CHIMIA **2004**, *58*, No. 4

Chimia 58 (2004) 219–221

ISSN 0009–4293

# Analysis of *Cannabis* Material by Headspace Solid-Phase Microextraction Combined with Gas Chromatography-Mass Spectrometry

Yara Ilias*, Serge Rudaz, Patrick Mathieu, Jean-Luc Veuthey, and Philippe Christen

*Abstract*: A headspace solid-phase microextraction method combined with gas chromatography/mass spectrometry was developed for the direct analysis of *cannabis* samples. Different parameters influencing the extraction of cannabinoids were studied and the optimised method was applied to the analysis of plants from two different regions in Switzerland. Applied to cannabinoid concentrations, a principal components analysis was applied and demonstrated sample discrimination.

**Keywords:** *Cannabis* · Headspace solid-phase microextraction · Principal Components Analysis

## Introduction

Solid-phase microextraction (SPME) is a solvent-free sample preparation technique introduced in 1990 by Arthur and Pawliszyn [1] in which several steps are combined in one simple device. Its simplicity, high sensitivity, possibility of automation and relatively low costs have encouraged a very extensive development of applications in several domains such as environmental [2], biomedical [3–5], pesticide residues [6], food [7] and medicinal plant analysis [8]. This technique is performed with a modified syringe which contains a microtubing with a 1 cm fused silica fibre tip coated with an organic polymer. The fibre can be moved between two positions, inside and outside the needle, with a simple plunger. SPME allows analysis in two steps. During the first step, a distribution of analytes between the matrix and the fibre coating occurs. In the second step, extracted compounds are desorbed into an analytical instrument, generally a gas chromatograph [9]. Two main modes of extraction can be used for SPME analysis: direct SPME through dipping the fibre into an aqueous sample and headspace-SPME (HS-SPME) which extracts the compounds from the sample headspace.

SPME was successfully applied to the analysis of *cannabis* components in biological matrices such as saliva [10][11] and hair [12][13].

In the present work, SPME, in headspace mode, was used for the extraction of cannabinoids directly from the *cannabis* plants. It should be noted that cannabinoids are characteristic compounds of *Cannabis sativa* L. Their occurrence and concentration as potential indicators of the geographical origin of the plant [14–16] is under discussion. With respect to possible legalization of the use of *cannabis* and derivatives in Switzerland, the identification of origin-linked indicators can be of great significance.

The different parameters affecting the extraction efficiency, such as the coating type, the extraction temperature and time, the equilibrium and desorption time influences were discussed. The optimal conditions were then used for the analysis of two series of samples from Switzerland. Chromatographic analyses were performed by GC/MS. Data obtained from the different samples were processed by principal components analysis (PCA) in order to study possible similarities and/or differences in the composition of the plants.

## Experimental

### *Samples*

Two different lots of Swiss marijuana samples of certified origin and cultivated outdoors were used. The first group (n = 8) was harvested in Zurich and the second one (n = 12) in Geneva. The method development was performed with one sample from Zurich (sample Z24).

### *SPME Procedure*

All tested fibres, as well as the manual holder, were purchased from Supelco (Bellefonte, PA, USA). Extractions were performed directly on 60 mg of grounded marijuana in 2 ml glass vials sealed with silicone/PTFE septa. The vials were allowed to heat in a thermostated bath at a controlled temperature. The fibres were then inserted into the headspace for a determined period of time in order to extract analytes of interest. After extraction, the fibres were removed and directly inserted into the injection port of the gas chromatograph where thermal desorption of the analytes occurred.

*Correspondence: Y. Ilias
Laboratory of Pharmaceutical Analytical Chemistry
University of Geneva
20, bd. d'Yvoy
CH-1211 Geneva 4
Tel.: +41 22 379 68 94
Fax: +41 22 379 68 08
E-Mail: yara.ilias@pharm.unige.ch

CHIMIA **2004**, *58*, No. 4

### *GC/MS Conditions*

GC/MS analyses were performed on an HP 5890 series II gas chromatograph coupled with an HP 5972 mass spectrometer (Agilent Technologies, Waldbronn, Germany) and equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d. with a 0.25 µm phase thickness). The injections were made in the splitless mode at 280 °C (except for the PDMS/DVB and DVB/CAR/PDMS fibres, for which the injection temperature was 270 °C). The temperature program was: 150 °C for 2 min, increase to 210 °C at 30 °/min, followed by an increase to 250 °C at 5 °/min, followed by an increase to 280 °C at 10 °/min and hold at 280 °C for 3 min. Helium was used as carrier gas at 1 ml/min constant flow rate. The transfer line was set at 280 °C. The mass spectrometer was operated in electron impact (EI) ionisation mode at 70 eV, in the scan range *m/z* 30–400. For the identification of cannabinoids, the NIST98 and WILEY spectral libraries were used, as well as literature MS data [14][15].

Table. Description of the studied parameters

| Parameter | Tested | Optimal value |
|---|---|---|
| **Coating type** | Polydimethlysiloxane 100 µm, apolar (PDMS)<br>Carboxen®/PDMS 85 µm, bipolar (CAR/PDMS)<br>PDMS/Divinylbenzene) 65 µm, bipolar (PDMS/DVB)<br>DVB/CAR/PDMS 50/30 1 cm length, bipolar | PDMS 100 µm |
| **Sampling temperature** | 80, 90, and 150 °C | 150 °C |
| **Sampling time** | 2, 5, 10, 20, and 30 min | 5 min |
| **Equilibrium time** | 10 and 15 min | none |
| **Desorption time** | 1, 2, 3, and 6 min | 3 min |

## Results and Discussion

A description of the different parameters studied is presented in the Table. Experiments were performed in triplicate on the same sample (Z24), varying one parameter at a time. The influence of these parameters on extraction efficiency was evaluated on the basis of mean absolute peak areas of the three major cannabinoids (cannabidiol, CBD; $\Delta^9$-tetrahydrocannabinol, $\Delta^9$-THC; cannabinol, CBN). The PDMS 100 µm coated fibre was selected as the best extraction support. On the other hand, due to the low volatility of cannabinoids, the effect of the sampling temperature was clearly demonstrated and 150 °C was thus selected for further analyses. Furthermore, study of the sampling time showed the same profiles for the three target analytes, with a steady state after 10 min. However, because of the sufficient sensitivity obtained, a sampling time of 5 min was selected. Since no gain in terms of total analysis time could be obtained, no preheating time was performed during the following analyses. Lastly, due to the high lipophilicity of cannabinoids, a 3 min desorption time was necessary to assure a total transfer of these compounds from the fibre coating to the gas chromatograph.

The method was then applied to the analysis of two series of *cannabis* samples from Switzerland (n = 20). Each sample was analysed in triplicate. A typical chromatogram is illustrated in Fig. 1, where two separation windows can be distinguished: the former corresponding to volatile compounds not taken into account for our study, and the latter related to cannabinoids. Ten different cannabinoids were identified and, based on the concentration of each compound, a principal components analysis was applied to the mean areas. Around 70% of the total variance was explained by the two first principal components axes. As shown in Fig. 2, samples could be clearly divided into two groups: samples from Geneva (D) and samples from Zurich (Z). In addition, one sample (Z6) was strongly differentiated. This plant is not a drug- but a fibre-type *cannabis* and hence contains only a small amount of THC.

As illustrated in Fig. 3, the three major cannabinoids showed little correlation, their vectors pointing in different directions. This means that these compounds do not furnish redundant information. On the other hand, a strong correlation was observed amongst cannabinoids participating to the first axis and characterising the samples from Zurich. Samples from Geneva are distributed along the second axis, for which THC, CBV and THCV present the largest contributions (31, 25 and 18%, respectively). Furthermore, THC, which is the psychoactive compound, seems to be in different concentrations and not correlated to the origin.

The results reported here showed the effectiveness of PCA in furnishing relevant information for sample description. In addition, an important discrimination of *cannabis* samples coming from the same country but cultivated in different regions was observed.

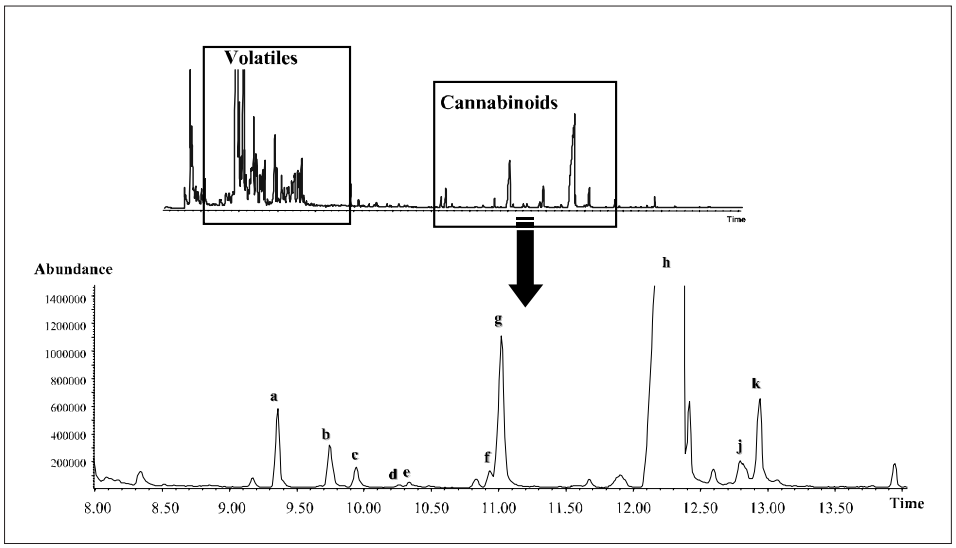


Fig. 1. Example of a chromatogram. Cannabinoids: a: cannabinoid 1 (Canna1); b: tetrahydrocannabivarine (THCV); c: cannabinoid 2 (Canna2); d: cannabicyclol (CBCL); e: cannabivarine (CBV); f: cannabidiol (CBD); g: cannabichromene (CBC); h: $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC); j: cannabigerol (CBG); k: cannabinol (CBN).

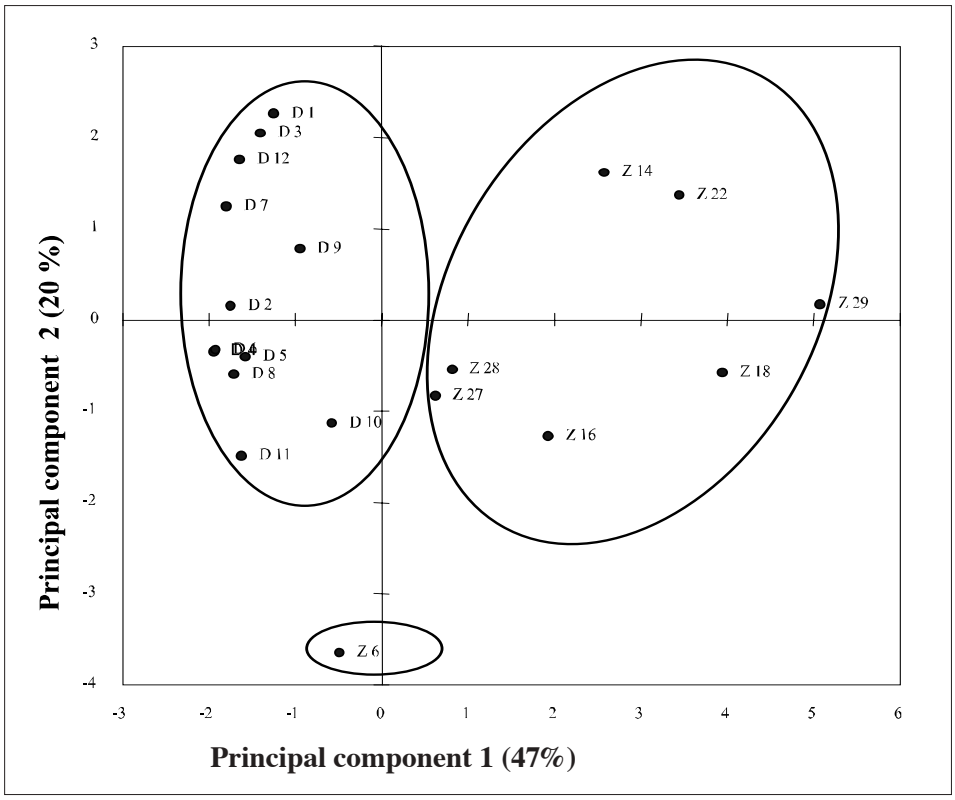


Fig. 2. Distribution of 20 samples by PCA. Representation of the scores plot: D = samples from Geneva, Z = samples from Zurich.

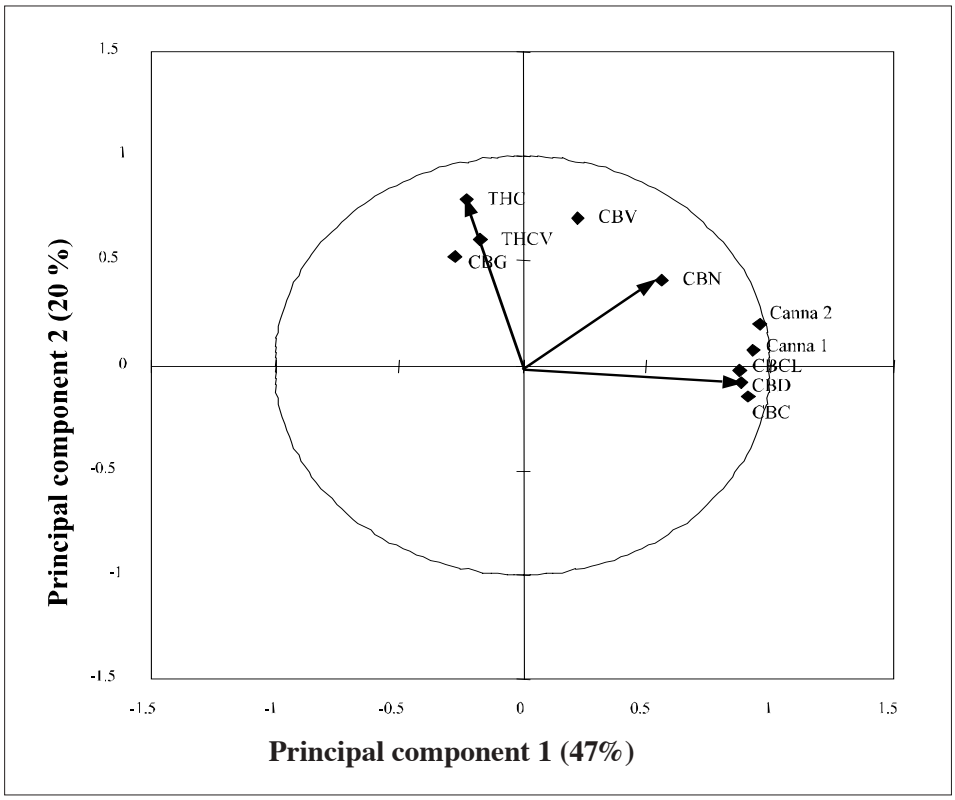


Fig. 3. Description of 20 samples by PCA. Representation of the loadings plot. Arrows indicate vectors of the three major cannabinoids (CBD, $\Delta^9$-THC and CBN).

## Conclusion

Compared to classical solvent extraction, SPME allowed a complete, rapid, and simple analysis of *cannabis* in which no sample preparation was needed. Furthermore, the method is solvent-free which is important for environmental purposes. And lastly, working in the headspace mode preserved the fibre coating which could therefore be employed for a larger number of extractions. Sensitivity can be considered as another central advantage of SPME, especially when it concerns the characterization of unknown samples.

Analyses of plants originating from different regions of Switzerland produced interesting information about cannabinoid content. Statistical description of the data by PCA showed a significant sensitivity of the plant to external factors since a discrimination between the two regions was observed on the basis of cannabinoid concentration. Work is in progress to determine if a correlation between cannabinoid content and geographical origin can be demonstrated.

Received: January 22, 2004

[1] C. Arthur, J. Pawliszyn, *Anal. Chem.* **1990**, *62*, 2145.
[2] M. Alpendurada, *J. Chromatogr. A* **2000**, *889*, 3.
[3] S. Ulrich, *J. Chromatogr. A* **2000**, *902*, 167.
[4] N.H. Snow, *J. Chromatogr. A* **2000**, *885*, 445.
[5] G. Theodoris, E.H.M. Koster, G.J. de Jong, *J. Chromatogr. B* **2000**, *745*, 49.
[6] J. Beltran, F. Lopez, F. Hernandez, *J. Chromatogr. A* **2000**, *885*, 389.
[7] H. Kataoka, H. Lord, J. Pawliszyn, *J. Chromatogr. A* **2000**, *880*, 35.
[8] C.W. Huie, *Anal. Bioanal. Chem.* **2002**, *373*, 23.
[9] H. Lord, J. Pawliszyn, *J. Chromatogr. A* **2000**, *885*, 153.
[10] B.J. Hall, M. Satterfield-Doerr, A.R. Parikh, J.S. Brodbelt, *Anal. Chem.* **1998**, *70*, 1788.
[11] N. Fucci, N. De Giovanni, M. Chiarotti, S. Scarlata, *Forensic Sci. Int.* **2001**, *119*, 318.
[12] S. Strano-Rossi, M. Chiarotti, *J. Anal. Toxicol.* **1999**, *23*, 7.
[13] F. Sporkert, F. Pragst, *Forensic Sci. Int.* **2000**, *107*, 129.
[14] M. Novotny, M.L. Lee, C.-E. Low, A. Raymond, *Anal. Chem.* **1976**, *48*, 24.
[15] R. Brenneisen, M.A. ElSohly, *J. Forensic Sci.* **1988**, *33*, 1385.
[16] C.E. Turner, M.A. ElSohly, E.G. Boeren, *J. Nat. Prod.* **1980**, *43*, 169.