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Cloud point extraction of Δ^9-tetrahydrocannabinol from cannabis resin

S. Ameur · B. Haddou · Z. Derriche · J. P. Canselier · C. Gourdon

Abstract A cloud point extraction coupled with high performance liquid chromatography (HPLC/UV) method was developed for the determination of Δ^9-tetrahydrocannabinol (THC) in micellar phase. The nonionic surfactant “Dowfax 20B102” was used to extract and pre-concentrate THC from cannabis resin, prior to its determination with a HPLC–UV system (diode array detector) with isocratic elution. The parameters and variables affecting the extraction were investigated. Under optimum conditions (1 wt.% Dowfax 20B102, 1 wt.% Na_2SO_4, T=318 K, t=30 min), this method yielded a quite satisfactory recovery rate (~81 %). The limit of detection was 0.04 $\mu g mL^{-1}$, and the relative standard deviation was less than 2 %. Compared with conventional solid–liquid extraction, this new method avoids the use of volatile organic solvents, therefore is environmentally safer.

Keywords Cannabis · Δ^9-Tetrahydrocannabinol · Cloud point extraction · Coacervate · HPLC · Diode array detector · Nonionic surfactant

Abbreviations

ASE	Accelerated solvent extraction
CBD	Cannabidiol
CBN	Cannabinol
CPE	Cloud point extraction
CPT	Cloud point temperature
DAD	Photodiode array detector
HPLC	High performance liquid chromatography
LLE	Liquid–liquid extraction
LOD	Limit of detection
RSD	Relative standard deviation
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
THC	Δ^9-Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid

Introduction

Cannabis (*Cannabis sativa* L.) is the most common illegal drug-producing plant in the world [1]. Fresh cannabis may be consumed orally, but more often, its herbal form, marijuana, is vaporized, and the vapor is inhaled. Besides, its resinous extract, hashish, is smoked or eaten in cannabis foods, as a mix with cannabis essential oils (“hash oil”). Cannabis finds uses in the medical field, as a drug against nausea, e.g., caused by chemotherapy, a stimulant of appetite, e.g., for the patients suffering from AIDS, a substance lowering intraocular pressure (effective for treating glaucoma), and a pain reliever [2, 3].

Several hundreds of constituents have been isolated and identified in the cannabis plant, but many factors have been found to affect the chemical composition of *cannabis* resin, including genetic factors, soil, climate, plant maturity at harvest, and storage conditions. Among these various components, terpenes and sesquiterpenes, terpenoids, flavonoids, nitrogenous compounds [4] and, more specifically, a group of terpenophenolic compounds known as cannabinoids have been identified. The four main ones are Δ^9-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), cannabinol (CBN), and cannabidiol (CBD), but large variations in their amounts and distribution have been found [5–11].

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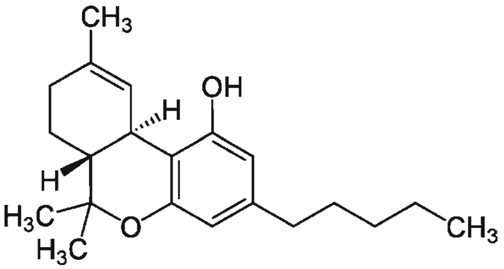
The THC content in hashish may average 5 wt.% [12–15]. Acting on the central nervous and cardiovascular systems, it is the compound to which most of the pleasant effects of cannabis are usually assigned: euphoria, the feeling of good being and relaxation. THC has been reported to prevent cerebral infarction [16], but at high doses, THC also produces tachycardia and hallucinations. THCA, which is present in abundance in some cannabis samples, is itself inactive but is converted by smoking into active THC; CBN and CBD, which may be present in large amounts, are not psychoactive but only possess sensory activity [17, 18]. The pharmacological aspects of CBD have been reviewed [19]. In particular, CBD has been shown to reduce or even counteract the anxiogenic effect of THC, possibly due to contrary actions (agonist vs. antagonist, respectively) at the cannabinoid receptor [20].

Marijuana chemistry has been first summarized in 1970 [21]. THC, also known as dronabinol, its synthetic form ((−)-(6a*R*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol, $C_{21}H_{30}O_2$, MW: 314.45, CAS number: 1972-08-3; Scheme 1), had been isolated from *C. sativa* L. and studied (chemical structure and partial synthesis) in 1964 [22]. It appears as a glassy solid at room temperature (M.P.=160.3 °C, B.P.=200 °C/2.0·10^{-2}Torr) [23], almost insoluble in H_2O (2.8 mg.L^{-1} at 23 °C) [24] but soluble in most organic solvents, especially alcohols and lipids. The reported log *P* values range from 3.78 [25] to 7.6 (predicted) [26]; a most likely estimate could be 6.4 (from high performance liquid chromatography (HPLC) method) [27].

Generally, the cannabinoids are extracted with different solvents, including methanol, diethyl ether, hexane [28], or their mixtures [5, 29, 30]. Although a new method for sample preparation has been proposed in the early 1990s (solid-phase microextraction (SPME)) [31], accelerated solvent extraction (ASE) [32] is more often used.

Conventional solid-phase extraction (SPE) and classical liquid–liquid extraction (LLE) are still employed extensively [29, 30]. Classical LLE consumes large amounts of toxic organic solvents, which are evaporated later. The main disadvantages of SPE are the time-consuming and multi-step features of the process, and the high cost of the columns [33].

An alternative approach to the sample preparation process is an extraction technique using surfactants above their cloud point. The application of cloud point extraction (CPE) in analytical chemistry has received much attention and has given rise to numerous research works. Firstly, the CPE technique, introduced by Watanabe and Tanaka in 1978 [34], was used for the preconcentration of metal ions from aqueous samples. It was then extended to the extraction of proteins, enzymes, and other biological substances [35]. Then, many authors highlighted the effectiveness of CPE for the elimination of organic pollutants [36–44].



Scheme 1 Chemical structure of Δ^9-tetrahydrocannabinol (THC)

CPE provides the possibility of extracting and preconcentrating analytes in a single step using a simple procedure [45–49]. The performance of a CPE process is influenced by many factors, such as the cloud point temperature (CPT) and concentration of surfactants and the physicochemical properties of solutes themselves. CPE from solid samples is another important area that needs more investigation. Hence, the aim of the present report is, for the first time, to give a comparative study of the extraction and preconcentration of THC from cannabis resin between conventional solid–liquid extraction and the competitive CPE technique from solid, prior to its HPLC determination with UV (diode array) detector. The final goal of our work is the analysis of THC in body fluids (work in progress).

Materials and methods

Chemicals

The nonionic surfactant used in this work was biodegradable: “Dowfax 20B102” (Dow Chemical Co.), belonging to the poloxamer family (ethylene oxide–propylene oxide copolymers).

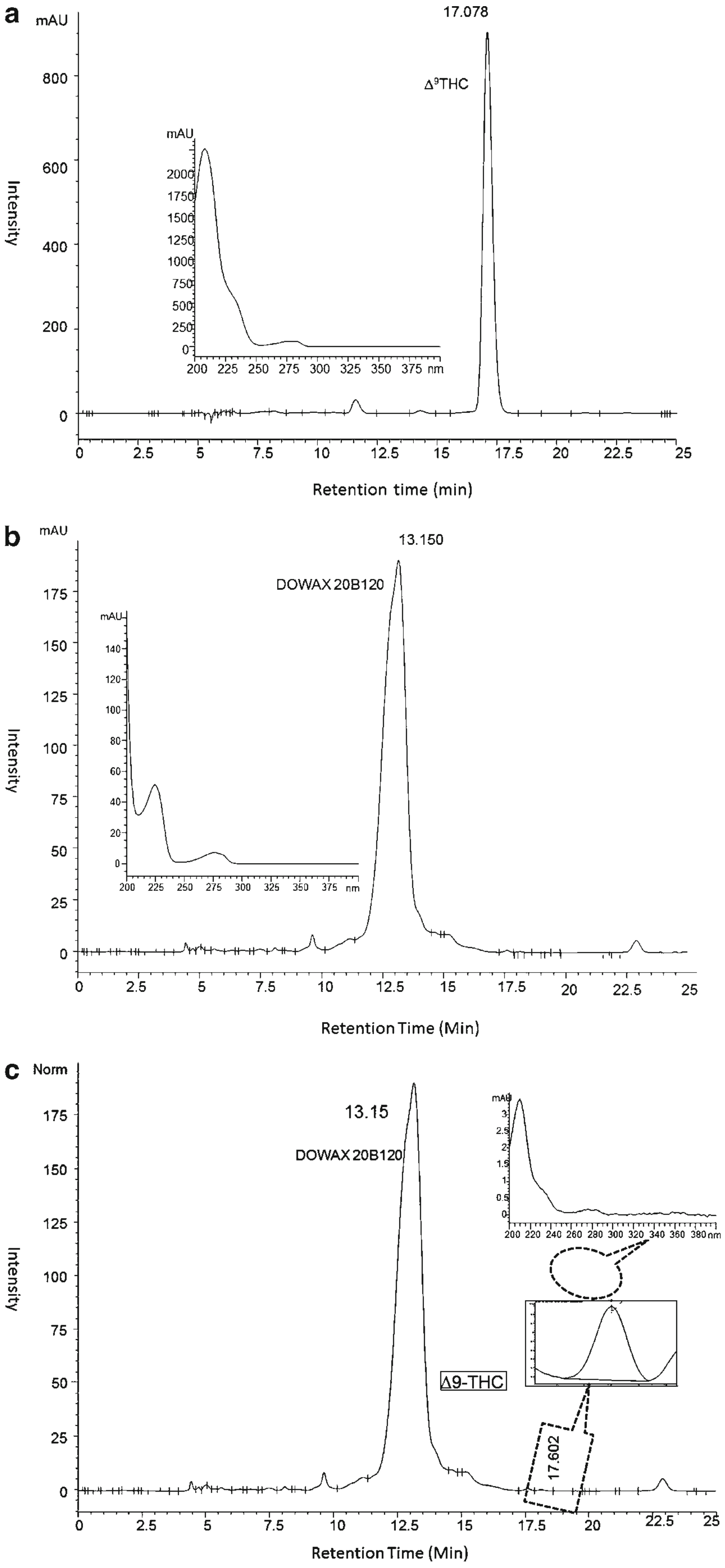
Reference THC was supplied by Lipomed (Arlesheim, Switzerland). An appropriate amount was diluted with methanol to prepare a 200-µg/mL mother solution, which was further diluted with methanol to prepare working solutions. As the distribution of the principal cannabinoids within bars of compressed cannabis resin is not homogeneous [5], a cannabis resin sample (UNC 491) from the UN Office on Drugs and Crime (Laboratory and Scientific Section, Vienna, Austria) was used in this work as a guide.

All solvents were purchased from Merck KGaA (Germany) and other chemicals from Fluka (Buchs, Switzerland). Deionized water was used to prepare all the solutions, which were filtered through a membrane (0.45 µm) before use.

Apparatus

The HPLC system consisted of a quaternary pump (model G1311A), an auto sampler (model G1313A), a Hypersil BDS C18 column (250×4.6 mm, particle size 5 µm) obtained from Thermo Electron Corporation (Waltham, MA, USA), and a photodiode array detector (DAD, model

Fig. 1 HPLC chromatogram of (**a**) Dowfax 20B102, (**b**) THC (standard solution), and (**c**) solution containing THC and Dowfax 20B102; direct injection of micellar phase into $CH_3CN:H_2O$ (83:17) mobile phase, acidified to pH 1.8 with 0.5 mL H_2SO_4 (2.5 M)



G1315B). The data acquisition and processing were performed with the Chemstation software. Unless stated otherwise, the whole equipment was purchased from Agilent Technologies (Santa Clara, CA, USA). A centrifugal machine (model CT20, Prolabo, France) was used to induce phase separation. A thermostated bath was used to maintain the desired temperature within ±0.5 °C.

Method

Cloud point extraction from cannabis resin

The experimental conditions for the CPE of THC from cannabis resin were optimized: 5 mg cannabis resin were placed in a flask with 0.1 to 0.7 g Dowfax 20B102 and, possibly, up to 0.2 g Na_2SO_4. The mixture was diluted to 10 mL with deionized water, shook for 10 min, and extracted at a temperature ranging from 40 °C to 90 °C for 1 to 4 h (see "Results and discussion" section below). The extract was filtered and placed in a graduate cylinder.

To induce phase separation of the aqueous surfactant solution and preconcentration of the cannabis extract into the surfactant-rich phase (coacervate), an appropriate amount of Na_2SO_4 was added to some sample solutions, which were then vigorously shaken for 10 min to dissolve the salt and kept in a constant temperature bath at 45 °C for half an hour. Separation of the cloudy solution into two distinct phases was then achieved via centrifugation for 10 min at 4,000 rpm.

HPLC–UV analysis

The separation and determination of THC were carried out by directly injecting 5 µl of the extract (coacervate) into the HPLC–UV (DAD) system under the following conditions: mobile phase $CH_3CN{:}H_2O$ (83:17) acidified with 2.5 M H_2SO_4 (final pH=1.8) with isocratic elution. The flow rate was 1 mL min^{-1}. THC was recorded at a wavelength of 231 nm.

Peaks in the chromatograms were identified by comparison with retention times and reference spectra of THC and Dowfax 20B102.

On the chromatogram of the aqueous surfactant Dowfax 20B102 solution containing THC, Dowfax 20B102 appears at a retention time of 13.7 min and THC at about 17.5 min, free from any interference (Fig. 1).

Results and discussion

Binary and pseudo-binary phase diagrams

To ensure CPE at the desired temperature, it is imperative to get detailed information on the clouding behavior and CPT of the surfactant solution.

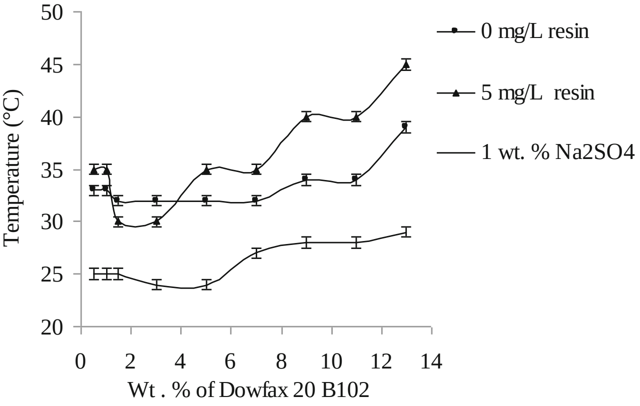


Fig. 2 Effect of cannabis resin and electrolyte (Na_2SO_4) on the cloud point temperature of Dowfax 20B102

Figure 2 shows the cloud point curve of pure Dowfax 20B102 in water (±0.5 °C). Below the curve, there exists only one liquid phase, i.e., a micellar phase commonly denoted as L_1, whereas two coexisting liquid phases are found in the region above the curve: a rich micellar phase L_1 (coacervate) and a dilute phase (W).

Near the ordinate axis of the phase diagram (pure H_2O), the CPT of a surfactant steeply decreases with increasing surfactant concentration, then gradually rises with a further increase in surfactant concentration, which is the typical clouding behavior of nonionic polyethoxylated surfactants [50–52]. However, no experimental data related to very low Dowfax 20B102 concentrations were collected.

The addition of salt or resin decreases the CPT. Na_2SO_4 lowers the CPT because of a further dehydration of ethylene oxide units, due to salt solvation [53]. This electrolyte is structure making, so that water is less available to hydrate micellar aggregates.

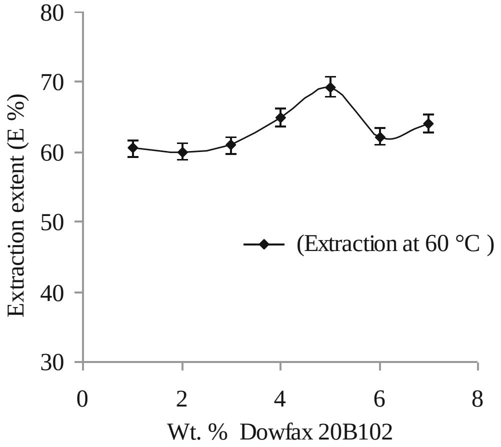


Fig. 3 Effect of Dowfax 20B102 concentration (weight percent) on extraction recovery (E%); CPE conditions: T=45 °C, t=30 min

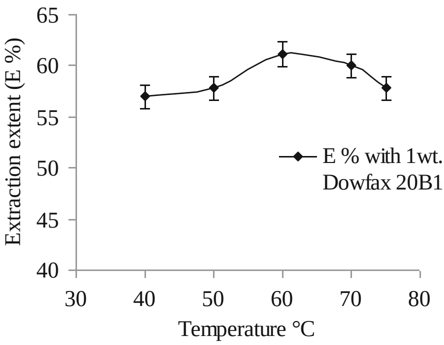


Fig. 4 Effect of temperature (*T*, degrees Celsius) on extraction recovery (*E*%)

Optimization of the extraction/preconcentration process

In the 0.05–200-μgmL^{-1} concentration range, the equation of the linear calibration curve (least squares regression) was

$$C = 0.008A + 4.711\ \left(R^2 = 0.994\right)$$

with *C*: THC concentration (micrograms per milliliter), and *A*: peak area (arbitrary unit).

The limit of detection (LOD) was 0.04 μgmL^{-1}. The precision of the quantitative analysis was evaluated through six replicate determinations (at C=5 μgmL^{-1}), and the relative standard deviation (RSD) was within 2 %.

Effect of surfactant concentration and temperature

The amount of surfactant required to achieve quantitative extraction of the analyte was studied. Figure 3 reveals that the extraction efficiency of THC from cannabis resin remains relatively constant (from 60.5 % to 63.7 %) when Dowfax 20B102 concentration increases. The effect of temperature on the efficiency of THC extraction from cannabis resin is illustrated in Fig. 4 for a 0.05 wt.% resin and 1 wt.% Dowfax 20B102 solution and an extraction duration of 1 h. Extraction of THC from cannabis resin at 60 °C appears adequate; above this temperature, THC degrades and the extraction extent decreases.

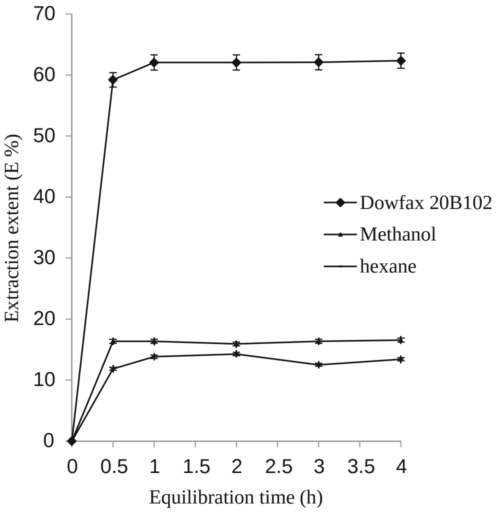


Fig. 5 Comparison of extraction kinetics and recoveries between Dowfax 20B102 (1 wt.%) methanol and hexane extractions. Effect of equilibration time (*t*) on extraction recovery (*E*%)

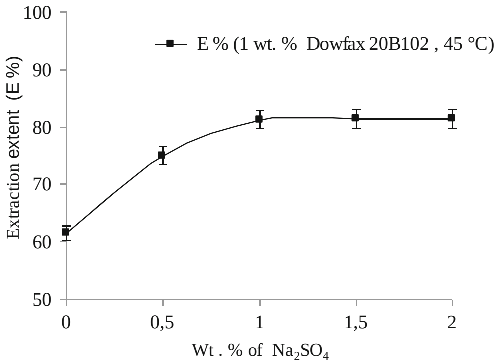


Fig. 6 Effect of salt (Na_2SO_4) on extraction recovery (*E*%)

Comparison of extraction kinetics and THC recovery between Dowfax 20B102 solution and organic solvents

Percentage of recovery (*E*%) and extraction kinetics experiments were performed: the amounts of THC (initially contained in the UNC 491 sample) obtained from single extractions, using aqueous surfactant solution or methanol as the extractant, were compared with those obtained from multiple extractions using methanol. The experimental conditions for multiple extractions were as follows: number of extractions, 3; volume of methanol per extraction, 10 mL; and maceration time, 24 h. The *E*% value for multiple extractions was considered as a reference (i.e., 100 % efficiency, equivalent to 7.71 wt.% THC). Figure 5 shows the variation in recovery of THC from cannabis resin as a function of extraction time (over a period of 4 h) for three different extractants: 1 wt.% Dowfax 20B102 solution, methanol, and hexane. Extraction volumes were identical. From the data shown in Fig. 5, it is interesting to note that a single extraction with the surfactant solution (62 % yield,

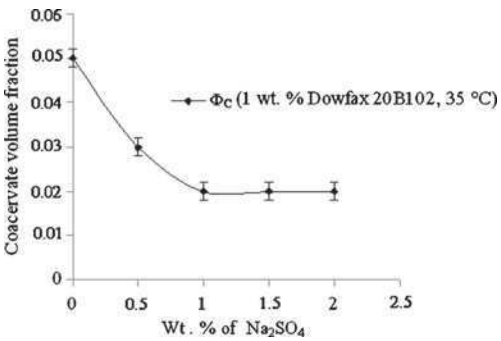


Fig. 7 Effect of salt (Na_2SO_4) on the volume fraction of coacervate, ϕ_c

equivalent to 5.0 wt.% THC extracted from UNC 491) is much more efficient than with methanol (16.5 % yield, equivalent to 1.3 wt.%) and even more than with hexane (14.2 % yield, equivalent to 1.1 wt.%).

In previous studies, different organic solvents were used: using methanol as an extractant (5.0 g sample in 100 mL solvent), Kaa [54] found 0.26 % to 4.89 % THC in cannabis plants illicitly grown in Jutland (Denmark). Besides, with various procedures (e.g., Soxhlet [7] or shaking [55, 56]), other authors used chloroform [55, 56], petroleum ether, *n*-hexane, ethyl acetate, or solvent mixtures: methanol:petroleum ether (1:9), methanol:chloroform (4:1 or 9:1). According to Lewis et al. [5], ethyl acetate and *n*-hexane gave the best performances. For a single extraction with hexane, our findings do not confirm those previous results. Anyway, the latter is no more recommended for safety reasons.

Effect of equilibration time

The effect of the equilibration time on *E*% was studied by analyzing the extract of THC solution in the presence of 1 wt.% Dowfax 20B102 and 1 wt.% Na_2SO_4 kept above the CPT (60 °C) for different periods of time ranging from 0.5 to 4 h. The results obtained show no significant variation of the recovery of the analyte vs. equilibration time after 30 min (Fig. 5), so a period of 30 min was chosen for further studies.

Effect of salt

Adding salt to an aqueous system can increase the incompatibility between the water structure in hydration shells of ions and surfactant molecules, which can reduce the concentration of "free water" in the coacervate and, consequently, reduce the volume of the phase [37–43]. Now, SO_4^{2-} is more effective in this respect than Cl^-, and the behaviors of NaCl and Na_2SO_4 have been compared [38]. In order to determine this effect on the extraction process, different amounts of Na_2SO_4, ranging from 0.5 wt.% to 2 wt.%, were tested. The results show (Figs. 6 and 7) that Na_2SO_4 improves the extraction extent (from 60.5 % to 81.34 %) and reduces the volume fraction of coacervate (from 0.05 to 0.02). These results are in good agreement with the literature [37–43].

Conclusion

In this study, the results obtained indicate that CPE of THC from cannabis resin (reference: UNC 491), using Dowfax 20B102, is quite efficient. Thus, this technique is a potentially powerful tool for the solubilization, purification, and preconcentration of active substances from solid extract. As an extraction technique, CPE can be a good alternative to other traditional processes and offers many interesting advantages, providing the possibility of extracting and preconcentrating analytes in a very simple, single-step procedure, without needing to use expensive and potentially toxic organic solvent. It is also cheaper than other conventional extraction processes like LLE and SPE: it is not necessary to evaporate the solvent, no analyte is lost as a result of the process, and the extract is compatible with the mobile phase used in HPLC.

Furthermore, the proposed method has also opened up new possibilities in the separation and concentration of other bioactive drugs.

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