SUPERCRITICAL FLUID EXTRACTION OF TETRAHYDROCANNABINOL FROM MARIHUANA. DETERMINATION OF TETRAHYDROCANNABINOL AND TETRAHYDROCANNABINOL ACID

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ABSTRACT: A two-stage sample preparation procedure was elaborated for the analysis of THC (delta-9-tetrahydrocannabinol) and THC-acid in marihuana samples which includes in the first step the supercritical fluid extraction (SFE) of THC and in the second step the thermal decarboxylation[1] of THC-acid to THC at conditions of static SFE and the subsequent extraction of THC formed. The THC and THC equivalent to the THC-acid content of the sample were determined by high performance liquid chromatographic (HPLC) analysis of extracts obtained in the first and second extraction step, respectively. The applicability of the proposed procedure is demonstrated by results obtained by analysis of different marihuana samples.

KEY WORDS: SFE; Marihuana; THC; THC-acid.

Problems of Forensic Sciences, vol. XLVII, 2001, 328–332
Received 19 April 2001; accepted 15 September 2001

INTRODUCTION

The marihuana prepared from fresh plant contains the tetrahydrocannabinol (THC) mostly as acidic derivative (THC-acid). The THC-acid has no biological activity but during smoking it easily turns into THC by decarboxylation. The psychotropic potency of the marihuana can be characterised by its total THC content that is the sum of the THC and THC chemically equivalent to the THC-acid. Gas chromatography is a suitable technique for the direct determination of total THC because of the decarboxylation of THC-acid at the injection temperature. In some cases the determination of both THC and THC-acid separately is of interest. For this reason the supercritical fluid extraction (SFE) with pure carbon dioxide is a suitable sample preparation technique. The supercritical carbon dioxide is a non-polar solvent that dissolves the THC since it is a relatively non-polar compound but does not dissolve the THC-acid because the later compound is too polar owing to its carboxyl group.

The aim of the present study to elaborate a two-stage sample preparation procedure for HPLC analysis, which allows production of samples suitable for the determination of THC and total THC separately. The difference of the two results is equal to the THC chemically equivalent to the THC-acid content of the sample.

EXPERIMENTAL

Chemicals, investigated plant material 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, CBD and CBN were received from the UN Narcotic Laboratory Section (Vienna, Austria). For the experiments the fiber-type plant material was received from the Fleischmann Rudolf Institute for Agricultural Research (Kompolt, Hungary), the wild--type material was collected in different geographical parts of Hungary and the drug-type material was 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 plant material was ground in an electric grinder and 50 mg amounts were weighed for SFE experiments. The samples were wrapped in a filter-paper (75 mm x 30 mm) in order to avoid the plugging of the frit with small particles at the outlet of the extractor chamber.

SFE of THC

The extractions were run with a 0.9 g/ml density of carbon dioxide at 40°C for 50 minutes. The flow rate of the extraction fluid was 1.5 ml/min. The ex-

tracted components were trapped at 30°C and then eluted with 1.5 ml of n-hexane at 40°C.

SFE of total THC

In order to decarboxylate the acids the sample was heated in the extractor chamber at 120°C for 10 minutes applying 0.2 g/ml density of carbon dioxide without flowing. The THC formed was extracted as described in the previous paragraph. The conditions needed for total decarboxylation of acids were systematically studied earlier [1].

HPLC monitoring of the cannabinoid content of samples obtained by SFE

The HPLC separation was achieved in normal-phase mode 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.

Calculation of THC chemically equivalent to THC-acid

The THC chemically equivalent to THC-acid was calculated by subtracting the THC content from the total THC content.

RESULTS

Typical chromatograms obtained for the three different types of plants are shown in Figure 1, where the upper chromatograms refer to extracts of non-heated samples, the lower chromatograms refer to extracts of heated samples. The heating, e.g. decarboxylation resulted in increased cannabinoid content in case of each sample. The dominant cannabinoid in the wild-type plant is CBD while in the drug-type contains mainly THC. The level of THC in fibre-type plants is close to that of the CBD.

In Table I the percent THC, THC equivalent to THC acid and total THC content of different types of cannabis are listed.

It can be stated that the THC equivalent to THC-acid is not negligible to the total THC content. The ratio of THC equivalent to THC-acid comparing to total THC content fluctuates between 8% and 78%, which is probably in connection with the age of the plant material, as well as its storage conditions. (For examples, the old plant contains THC-acid in low concentration as well as the material stored in a warm place even it had been collected from a fresh batch).

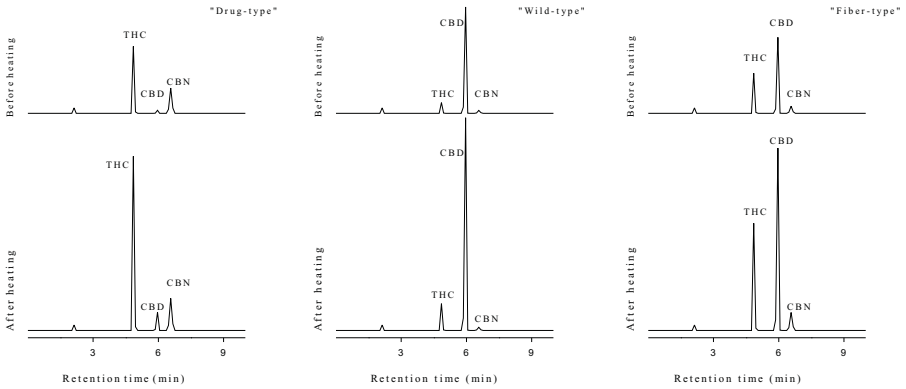

Fig. 1. Typical HPLC chromatograms obtained by analysis of "drug-type", "wild-type" and "fibre-type" cannabis before and after heating of the samples, respectively.

TABLE I. PERCENT THC, THC EQUIVALENT TO THC-ACID AND TOTAL THC CONTENT OF DIFFERENT TYPES OF CANNABIS

Type	Sample name	THC [%]	THC^{acid} [%]	THC^{total} [%]
Wild-type	*Szentendre[1]	0.008	0.016	0.024
	*Szentendre[2]	0.012	0.043	0.055
	*Szentendre[3]	0.028	0.023	0.051
	**Gyömrői út	0.014	0.036	0.050
	***Ócsa	0.027	0.019	0.046
Fiber-type	Bitrobrezkie	0.277	0.089	0.366
	Fedora	0.058	0.015	0.073
	Fibrimon	0.194	0.117	0.311
	Futura	0.063	0.098	0.161
	Kompolti	0.027	0.023	0.050
	$Kompolti^{yellow}$	0.111	0.009	0.120
	$Chinese^{female}$	0.047	0.129	0.176
	$Chinese^{unisex}$	0.023	0.070	0.093
	Tiborszállási	0.143	0.093	0.236
Drug-type	In-door[1]	0.712	0.222	0.934
	In-door[2]	1.681	0.969	2.650

* The samples were collected in three different regions of the Szentendre Island (The Szentendre Island is surrounded by the river Danube).
** 10 district of Budapest.
*** Ócsa is a village in central geographical area of Hungary.

SUMMARY

An analytical procedure was elaborated for the determination of THC and THC-acid in marihuana. The procedure is based on a two-stage sample preparation consisting of SFE of THC and total THC and subsequent HPLC analysis of the two extracts. The total THC can be extracted after decarboxylation of THC-acid, which can be effected without waste by heating of the sample in the extraction chamber at conditions of static SFE.

The difference between the contents of THC and total THC is equal to the THC chemically equivalent to the THC-acid content of the sample.

The applicability of the proposed procedure is demonstrated by analysis of cannabis samples of different types. The procedure is utilisable in comparative studies and in the estimation of the age of samples.

References:

1. Veress T., Sample Preparation by SFE for the HPLC determination of cannabinoids, *LC-GC International* 1997, vol. 10, pp. 114–122.