Macedonian pharmaceutical bulletin, **62** (suppl) 239 - 240 (2016)
ISSN 1409 - 8695
UDC: 633.888:615.212.7.074
Short communication

Determination of cannabidiol and Δ^9tetrahydrocannabinol in *Cannabis sativa* L. preparations present in the European market by HPLC/DAD

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Introduction

Cannabis sativa L. is a medicinal plant, known and used for a long time. There are 400 – 500 compounds that have been identified in its extracts, and among them approximately 70 are C_{21} terpenophenols, members of a group known as cannabinoids, specific for this plant (ElSohly and Slade, 2005; Fischedick, et al., 2009; Turner, et al., 1980). The constituent of *Cannabis sativa* L., Δ^9-tetrahydrocannabinol (Δ^9THC), is the primary psychoactive cannabinoid. Given that the main pharmacological and psychoactive effects have been attributed to this compound, the most of the studies have been focused on its effects (Costa, 2007). The effects of *Cannabis sativa* L. are not solely due to Δ^9THC because cannabidiol (CBD) was found to cause pharmacological effects (Russo and Guy, 2006). It was shown that CBD and other cannabinoids achieve synergy with Δ^9THC causing potentiation of benefits, antagonism of adverse effects, summation, pharmacokinetic advantages, and metabolism (Russo and Guy, 2006).

Many countries became more liberal towards medicinal use of *Cannabis sativa* L. (Baker et al., 2003). Recently there has been an increasing interest in development of cannabinoids and *Cannabis sativa* L. preparations as legitimate medicines for a variety of medical applications. Some of them include, but are not limited to, multiple sclerosis, chronic pain, glaucoma, asthma and cardiovascular conditions, and as an antiemetic (Williamson and Evans, 2000). Cannabidoids are very potent compounds and their control in *Cannabis sativa* L. preparations is very important, especially because their potential users are patients which already have serious health problems.

The aim of this study was to apply our in-house HPLC/DAD method on the *Cannabis sativa* L. preparations present in the European market and to control their quality.

Materials and methods

The samples of *Cannabis sativa* L. preparations were purchased from the European market, produced by Endoca (Pharmaceutical Company, Denmark). The standard substances were obtained from Lipomed AG (Switzerland). The samples of standard substances were: Δ^9-THC (3-pentyl-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-dibenzo(b,d) pyran-1-ol), delivered as 1 mL ampule solution (5 mg/ mL prepared in ethanol, with purity 98.52%, *m/m*) and CBD ((-)-*trans*-2-p-mentha-1,8-dien-3-yl-5-pentylresorcinol) as the solid substance (99.73%, *m/m*). The solvents used during the analysis were with HPLC grade.

For the chromatographic analysis Agilent Technologies HPLC system 1200 series (Germany) was used. The analysis was performed on HPLC-column: Purospher® Star RP18e (150 mm x 4.6 mm ID, 5 µm) from Merck KGaA (Germany) using mobile phase composed of acetonitrile and water in gradient mode with acetonitrile from 50%, *V/V*, to 80%, *V/V*, at flow rate 1.5 mL/min, temperature 30 °C, detection at 220 nm, injection 10 µL, in 31 min run time.

The working solutions of standard substance CBD were prepared in methanol in two concentration ranges:

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95.48 µg/mL – 286.43 µg/mL and 103.13 µg/mL – 309.40 µg/mL. The Δ^9THC working solutions were prepared in methanol in concentration range: 9.81 µg/mL – 19.63 µg/mL.

The sample solutions were prepared by liquid/liquid extraction technique using a mixture of methanol and chloroform (9:1). When the layers were clearly separated, the sample from the methanolic layer was applied in HPLC/DAD system.

Results and discussion

Five different *Cannabis sativa* L. preparations purchased from the European market were analyzed. All were prepared from hemp oil with various amounts of CBD as active compound and naturally contains Δ^9-THC, as an impurity. According to the certificates the amount of CBD was: 3%; 5%; 10 %; 15% and 30%, *m/m*, and expected impurity as Δ^9THC was limited at maximum of 0.20%, *m/m*.

For preparation of the samples we used the recommended United Nations Office on Drugs and Crime method (UNODC Manual, 2009). For identification and quantification of cannabinoids we used our in-house chromatographic gradient method which we have proposed for hemp seed oil analysis (Shishovska et al., 2014) because of the similarity of samples in origin and form.

In the analyzed samples of hemp oil preparations cannabidiol and Δ^9THC were identified and quantified. Identifications were done by comparing of the retention times and UV spectra of standard compounds with retention times of peaks at the chromatograms of samples and their UV spectra. The retention times of CBD and Δ^9THC peaks were 15.9 min and 21.0 min, respectively.

For the quantification, data obtained from the chromatograms were calculated using the method of the calibration curve (constructed as concentration, c (µg/ml), versus peak area, A (mAU)). The analyzed working solutions of the active compound CBD and the trace compound Δ^9THC showed high linearity in the working ranges. The estimated coefficient of the linearity for CBD working solutions at concentration range: 95.48 µg/mL – 286.43 µg/mL was 1.000 ($A = 23688\ c + 16.188$); while for the CBD working solutions at concentration range: 103.13 µg/mL – 309.40 µg/mL it was 0.9998 ($A = 39613\ c + 31.129$), and for the working solutions of standard substance Δ^9THC at concentration range: 9.81 µg/mL – 19.63 µg/mL it was 0.9997 ($A = 25269\ c - 7.0738$).

It was found that content of cannabidiol in analyzed samples is from 109.7% to 125.4%, *m/m* of the declared values. The assays of Δ^9THC traces at the samples vary according the concentration of the active compound. As quantities of CBD at samples were lower (3 – 5%), the assay of Δ^9THC is under the allowed maximum limit (0.02% – 0.14%), but at samples with higher concentrations of CBD (10 – 30%) the Δ^9THC traces are above the allowed maximum limit (0.45% – 1.14%).

Conclusion

The satisfactory chromatographic data proved that our in-house gradient HPLC/DAD method can be successfully used for determination of the active substance CBD and traces of Δ^9THC in *Cannabis sativa* L. preparations. The results obtained for the analyzed samples of *Cannabis sativa* L. preparations showed higher amounts of CBD than declared value in the certificate (differences range from 9.7% *m/m* to 25.4%, *m/m*). In two samples, the amount of Δ^9THC found was under allowed maximum limit of 0.20%, while in three samples the amount was above that value. Not all of the samples tested have composition as declared in the certificate, although they all origin from a European manufacturer and are present in the European market.

References

Baker, D., Pryce, G., Giovannoni, G., Thompson, A.J., 2003. The therapeutic potential of cannabis. Lancet Neurol. 2(5), 291–298.

Costa, B. 2007. On the pharmacological properties of Δ^9tetrahydrocannabinol (THC). Chem Biodiv. 4, 1664–1677.

ElSohly, M.A., Slade, D., 2005. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci. 78, 539–548.

Fischedick, J.T., Glas, R., Hazekamp, A., Verpoorte, R., 2009. A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in Cannabis sativa L. Phytochem Anal. 20, 421-426.

Russo, E., Guy, G.W., 2006. A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses 66, 234–246.

Shishovska, M., Doneva, D., Starkoska, K., Arsova-Sarafinovska, Z., 2014. Determination of Δ^9-tetrahydrocannabinol by HPLC/DAD in food supplement samples of hempseed oil. Presented at: XXIII Congress of Chemists and Technologists of Macedonia, Ohrid, Macedonia, AC-018.

Turner, C.E., ElSohly, M.A., Boeren, E.G., 1980. Constituents of Cannabis sativa L. XVII. A review of the natural constituents. J Nat Prod. 43(2), 169–234.

United Nations Office on Drugs and Crime, 2009. Recommended methods for the identification and analysis of cannabis and cannabis products; Manual for use by national drug analysis laboratories, United Nations, New York, pp. 41-43.

Williamson, E.M., Evans, F.J., 2000. Cannabinoids in clinical practice. Drugs 60(6), 1303–1314.

Poster presentations