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# ON-LINE HIGH-PRESSURE EXTRACTION–HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

## I. EQUIPMENT DESIGN AND OPERATION VARIABLES

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SUMMARY

A coupling unit is described that allows on-line operation of a high-pressure extraction apparatus with a high-performance liquid chromatograph. The unit consists of two high-pressure sample-injection valves, connected in series. The first valve operates as a switching valve to the loop and controls the release of pressure along a packed microbore column. Two short, packed microbore columns are positioned between the first and second valve, sampling the extracted compounds over a desired period and simultaneously operating as sample loop for the second valve; the latter is the injector for the high-performance liquid chromatographic column. The unit was tested by continuous extraction of valtrate and didrovaltrate from *Radix valerianae* with carbon dioxide at 313°K and 96 bar.

## INTRODUCTION

High-pressure extraction (HPE) by liquefied and supercritical gases has developed into a powerful method for the isolation and production of a variety of compounds from raw materials under mild conditions[1,2]. Carbon dioxide is preferred among the supercritical extraction agents, because it is non-toxic, non-flammable, non-polluting and inexpensive. The extraction properties can best be understood by inspection of a reduced pressure–density diagram. The region indicated as supercritical fluid in Fig. 1 defines the usual range of working conditions, where the critical temperature, $T_c$, is 304.20°K, the critical pressure, $p_c$, 73.86 bar and the critical density $\rho_c$, 468 kg/m$^3$ (ref. 3). In the supercritical fluid state carbon dioxide exhibits a density of up to 1000 kg/m$^3$, which is considerable compared with liquid carbon dioxide[5]. A second favourable property is its miscibility with liquids. In addition, supercritical carbon dioxide possesses a diffusivity much higher than that of common liquids and a viscosity comparable to that of gases[6]. These properties ensure rapid mass transfer of solute in the supercritical extraction process.

Much attention has been paid to the assessment of solubility data and to the establishment of fluid-phase equilibria[5]. In the extraction of compounds from solid

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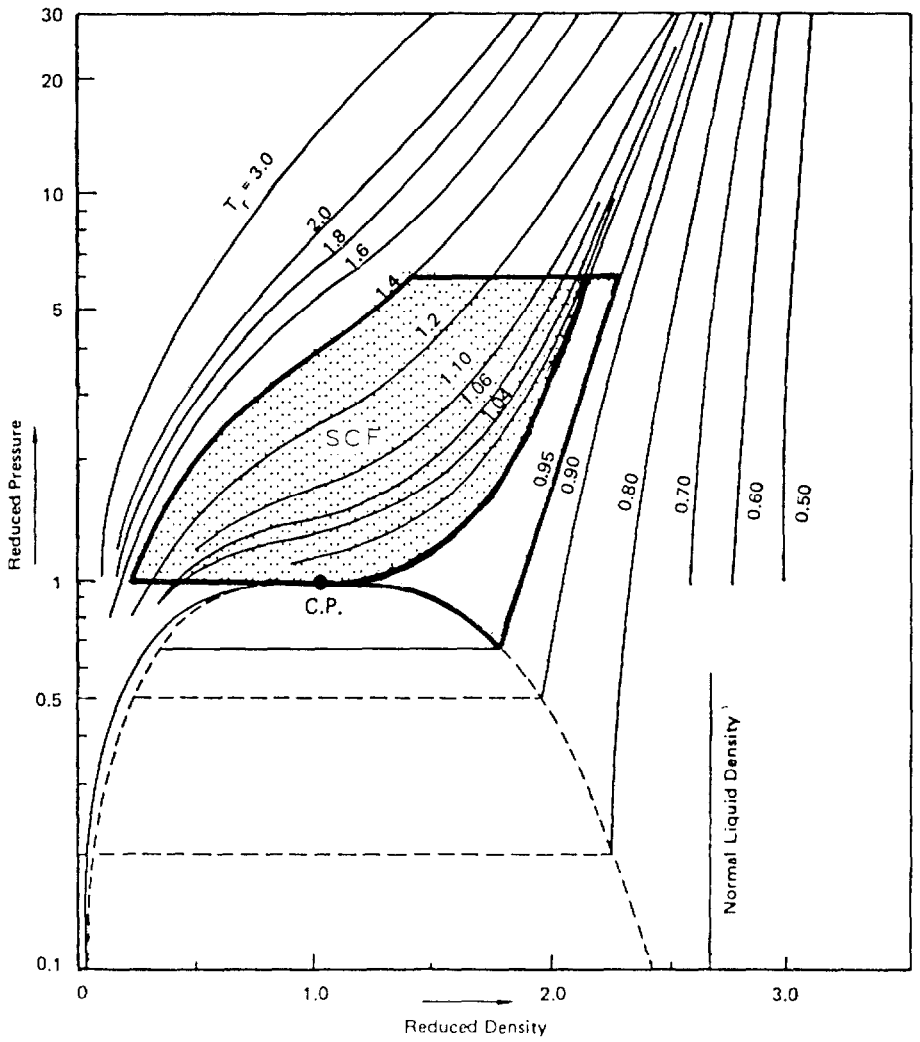


Fig. 1. Reduced pressure *versus* reduced density diagram for carbon dioxide (from ref. 4, with permission).

raw material, solubility (and hence purity and yield) is difficult to predict, and experiments must be conducted at appropriate levels of temperature and pressure, followed by an analysis of the extract. Taditionally, extraction analysis is performed off-line. Stahl and co-workers[2,7–10] developed a mini-extraction apparatus that allowed on-line detection of the extract by thin-layer chromatography. The solubility of organic substances in liquid carbon dioxide has been thoroughly examined by Nieass and co-workers[11,12], who used a system that connects the high-pressure cylinder containing the loaded liquid carbon dioxide with conventional high-performance liquid chromatography (HPLC) equipment.

This paper describes the design and operation of a coupling unit, linking (HPE) with HPLC. The various aspects of the on-line extraction are demonstrated with *Radix valerianae* as raw material by analysing the valepotriates in the extract.

## EQUIPMENT DESIGN[13]

The successful operation of an HPE–HPLC coupling unit requires the performance of three basic functions:

(i) separation of the extract and the gas by releasing the pressure and/or decreasing the temperature;

(ii) sampling and deposition of the extract in such a way that defined aliquots of the material are loaded into a loop, which is a constituent of an HPLC injection system;

(iii) reproducible injection of the extracted compounds by flushing the loop with eluent.

As discussed by Hubert and Vitzthum[1], the separation of extract and gas may be accomplished in three different ways: by releasing the pressure, by decreasing the temperature or by adsorption at constant pressure and temperature. In this study, isothermal conditions were chosen. The temperatures of extraction and separation, $T_e$ and $T_s$, were the same, but were above the critical temperature of carbon dioxide, $T_c$, *i.e.*,

$$T_e = T_s > T_c$$

The separation pressure, $p_s$, was below the critical pressure, $p_c$; and $p_c$ was lower than the extraction pressure, $p_e$, *i.e.*,

$$p_e > p_c > p_s$$

Inspection of the literature on supercritical gas extraction shows that the preferred range for $T_e$ is between 308 and 373°K and for $p_e$ between 80 and 300 bar[1]. As the extraction experiments were carried out at 313°K and 96 bar, it seemed sensible to incorporate the extraction vessel and the injection system of the HPLC apparatus into the thermostated coupling unit. However, other versions are also feasible.

Carbon dioxide of 99.998% purity (Linde Mainz-Kostheim, F.R.G.) was fed into a diaphragm-type compressor (No. 560.0105; Nova Werke, Effretikon, Switzerland), operating at up to 1000 bar with a pressure control unit (back-pressure regulator, Type 26-1700, from Tescom, Minneapolis, MN, U.S.A; No. 1 in Fig. 2). The laboratory-constructed coupling unit is shown in Fig. 2. The stainless-steel extraction vessel (2) had volumes between 0.4 and 10 ml. Depending on its volume, the vessel was loaded with 10–1000-mg amounts of the raw material (powdered *Radix valerianae* Mex., No. 007/042, kindly supplied by E. Scheurich, Pharmwerk, Appenweier, F.R.G.).

The vessel outlet was connected to a high-pressure two-way angle valve (Type 530.0111; Nova Werke) (3) for expansion. The heart of the coupling unit was made up of two high-pressure six-port valves, slightly modified in their functions and connected in series. The first valve was a six-port 200-bar external sample-injection valve (Valco Instruments, Houston, TX, U.S.A.) (4) and was employed in a two-fold fashion, *viz.*, for release and for feeding the sample loop. Hence, only two segments were used. The outlet of one segment (Fig. 2) was connected to a packed microbore

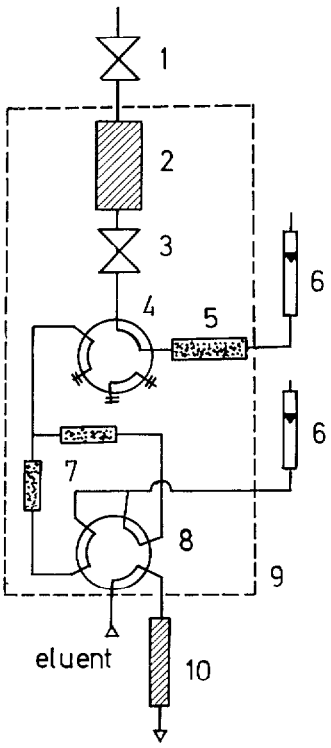


Fig. 2. Schematic diagram of coupling unit. 1 = Back-pressure regulator; 2 = extraction vessel; 3 = high-pressure two-way angle valve; 4 = six-port external sample valve; 5 = packed microbore column for release and waste deposit; 6 = rotameter; 7 = microbore columns, serving for deposit and as loop; 8 = sample injector; 9 = thermostat; 10 = HPLC column.

column (5), which served as a waste column. The flow-rate of the carbon dioxide evolved was measured by a rotameter (6). The outlet of the second segment was split by a joining-T into two capillaries, each leading to a packed microbore column (7). The second valve (8) (a syringe-loading type from Rheodyne, Model 7120, supplied by Latek, Heidelberg, F.R.G.) functioned essentially as the injection system, in which the two segments connecting the microbore columns operated as a loop. The third segment of the valve contained the inlet of the eluent and the outlet to the HPLC column (10).

The whole unit was thermostated (9). The eluent was dichloromethane–methanol (99.5:0.5, v/v) and the column (120 × 4 mm I.D.) was packed with LiChrosorb Si 100, particle diameter $d_p$ = 5 $\mu$m (E. Merck, Darmstadt, F.R.G.). The HPLC apparatus was made up of a DuPont 8800 liquid chromatograph solvent delivery pump (DuPont de Nemours Deutschland, Bad Nauheim, F.R.G.), a fixed-wavelength UV detector (254 nm) with a 10$\mu$l cell volume (1203 UV Monitor III from Latek) and a Hewlett-Packard integrator, Model 3385A (Hewlett-Packard, Waldbronn, F.R.G.).

## OPERATION AND PROCESS VARIABLES

The operation of the system involved three consecutive steps (release, load, inject), which were carried out by switching the rotors of the two valves to the positions indicated in Fig. 3. In the release position, expansion of the loaded carbon dioxide took place along the waste column from $p_e$ to $p_s$, the latter being atmospheric pressure. The speed of extraction and separation in this part (expressed in ml (STP) of carbon dioxide per unit time) was regulated by the length and inner diameter of the column and the particle size of the packing. During expansion, the column was loaded with the extracted compounds and was replaced from time to time after regeneration.

By switching the valve into the sampling position (90° rotation), the extract flowed through the packed microbore columns, positioned between the two valves. As both columns had a permeable volume of about 20 $\mu$l and were packed with porous microparticles, they simultaneously served as sampler and loop. The loading period was between 10 and 60 sec. After switching valve 4 back into the release position, the pressure in the loop decreased to atmospheric pressure. Then, valve 8 was rotated into the inject position and the loop was rinsed with the eluent at a low flow-rate to avoid too high a back-pressure. The extracted compounds were dissolved by the eluent and entered the chromatographic column. At that point, the flow-rate of the eluent was increased to the desired rate. Stainless-steel capillaries of 0.2 mm I.D. were used for connections. Further improvements are possible in the automation of the two valves (4 and 8). The six-port Valco valve (4) will be exchanged for a four-port valve in future experiments.

Five variables were identified as parameters in handling the system: the mass of solid material to be extracted, the pressure and the temperature of extraction, the speed of extraction and separation and the mass of extract deposited in the loop for injection.

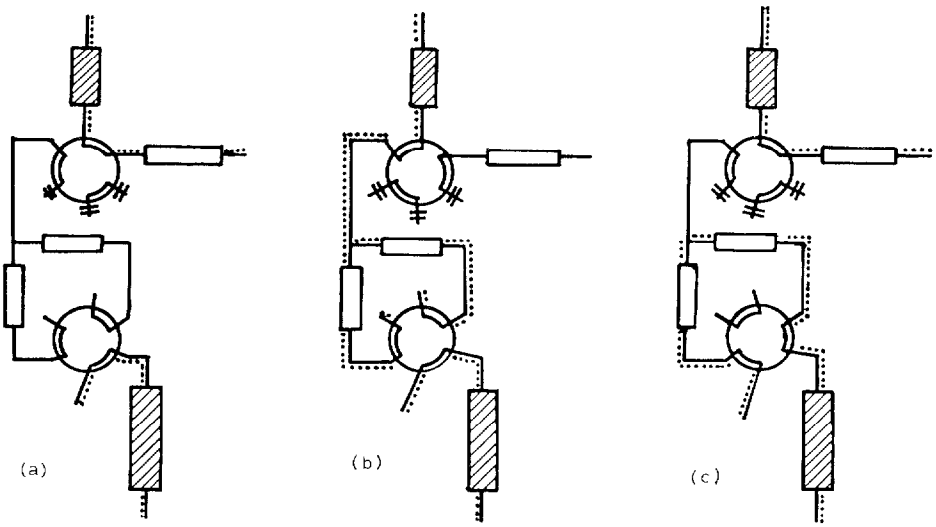


Fig. 3. Handling of coupling system. (a) Release position; (b) load position; (c) inject-and-release position.

For an analytical technique, small sample sizes, of the order of 10–100 mg of solid material, are required unless the extraction yield is far below 1%. The pressure is automatically checked and maintained constant by means of the pressure control unit (reproducibility ± 1%). In quantitative work, attention should be paid to possible leaks in the connections of the coupling unit. The temperature of the unit was adjusted to ± 1°C. The optimum flow-rate (*i.e.*, the speed of extraction) was about 150 ml (STP) of carbon dioxide per minute when a microbore column (250 × 1 mm I.D.) packed with 5-μm particles was used. However, longer assemblies of 250-mm columns were also tested.

The microbore columns comprising the loop (2.5 × 1 mm I.D.) were also packed with 5-νm particles. The speed of extraction was higher by a factor of 5 than that of a 250 × 1 mm I.D. waste column. The mass deposited on to the packings was controlled by the loading period, which was between 10 and 30 sec.

The system provides two options for operation, as follows.

*Continuous extraction mode*

The solid material is continuously extracted, with the unit set at the release position. Samples are taken at desired time intervals by loading the loop, and analysis of the extract is carried out with an appropriate HPLC system. The frequency of analysis is limited by the time required to develop the chromatogram.

*Discontinuous extraction mode*

In this mode two variations are considered, as follows.

(a) After a certain extraction period at constant pressure and temperature, equivalent to conditioning, the high-pressure valve is opened for a short period to load the two microbore columns. This is followed by an analysis of the extract. The carbon dioxide lost during release and loading is not replaced.

This method allows the total amount of components extracted to be calculated, provided a careful calibration of flow-rate and an adjustment of experimental conditions are carried out. Analyses of this type were also performed in this study. High accuracy is achieved when dead-volume contributions are kept negligibly small and the system is free from leaks.

(b) After conditioning for a desired period of time, the extract is continuously released via the waste column, and repeated loading and analysis is performed while the carbon dioxide consumed is fed to the extraction vessel.

The continuous extraction mode is probably preferable, as changes in the concentration of extracted compounds as a function of time are easily recognized. Furthermore, information on the carbon dioxide consumed is obtained when the flow-rates at the outlet of the waste and the loop column are measured.

## EXTRACTION AND ANALYSIS OF *RADIX VALERIANAE*

*Radix valerianae* was chosen as a model for testing the capability of the HPE–HPLC system. Earlier extraction studies by Stahl and Schütz[14] have shown that didrovaltrate and valtrate could be extracted with supercritical carbon dioxide (3.0 and 0.3% w/w, respectively).

Continuous extraction with supercritical carbon dioxide was conducted at

313°K and 96 bar. Several types of valepotriates were extracted and monitored by means of HPLC with a LiChrosorb Si 100 column (120 × 4 mm I.D.) and dichloromethane–methanol (99.5:0.5, v/v) as eluent. The analysis time was 15 min. As the valepotriates differ significantly in their UV absorptions, two wavelengths (229 and 254 nm) were chosen for detection; 254 nm is particular suitable for valtrate and 229 nm for didrovaltrate. A typical time course of the extraction of 20 mg of *Radix valerianae* is shown in Fig. 4 for valtrate, indicating strong extraction within 30 min. Extraction is completed in *ca.* 1 h. The total number of points obtainable for assessing the course of the extraction curve is limited by the analysis time, *i.e.*, only after the chromatogram has been developed can the injection be repeated. This also causes problems in the accurate determination of the starting point of the extraction, where the amount of compound extracted is at a maximum. The release time at the first point of measurement must be adjusted in such a way that it corresponds exactly to the dead-time, *i.e.*, the dead-volume must be completely flushed out before the next loading and injection. As the flow-rate of carbon dioxide was measured, the amount of carbon dioxide used to extract a given mass of valtrate could be calculated easily.

Although standards of valtrate and didrovaltrate were supplied by several institutions, careful checks of their purity by HPLC strongly indicated that none of

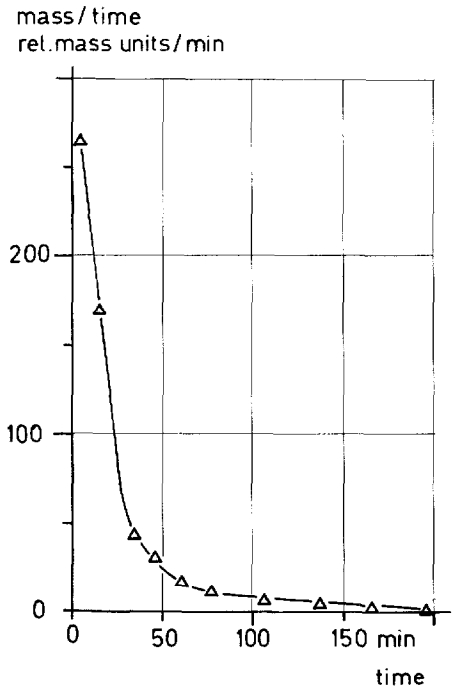


Fig. 4. Kinetics of extraction of valtrate from *Radix valerianae*. Weight of solid material, 20 mg; pressure, 96 bar; temperature, 313°K; extraction vessel, 50 × 4 mm; waste column, 250 × 1 mm I.D., packed with LiChrosorb RP-18, $d_p$ = 5 μm; microbore colums, serving as loop, each 2,5 × 1 mm I.D., each, packed with LiChrosorb RP-18, $d_p$ = 5 μm; eluent, dichloromethane methanol (99.5 : 0.5, v/v); column, 120 × 4 mm I.D., packed with LiChrosorb Si100, $d_p$ = 5 μm.

them was pure and suitable for calibration. The ordinate values of the extraction curve in Fig. 4 are therefore given in relative mass units per unit time.

When the extraction experiments under the conditions mentioned were repeated, a reproducibility of $\pm 6\%$ was observerd.

## CONCLUSIONS

With the aid of the coupling system described here the time course of a high-pressure extraction under given conditions was monitored analytically (type of compounds, concentration). The procedure also permitted the evaluation of costs, *i.e.*, the carbon dioxide consumed.

The system is ideally suited for extracting solids with supercritical fluids in order to isolate and identify the extracted compounds. The ability to search for optimum extraction conditions with respect to yield and purity is a further advantage provided by the coupling unit; a basis is provided for scaling up the extraction experiments. When built to the appropriate scale, the system may be connected to pilot or industrial extraction plants for monitoring the composition of extracts.

## ACKNOWLEDGEMENT

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