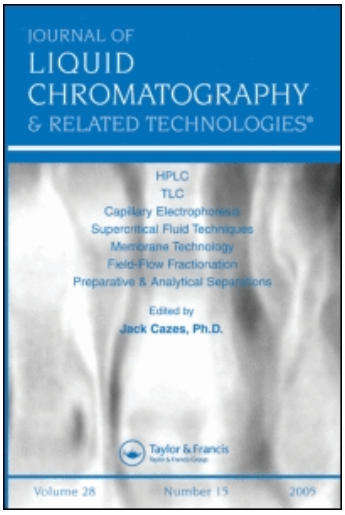

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Profiling of Cannabis Products

T. Lehmann[a]; R. Brenneisen[a]
[a] Institute of Pharmacy University of Berne Baltzerstrasse, Switzerland

To cite this Article Lehmann, T. and Brenneisen, R.(1995) 'High Performance Liquid Chromatographic Profiling of Cannabis Products', Journal of Liquid Chromatography & Related Technologies, 18: 4, 689 — 700
To link to this Article: DOI: 10.1080/10826079508009265
URL: http://dx.doi.org/10.1080/10826079508009265

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(4), 689-700 (1995)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC PROFILING OF CANNABIS PRODUCTS

T. LEHMANN AND R. BRENNEISEN
Institute of Pharmacy
University of Berne
Baltzerstrasse 5
CH-3012 Berne, Switzerland

ABSTRACT

An HPLC method with photodiode array detection (DAD) is described for the qualitative and quantitative determination of neutral and acidic cannabinoids in Cannabis sativa L. The complex chromatographic pattern can be used for the classification of Cannabis chemotypes, the monitoring of the psychotropic potency and the comparison of Cannabis products of different origin.

INTRODUCTION

Psychotropic products of the chemotype I (drug type) (1,2) and chemotype II (intermediate type) of Cannabis sativa L. - like herbal Cannabis (marijuana), Cannabis resin (hashish) and extracts of Cannabis resin (hashish oil) - are the most abused illicit drugs of the world. On the other hand, the chemotype III (fiber/industrial type) becomes in Europe

more and more important as a renewable, fast growing fiber producing plant. It is an economically and ecologically interesting alternative source for the production of natural fibers, which can be used for example as an inexpensive, rugged raw material in the paper, car or building industry.

For the last ten years our laboratory has analyzed several hundred samples of Cannabis sativa L. of different origin as part of botanical, phytochemical and forensic research projects, using an isocratic HPLC method (1-3). Due to the lack of suitable standards this method did not allow to measure the main neutral *and* acidic cannabinoids by direct quantitation. The same holds true for other published HPLC methods (4-8). This paper describes the first HPLC method with photodiode array detection (DAD) for the aquisition of full cannabinoid profiles.

MATERIALS AND METHODS

Instrumentation

All HPLC analyses were performed on a Hewlett-Packard (HP, Waldbronn, Germany) HPLC system consisting of a 1090M liquid chromatograph, a 1090L autosampler, a 1040M photodiode array detector, a Vectra 486/33N computer with HPLC Chemstation Rev. A.02.00 software and a Desk Jet 550C printer.

Chromatographic Conditions

The HPLC separation of Cannabis extracts was performed at 40°C oven temperature on a 200 x 2.0 mm i.d. column with a 20 x 2.0 mm i.d. precolumn, packed with Spherisorb ODS-1, 3 µm (Stagroma, Wallisellen,

Switzerland). Solvent A was water, containing 8.64 g/L orthophosphoric acid (85%), solvent B was acetonitrile. The gradient profile was as follows: 0-38 min, 47-60% B, linear; 38-48 min, 60-70% B, linear; 48-50 min, 70-47% B, linear; 50-60 min, 47% B, isocratic. The flow rate was 200 µL/min. The solvents were filtered under vacuum through a 0.45-µm nylon membrane filter and degassed by sonication prior to use and by a constant flow of helium during use. After use the column was washed with acetonitrile. Precolumn and column have been replaced after 50 and 150 runs, respectively. The quantitation of THCA-B, CBD, CBG and THC was performed at 210 nm, CBDA, CBN and THCA-A at 224 nm. The peak identity was ascertained by on-line scanning of UV spectra from 192 to 350 nm at a sampling rate of 3.125 spectra/sec.

Chemicals and Reagents

(-)-Δ^9-(*trans*)-tetrahydrocannabinolic acid A (THCA-A) was isolated in our laboratory (9). (-)-Δ^9-(*trans*)-tetrahydrocannabinol (THC) and cannabichromene (CBC) were donated by the UN Narcotics Laboratory (Vienna, Austria). Cannabidiol (CBD), cannabigerol (CBG) and cannabinol (CBN) were obtained by the Swiss Federal Office of Public Health (Berne, Switzerland). (-)-Δ^9-(*trans*)-tetrahydrocannabinolic acid B (THCA-B), cannabidiolic acid (CBDA), (-)-Δ^9-(*trans*)-tetrahydrocannabivarin (THV), (-)-Δ^9-(*trans*)-tetrahydrocannabivarinic acid (THVA), cannabiripsol (CBR), cannabielsoin (CBE) and cannabicyclol (CBL) were kindly supplied by the Research Institute of Pharmaceutical Sciences, University of Mississippi (Oxford, MS, USA). Chemicals and reagents were of HPLC or analytical grade, purchased from Merck (Basel, Switzerland).

Cannabis Samples

Cannabis of chemotype I and II was collected from own Cannabis plantations, authorized by the Swiss Federal Office of Public Health. Chemotype III was collected from plantations of the Swiss Federal Office of Agriculture. Hashish and hashish oil have been confiscated by police and customs authorities.

Sample Preparation

100 mg dried (40°C, 24h), pulverized herbal Cannabis (Cannabis, marijuana), 50 mg Cannabis resin (hashish) or 50 mg Cannabis oil (hashish oil) was extracted with 1.0 mL methanol-chloroform (9:1, v/v) by sonication during 15 min. 100 µL of the filtered extract was diluted with 300 µL of methanol and aliquots of 1 µL were used for HPLC.

Quantitation

HPLC quantitation was performed by the external standard method, measuring the peak areas of the cannabinoids at their maxima of 210 or 224 nm, respectively. The calibration graphs (linear regression analysis) were obtained by triple analysis of different injection volumes (0.5 - 5 µL) of standard mixtures containing 10, 100 and 1000 ng/µL of each cannabinoid.

RESULTS AND DISCUSSION

The *sample preparation* is much faster and simpler than the previous method used routinely in our laboratory (3). As isolated

cannabinoid acids, like THCA-A and CBDA, the dominant biogenic cannabinoid acid of Cannabis drug type and fiber/industrial type, respectively, are now available as standards, the evaporation and thermal decarboxylation of the Cannabis extracts can be avoided and direct quantitation is possible. Previously, these steps were necessary for the indirect quantitation of THCA-A, THCA-B and CBDA by measuring the amount of the corresponding neutral cannabinoids (THC, CBD) before and after decarboxylation of the acids at high temperature. The efficiency of the extraction was checked by duplicate extraction of two samples and showed a *recovery* of 97.6 to 99.4% for the main cannabinoids.

The *chromatographic system* is based on acetonitrile-water-phosphoric acid, a solvent with low UV cut-off and allowing the detection down to 192 nm, and 3-µm spherical reversed phase material in a low-diameter column, allowing to reduce the flow rate to 200 µL/min. A sharp symmetrical peak-shape and sufficient separation of the cannabinoids from the complex plant matrix can be achieved (see Fig. 1 and 2). The large polarity range of the cannabinoids requires the use of a fine tuned solvent gradient program to limit the run to 60 min for a full cannabinoid profile. Co-extracted lipophilic plant constituents necessitate the replacement of the column and precolumn after 150 and 50 runs, respectively, because of deteriorating peak-shapes and -resolution (especially between CBC and THCA-A).

Peak identification was achieved by standards and their characteristic DAD-UV spectra (see Fig. 3) with a library match of > 990 within a retention time window of ± 0.2 min. Retention time windows were adjusted every 10th run with a standard mixture, if necessary. An indication of peak homogeneity was given by peak purity check demonstrated by an up-slope, apex and down-slope peak spectra match of 990 or more. The *limit of detection*, measured with standard mixtures,

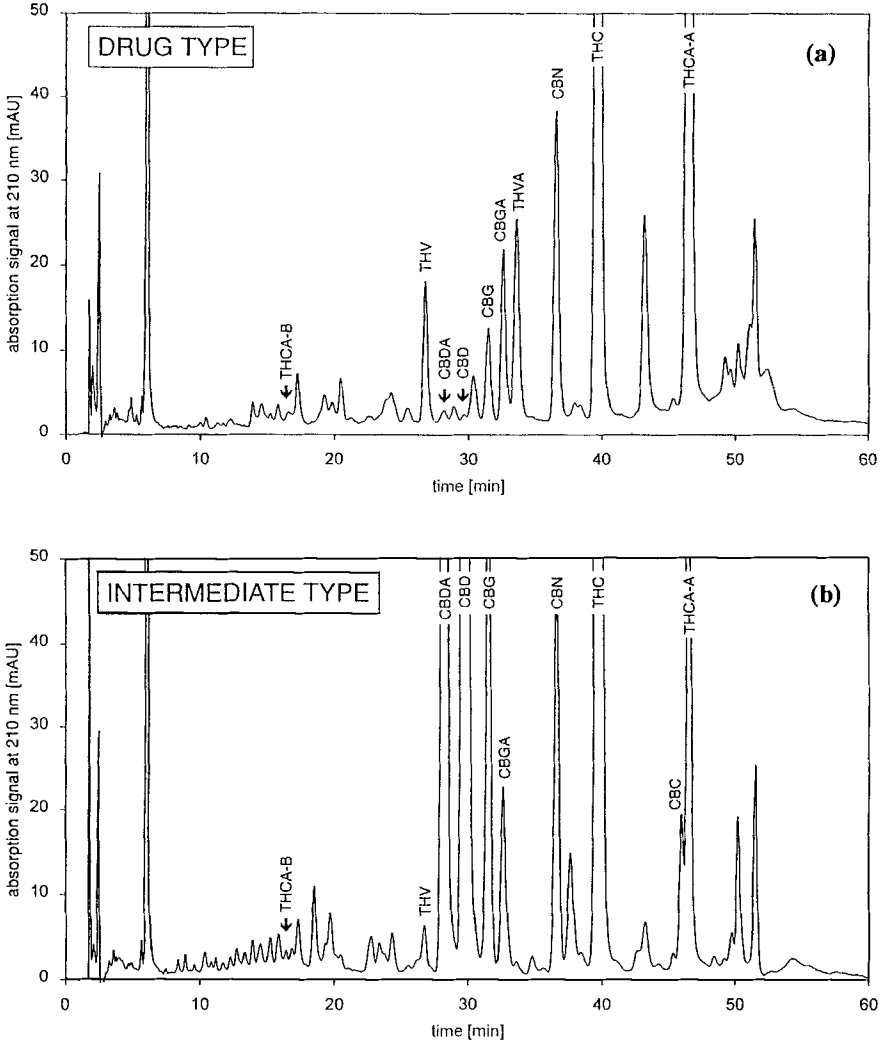

FIGURE 1. HPLC profiles of chemotypes of Cannabis sativa L.: (A) Chemotype I (drug type); (B) chemotype II (intermediate type); (C) chemotype III (fiber/industrial type).

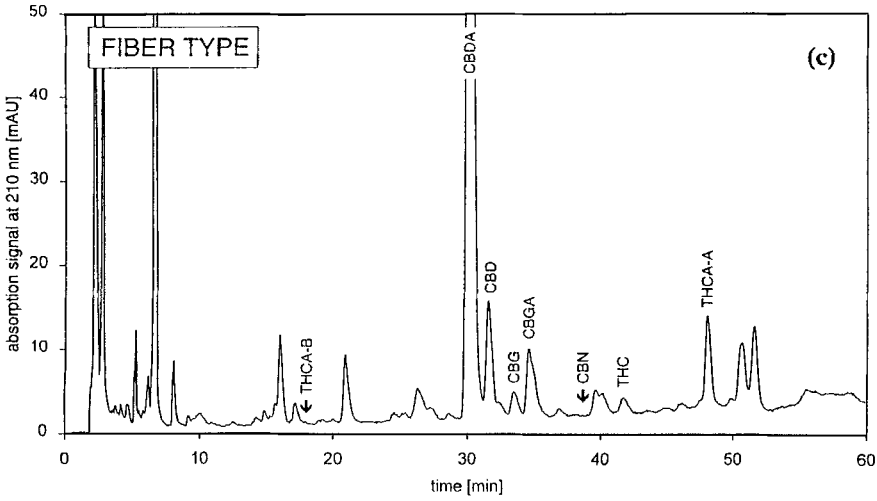

FIGURE 1 (Continued)

was 1 ng cannabinoid on the chromatographic system with a signal to noise ratio of 3 or higher. The *limit of quantitation* was about 25 ng cannabinoid per 1 µL extract, corresponding to 0.1% cannabinoids per drug sample and allowing to acquire full UV spectra. The *linearity* was determined in the range of 5 to 5000 ng for each cannabinoid. A linear relationship was found between the peak area and the concentration of THC, CBG, CBD, CBN, THCA-A, THCA-B and CBDA. The correlation coefficient r of these cannabinoids was > 0.9999. The inter-day precision of the calibration was checked by analyzing two replicates of solutions containing 100 and 1000 ng/µL cannabinoid standards at five different days within one month. The relative standard deviations were between 3.0 and 5.9% at the low (100 ng/µL) concentration level and between 1.3 and 4.3% at the high (1000 ng/µL) concentration level. The calibration solutions are stable over five weeks if stored at -20°C. The over-all

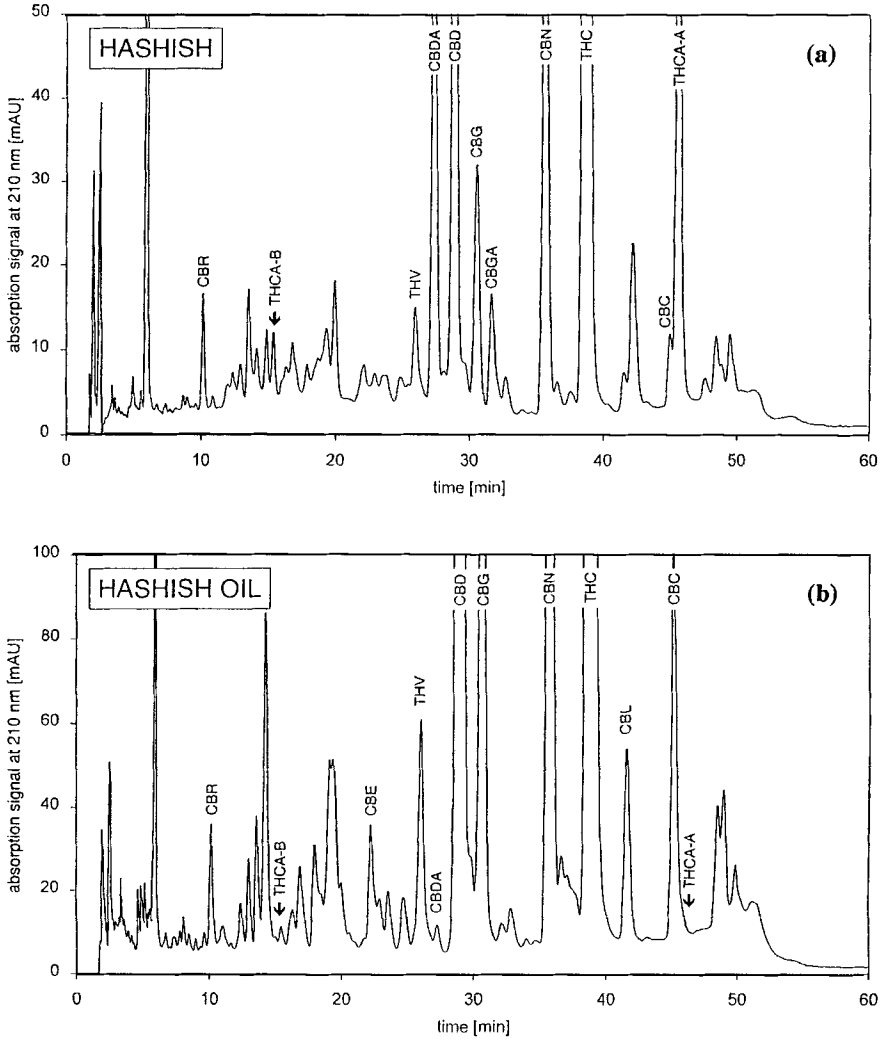

FIGURE 2. HPLC profiles of other Cannabis products: (A) Cannabis resin (hashish); (B) Cannabis resin extract (hashish oil).

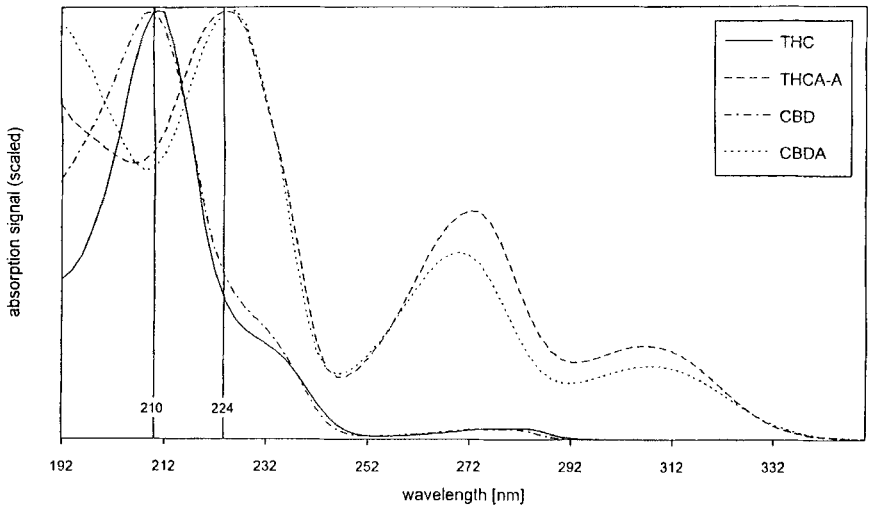

FIGURE 3. DAD-UV spectra of neutral and acidic cannabinoids.

precision of the method was determined by analyzing two replicates of five Cannabis extracts within one day and on five different days within two months. The relative standard deviations for the intra-day and inter-day variation were 2.3 to 5.8% and 2.7 and 5.3%, respectively.

As can be seen in Figures 1 and 2, the HPLC profiles of extracts of Cannabis products show up to 40 different peaks. With standards and DAD-UV spectra, 13 major or minor cannabinoids could be identified as CBR, THCA-B, THV, CBDA, CBD, CBG, CBGA, THVA, CBN, THC, CBL, CBC, and THCA-A. The quantitation included the key cannabinoids THC, THCA-A, CBD, and CBDA as well as THCA-B, CBG and CBN. The resulting characteristic chromatographic pattern makes the forensically and pharmacologically important differentiation of chemotype I-III of herbal Cannabis feasible. Chemotype I, the so called drug type, is characterized by the dominant key cannabinoids THC and THCA-A. The THC and THCA-A concentrations varied from 0.1 to 2.5% and from 0.1 to 8.2%,

respectively. Chemotype II, the intermediate type, shows high concentrations of THC/THCA-A as well as CBD/CBDA. Both chemotypes are psychoactive. A typical specimen of this type contained 4.31% (± 0.12%) CBDA, 2.30% (± 0.09%) CBD, 0.17% (± 0.01%) CBG, 0.33% (± 0.01%) CBN, 1.19% (± 0.06%) THC and 1.95% (± 0.10%) THCA-A. CBD and CBDA are the major cannabinoids of the psychoinactive chemotype III, the fiber or industrial type, whereas the THC/THCA-A content is < 0.5%. Only traces of THCA-B could be detected in all analyzed samples. Chemotype IV, the propyl isomer/C3 type (10, 11), can be differentiated by the dominant key cannabinoids (-)-Δ^9-(*trans*)-tetrahydrocannabivarin (THV) and its corresponding acid (-)-Δ^9-(*trans*)-tetrahydrocannabivarinic acid (THVA). This least frequent chemotype, originating mainly from South Africa (11, 12), contains also remarkable amounts of THC/THCA-A (THV/THVA : THC/THCA-A > 1) and exhibits psychoactivity. Low concentrations of THV/THVA can be detected in most Cannabis samples of chemotype I and II. Figure 2 demonstrates that HPLC profiles of the resin (hashish) and the resin extract (hashish oil) are usually more complex than those of herbal Cannabis (marijuana). Resin extracts which are produced by solvent extraction or more often by direct distillation of the resin using high temperature resulting in a reduction of the acids and decomposition of unstable cannabinoids.

CONCLUSION

The described HPLC-DAD method can be used for classifying Cannabis chemotypes, for monitoring the psychotropic potency of Cannabis products by quantitation of THC/THCA, for checking the identity of Cannabis specimens of different origin by comparing subtle differences

in their chromatographic pattern and within other applications, where the information of a full cannabinoid profile is useful. It completes our earlier published GC/MS procedure, which was developed for profiling mainly the non-cannabinoid constituents of Cannabis products and used for the determination of the geographical origin of confiscated Cannabis samples (13, 14).

ACKNOWLEDGEMENTS

This work was supported by grants from the Swiss Federal Office of Public Health (Foundation for Narcotics Research) and the Ernst Steinegger Foundation for Medicinal Plant Research. The authors wish to thank the Swiss Federal Office of Public Health, the Research Institute of Pharmaceutical Sciences, University of Mississippi, and the UN Narcotics Laboratory in Vienna for providing cannabinoid standards.

REFERENCES

1. R. Brenneisen, Arch. Kriminol., 177: 95-104 (1986)
2. R. Brenneisen, T. Kessler, Pharm. Acta Helv., 62: 134-139 (1987)
3. R. Brenneisen, Pharm. Acta Helv., 59: 247-259 (1984)
4. Y. Nakahara, H. Sekine, J. Anal. Toxicol., 9: 121- 124 (1985)
5. T. Veress, J. I. Szanto, L. Leisztner, J. Chromatogr., 520: 339-347 (1990)
6. G. Ray, M. Crook, N. West, M. Kwoka, G. Rehagen, J. Cox, E. Murrill, K. Flora, J. Chromatogr., 317: 455-462 (1984)

7. J. C. Turner, P. G. Mahlberg, J. Chromatogr., 283: 165-171 (1984)

8. K.-A. Kovar, H. Linder, Arch. Pharm. 324: 329-333 (1991)

9. T. Lehmann, R. Brenneisen, Phytochem. Anal., 3: 88-90 (1992)

10. C.E. Turner, K. Hadley, P.S. Fettermann, J. Pharm. Sci., 62: 1739-1741 (1973)

11. E. Stephanou, C. Lawi-Berger, I. Kapetanidis, Pharm. Acta Helv., 59: 216-224 (1984)

12. J.E. Pitts, J.D. Neal, T.A. Gough, J. Pharm. Pharmacol., 44: 947-951 (1992)

13. R. Brenneisen, M. A. ElSohly, J. Forens. Sci., 33: 1385-1404 (1988)

14. T. Lehmann, C. Affolter, A. Bezegh, R. Brenneisen, J. T. Clerc, M.A. ElSohly, Proceedings of the 44th Annual Meeting of the American Academy of Forensic Sciences, 1992, p. 82

Received: July 20, 1994
Accepted: September 7, 1994