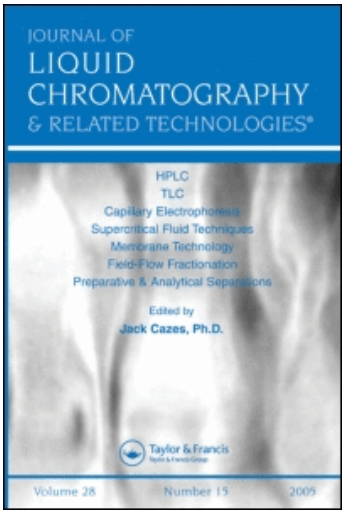

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COLLECTION CAPACITY OF A SOLID PHASE TRAP IN SUPERCRITICAL FLUID EXTRACTION FOR THE EXTRACTION OF LIPIDS FROM A MODEL FAT SAMPLE

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ABSTRACT

Collection capacity of lipids from a model fat in supercritical fluid extraction using a solid phase traps was investigated. It was found that 0.6 g of an octadecylsilica material efficiently trapped 80-100 mg of fat. By developing a fractionated extraction/elution procedure, samples containing up to 500 mg of fat could easily be trapped. Losses due to the vapor pressure of the fat components were negligible, even after several hours of purging with gaseous carbon dioxide at a flow rate of ca 1 L/min. The trapping efficiency was found to be independent of the flow rate up to at least ca 1 L/min of gaseous carbon dioxide, and of modifiers (ethanol, methanol) in concentrations up to at least 10% if the temperature in the trap was sufficiently high to prevent modifier condensation.

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INTRODUCTION

The reliability of a supercritical fluid extraction (SFE) procedure depends not only on a quantitative elution of the target substances from the extraction cell, but also on a quantitative collection of these substances. A considerable amount of work has been devoted to this problem. Two main principles dominate the collection procedures; either collection in a solvent[1] normally using a test tube of appropriate size, or onto a solid phase trap followed by an elution step.[2] Occasionally, a combination of these two principles has been used as described by Kleiböhmer et al.,[3] where the extracted analytes passed a small adsorbent column before being collected in a solvent. A similar approach was used by Eckard and Taylor[4] in their investigation of the trapping capacity of different solid phases. In some cases, quantitative collection in solvent free test tubes have been sufficient. Total fat determination after SFE, utilising this collection principle, gave results in good agreement with those obtained using conventional liquid-liquid extraction.[5]

A careful examination of results obtained by using different collection principles shows, that in many cases, the recoveries agree well with each other.[6] Extraction of pollutants from soil,[7] and even fruits and vegetables,[8] using solvent collection or solid phase traps, were shown to give very similar results with close to 100% recoveries. One exception was substances with high volatility. Several authors[5,6,8] have pointed out the larger loss for volatile pollutants using solvent collection. Here a solid phase trap should be the preferable trapping device. The temperature can easily be kept below zero and, furthermore, the analytes will undergo a chromatographic retention process before breakthrough at the outlet of the trap occurs.

Generally, not unexpectedly, a combination of collection on a solid trap followed by collection in a solvent as in reference 3 and 4, seems to be most efficient. The possibility for automation using this approach will be restricted, which means that for routine measurements the use of either solvent collection or a solid phase trap will be preferred. When choosing between these two approaches it should be noted, that the more limited sample capacity of a solid phase trap compared to collection in a solvent may become a problem.

In almost all papers presented up to now concerning different trapping techniques, the amounts of eluted target compounds have been small and the sample capacity of the trap then becomes unimportant. However, there are important applications where, also, the major components need to be determined. For example, in the calculation of bioconcentration factors of fat-soluble pollutants in a specimen, the amount of the pollutant, as well as the amount of fat in the sample is required. In this paper, a more detailed study of the possibility of using solid phase traps for the determination of major components in a sample

has been performed. A lipid mixture has been used as a model matrix in our studies of the solid phase trapping procedure.

EXPERIMENTAL

All extractions were carried out on a Hewlett Packard 7680T SFE unit, equipped with a HP 1090 LC pump for modifier addition (Wilmington, DE, USA). In all experiments, standard HP 7 mL extraction cells were used. The SFE system was controlled by a Hewlett-Packard 386/25N personal computer with Windows based software (Hewlett-Packard, G1225C, version 4.01).

The extraction temperature was always set to 40°C with a density of 0.90 g/mL (corresponding to a pressure of 281 bar). The solid phase trap temperature was in the range of 40°C to 90°C depending on the boiling point and the concentration of the added modifier. In all extractions, the nozzle temperature of the SFE unit was set 5°C above the trap temperature. Stainless steel beads (diameter 50-500 μm) used as sample support in the extraction cell were kindly donated from Anval (Anval, Torhälla, Sweden). Extracted compounds were collected on a standard HP trap packed with 0.6 g of octadecyl silica (ODS) (HP part no. 79903-85031).

After the extraction step, the fat components were eluted from the trap with cyclohexane into standard 1.8 mL sample vials (Chromacol Ltd., Welwyn Garden City, UK). The amount of fat was determined according to a previous publication.[9]

Carbon dioxide for extraction (99.998%) and cooling (food quality) was delivered by AGA Gas AB (Lidingö, Sweden). Cyclohexane (HPLC quality) was purchased from LabScan (Dublin, Ireland). Swedish Meats R&D (Kävlinge, Sweden) donated the lard fat used in all experiments. Ethanol (99.5%) was delivered by Kemetyl AB (Stockholm, Sweden), and methanol (p.a.) by Merck (Darmstadt, Germany).

RESULTS AND DISCUSSION

Trapping Capacity

Extraction with Supercritical Carbon Dioxide

In all experiments, the solid fat was melted on a water bath and pipetted directly onto 6 g (2 mL) of stainless steel beads in the pre-weighed extraction thimble. The thimble was weighed again and the amount of applied fat was cal-

culated. In a typical extraction, the size of the fat sample was 100 mg. The amount leaving the extraction cell was compared to the amount collected on the trap. Results of such experiments are shown in Figure 1.

Obviously, the extracted and the collected amounts agree quite well for sample sizes up to at least 100 mg. The trapping efficiency was independent of the flow rate in the interval 0.5 to 4 mL/min as long as the capacity of the trap was not exceeded. The results in Figure 1 shows that at least 50 mg fat can be trapped in a single extraction step.

To evaluate the maximum amount of fat possible to trap, fat samples with masses assumed to be larger than the trap capacity were extracted. It was found when using 500 and 1000 mg samples, that after 15 min extraction the trapped amount in both cases was ca 135 mg, although the extracted amounts exceeded 200 mg. To investigate how tightly bound this fat amount was to the packing material, the experiments continued for another 35 minutes with pure carbon dioxide entering the solid phase trap (the sample-containing thimble was replaced with an empty extraction cell). Such experiments performed for sample sizes of 100, 500, and 1000 mg are shown in Figure 2.

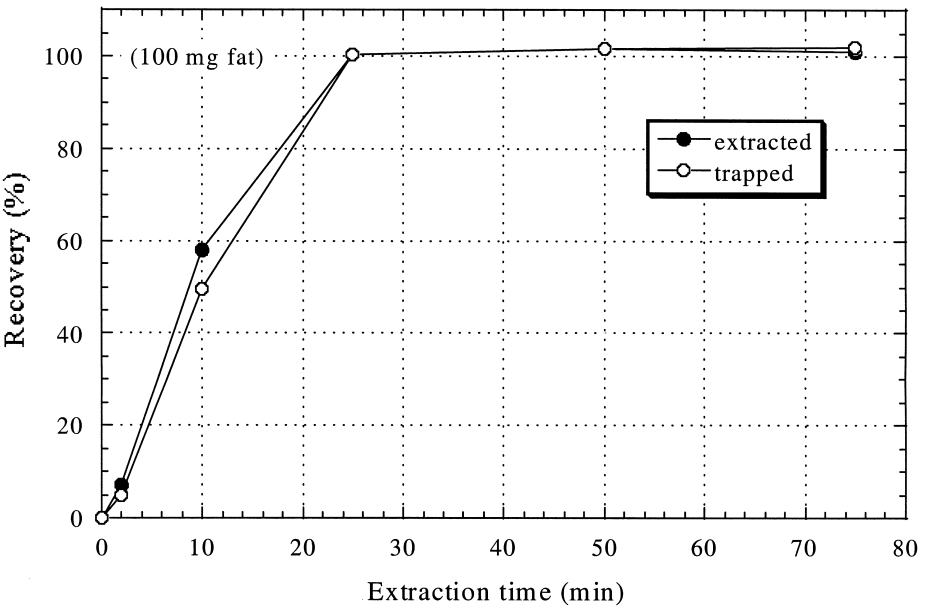

Figure 1. Comparison of extracted and trapped amounts of fat. Sample size 100 mg fat, applied on stainless steel balls. Extraction conditions: Temperature: 40°C, Density: 0.90 g/mL (pressure: 281 bar), Flow rate: 2.0 mL/min. Trap conditions: Nozzle 45°C, Trap 40°C.

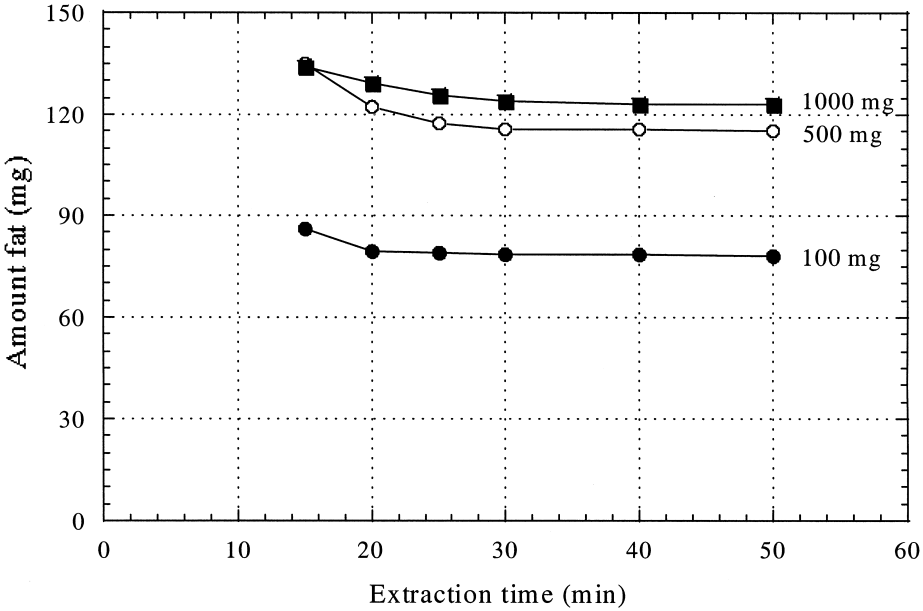

Figure 2. Comparison of total amount trapped fat during an extraction time of 15 minutes, with different amounts of fat applied on stainless steel beads (100, 500 and 1000 mg), followed by a continued extraction with pure carbon dioxide (35 minutes, empty extraction cell) to evaluate the binding strength of trapped fat. Each point is the average of three extractions. Extraction and trap conditions as in Figure 1.

The first larger decrease of the curves to 30 min, probably depends on a small amount of fat in the fat filled trap being pressed out as a liquid by the high flow rate of gaseous carbon dioxide *(ca* 1 L/min). After this time period, the losses are very small and most probably essentially depend on the vapour pressure of the fat components. From the average slopes of the lines calculated for the remaining 20 minutes (30-50 min in the time scale of Figure 2), for all three sample sizes, the losses due to the vapour pressure were determined to be less than 1.4 mg for a 20 minutes extraction. Thus, the losses due to evaporation of the fat components are very small. This means that, provided the sample capacity is not exceeded, the eluted amount of fat will correspond well to the amount found in the extracted sample.

That fat samples in the order of at least 80-90 mg can be safely trapped, is in agreement with results obtained for a 500 mg sample below (Figure 4). Here, ca 70 mg of fat was efficiently trapped in each one of 7 steps in a fractionated extraction procedure.

There are very few investigations devoted towards extraction of large amounts of target substances. However, Eckard and Taylor[4] have investigated the capacity of different types of trap packings using five test substances; acetophenone, dimethylaniline, naphthalene, 2-naphthol, and n-tetracosane. With a total sample size of 100 mg (20 mg of each component) the trapped amount was ca 64 mg for ca 0.5 g ODS. These values agree quite well with our values of ca 80-100 mg for ca 0.6 g ODS, taking into consideration the large differences in volatility between their sample and our fat sample. Using 0.4 g of Porapak Q, they found very small losses when extracting a 100 mg sample of their test substances, which must be attributed to the higher sample capacity of this type of packing compared to ODS.

Extraction with a Modifier Added to the Supercritical Carbon Dioxide

In some cases, for example in extraction of phospholipids, the solvent strength for carbon dioxide towards polar molecules is insufficient for a complete extraction, and a polar modifier needs to be added. This means that both carbon dioxide and modifier will pass through the trap altering the trapping conditions. Figures 3a and 3b illustrates the trapping efficiency for the extraction of 10 mg of a phospholipid mixture using ethanol or methanol as modifiers.

With suitable conditions it is, obviously, possible to also approach 100% recoveries when using a modifier. The vapour pressure of the modifier is important. This is illustrated in Figure 3, where a trap temperature of 80°C is sufficient for 100% recovery using 5% of methanol, while 90°C is needed with 10% of ethanol as modifier. The temperature of the trap must be so high that any condensation of the modifier is avoided. Otherwise, a large part of the sample components will elute to the waste in a liquid chromatographic process during the extraction/collection step. This is illustrated in Figure 3b with 15% of methanol as modifier at different trap temperatures. When increasing the trap temperature from 80°C to 90°C, the trapping efficiency increased from *ca* 33% to *ca* 95%. Generally, modifiers with high vapour pressures are preferable. This makes it possible to keep the trap at a relatively low temperature even at a relatively high modifier concentration. This is favourable for the extraction of thermolabile substances.

In the experiments above, 10 mg of a phospholipid mixture was used. To ensure that the sample size did not significantly influence the trapping efficiency up to the sample capacity of the trap (shown above to be ca 100 mg), a lipid mixture of triglycerides and phospholipids with a sample size of 100 mg was extracted with 5% methanol as modifier. Three different lipid mixtures were used with 1, 2, and 12% of phospholipids, respectively. For each mixture, the extraction was repeated three times. The results are shown in Table 1.

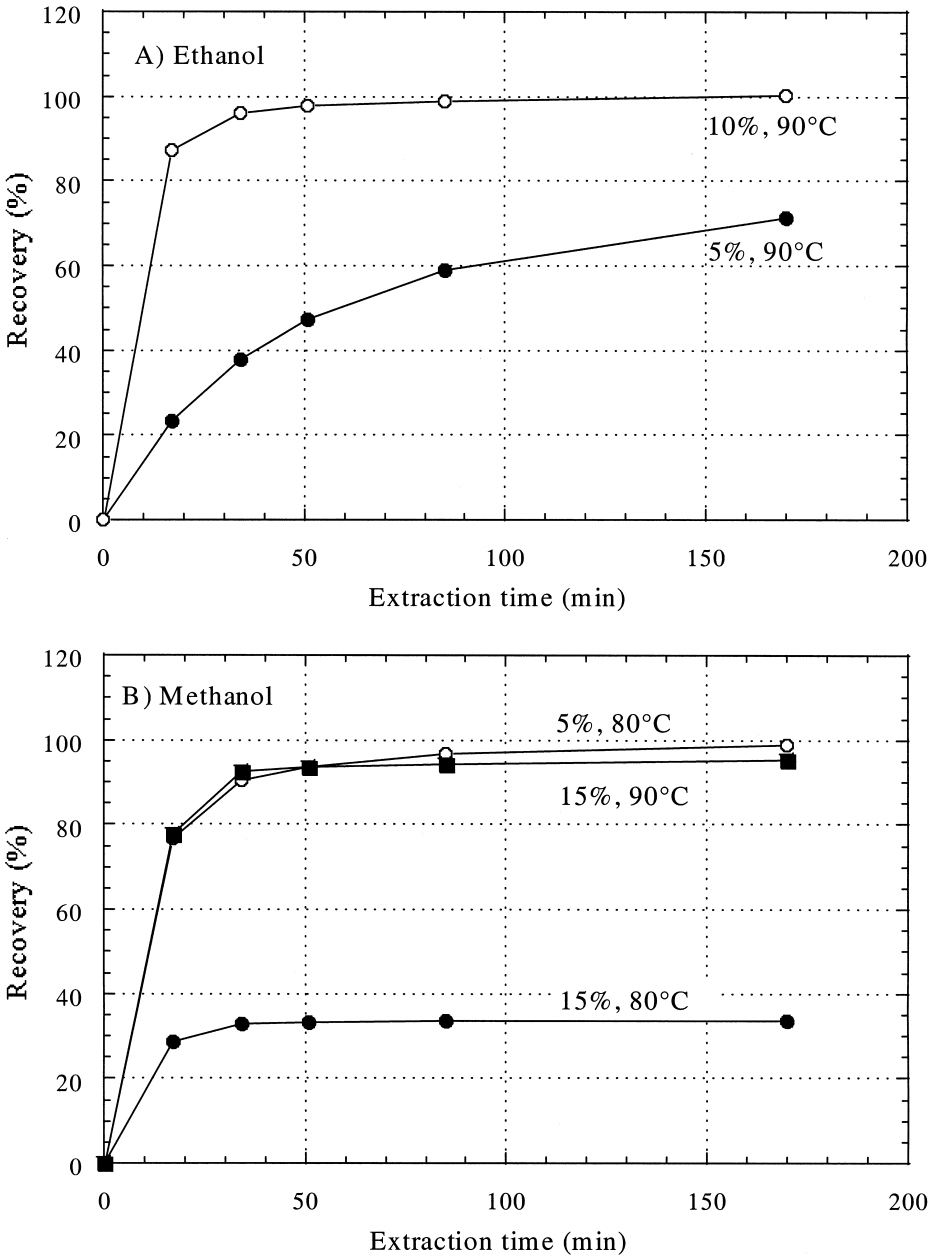

Figure 3. Trapping efficiency during extraction of 10 mg of a phospholipid mixture using ethanol (3a) or methanol (3b) as modifier (n=3).

Table 1. Recovery of Fat Samples Containing Triglycerides with Different Amounts of Phospholipids

Phospholipids (%)	Recovery (%)
	(104.33)*
12	98.90
	101.77
	100.15
2	99.65
	99.75
	99.87
1	99.43
	99.89
Average	99.93
RSD	0.83

* Outlier at the 90% significance level according to Dixon's R-test.
Extraction was made in two steps with pure carbon dioxide for the extraction of the glyceride fraction and with 5% of methanol as modifier for the extraction of the phospholipids. Sample size 100 mg. Density 0.90 g/ml. Flow rate 2 mL/min (triglycerides) and 4 mL/min (phospholipids). Extraction temperature 40°C. Trap 40°C (triglycerides), 80°C (phopholipids).

No significant difference at the 95% significance level was found for fat samples with different amount of phospholipids.

Handling of Large Samples

When the sample size is expected to exceed the capacity of the trap, the possibility of fractionated extraction/elution, which can be performed automatically in modern equipment, should be utilised. This is illustrated in Figure 4.

Using this approach, quite large amounts of fat can be handled. The time needed for quantitative extraction will, however, increase from ca 30 minutes for a 100 mg sample (Figure 1), to ca 70 minutes for a 500 mg sample (Figure 4). This approach is presently being successfully used for the extraction of fat-soluble vitamins in food samples [H. Berg and L. Dahlberg, pers. comm.]. In these cases, fat samples between 0.5-1 g, with a fat content up to ca 30% have been extracted.

Speed of Extraction

Increasing the flow rate is a method of decreasing the analysis time, provided that this does not change the trapping efficiency. In separate experiments

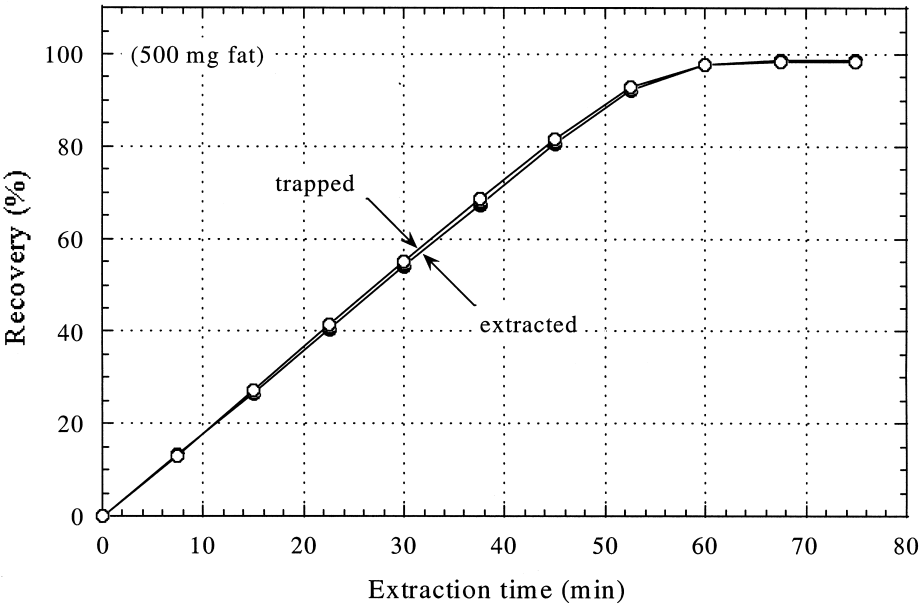

Figure 4. Comparison of extracted and trapped amount of fat. Sample size 500 mg fat, applied on stainless steel balls. Each extraction step has a length of 7.5 minutes. Extraction and trap conditions as in Figure 1.

with extractions of 100 mg fat samples at different flow rates, it was found that the trapping efficiency was unaffected up to 4 mL/min. The recoveries in three repeated experiments at flow rates of 2 mL/min and 4 mL/min were 99.9% (RSD 0.1%) and 99.8% (RSD 0.2%), respectively. Extraction rates of fat samples at different flow rates are illustrated in Figure 5. Values of extracted amounts were obtained from different weighings of the extraction cell.

The increase in the extraction rate from a flow rate of 2 mL/min to 4 mL/min calculated from the slopes of the corresponding lines (11.5 and 22.0 mg/min, respectively) will be roughly 1.9 times, which would lead to a corresponding decrease in the extraction time. This means that the system is controlled by solubility.[10] When extracting samples with a size exceeding the trap capacity, the information in Figure 5 can be used to determine the time difference between the consecutive extractions in the fractionated extraction procedure, which is then needed.

In Figure 4, the aim was to illustrate the shape of the extraction/elution curve. To get sufficient number of points, the extraction steps were evenly spread out. In a further optimization of the time needed for the extraction procedure, knowledge of the curve shape could be utilised to reduce the number of steps by

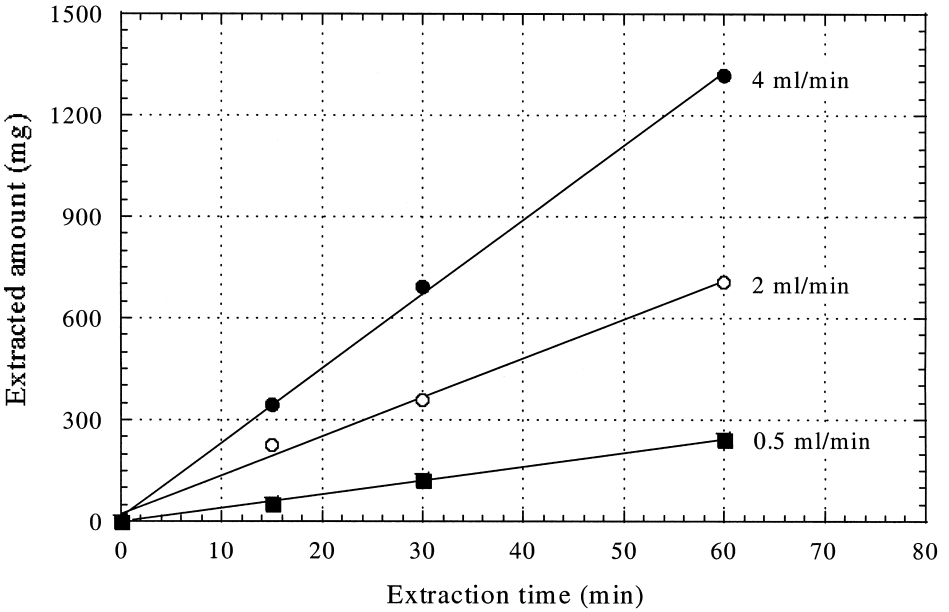

Figure 5. Effect of flow rate on extracted fat amount, when applied on stainless steel beads. Each point represents an independent extraction. Extraction and trap conditions as in Figure 1.

using different time increments between the extraction/elution steps. For example, in the last four steps in Figure 4 the amount of fat entering the trap is so small that one extraction/elution step would be sufficient.

In Figure 4, the extraction rate is almost constant until most of the sample has been extracted. This extraction system, with the sample applied on stainless steel balls, represents a very simple system without delaying chromatographic mass transfer processes of the analytes from the sample to the bulk extraction fluid. In samples with complicated matrices and, also if adsorbents and/or water trapping material has been added to the extraction cell, the shape may differ considerably compared to the one shown in Figure 4. One common feature in these cases is retardation of the fat (the occurrence of a fat free time window, i.e. a delay time before the fat starts to elute from the extraction cell), as demonstrated by Järemo et al.[9] Obviously, extraction profiles give valuable information about important extraction parameters and should always be obtained during the method development of new types of samples.

CONCLUSION

It has been shown that as long as the sample capacity of the trap is not exceeded, 100% recovery of major components, in this case fat, can be achieved even with a modifier present in the supercritical carbon dioxide. However, the trap temperature must be set sufficiently high to avoid condensation of the modifier. With a fractionated extraction technique with short consecutive extraction steps, large amounts of major components can be quantitatively trapped.

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