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Medicinal cannabis: Principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method

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ABSTRACT

In the last few years, there has been a boost in the use of cannabis-based extracts for medicinal purposes, although their preparation procedure has not been standardized but rather decided by the individual pharmacists. The present work describes the development of a simple and rapid high performance liquid chromatography method with UV detection (HPLC-UV) for the qualitative and quantitative determination of the principal cannabinoids (CBD-A, CBD, CBN, THC and THC-A) that could be applied to all cannabis-based medicinal extracts (CMEs) and easily performed by a pharmacist. In order to evaluate the identity and purity of the analytes, a high-resolution mass spectrometry (HPLC-ESI-QTOF) analysis was also carried out. Full method validation has been performed in terms of specificity, selectivity, linearity, recovery, dilution integrity and thermal stability. Moreover, the influence of the solvent (ethyl alcohol and olive oil) was evaluated on cannabinoids degradation rate. An alternative extraction method has then been proposed in order to preserve cannabis monoterpene component in final CMEs.

1. Introduction

In the last few years, the use of cannabis-based extracts for therapeutical purposes has greatly increased. Nonetheless, cannabis is out of tune with the conventional paradigm of design, development and testing which generally applies to medicines. Indeed, it was pioneered and promoted by patients and their caregivers, instead of scientific researchers or physicians. It is often consumed in its herbal form, using unconventional modes of intake such as smoking, vaporizing, tea or brownies [1]. Moreover, despite the release onto the market of the main cannabis active principle, (−)-Δ^9-*trans*-tetrahydrocannabinol ((6*aR*,10*aR*)-delta-9-tetrahydrocannabinol or Δ^9-THC, Fig. 1), as a synthetic derivative, the employment of the drug or its medicinal extracts is still widespread. This is due to the higher efficacy towards the treatment of specific pathologies and to a lower onset of side effects compared to the synthetic drug [2,3]. In fact, cannabis-based medicinal extracts (CMEs) can improve neurogenic symptoms unresponsive to standard treatments [4]. Even though still very little is known about the chemical composition of CMEs, more and more in-depth studies of the pharmacological properties of the different active principles present in cannabis inflorescences have allowed for their application in several pathologies [5,6]. Indeed, cannabis is used to reduce nausea and vomiting during chemotherapy, to improve appetite in people with HIV/AIDS, to treat chronic pain and help with muscle spasms [7,8].

Since the chemical composition largely varies among the cannabis varieties employed the Bedrocan BV company has recently developed a few cannabis strains with a standardized composition of cannabinoid active principles. In particular, the

Abbreviations: ACN, acetonitrile; CBD, cannabidiol; CBD-A, cannabidiolic acid; CBN, cannabinol; CBN-A, cannabinolic acid; CME, cannabis-based medicinal extract; CMER, cannabis-based medicinal extracts prepared under refluxing; DAD, diode array detector; ESI, electrospray ionization; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; THC, (−)-Δ^9-*trans*-tetrahydrocannabinol; THC-A, tetrahydrocannabinolic acid; UV, ultraviolet.

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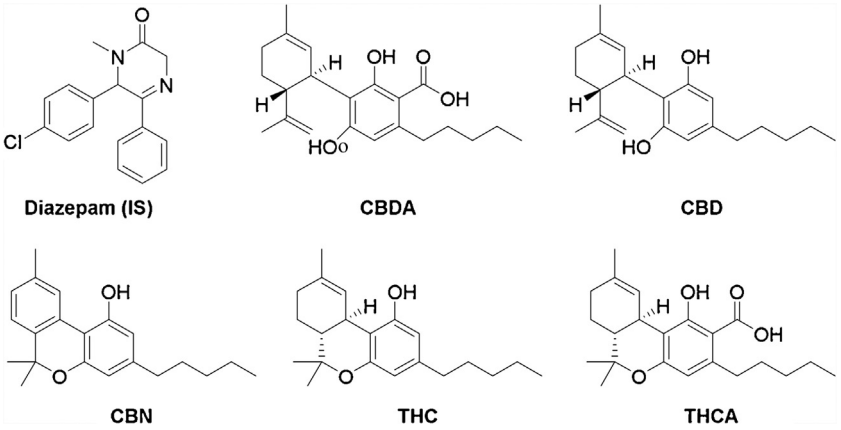

Fig. 1. Molecular structure of Diazepam (IS), CBD-A, CBD, CBN, THC and THC-A.

Bedrocan® strain contains 22% (w/w) THC, while the Bediol® strain contains 6.3% (w/w) THC and 8% (w/w) cannabidiol (CBD, Fig. 1). Other strains are Bedrobinol® with 13.5% (w/w) THC, Bedrolite® with <0.4% (w/w) THC and 9% (w/w) CBD and Bedica® with 14% (w/w) THC. The main cannabinoids THC and CBD are found in the plant in the carboxylated form (tetrahydrocannabinolic acid or THC-A and cannabidiolic acid or CBD-A, Fig. 1). Although these compounds possess several pharmacological properties, they do not display psychotropic activity simply because they are not able to cross the blood-brain barrier. When subjected to heat they undergo decarboxylation to give the psychoactive THC and CBD. THC is the main responsible for the psychotropic activity known for cannabis, whilst CBD seems to have analgesic and antioxidant activity and to reduce THC side effects [9–11]. If cannabis is exposed to air for a prolonged period of time, THC-A will convert to cannabinolic acid (CBN-A). Decarboxylation of CBN-A will then give cannabinol (CBN). CBN (Fig. 1) is a weak psychoactive cannabinoid with mostly anticonvulsant activity [12] and mild analgesic properties [13].

Anyway, it has been widely demonstrated that the effect of the single cannabinoids is different from that of the whole CME [14,15]. It has been suggested that cannabinoids in their acidic form present in CMEs can also intervene in the pharmacological activity [16]. Hence, it is important to determine the main cannabinoids in both their forms in order to correlate the individual amount present in the CMEs with the pharmacological effects. To this end, the most widely employed methodology is gas chromatography [17,18]. Anyway, this method involves the heat of the mixture prior to its chromatographic separation resulting in the partial decarboxylation of the cannabinoids in their acid form. Therefore, this methodology cannot be employed to evaluate the actual composition of cannabinoids present in the cannabis-based preparations without a preliminary derivatization step. Liquid chromatography (LC) based methods, instead, do not cause any sample decomposition since the analysis is conducted at room temperature. The literature reports various LC-based methods with either ultraviolet (UV) or mass spectrometry (MS) detection for the qualitative and quantitative determination of cannabinoids [18–22]. Although liquid chromatography enables the determination of the cannabinoid composition, it does not allow to establish the terpenoid composition of CMEs. In this case, the use of gas chromatography is mandatory.

Even though there is a large increase in the use of cannabis-based preparations for medicinal purposes [23], their preparation procedure has not been standardized but rather decided by the individual pharmacists. Recently, the Italian legislation has imposed the qualitative and quantitative determination of the main cannabinoids in each CME by a specific and selective chromatographic method [24]. The primary goal of this work was to develop a simple and cost effective chromatographic method that can be easily performed by a pharmacist who dispenses his own CMEs. The method developed has allowed to evaluate how the cannabinoids composition (acid and neutral form) can change depending on the temperature and time of extraction of the drug. In order to evaluate the identity and purity of the analytes, a high-resolution mass spectrometry analysis was also carried out.

It has been recently suggested that the volatile terpenoid composition can also contribute to the pharmacological activity of cannabis-based drugs [2,25]. Therefore, it is important to avoid any loss in terpenes, which is caused by their evaporation. Hence, a simple way to preserve such component in CMEs has been proposed. Gas chromatography coupled to mass spectrometry (CG-MS) analyses were performed in order to evaluate the variation in the terpenoid composition during the preparation.

2. Material and methods

2.1. Materials

All chemicals and reagents, except those specifically noted, were purchased from Sigma-Aldrich. Acetonitrile, water, 2-propanol, formic acid LC–MS grade was purchased from Carlo Erba (Milan, Italy). Special refined olive oil was bought from Fagron Italia Srl (Bologna, Italy). Ethyl alcohol was of pharmaceutical grade bought from Carlo Erba (Milan, Italy). THC and CBD were purchased from SALARS (Como, Italy). CBN, THC-A and CBD-A from Echo Pharmaceuticals B.V. (Weesp, The Netherlands). All CMEs (Bediol® olive oil, ethyl alcohol and carbon dioxide medicinal extracts) were prepared by Farmacia Tundo Dr. Alfredo (Alliste, Lecce, Italy) employing a final ratio of 1 g of cannabis inflorescence in 10 mL of either olive oil or ethyl alcohol.

2.2. LC-UV analysis

High performance liquid chromatography (HPLC) analyses were performed on an Agilent Technologies (Waldbronn, Germany) modular model 1200 system, consisting of a vacuum degasser, a binary pump, a thermostated autosampler, a thermostated column compartment and a diode array detector (DAD). The chromatograms were recorded using an Agilent Mass Hunter software (Rev. B.01.04). A Poroshell 120 C18 column (Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 μm, Agilent, Milano, Italy) was used with a mobile phase composed of 0.1% formic acid in both (A) water and (B) acetonitrile (ACN). The isocratic elution was set at a flow rate of 0.5 mL/min. The run time was 10 min. The column temper-