikes the thin layer, held horizontally and at a distance of 1–5 mm, depending on the pressure. The TLC plate is moved backwards and forwards and the extracted substances are precipitated as a starting band of length 2 cm.

Fig. 4 shows the front of the apparatus. In the temperature-controlled lower part is the diaphragm compressor with the corresponding connections and also the
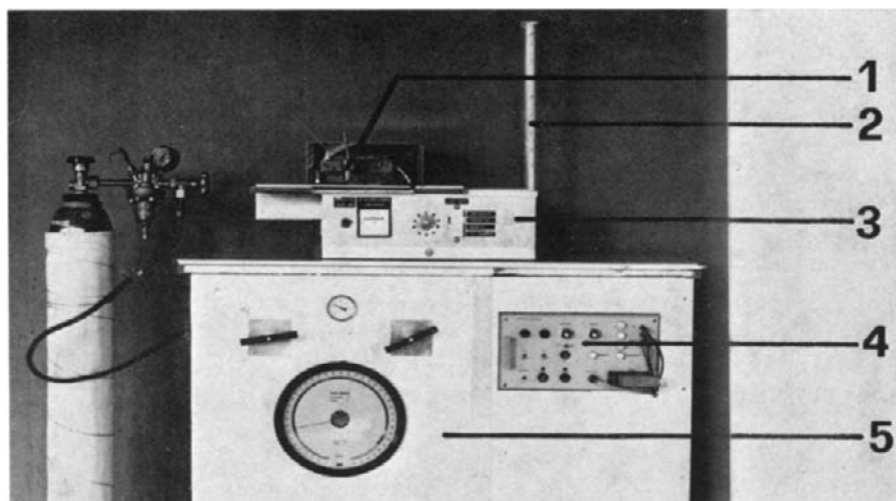

Fig. 4. Front view of the apparatus for fluid extraction coupled with TLC. 1 = Micro-autoclave for extraction; 2 = soap bubble meter; 3 = TLC receiving part; 4 = pressure control unit; 5 = high-pressure generating unit.

precision manometer and thermostating device. Above this is the collecting system for TLC, together with the heated micro-autoclave and the moveable slide that displaces the TLC plates backwards and forwards. Generally, we begin the extraction of a sample at 70 bar and allow 1 Nl of gas to flow through. The pressure is then increased stepwise by 5, 10 or 20 bar, the TLC plate appropriately displaced and the sample extracted again with 1 Nl of gas. It is then possible, after developing the chromatogram, to recognize the pressure at which a substance has been extracted. By comparing the zone intensities one can judge whether an increase in pressure has led to more or less of a substance being extracted. A stepwise pressure gradient is thus used in this procedure.

RESULTS AND DISCUSSION

Before attacking our real problem of the extraction of natural drugs, we investigated model substances from various groups of naturally occurring materials, hoping in this way to find relationships between extractibility with supercritical carbon dioxide and chemical structure. The results obtained so far can be summarized in the general rules given below.

General rules

(a) Hydrocarbons and other typically lipophilic organic compounds of relatively low polarity, such as esters, ethers, lactones and oxides, can be extracted in the lower pressure region, that is, between 70 and 100 bar.

(b) The extractibility is rendered difficult by the introduction of strongly polar functional groups such as –OH or –COOH. In the aromatic series, substances with up to three phenolic hydroxyl groups or with one carboxyl and two hydroxyl groups can still just be extracted. Aromatic compounds with one carboxyl and three or more hydroxyl groups cannot be extracted.

(c) More polar compounds, such as sugars, amino acids and similar compounds, cannot be extracted in the pressure region up to 500 bar.

(d) Fractionation in the pressure gradient takes place when there are marked differences in the temperatures at which boiling or sublimation begins and/or in the polarities of the substances (see the dielectric constants in Table I). The fractionation effects are most evident in the region of a rapid increase in density or dielectric constant of the supercritical carbon dioxide.

This is illustrated by the schematic fluid extraction chromatograms shown in Figs. 5 and 6.

Our results are also useful for "fluid" chromatography, as the solubilities determined in supercritical carbon dioxide enable a forecast to be made about which substances are amenable to this procedure.

We are now continuing our work in several directions. For instance, we are interested in the possibilities of extraction at higher pressures (up to 3000 bar). Our procedure of direct coupling with TLC fails, however, in this higher pressure region, and we have employed a different collecting procedure here. Another domain which interests us is the determination of the extraction yields at various pressures. We are working with model mixtures and our first results are reported below.

Fig. 7 shows the quantitative course of extraction of *p*-hydroxybenzoic acid
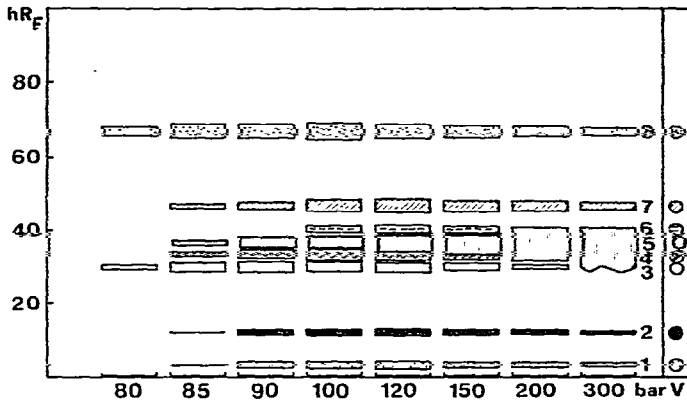
Fig. 5. Thin-layer chromatogram after fluid extraction of a vitamin–oil mixture (200 μg each). 1 = Cholesterol; 2 = vitamin D₃; 3 = vitamin K₃; 4 = α-tocopherol; 5 = triglyceride; 6 = vitamin A acetate; 7 = α-tocopheryl acetate; 8 = steryl ester.
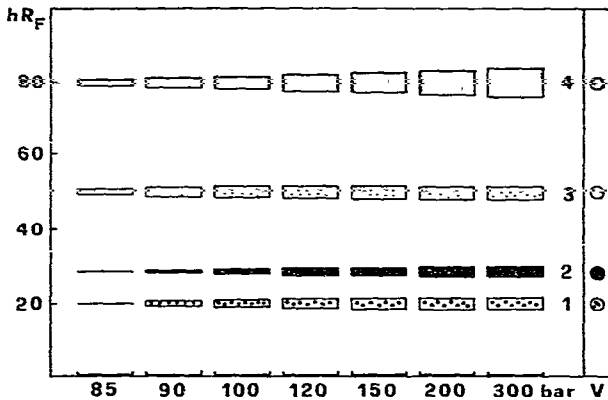

Fig. 6. Thin-layer chromatogram after fluid extraction of the alkaloid drug *Strychnos nux vomica*. 1 = Brucine; 2 = strychnine; 3 = fatty oils. The powdered drug was moistened with a drop of concentrated ammonia solution before extraction.
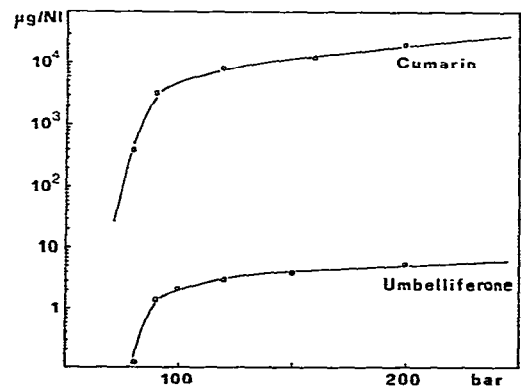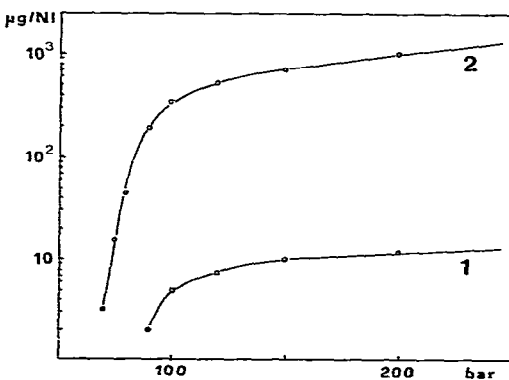

Fig. 7. Course of extraction with supercritical carbon dioxide in relation to the pressure at 40°. 1 = *p*-Hydroxybenzoic acid; 2 = *p*-hydroxybenzoic acid ethyl ester. The ester is extracted 100 times more strongly than the acid.

Fig. 8. Course of extraction with supercritical carbon dioxide in relation to the pressure at 40°. Coumarin is extracted 4000 times more strongly than umbelliferone.
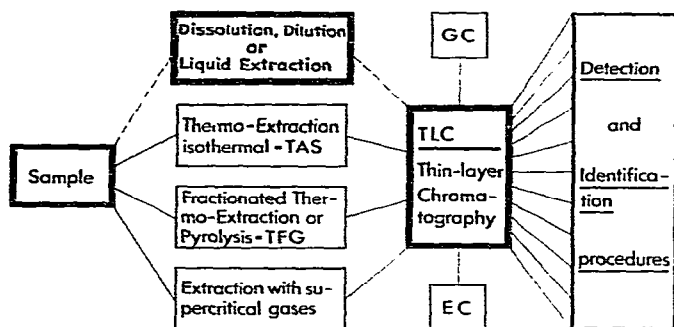
Fig. 9. The possibilities for extraction of substances from complex mixtures coupled with TLC. Boxes with thick lines indicate classical procedures.

and its ethyl ester with supercritical carbon dioxide under increasing pressure. It can be seen that the ester is extracted about 100 times more strongly than the acid. Fig. 8 shows the course of extraction of coumarin and of umbelliferone, which has one more hydroxyl group. The latter is extracted 4000 times less well than coumarin. These somewhat time-consuming experiments are being continued[10].

This coupling method provides a rapid and simple insight into the course of extraction and saves many costly trials. We consider that this method is paving the way towards the promising future possibility of industrial extraction procedures with supercritical gases. The coupling of this elegant extraction method with TLC is the keystone of our attempts to achieve solvent-free extraction before chromatography. Progress to date is summarized in Fig. 9.

REFERENCES

1 E. Stahl (Editor), *Thin Layer Chromatography, A Laboratory Handbook*, Springer, Berlin, Heidelberg, New York, 1st ed., 1962, 2nd ed., 1967.
2 E. Stahl, *J. Chromatogr.*, 37 (1968) 99.
3 E. Stahl, *Analyst (London)*, 94 (1969) 723.
4 E. Stahl, *Z. Anal. Chem.*, 261 (1972) 11.
5 E. Stahl, *Accounts Chem. Res.*, 9 (1976) 75.
6 J. B. Hannay and J. Hogarth, *Proc. Roy. Soc. London*, 29 (1879) 324.
7 E. Stahl and W. Schilz, *Chem.-Ing.-Tech.*, 48 (1976) 773.
8 T. H. Gouw and R. E. Jentoft, *J. Chromatogr.*, 68 (1972) 303.
9 E. Stahl and W. Schilz, *Z. Anal. Chem.*, 280 (1976) 99.
10 W. Schilz, *Thesis*, in preparation.