SUPERCRITICAL FLUID EXTRACTION OF TETRAHYDROCANNABINOL FROM MARIHUANA STUDY OF THE EFFECT OF PARTICLE SIZE

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ABSTRACT: In order to obtain reproducible quantitative result for constituents of plant materials, the grinding of sample and extraction of a sieve fraction with a predefined average particle size is advised. The aim of the present study is to investigate the influence of particle size of marihuana on the extraction recovery of THC (delta-9-tetrahydrocannabinol) applying supercritical fluid extraction as well as the study of the effect of sieving of the sample on the quantitative results obtained by high performance liquid chromatography. It could be stated that marihuana samples can not be characterised in terms of potency by determination of the THC content of a selected sieve fraction of the ground material and subsequent calculation for the content of the whole sample, because owing to the significant inhomogenity of the different sieve fractions as far as the morphological composition is concerned, false THC content can be calculated for the whole sample.

KEY WORDS: SFE; Marihuana; THC; Particle size; Extraction recovery.

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INTRODUCTION

Marihuana is the most frequently encountered illicit drug in Hungary. The quantification of its psychotropic principle, the delta-9-tetrahydrocannabinol (THC) is often requested. Supercritical fluid extraction (SFE) is a versatile automated sample preparation technique assuring reproducible recovery needed for reliable quantification. SFE is suitable for the extraction of THC from both marihuana and hashish by using carbon dioxide as extraction agent. Previously a procedure [3] for the determination of the systematic error caused by the non-extracted ratio of THC by SFE was reported but the effect of particle size on the extraction recovery was not investigated.

For the extraction of plant materials, as a rule of thumb, the grinding of sample and extraction of a sieve fraction with a predefined average particle size is advised in most of the analytical determinations [1, 2, 4]. The aim of

the present study is to investigate the influence of particle size of marihuana on the extraction recovery of THC.

EXPERIMENTAL

Chemicals and equipment

The 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 THC was received from the UN Narcotic Laboratory Section (Vienna, Austria). The plant materials extracted were sampled from marihuana seized by the Hungarian drug enforcement agencies.

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. For analyte trapping, a Hypersil ODS octadecylsilica (dp 30–40 μm) (Shandon Scientific, Runcorn, UK) packed column was used.

The HPLC separation and chromatographic data handling were performed on a Kontron 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.

Preparation of plant material for extraction

The air-dried marihuana samples were ground in an electric grinder, sieved and from the fractions of different particle size (d) ranges (0.800 mm < d, 0.315–0.800 mm, 0.250–0.315 mm, 0.200–0.250 mm, 0.125-0.200 mm, 0.063–0.125 mm, d < 0.800 mm) 50 mg amounts were weighed for SFE experiments. The samples were wrapped in a filter-paper (75 mm × 30 mm) in order to avoid the plugging of the frit with small particles at the outlet of the extractor chamber.

SFE

The extractions were run with a 0.9 g/ml density of carbon dioxide at 40°C. The flow rate of the extraction fluid was 1.5 ml/min. For studying the extraction profiles for the different particle size samples the total extraction time was 130 min and within this period the fractions extracted serially after 5; 5; 7; 8; 10; 15; 20; 30 and 30 min, were trapped at 30°C and then eluted

with 1.5 ml of n-hexane at 40°C. Each experiment was run at least duplicate. The THC content of the extracts was monitored by HPLC.

HPLC monitoring of the cannabinoid content of samples obtained by SFE

For HPLC, the isocratic normal-phase separation was achieved using aminopropylsilica stationary phase and n-hexane-ethanol (97:3 v/v) as a mobile phase at a flow-rate of 1.3 ml/min. The cannabinoids were detected at 215 nm. The injection volume was 20 μl. A typical chromatogram of an extract is shown in Figure 1.

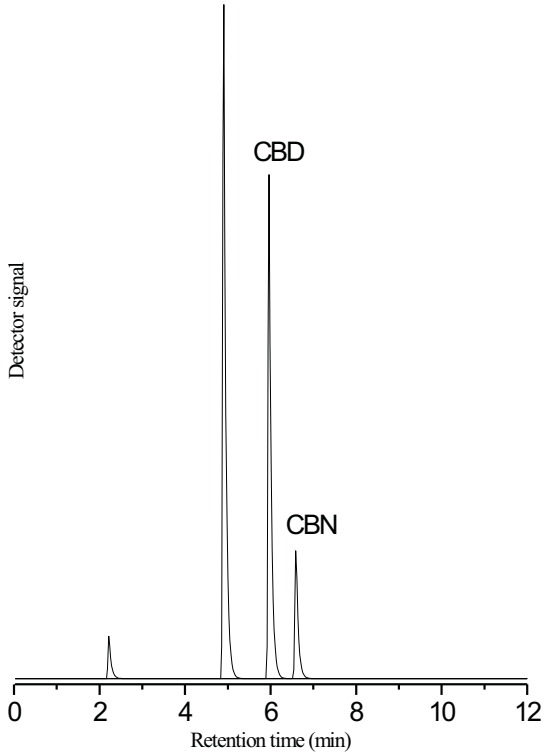

Fig. 1. Typical HPLC chromatogram of a marihuana extract.
THC – tetrahydrocannabinol, CBD – cannabidiol, CBN – cannabinol.

RESULTS

The extraction curves obtained for the samples with different average particle size are shown in Figure 2. According to the similarity of curves the same extraction mechanism can be assumed for all of the sieve fractions investigated. For each sample application of 40 min extraction time resulted in practically exhaustive extraction of THC. However, in the THC content of the different sieve fractions significant differences can be observed. In Figure 3, the THC concentrations of the different sieve fractions are shown, where the highest value can be observed for sieve fraction of particle size range of 0.063–0.125 mm. The lowest THC concentration could be determined for the fraction consisting of particles with size larger than 0.8 mm. By microscopic analysis of different sieve fractions morphological inhomogeneity can be stated for the different fractions which causes the deviations in the THC contents. The majority of this sample consists of bracteal parts of the cannabis plant, which parts are rich in resinous material. The sieve fractions with greater average particle size than 0.200 mm contain relatively high portions of leaves and other parts being poor in resin, resulting in lower THC content for these samples. The mass balance constructed for the THC according to the content of different sieve fractions and non-sieved ground sample, respectively, confirms the reliability of results. In Figure 4, the good agreement between the sum of THC content of different sieve fractions and that of the non-sieved sample is demonstrated.

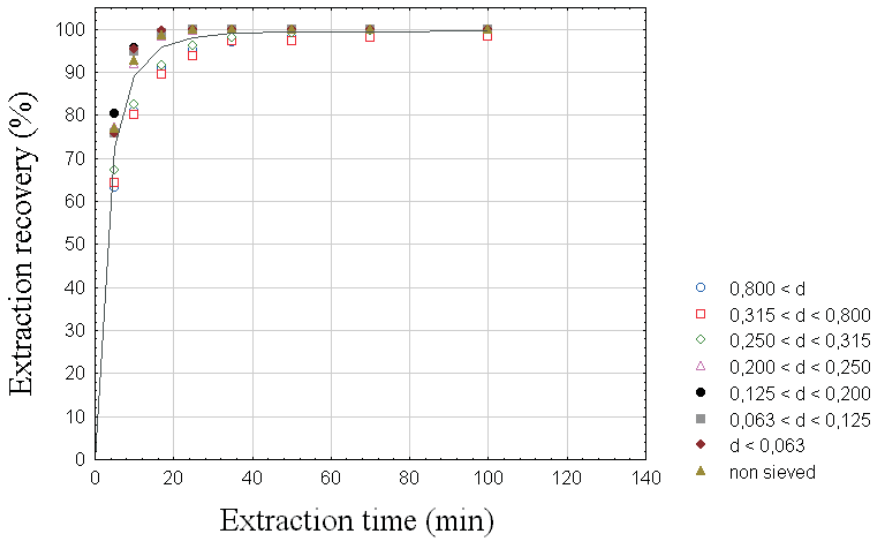

Fig. 2. Extraction recovery of THC vs. extraction time, obtained for different sieve fractions of marihuana.

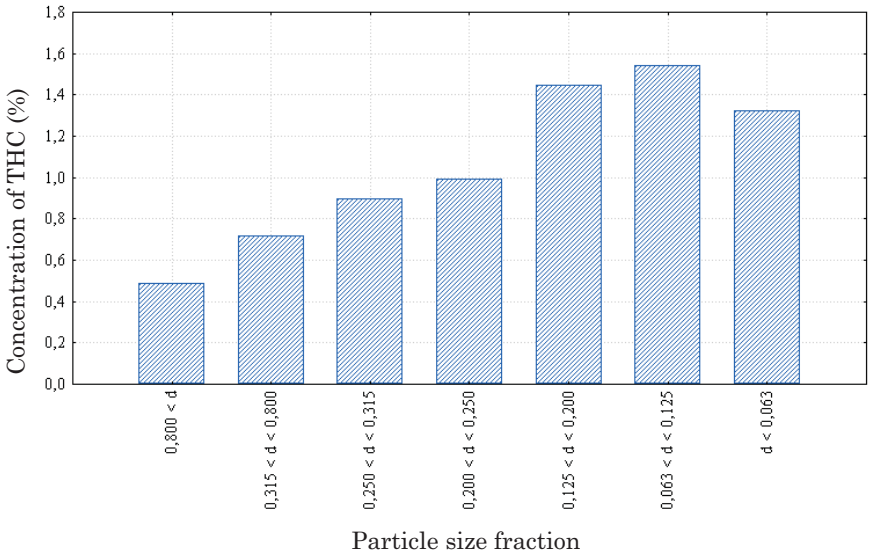

Figure 3. THC concentration of different sieve fractions of marihuana.

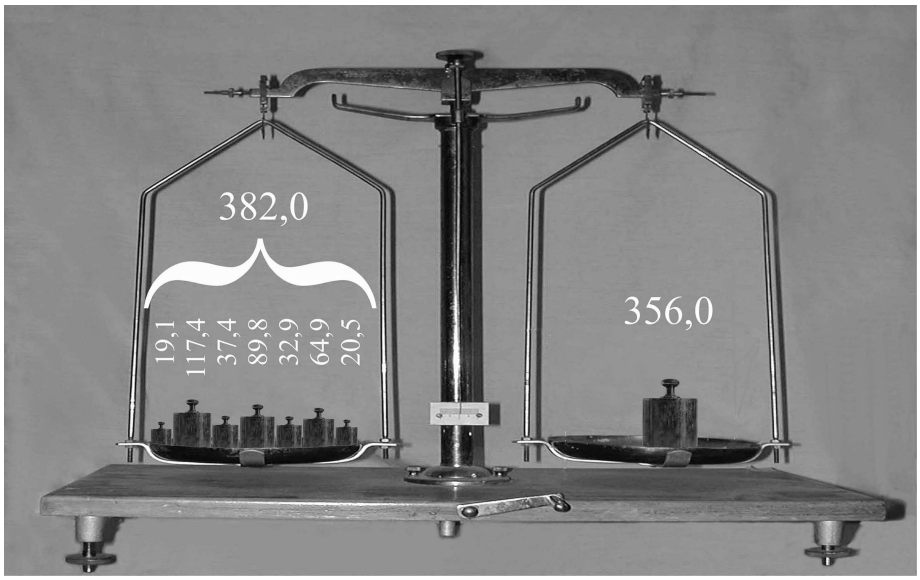

Fig. 4. Scheme of THC balance constructed according to THC content of different sieve fractions of marihuana and the non-sieved sample, respectively.

The left-side pan represents the sum of THC content of different sieve fractions of marihuana, the right-side pan symbolises the THC content of the sample before sieving in milligrams.

SUMMARY

Supercritical fluid extraction with pure carbon dioxide is a suitable technique to remove the THC from marihuana regardless of the particle size of the sample. Using a 0.9 g/ml density of carbon dioxide applying flow rate of 1.5 ml/min at 40°C, practically results in exhaustive extraction.

The marihuana samples can not be characterised in terms of potency by determination of the THC content of a selected sieve fraction of the ground plant material and subsequent calculation for the content of the whole sample because of the significant inhomogeneity of the different sieve fractions. By sieving of the ground marihuana inhomogeneous fractions with different THC content can be obtained. For reliable determination of the THC the sieving of the ground marihuana is not necessary.

References:

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