DETERMINATION OF Δ^9-TETRAHYDROCANNABINOL BY HPLC/DAD IN FOOD SUPPLEMENT SAMPLES OF HEMP SEED OIL

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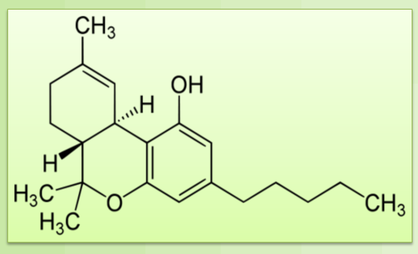
INTRODUCTION

The EU member states have different regulation in allowed limit of controlled compound Δ^9-tetrahydrocannabinol (THC) in hemp seed oil, produced for consumption or as food supplement.



Formula of Δ^9-tetrahydrocannabinol (THC)

EXPERIMENTAL

The THC traces were analyzed in few samples by recommended HPLC isocratic method[1] using different HPLC columns (C8 and C18):
C8 (250 mm x 4.6 mm I.D., 5 μm) from Agilent Technologies, Inc. (Germany);
BDS RP18 (150 mm x 4.6 mm I.D., 5 μm) and
Discovery® C18 (250 mm x 4.6 mm I.D., 5 μm) from Supelco;
Purospher® Star RP18e (150 mm x 4.6 mm I.D., 5 μm) and
Purospher® Star RP18e (250 mm x 4.6 mm I.D., 5 μm) from Merck KGaA (Darmstadt, Germany).
During analysis all columns were garded by analytical guard columns Eclipse XDB-C18 (4.6 mm x 12.5 mm I.D., 5 μm) from Agilent Technologies (USA).

Chromatographic procedures

The first, recommended method[1] propouse following conditions: column type RP8 (250 mm x 4.0 mm I.D., 5 μm); pre-column type RP8 (4 mm x 4 mm I.D., 5 μm); mobile phase composed of acetonitrile and water in ratio 80/20, *V/V*, in isocratic mode, at flow rate of the mobile phase 1.0 ml/min, column temperature at 30°C; detection at 220 nm and 240 nm, injection of 10 μl and stop time 8 min.

The second method[2] propouse following conditions: column type RP18 (125 mm x 2.0 mm I.D., 5 μm); pre-column type RP-8 (4 mm x 4 mm I.D., 5 μm); mobile phase composed of acetonitrile and water acidated with 85 % phosphoric acid (8.6 g/L) in gradient program (acetonitrile in 25 min change from 55 %, *V/V*, to 80 %, *V/V*, flushing at 90 %, *V/V*, for 1 min and next 4 min at 90 %, *V/V*, for 1 min beck to 55 %, *V/V*, and at the end equilibrium for 9 min at 55 %, *V/V*), flow-rate 0.2 ml/min, column temperature 26°C; detection at 210 nm; injection of 10 μl; in 40 min runtime.

The most efficient method is as follows: column type Purospher Star RP18e (150 mm x 4.6 mm I.D., 5 μm); mobile phase composed of acetonitrile and water in gradient mode (acetonitrile quantity change from 50 %, *V/V*, to 80 %, *V/V*, in 20 min, 5 min at 80 %, *V/V*, and back from 80 % to 50 % in 1 min and equilibrium for 5 min at 50 % , *V/V*), at flow rate 1.5 ml/min, column temperature at 30°C; detection at 220 nm and 240 nm, injection of 10 μl; in runtime 31 min.

Using the recommended method[1] It was not possible to separate THC from one component also present in the other plant oils (flax seed oil and rape seed oil), used as blank samples.

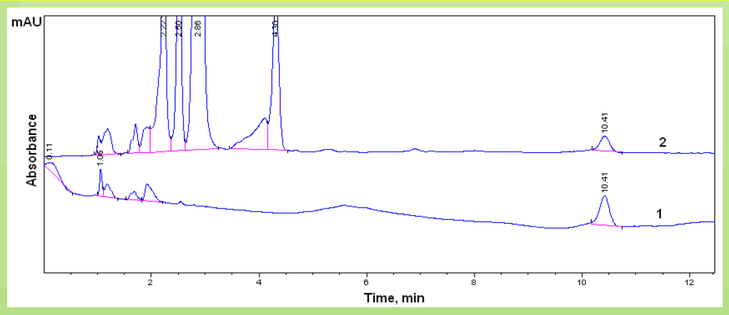


Fig. 1 Comparative chromatograms of: solution of standard substance THC (1) and flax oil solution used as blank sample (2), obtained using the first recommended chromatographic procedure for THC determination on C18 column (250 mm x 4.6 mm I.D., 5 μm)

This unidentified compound does not have quite characteristic UV spectrum and it is similar with the UV spectrum of THC.

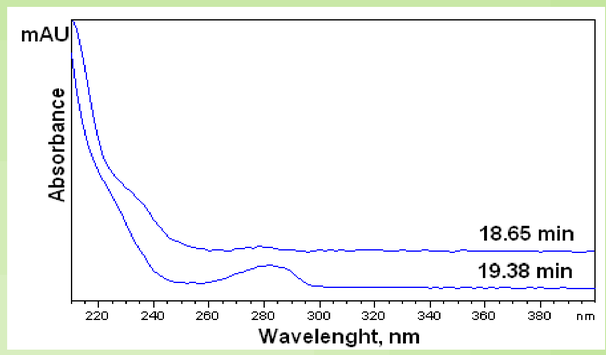


Fig. 2 UV spectra of chromatographic peaks of standard substance THC (18.65 min) and x compound from the solution of flax seed oil solution sample (19.38 min)

Then the gradient mode HPLC method was developed and we succeeded to separate THC (t_R = 18.6 min) from this unknown intereferent (t_R = 19.4 min) with suitable resolution (R_s = 2.36).

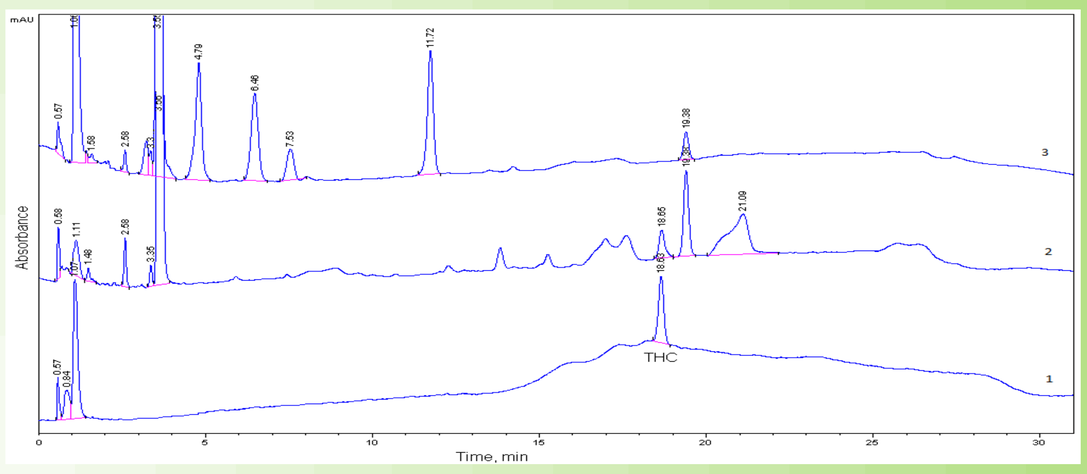


Fig. 3 Comparative chromatograms of: solution of standard substance THC (1); sample hamp seed oil solution (2); and flax oil solution used as blank sample (3) obtained using here recommended gradient chromatographic procedure for THC determination

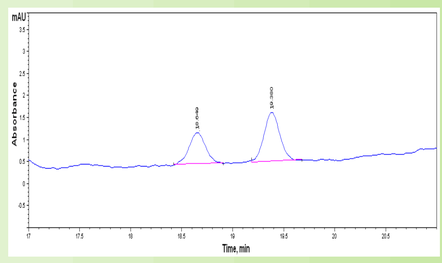


Fig. 4 Chromatogram of extract of hempseed oil obtained using gradient chromatographic procedure: THC (18.649 min) and X compound (19.380 min)

The obtained results for determined quantity of THC in tested samples of hemp seed oil were in range from 2.66 mg/L to 9.84 mg/L.

Table 1. Results for THC content for hempseed oil samples obtained by gradient HPLC method

Sample	Color	THC mg/L
Sample 1	Green	9.55
Sample 2	Yellow	2.33
Sample 3	Yellow	3.22

These results are in agreement with the already published data for this kind of samples.

References

1. United Nations Office on Drugs and Crime: Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products; Manual for use by national drug analysis laboratories; New York 2009, p 60.
2. Zoller O, Rhyn P, Zimmerli B, High-performance liquid chromatographic determination of Δ^9–tetrahydrocannabinol and corresponding acid in hamp containing foods with special regard to the fluorescence properties of Δ^9–tetrahydrocannabinol, *Journal of Chromatography A*, **872** (2000), 101-110.
3. Council regulation (EC) No 1420/98 of 26. June 1998 amending Regulation (EEC) No 619/71 laying down general rules for granting aid for flax and hemp.
4. Common catalogue of varieties of agricultural plant species; 28th complete edition; Official Journal of European Union (2009/C 302 A/01)
5. Commission Regulation (EC) No 796/2004 of 21 April 2004 laying down in Council Regulation (EC) No 1782/2003 provides for cross-compliance, modulation and the integrated administration and control system, detailed rules

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