ELSEVIER

Journal of Chromatography A, 668 (1994) 285–291

JOURNAL OF CHROMATOGRAPHY A

Sample preparation by supercritical fluid extraction for quantification
A model based on the diffusion-layer theory for determination of extraction time

T. Veress

Institute of Forensic Sciences, P.O. Box 314/4, H-1903 Budapest, Hungary

Abstract

A mathematical model based on the diffusion-layer theory was elaborated in order to calculate the extraction time in dynamic supercritical fluid extraction required to reach a predefined level of extraction recovery. The goodness of the model is demonstrated by application to the extraction of the main neutral cannabinoids from marihuana and hashish samples. For monitoring of the cannabinoid content of extracts normal-phase HPLC was applied. To obtain reliable quantitative results, the extraction time ensuring a predefined level of recovery should be calculated for each individual sample according to the model because the extraction recovery depends on the sample matrix. The systematic error caused by the unextracted compounds can be eliminated by correction of the experimental data. For semi-quantitative determinations, where a knowledge of the correct value of the extraction recovery is not important, as a rule of thumb the extraction of marihuana with carbon dioxide of density 0.9 g/ml at 40°C for 34 min and of hashish for 18 min can be suggested. The application of the proposed extraction times ensured at least a 95% recovery for the main neutral cannabinoids.

1. Introduction

Supercritical fluid extraction (SFE) is a versatile method for sample clean-up and trace enrichment. For qualitative analysis the selection of suitable conditions to extract a given analyte even from a complex matrix is not so difficult, because the selectivity and solubility can easily be controlled by the composition, density and temperature of the extraction fluid [1–6]. However, the determination of the conditions required for reliable quantification is much more complicated because the efficiency of extraction is dependent on both the properties of the sample (water content, matrix, particle size, etc.) and also the operating parameters (void volume, flow-rate, extraction time) [1–3,7,8].

The effect of the sample properties on the extraction efficiency will not be discussed here. The scope of this study was the investigation of the operating parameters, especially the extraction time, in dynamic SFE in order to select the optimum values required for reliable chromatographic quantification.

Among the operating parameters, the volume of extractor chamber is determined by the instruments commercially available. It is advisable to keep the void volume of the extractor as low as

0021-9673/94/$07.00
SSDI 0021-9673(93)E1221-K

possible [7]. The density of the extraction fluid required for sufficient solubility can be calculated by Chrastil's method [9]. Logic predicts that a higher flow-rate of the extraction fluid will give a more rapid extraction. In practical analytical-scale SFE the range 1–4 ml/min is generally accepted. However, a convenient method for the determination of the extraction time required for a predefined level of recovery of the analyte from a given matrix using a particular instrument and extraction fluid at a selected flow-rate has not yet been developed. It is well known that a 100% extraction recovery cannot be achieved theoretically but the extraction of 95–98% of the analyte is possible even within 1 h, which is acceptable for analytical work. Different models [10] have been reviewed for the description of the kinetics of the SFE of various substrates. An approach developed by Newman [11] according to Fick's second law is applicable for the calculation of the extraction time needed to reach a predefined level of recovery. Andersen *et al.* [3] demonstrated the applicability of Newman's method for the prediction of extraction time, *e.g.*, assuming a diffusivity value of the order of $1 \cdot 10^{-10}$ m^2/s, particle sizes averaging 0.5 mm diameter should provide a 99% recovery in a 5-min dynamic extraction, which seems to be unlikely.

The aim of this study was to develop a procedure for the prediction of the extraction time required to reach a predefined level of extraction recovery with dynamic SFE of compounds to be determined chromatographically. The procedure elaborated is based on a mathematical model created according to the diffusion-layer theory [12,13], which was successfully applied to the description of the dissolution process of solids in liquids [14]. To demonstrate the applicability of the proposed model to the prediction of extraction time, the extraction of some neutral cannabinoids from hashish and marihuana was studied. These illicit preparations contain more than 400 compounds of different polarities [15], representing sufficiently complex matrices to use them for demonstration purposes as real samples.

2. Theoretical

It is assumed that during dynamic SFE two processes occur simultaneously: transport of the analyte from the matrix to the bulk of the extraction fluid by dissolution and the flushing out of the dissolved analyte from the extractor by the extraction fluid. For the description of the process of dissolution of a solid in a liquid, one of the simplest models is the diffusion-layer theory, which is based on Fick's first law. According to this theory, the dissolution rate is controlled by the rate of diffusion of the solute molecules across a diffusion layer of thickness h. The dissolution rate (dm/dt), *e.g.*, the mass of solute dissolved per unit time, is given by the following equation:

$$dm/dt = (AD/h)(c_s - c_b) \quad (1)$$

where A is the surface area of the solid, D is the solute diffusivity, c_s is the concentration of the dissolving solute, which is equal to the solubility, and c_b is the concentration in the bulk solution.

To describe the flushing out process, assuming a solute concentration c_0 at the beginning of the extraction without mass transfer from the matrix to the fluid and total mixing inside the extractor chamber, the time dependence of concentration can be represented by with the following differential equation:

$$dc/dt = (F/V)(c_0 - c) \quad (2)$$

where c is the actual concentration at time t, F is the flow-rate of the extraction fluid and V is the void volume of the extraction chamber.

Omitting the detailed derivatization according to Eq. 1 and 2, the concentration profile in dynamic SFE can be represented by the expression

$$c = \frac{M\beta}{\beta V - F}(e^{-Ft/V} - e^{-\beta t}) \quad (3)$$

where β is a constant relating to the analyte transport from the matrix to the fluid, defined to be proportional to the term AD/h, M is the mass

of analyte to be extracted and present in the matrix and F, V, c and t are as in Eq. 1 and 2.

The equation describing the time dependence of the recovery in dynamic SFE can be derived from the integral of the product of c and F by taking into consideration that total recovery could only be achieved after an infinite time of extraction. According to this, the recovery r can be expressed in terms of the previously used variables as follows:

$$r = 1 - \frac{\beta F}{\beta V - F}\left(\frac{V}{F}\cdot e^{-Ft/V} - \frac{1}{\beta}\cdot e^{-\beta t}\right) \quad (4)$$

In Eq. 4, V and F are known, as measurable operating parameters. The term β, representing the analyte transport from the matrix to the fluid, is assumed to be constant for a given matrix–analyte–extraction fluid system. By knowing β the extraction time required to reach a predefined level of extraction recovery can be calculated according to Eq. 4.

3. Experimental

3.1. Materials and equipment

The organic solvents *n*-hexane and ethanol were of LiChrosolv grade (Merck, Darmstadt, Germany). The carbon dioxide extraction agent was of 99.996% purity (Union Carbide, Westerlo, Belgium).

The cannabinoid standards Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) were obtained from the UN Narcotic Laboratory Section, (Vienna, Austria). Marihuana and hashish, applied as test materials, were samples seized by the Hungarian drug enforcement agencies. Cannabinoid-free, blank plant matrices were prepared by removing the cannabinoids from a marihuana sample by multiple extraction.

SFE experiments were performed on a Hewlett-Packard (Avondale, PA, USA) Model 7680T supercritical fluid extractor controlled by a Hewlett-Packard Vectra 386/16N personal computer. For the extraction, 7-ml thimbles were used as extractor chambers. The void volume of the extractor was decreased by filling the empty space with 2-mm diameter nickel balls, which resulted in an interstitial volume of 4.6 ml. The void volume was measured by adding a known volume of *n*-hexane to fill the interstices inside the extractor. For analyte trapping, a Hypersil ODS octadecylsilica (d_p 30–40 μm) (Shandon Scientific, Runcorn, UK) packed column was used.

The HPLC separation and chromatographic data handling were performed on a Kontron (Milan, Italy) HPLC System 400 liquid chromatograph with the following configuration: two Model 420 HPLC pumps, a Model 460 autosampler, a Model 480 column oven, a Model 430 rapid-scanning UV–Vis detector and an IBM/AT-compatible Model 450 data system. For evaluation of experimental data, SigmaPlot Scientific Graphing System V.4.02 software (Jandel Scientific, San Rafael, CA, USA) was applied.

3.2. Supercritical fluid extraction

The air-dried marihuana and hashish samples chosen as test materials were ground in an electric grinder. Fractions from the particle size range 0.4–0.6 mm were used for the experiments. From marihuana 50-mg and from hashish 10-mg amounts were weighed on to 75 mm × 30 mm filter-papers. The papers were folded to hinder the plugging of extractor frits with solid particles and inserted into the thimbles. The extractions were made with carbon dioxide of density 0.9 g/ml at 40°C. The flow-rate of the extraction fluid was 1.5 ml/min. The total extraction time was 100 min and within this period the fractions extracted serially for 5, 5, 7, 8, 10, 15, 20 and 30 min were trapped at 25°C and then eluted with 1.5 ml of *n*-hexane at 40°C. Each experiment was run at least in duplicate. The main neutral cannabinoid contents of the fractions obtained from the different intervals of extraction were monitored by HPLC in the normal-phase mode, as described previously [16].

3.3. Recovery experiments

In order to investigate the extraction recovery, 50-mg amounts of cannabinoid-free marihuana were spiked with known amounts of cannabinoids in the range 40–611 μg by adding 0.2 ml of *n*-hexane solutions of the compounds. The solvent was left to evaporate at ambient temperature and the spiked samples were extracted. The re-extracted cannabinoids were determined by HPLC [16].

Both the dependence of the recovery on the amounts of cannabinoids, applying a 30-min extraction time, and the time dependence of cannabinoid recovery, applying the same extraction time programme as detailed in Section 3.2, were studied. In the latter experiments 540 μg of CBD and 416 μg of THC were added to the blank plant matrices.

4. Results and discussion

Typical results for the dynamic SFE of a marihuana sample for THC and CBD are shown in Figs. 1 and 2, respectively. The percentage recoveries calculated from experimental data and percentage recoveries calculated according to

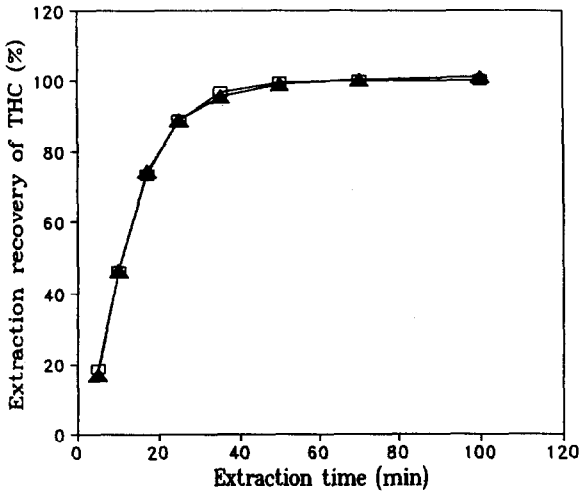

Fig. 1. Typical plot of extraction recovery of THC *versus* extraction time. For extraction conditions, see text. ▲ = Measured; □ = calculated.

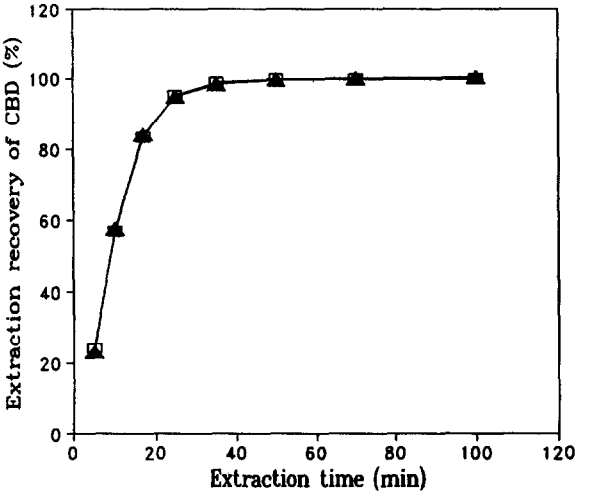

Fig. 2. Typical plot of extraction recovery of CBD *versus* extraction time. For extraction conditions, see text. ▲ = Measured; □ = calculated.

Eq. 4, as detailed below, are plotted against the extraction time. To calculate the percentage recoveries from experimental data, the cumulative values were determined within the total extraction time interval by summing the appropriate cannabinoid contents obtained by consecutive extractions and these values were normalized to the highest cumulative value. The latter, which relates to the total extraction time, was assumed to be equal to the maximum extractable amount of cannabinoid from the given matrix. As Figs. 1 and 2 show, these maximum values could be approached 30–50 min before the total extraction time. Fig. 1 shows that after extraction for 70 min the increase in THC recovery is negligible. The results in Fig. 2 indicate that CBD can be extracted faster than THC, *e.g.*, the previously mentioned limit could be reached after extraction for 50 min.

In order to determine the term β in Eq. 4, required for the calculation of the extraction time needed for a predefined level of extraction recovery, the experimental data were fitted to Eq. 4 by using the SigmaPlot software. The calculated results for six marihuana and two hashish samples are given in Table 1, together with the standard deviations of the calculated β values. The good quality of the curve fitting can

Table 1
β Values of THC and CBD, calculated[a] according to Eq. 4 and their standard deviations for six marihuana and two hashish samples

Sample	No.	β_{THC} (min^{-1})	β_{CBD} (min^{-1})
Marihuana	1	0.168 ± 0.005	0.280 ± 0.006
	2	0.118 ± 0.003	0.172 ± 0.004
	3	0.118 ± 0.003	0.176 ± 0.003
	4	0.100 ± 0.003	0.152 ± 0.002
	5	0.124 ± 0.004	0.191 ± 0.003
	6	0.119 ± 0.007	0.170 ± 0.009
Hashish	1	0.243 ± 0.011	0.347 ± 0.007
	2	0.229 ± 0.009	0.363 ± 0.008

[a] Number of data pairs used for the calculation = 8.

be observed in Figs. 1 and 2, where the recoveries recalculated according to eq. 4 by using the β values are very close to data obtained experimentally. It can be seen in Table 1 for both THC and CBD that the β values for hashish samples are nearly double those for marihuana samples. The higher β values obtained for hashish samples mean that the transfer of cannabinoids from hashish to the extraction fluid is faster than that from marihuana. This finding can easily be understood by considering that hashish is a preseparated material, which consists mainly of resinous matrix obtained from the surface of the plant, whereas marihuana is a plant material containing resin with compounds to be extracted both inside and outside the plant. As the same compounds were extracted under the same extraction conditions from different matrices, the differences between the calculated β values are obviously due to the different matrix effects, so β mirrors the effect of the matrix. According to the data in Table 1, it can also be seen that the β values for CBD are higher than those for THC, indicating that supercritical carbon dioxide dissolves CBD more effectively than THC.

The relative standard deviation of the β values calculated by using data obtained from five parallel extractions of a marihuana sample did not exceed 9%.

In Table 2 the calculated extraction times required to reach 95% and 99% recoveries of cannabinoids are shown for the samples listed in Table 1. The calculation was done according to Eq. 4 for each sample by using the β values given in Table 1. It can be seen in Table 2 that 95% of THC can be extracted from most of the marihuana samples within 30 min, whereas a 99% recovery requires about 45 min. The corresponding extraction times for CBD are *ca.* 20 and 30 min, respectively. Owing to the high β values for hashish samples, the cannabinoid

Table 2
Calculated extraction times required for 95% and 99% recoveries of THC and CBD according to Eq. 4 using β values listed in Table 1

Sample	No.	Extraction time (min)			
		THC		CBD	
		95% Recovery	99% Recovery	95% Recovery	99% Recovery
Marihuana	1	22	32	16	22
	2	29	43	22	31
	3	29	43	21	31
	4	34	50	24	34
	5	28	41	20	29
	6	29	43	22	31
Hashish	1	17	24	14	20
	2	18	25	14	19

content can be extracted exhaustively within 30 min. The shorter extraction time for hashish compared with marihuana is in accordance with the previous considerations regarding the matrix quality.

4.1. Recovery experiments

The results of recovery experiments at different levels of added cannabinoids are given in Table 3. It can be seen that for levels of added cannabinoids >100 μg the recovery is 95–98%, whereas with a level of *ca.* 40 μg the recovery is only 90–91%. The lower recovery in the lower concentration range might be due to the constant error probably caused by the irreversible adsorption inside the extraction system, which causes a higher relative error in the lower than in the higher concentration range.

The β values calculated according to Eq. 4 using the experimental data obtained from the sequential extraction of spiked samples for different times are higher by a factor of 5 than those listed in Table 1, *e.g.*, $\beta_{THC} = 0.538$ min^{-1} and $\beta_{CBD} = 1.081$ min^{-1}. These significant differences between β values obtained for spiked and non-spiked natural samples indicate that the mass transfer of cannabinoids from spiked samples to the extraction fluid is quicker than that from natural samples. A possible explanation of this phenomenon could be that by spiking the cannabinoid-free blank marihuana with solutions of cannabinoids the compounds cannot be placed in the same matrix environment as the original non-spiked sample. Consequently, the interactions between the added compounds and the matrix differ from those acting in the original sample, resulting in a change in the kinetics of component transport. According to these results, it should be noted that the determination of systematic errors of the extraction procedure must not be based on measurements of spiked samples.

Table 3
Recoveries and standard deviations[a] obtained for extractions of spiked marihuana

THC		CBD	
Spiked amount (μg)	Recovery (%)	Spiked amount (μg)	Recovery (%)
40	90 ± 12	45	91 ± 9
122	97 ± 6	131	97 ± 7
376	98 ± 5	420	95 ± 6
581	97 ± 5	611	97 ± 4

For the extraction conditions, see text.
[a] Number of parallel measurements = 5.

5. Conclusions

The elaborated mathematical model based on the diffusion-layer theory is suitable for the description of the concentration profile of dynamic supercritical fluid extraction using pure carbon dioxide as the extraction fluid. According to the model the extraction times to extract either 95% or 99% of the main neutral cannabinoids from hashish and marihuana samples were calculated. The extraction times calculated for six different marihuana samples were scattered with a relative standard deviation of more than 10%, which indicates that the matrix has a significant effect on the extraction recovery. According to the equation describing the concentration profile of dynamic SFE, the effect of the matrix is taken into account by the term β. As β is a constant regarding the analyte transport from the matrix to the fluid, the magnitude of β represents the effect of matrix quality on the extraction recovery, *e.g.*, under constant extraction conditions the higher is β the greater is the extraction recovery per unit time. These previous statements were experimentally verified for hashish and marihuana samples; for hashish samples the β values obtained were nearly double those for marihuana samples, indicating that owing to the different matrix effects of marihuana and hashish the transport of cannabinoids from hashish to the extraction fluid is faster than that from marihuana. According to the experimental results obtained for hashish and marihuana, it can be seen that an increase in β by a factor of two resulted in a decrease in the time required for extraction by *ca.* 30%. Because

the quality of the sample matrix might be different from sample to sample, in order to obtain reliable quantitative results the extraction time should be calculated for each individual sample according to the model. The systematic error caused by the unextracted proportion of the analyte can then be eliminated by the correction of the experimental data. It was also found in the extraction of cannabinoids that the determination of systematic errors of the extraction procedure must not be based on measurements of spiked samples because by spiking blank matrices with the analytes to be extracted the compounds cannot be placed in the same matrix environment as the original non-spiked sample.

For semi-quantitative determinations, where the correct value of the extraction recovery is not of interest, as a rule of thumb the extraction of marihuana with carbon dioxide of density 0.9 g/ml at 40°C for 34 min and of hashish for 18 min can be suggested. The application of the proposed extraction times ensured at least a 95% recovery of the main neutral cannabinoids.

It should be emphasized that for application of the proposed procedure to unknown samples, the appropriate extraction conditions should previously be determined experimentally to ensure the dissolution of the analyte from the given matrix. Then the concentration profile of the extraction should be determined experimentally and the β value should be calculated by using the experimental data as described previously. The validity of the model for the actual extraction system should be checked either graphically or by other methods (*e.g.*, by analysis of residuals). For a valid model the calculated β value can be used to determine the extraction time required to reach a predefined level of recovery.

As the samples of natural origin have different matrix qualities with unknown composition and with unknown interactions between the matrix components, the extraction conditions required for a particular level of recovery cannot be predicted theoretically. Therefore, the steps of the developed procedure described above should be followed for each individual sample in order to obtain reliable quantitative results.

6. References

[1] T.P. Zhuze, G.N. Jushkevich and J.E. Gekker, *Maslo-Zhir. Promst.*, 24 (1958) 34.
[2] E. Stahl and W. Schilz, *Talanta*, 26 (1979) 675.
[3] M.R. Andersen, J.T. Swanson, N.L. Porter and B.E. Richter, *J. Chromatogr. Sci.*, 27 (1989) 371.
[4] T.S. Oostdyk, R.L. Grob, J.L. Snyder and M.E. McNally, *Anal. Chem.*, 65 (1993) 596.
[5] J.C. Giddings, M.N. Myers, L. McLaren and R.A. Keller, *Science*, 162 (1968) 67.
[6] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller and J. Pawliszyn, *Anal. Chem.*, 65 (1993) 338.
[7] S.B. Hawthorne, *Anal. Chem.*, 62 (1990) 633A.
[8] W. Pipkin, *LC·GC Int.*, 5, No. 1 (1992) 8.
[9] J. Chrastil, *J. Phys. Chem.*, 86 (1982) 3016.
[10] M.L. Lee and K.E. Markides (Editors), *Analytical Supercritical Fluid Chromatography and Extraction*, Chromatography Conferences, Provo, UT, 1990, p. 324.
[11] A.B. Newman, *Trans. Am. Inst. Chem. Eng.*, 27 (1931) 203.
[12] W. Nerst, *Z. Phys. Chem.*, 47 (1904) 52.
[13] E. Brunner, *Z. Phys. Chem.*, 47 (1904) 56.
[14] D.J.W. Grant and T. Higuchi, in A. Weisberger and W.H. Saunders (Editors), *Solubility Behavior of Organic Compounds*, Wiley, Toronto, 1990, p. 478.
[15] C.E. Turner, *Marihuana Research Findings: 1980*, National Institute on Drug Abuse, Rockville, MD, 1980, p. 81.
[16] T. Veress, J.I. Szántó and L. Leisztner, *J. Chromatogr.*, 520 (1990) 339.