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Extraction of major alkaloids from poppy straw with near-critical mixtures of carbon dioxide and polar modifiers

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SUMMARY

Near-critical extraction of major alkaloids from poppy straw was performed successfully with a simple device consisting mainly of two chromatographic pumps and a pressure regulator. The optimum extractant, consisting of carbon dioxide, methanol and water, gave a quantitative extraction of thebaine, codeine and morphine in 20 min. The method was compared with a classical liquid–solid extraction procedure and carbon dioxide was shown to act as a transporting agent of the extraction solvent (methanol–water) into the vegetable matrix.

INTRODUCTION

The use of supercritical fluids for analytical extraction has recently attracted increasing attention and several new applications have been reported[1–16]. In comparison with classical liquid–solid extraction methods, supercritical fluid extraction (SFE) offers many potential advantages[17,18]. *e.g.*, faster and more efficient extractions, increased selectivity, easier analyte fractionation and coupling with on-line analytical methods[19].

These potential advantages of SFE are due to the properties of fluids above their critical pressure and temperature. Such fluids have densities 100–1000 times greater than those of gases and solvating properties comparable to those of liquids. Their viscosities and diffusion coefficients are intermediate between those of liquids and gases. Solvent mixtures can also be used to achieve higher solvent strength and increased selectivity. Further, by using a fluid such as carbon dioxide with a relatively low critical temperature (31°C), extractions can be performed under mild thermal conditions. Finally, supercritical fluids have a solvent power close to that of liquids and offer better mass transfer. This clearly provides the potential for more rapid and more efficient extractions than classical liquid phases owing to a more rapid and more complete penetration into a solid matrix.

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Many experimental data are available on the solubility and extractibility of natural products such as steroids, alkaloids, anti-cancer agents, flavours and aromas, oils from seeds, caffeine from coffee beans and fatty acids from fish oils[17,20]. To determine the solubilities of compounds in supercritical fluids, Stahl and co-workers[21,22] developed a microextraction procedure directly coupled with thin-layer chromatography. Using this apparatus, they compared the capacities of carbon dioxide and nitrous oxide for extracting opium alkaloids from plant material. Nitrous oxide appeared to be a better extractant than carbon dioxide, but its extracting power remained weak. It was concluded that it was worth investigating the influence of polar modifiers which, when added to a supercritical fluid, could increase its solvating power.

In this paper, the near-critical extraction of alkaloids from poppy straw is described. As classical liquid–solid extraction procedures are often time consuming and tedious[23–25], it was of interest to develop a rapid, near-critical fluid extraction procedure that is readily applicable in routine analysis. The study resulted in a quantitative extraction of the three major alkaloids in 20 min. Special efforts were made to define the respective roles of carbon dioxide and polar modifiers in the extraction process.

EXPERIMENTAL

Chemicals and reagents

Carbon dioxide was of technical grade (Air Liquide, Lyon, France), methanol of high-performance liquid chromatographic grade (Prolabo, Paris, France) and methylamine was used as a 40% aqueous solution (Merck, Darmstadt, F.R.G.). Thebaine, codeine and morphine standards were obtained from Francopia (Paris, France).

Extraction procedure

Extraction was effected by percolation of a near-critical mixture (carbon dioxide–polar modifier) through a column containing poppy straw (obtained from Sanofi-Chimie, Aramon, France) previously ground and sieved. After decompression, the extract was collected by inserting the outlet tubing in a three-necked flask which contained *ca.* 5 ml of methanol. The methanolic solution was then analysed off-line by subcritical fluid chromatography.

The extraction cells were stainless-steel columns with stainless-steel frits maintained at both ends by Swagelock fittings. Two columns were used, 25 × 0.75 cm I.D. and 25 × 0.46 cm I.D., containing 4 and 2 g of poppy straw, respectively. For all experiments, the particle size of the poppy straw was between 250 and 500 μm.

To maintain near-critical pressures inside the extraction cells, we first used fused-silica capillaries and tapered stainless-steel tubes as the outlet. With carbon dioxide–polar modifier mixtures, these two kinds of restrictors did not work properly as they became plugged and did not ensure a constant pressure in the system. Finally, we used the same pressure regulator as in packed-column supercritical fluid chromatography (Model 26-3220-24004 valve; Tescom, Minneapolis, MN, U.S.A.). The whole extraction apparatus is shown in Fig. 1. The two pumps were the same as those used in our chromatographic studies[26].

It must be noted that this pressure-regulating system can only be used with

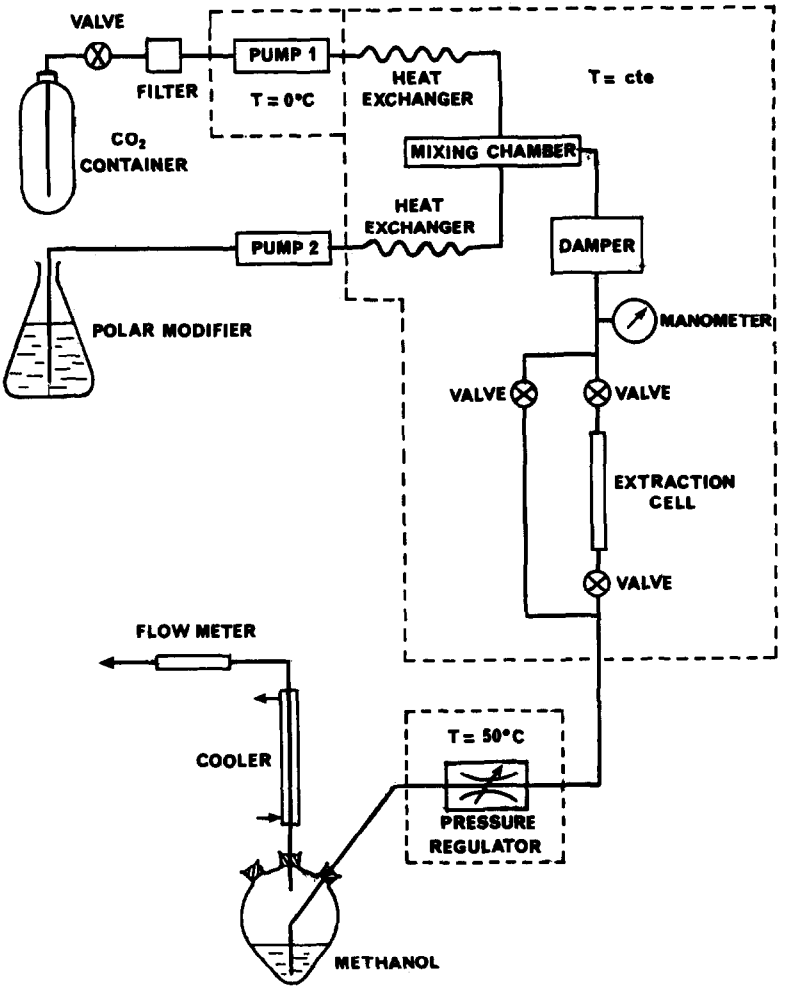


Fig. 1. Experimental set-up.

extraction phases containing a polar modifier. After decompression, gaseous carbon-dioxide and liquid modifier are obtained; the transfer of the extract is then due only to the liquid phase.

Chromatographic analysis of extracts

The chromatographic system used has been described elsewhere[26]. All chromatographic analyses were effected with a stainless-steel column (23 × 0.46 cm I.D.) packed with 5-μm LiChrosorb Si 60 silica (Merck). The mobile phase was a subcritical mixture of carbon dioxide–methanol–methylamine–water (83.00:15.90:0.14:0.96, w/w). A typical chromatogram of the extract aliquot is shown in Fig. 2, revealing three major alkaloids (thebaine, codeine and morphine) and two unidentified constituents.

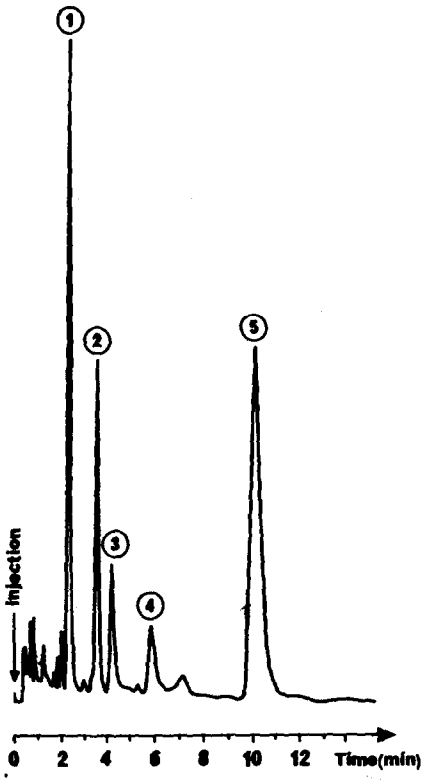


Fig. 2. Subcritical-fluid chromatography of a poppy straw extract. Column, 23 × 0.46 cm I.D.; stationary phase, bare silica LiChrosorb Si 60, 5 μm; mobile phase, carbon dioxide–methanol–methylamine–water (83.00:15.90:0.14:0.96, w/w); flow-rate, 8 ml min^{-1} CO_2 at 0°C; mean pressure, 220 bar; temperature, 41°C; detection, UV at 220 nm. Solutes: 1 = thebaine; 2, 4 = unidentified; 3 = codeine; 5 = morphine.

RESULTS AND DISCUSSION

The first experiments were performed with pure carbon dioxide, fused-silica capillaries and tapered 1/16-in. stainless-steel tubes maintaining the pressure above 200 bar in the extraction cell. No significant extraction was observed, in agreement with results of Stahl *et al.*[21]. Therefore, we studied the influence of various polar modifiers which could increase the dissolving power of the extractant for alkaloids. Owing to the high polar modifier contents, it must be noted that the extraction phase was in a near-critical but subcritical state rather than in a supercritical state[27].

Influence of polar modifiers

Three modifiers were considered: methanol, methylamine and water. All experiments were performed with a constant mass flow-rate through the extraction cell.

Influence of methanol. Mobile phases consisting of mixtures of carbon dioxide and methanol from 90:10 to 50:50 (w/w) were percolated through the vegetable

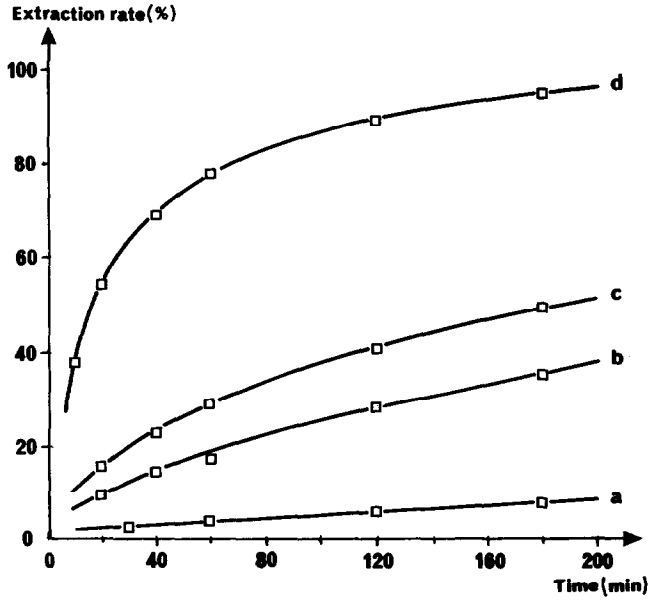


Fig. 3. Influence of methanol on the extraction curves of morphine at constant mass flow-rate (the extraction rate is the ratio of the amount extracted to the maximum extractable amount). Poppy straw granulometry, 250–500 μm; extraction pressure, 200 bar; temperature, 40.5°C; mass flow-rate, 2.11 g min^{-1}. Extractant, carbon dioxide–methanol: (a) 90:10; (b) 82:18; (c) 75:25; (d) 50:50 (w/w).

material. Kinetic extraction curves for morphine are given in Fig. 3. They show that very high percentages of methanol are necessary to ensure quantitative extraction in less than 20 min. The extraction phase would then be nearly a conventional liquid and all the potential gain in mass transfer would be lost. It was therefore necessary to find a polar modifier efficient at lower concentrations.

Influence of methylamine. In the subcritical fluid chromatography of opium-alkaloids, methylamine has been proved to be a very efficient polar modifier even at low concentrations[26], so we added methylamine to the extraction mixture. As shown in Fig. 4, the addition of a small amount of methylamine and water to the mobile phase considerably increased the extraction power: a mixture of 25% methanol, 0.22% methylamine and 0.34% water having the same effect as 50% methanol.

In spite of its strong extraction power, the methylamine–water mixture had the severe drawback that morphine was particularly sensitive to light in the presence of the amine (90% degradation after exposure to light for 3 h). Therefore, the methylamine–water mixture was not chosen as the final polar modifier.

Influence of water. Increasing the water content in the extraction fluid increased the extraction rate at a given time for alkaloids, as shown in Fig. 5 for thebaine. A similar behaviour was observed for all alkaloids. With 10% (w/w) water in the mobile phase, the time necessary to obtain a quantitative extraction is less than 30 min. Without water, even after 180 min quantitative extraction is not achieved.

However, even with methanol in the mobile phase, 10% water appeared to be the maximum value, higher concentrations resulting in degradation of the vegetable

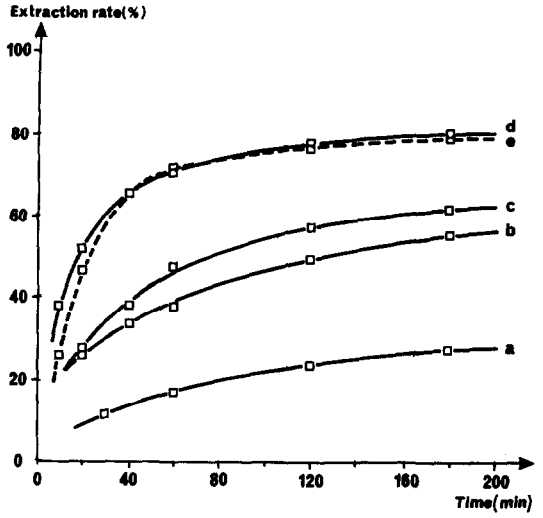


Fig. 4. Influence of methanol and methylamine on the extraction curves of codeine at constant mass flow-rate. Conditions as in Fig. 3. Extractant for curves (a)–(d) as in Fig. 3; (e) carbon dioxide–methanol–methylamine–water (75.00:24.44:0.22:0.34, w/w).

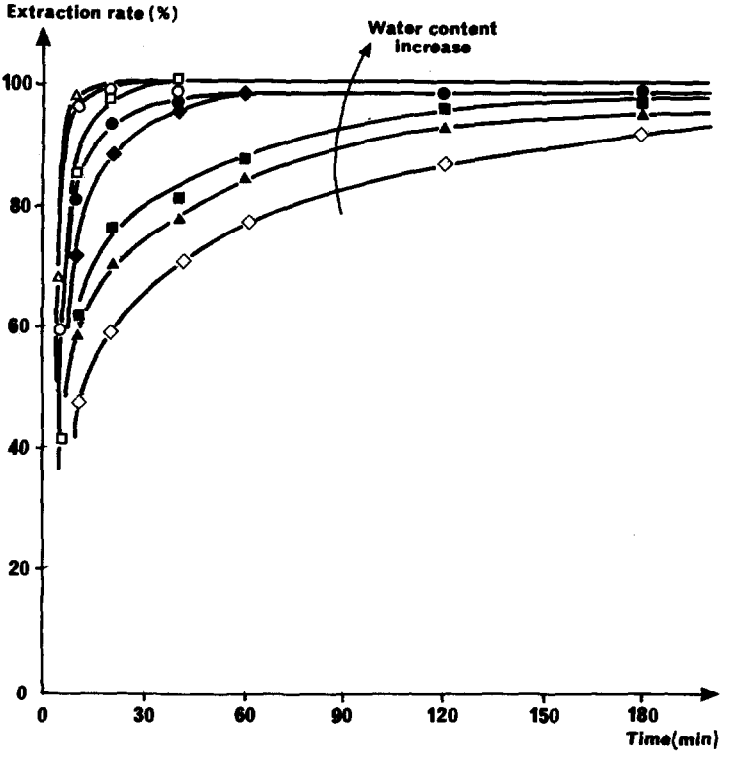


Fig. 5. Influence of water on the extraction curves of thebaine at constant mass flow-rate. Conditions as in Fig. 3 except extractants, carbon dioxide–methanol–water (◇) 50:50:0; (▲) 50:49.5:0.5; (■) 50:49:1; (◆) 50:46:4; (●) 50:44:6; (□) 50:40:10; (○) 50:36:14; (△) 50:32:18 (w/w/w).

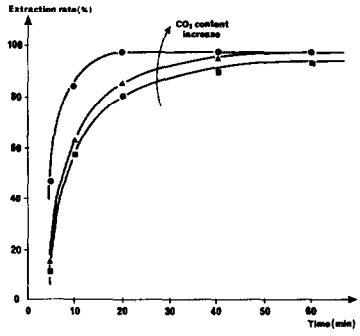
material with solid particles being collected in the three-necked flask. In addition, with more than 10% water in the extraction phase, the pressure regulator did not work properly.

Role of carbon dioxide

As morphine alkaloids are polar, it was clear that the higher the polar modifier flow-rate the faster would be their quantitative extraction. However, it was of interest to determine the extent to which the addition of carbon dioxide to the extraction phase could improve the transport phenomena. Fig. 6 shows the evolution of the kinetic extraction curves for morphine when the modifier flow-rate was maintained constant at 1.28 ml min^{-1} and the carbon dioxide flow-rate was increased from 0 to 5.2 ml min^{-1}. From 0 to 2.6 ml min^{-1}, the addition of carbon dioxide gave a reduction of a factor of 3 in the time required for quantitative extraction (from 60 to 20 min). Above 2.6 ml min^{-1}, carbon dioxide appeared to have no significant influence.

We also studied the influence of carbon dioxide during the extraction. The methanol flow-rate was maintained constant, but its concentration varied from 100 to 8% owing to the increase in the carbon dioxide flow-rate. Experiments were performed for three extraction times and the results are presented for thebaine in Fig. 7. It can be seen that the shorter the extraction time, the greater is the possible gain achieved by the addition of carbon dioxide to pure methanol. For an extraction time of 15 min, 72% carbon dioxide in the extraction phase tripled the extraction rate obtained with pure methanol; at a time of 60 min, the maximum gain was obtained with only 35% carbon dioxide in the extractant and the extraction rate increased by a factor of 1.2.

From this study, it can be deduced that the contributon of carbon dioxide to the extraction is probably only a volume effect; at the beginning of the extraction, carbon dioxide improves the transport of the modifier into the vegetable matrix and hence is favourable for the extraction. In contrast, at the end of the extraction, the solvating



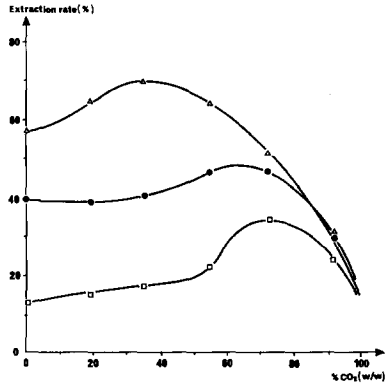


Fig. 6. Influence of carbon dioxide on the extraction curves of morphine at constant flow-rate of polar modifier, methanol–water (84:16, v/v) at 1.28 ml min^{-1}. Carbon dioxide flow-rate: (■) 0; (▲) 0.6; (●) 2.6, 3.9 and 5.2 ml min^{-1} at 0°C. Other condition as in Fig. 3.

Fig. 7. Influence of carbon dioxide content on the extraction rate of thebaine at constant flow-rate of methanol for three extraction times. Operating conditions as in Fig. 3 except flow rates: methanol, 0.35 ml min^{-1}; carbon dioxide, 0.07–3.30 ml min^{-1} at 0°C. Extraction times: (□) 15; (●) 30; (△) 60 min.

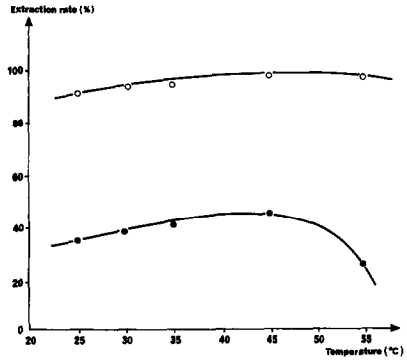


Fig. 8. Influence of temperature on morphine extraction rates. Extraction pressure, 200 bar; extractant, carbon dioxide–methanol–water (70:24:6, w/w/w); flow-rates, carbon dioxide, 2.6 ml min^{-1} at 0°C and polar modifier 1.28 ml min^{-1}. Extraction times: (●) 5; (○) 40 min.

power of the extractant is the major parameter and carbon dioxide which has a low polarity, is less beneficial for the extraction process.

These hypotheses were confirmed by thermodynamic results. Pressure (from 130 to 290 bar) was found to have no real influence on the curves shown in Fig. 7 (slightly higher extraction rates being obtained, however, near 200 bar). The influence of temperature was studied from 25 to 65°C with a carbon dioxide–methanol–water (70:24:6, w/w/w) extraction phase at 200 bar. The results are shown in Fig. 8. For a 5-min extraction, a maximum appears at *ca.* 45°C; for a 40-min extraction, in contrast, the temperature seems to have no real influence. These results confirm that the physical state of carbon dioxide only plays a role during the first few minutes of the extraction.

Optimized extractant

From the preceeding results two main conclusions can be drawn: the solvating power of the extractant is due only to the polar modifier, and the greater the modifier flow-rate the greater is the extraction rate at a given time; carbon dioxide at 45°C and 200 bar can increase the extraction rate at the beginning of the extraction by improving the transport of the polar modifier into the vegetable matrix.

These considerations are illustrated in Fig. 9. It can be seen that quantitative extraction can be achieved with 1.28 ml min^{-1} of pure modifier in about 60 min. This time can be reduced to 20 min by tripling the volume flow-rate, either with pure modifier or with carbon dioxide. It must be noted that, although carbon dioxide has a low polarity, it does not affect the extraction power at the end of the extraction.

Finally, with 1.28 ml min^{-1} of polar modifier [methanol–water (84:16, v/v)] quantitative extraction of the three major alkaloids in the poppy straw sample was achieved under optimum conditions in 20 min with an extraction phase composed of carbon dioxide–methanol–water (70:24:6, w/w/w). An increase in the carbon dioxide concentration did not improve the efficiency of the extraction. Quantitative results obtained for thebaine, codeine and morphine with this procedure were in very good agreement with those given by the classical liquid–solid extraction method (less than a 3% difference).

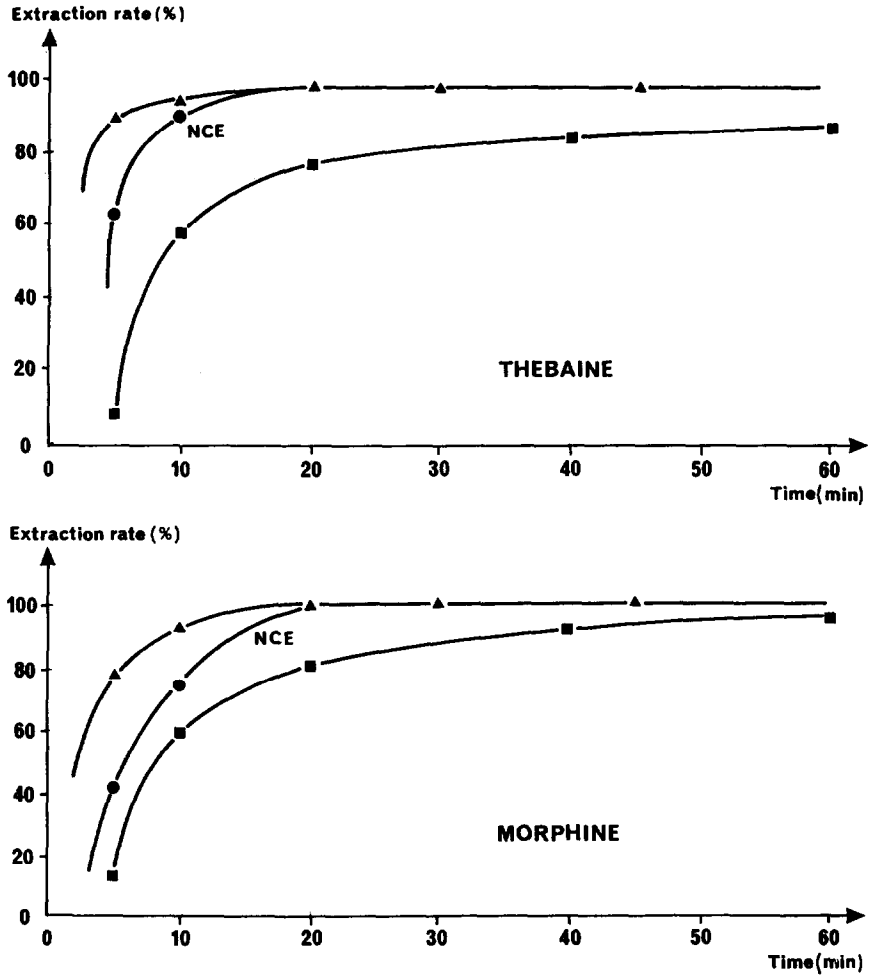


Fig. 9. Comparison of liquid and near-critical (NCE) extractions of thebaine and morphine. Pressure, 200 bar; temperature, 45°C; polar modifier, methanol–water (84:16, v/v). Flow-rates: liquid extraction (pure polar modifier), (■) 1.28 and (▲) 3.88 ml min^{-1}; near-critical extraction (●), polar modifier 1.28 ml min^{-1} and carbon dioxide 2.6 ml min^{-1} at 0°C.

CONCLUSION

It has been demonstrated that carbon dioxide cannot be considered as an extractant of morphine alkaloids from vegetable material but as a cheap and efficient transporting agent for the extraction solvent. Because of its high diffusivity, carbon dioxide improves the penetration of the extractant into the vegetable matrix and then, by decompression, the gas is very easily removed from the extracts. In 20 min, thebaine, codeine, and morphine can be extracted quantitatively by an extractant consisting of carbon dioxide–methanol–water (70:24:6, w/w/w) at 45°C and 200 bar.

As a similar extraction can be achieved with the pure liquid polar modifier at the same total volume flow-rate, near-critical fluid extraction may appear to be more difficult to perform. However, the entire extraction process was performed using a current chromatographic device and there are two main advantages of a near-critical extractant: from an economical point of view, it is of interset to replace a significant fraction of methanol in the extraction phase (up to two thirds) by carbon dioxide; and the evaporation of carbon dioxide after decompression leads to an extract three times more concentrated than that with the liquid extraction procedure.

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