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Green cannabigerol purification through simulated moving bed chromatography

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ABSTRACT

Cannabigerol (CBG) is a minor cannabinoid present in *Cannabis sativa* L. This molecule is gaining increasing popularity thanks to its antibacterial, antimicrobial, antidepressant and antitumoral properties. In parallel, there is growing attention towards the search of efficient, cost-effective, rapid, high-throughput, and green purification techniques.

In this work, CBG has been purified from a real cannabis extract by means of simulated moving bed chromatography. The proposed application is very promising, allowing to achieve a CBG extract free of tetrahydrocannabinol (a psychoactive cannabinoid) with 100% recovery and 97% final purity by using a faster and greener method if compared to traditionally used ones.

1. Introduction

Due to the progressive decriminalization of recreational cannabis and the therapeutic use of cannabis-based drugs, more and more attention has been paid on the properties of this controversial plant. To date, the total number of phytochemicals identified in *Cannabis sativa* L. exceeds 500 [1]. Among those, natural occurring cannabinoids, also known as phytocannabinoids, are the most studied ones. Cannabinoids are a group of terpenophenolic compounds with a characteristic C_{21} backbone (or C_{22} if in acidic form) that are responsible for many therapeutic effects in different types of diseases and disorders [2–7]. Undoubtedly, the most well-known and investigated ones are the non-psychoactive cannabidiol (CBD) and the psychoactive Δ^9-tetrahydrocannabinol (THC), thanks to their natural abundance in the plant.

In addition to CBD and THC, other minor non-psychoactive cannabinoids, such as cannabigerol (CBG) and cannabichromene (CBC), have shown interesting positive features. According to the amount (or potency) of these cannabinoids, different phenotypes of *Cannabis sativa* can be identified [2]: chemotype I (drug type), with a high THC content (THC/CBD>1); chemotype II (intermediate), with roughly the same CBD and THC content; chemotype III (fiber), with a high CBD content and THC < 0.3%; chemotype IV, with a prevalence of CBG (> 0.3%) and CBD (< 0.5%); and chemotype V, with undetectable content of cannabinoids. CBD and THC are used to treat chronic pain and neurological disorders, or as antioxidant and anti-inflammatory agents [2]. CBG was demonstrated to have antibacterial, antimicrobial, antidepressant and antitumoral effects, but only in *in vitro* and *in vivo* studies on animals [2,8,9]. This opens new perspectives and possibilities regarding the potentiality of using CBG also for humans. However, clinical trials in humans require large amounts of pure product (from mg to g).

The purification of phytocannabinoids can be achieved through different techniques, including medium-pressure (flash) chromatography, single-column (batch) and multi-column (continuous or semi-continuous) chromatography [3]. However, the development of efficient, fast and cost-effective methods for the purification of cannabinoids from real samples is still a difficult and challenging task, due to the complexity of the matrix, which includes terpenes, waxes, other cannabinoids, chlorophylls, etc. [10].

Overall, current purification processes are based on a combination of different steps, generally consisting in [11–16]: (i) decarboxylation, (ii) extraction, (iii) precipitation, (iv) filtration, (v) re-dissolution, (vi) purification and (vii) crystallisation steps. Steps (i-v) are necessary in order to convert acidic cannabinoids to neutral cannabinoids and to remove undesirable materials from the sample (such as chlorophylls and waxes). The purification step (vi) is usually obtained via multiple

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chromatographic separations. Solid-liquid techniques include single-column (batch) and multicolumn countercurrent chromatography (MCCC); liquid-liquid techniques include countercurrent chromatography (CCC) or centrifugal partition chromatography (CPC), in which no solid phase is used. Concerning column chromatography, the most used stationary phases consist of modified activated carbon adsorbents, modified hydrophobic adsorbents (styrene-divinylbenzene resin or poly methyl methacrylate resin) or modified hydrophilic adsorbents (silica or activated alumina).

Regarding CBG purification, only two methods have been proposed and patented for its isolation [14,17]. Patent [17] discloses methods able to produce substantially pure CBG (> 90%) using subsequent recrystallizations with a non-polar solvent. In order to increase the purity up to 98%, chromatographic steps can be carried out prior or after each crystallization step. On the other hand, Patent [14] describes a method for preparing enriched CBG extracts or substantially pure CBG preparations starting from cannabis plant material.

Undoubtedly, the methods proposed in Patents [14,17] have high effectiveness in producing highly pure CBG (> 95%). However, they are based on several time consuming steps, that require days or even weeks to obtain the final pure product, and they involve the use of large amounts of toxic solvents, such as chloroform, dichloromethane, hexane, etc., which need to be removed multiple times through evaporation, making these processes highly energy demanding and not environmentally friendly. Moreover, depending on the complexity of the sample, one or more chromatographic steps are required to obtain sufficiently pure CBG. All these drawbacks contribute to making the entire purification process unsustainable.

This points the attention on the need of new methods, applicable to a variety of matrices and effective independently from the initial purity of the target cannabinoid. Moreover, following the current trends of industrial processes and the principles of green analytical chemistry [37], in the last few years more and more attention has been paid on the environmental impact of separation process, focusing on the selection of greener solvents, fast and high performance methods, and processes with an overall small energy requirement. In this regard, the use of high performance column chromatography with stationary phases different from the traditional ones would be beneficial for the intensification of the purification process, in terms of performance parameters (consumption, recovery) and separation efficiency (resolution, selectivity). Indeed, it could allow to reduce the number of steps necessary to reach the targeted product purity with respect to low performance chromatographic methods used in Patents (i.e. flash, liquid/liquid or low pressure gel permeation chromatography). Moreover, the coupling of high performance column chromatography and continuous countercurrent techniques, such as simulated moving bed (SMB), will certainly contribute to making the purification process cost-effective, faster and greener.

In this regard, a recent publication, by some of the authors of this paper, showed the potentiality of some commercial polar stationary phases towards the separation of cannabinoids under normal phase (NP) elution mode [18]. It has been demonstrated that NPLC coupled with polar columns could offer several advantages in terms of (chemo) selectivity, resolution, sample preparation, analysis time, solvent evaporation and sample concentration in preparative conditions.

In this work, a method for the purification and the isolation of CBG from cannabis extracts through continuous multicolumn simulated moving bed chromatography is presented. To the best of our knowledge, this is the first time that a SMB procedure is described for CBG purification.

2. Theory and modeling of simulated moving bed chromatography

SMB is a multicolumn continuous countercurrent chromatographic process, where the simulated countercurrent movement of the stationary phase is achieved thanks to the use of a series of switching valves operated synchronously, that (apparently) change columns positions after a defined switching time. In SMB, the number of columns usually runs from 4 to 16 and the feed is continuously loaded onto the system. SMB has shown higher productivity and lower solvent consumption compared to batch chromatography and fixed-bed adsorption processes [19–22,25].

SMB has been used for different applications in the petrochemical, pharmaceutical, chemical and food sectors, to name but a few, primarily for the separation of binary or pseudo binary mixtures [26,27].

The SMB system is divided into four zones (j= I, II, III, IV) with two inlets (mobile phase and feed) and two outlets (extract and raffinate), as schematically shown in Fig. 1. The feed enters the system continuously between zone II and zone III, that are directly responsible for the separation, while the mobile phase is pumped between zones I and IV, where the complete regeneration of the solid or liquid phase occurs. If a binary mixture is considered, the more strongly retained component will be eluted in the extract fraction, while the less retained one will be obtained at the raffinate outlet (brown and green points, respectively, in Fig. 1).

For the correct design of the SMB process, a numerical model is necessary in order to optimize experimental parameters. Finding the optimal flow rates in each zone and the optimal switching time are the most critical points. The separation and adsorption process can be easily modeled with the help of several simplifying assumptions, starting from the more detailed lumped solid diffusion model to the simplified equilibrium theory model [22,23,28]. The design of the SMB process can be obtained by considering the simplest model of chromatography, the ideal model, where axial dispersion and mass-transfer are negligible and a permanent equilibrium between the solid and liquid phases is assumed [19,22,26,28]. In this case, analytical solutions can be derived for SMB separations. The experimental parameters can then be obtained with the so-called *triangle method* (see later), that indicates the separation region between raffinate and extract [23,28]. Considering the ideal model for all the columns in the SMB unit, the mass balance equation that accounts for the movement of the solid phase and describes the component i in zone j can be written as [28]:

$$\frac{\partial C_{i,j}}{\partial t} + F\frac{\partial C_{s,i,j}}{\partial t} + u_j\frac{\partial C_{i,j}}{\partial z} - u_s\frac{\partial C_{s,i,j}}{\partial z} = 0 \quad \text{with } i = a, b \tag{1}$$

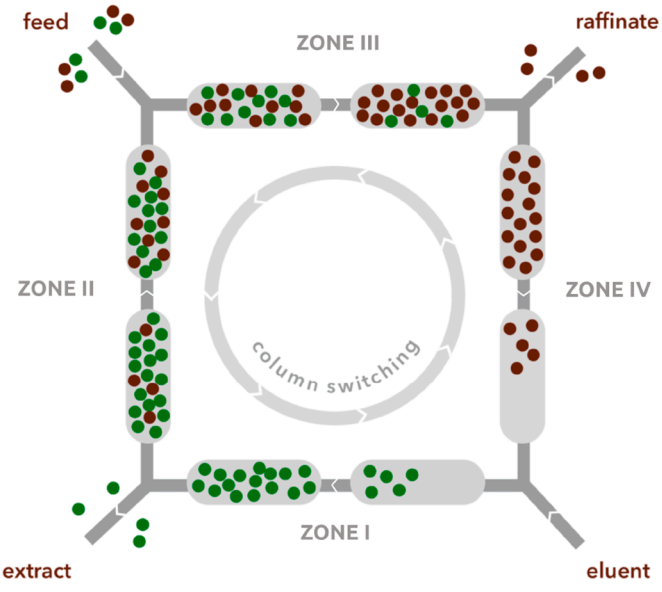

Fig. 1. Schematic representation of SMB chromatography [38]. Brown points: raffinate fraction, green points: extract fraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with a and b raffinate and extract components, z the axial coordinate, $C_{s,i,j}$ and $C_{i,j}$ are the concentrations of the ith analyte in the solid phase and in the bulk mobile phase, respectively, in zone j, $F = (1-\epsilon_t)/\epsilon_t$ the phase ratio, ϵ_t the total porosity of the packed bed given by the ratio between the hold-up, V_0, and the geometric volume, V_{col}, of the column. u_j and u_s are the liquid and solid phase velocities. u_j is directly related to the volumetric flow rate, Q_j, through the following equation:

$$u_j = \frac{Q_j}{A\epsilon_t} \tag{2}$$

with A the cross sectional area of the column. The term $C_{s,i,j}$ in Eq. (1) can be defined by a proper adsorption isotherm. If linear isotherms are considered, *i.e.* assuming no competition for adsorption, $C_{s,i,j}$ can be written as [22,28]:

$$C_{s,i,j} = q_{i,j} = K_i C_{i,j} \tag{3}$$

where K_i is the Henry constant. Since component b is more retained (extract fraction) than component a (raffinate fraction), it follows $K_b > K_a$.

For the modeling of the separation process, boundary conditions need to be defined. Indeed, since columns in the four zones are directly connected, the composition of the fluid phase eluting from the previous column, $C_{i,j}^{out}$, affects the composition of the fluid phase at the inlet of the subsequent column. As a consequence, the inlet concentration of component i in zone (or column) $j+1$, $C_{i,j+1}^{in}$, is dependent on both the node flow and the mass balance equations of node j, based on the following equation:

$$C_{i,j+1}^{in} = \frac{C_{i,j}^{out} Q_j + \delta_{i,j}}{Q_{j+1}} \tag{4}$$

with $\delta_{i,j}$ a parameter related to the mass flow of component i based on the node j [24]. Boundary conditions must be constantly updated, since they change after each switch, operated at a defined switching time (t^*). However, the solution of the ideal model suggests that each component is associated with a constant migration velocity.

The SMB process is characterized by four internal (Q_j where j=I,II,III, IV is the SMB zone) and four external (Q_D=desorbent, Q_E= extract, Q_F= feed, and Q_R= raffinate) volumetric flow rates, which are linked through the following overall fluid phase balances (for the complete description of integral mass balance equations the reader is referred to [23,28]):

$$Q_I = Q_{IV} + Q_D \tag{5a}$$

$$Q_{II} = Q_I - Q_E \tag{5b}$$

$$Q_{III} = Q_{II} + Q_F \tag{5c}$$

$$Q_{IV} = Q_{III} - Q_R \tag{5d}$$

These flow rates are pivotal in the simulation of the separation process for the definition of the component mass balances. Thus they represent the key point to achieve the complete separation between raffinate and extract fractions.

Under these conditions, one of the most widely established method for the identification of the most suitable flow rates reported in Eq. (5) is the so-called *Triangle theory* [29–33]. This method defines a triangular separation region obtained by plotting the dimensionless number, m_j, relative to zones II and III (see Fig. 2). m_j is defined as the ratio of liquid (Q_j) to solid (Q_s) flow rates in zone j [34]:

$$m_j = \frac{Q_j}{Q_s} = \frac{Q_j t^* - V_{col}\epsilon_t}{V_{col}(1-\epsilon_t)} \tag{6}$$

where V_{col} is the volume of the chromatographic column and t^* is the switch time of the SMB unit. It follows that, for linear isotherms, the complete separation of the binary mixture can be achieved only if the following inequalities are satisfied:

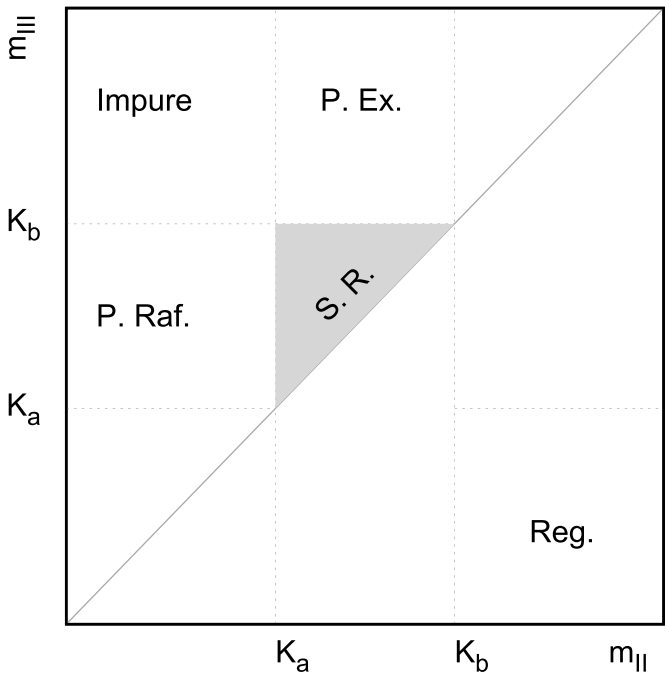

Fig. 2. Triangle theory obtained for linear adsorption isotherms for the separation of a binary mixture of a and b components, with b the more retained one. K_a and K_b are the Henry constants of adsorption and m_j the dimensionless internal flow ratios for zone j. P. Raf.: region where pure raffinate is obtained, P. Ex.: region where pure extract is obtained, S. R.: separation region, where both raffinate and extract are pure, Impure: region where no pure outlet is obtained, Reg.: regeneration region of zones I and IV.

$$\begin{aligned} K_b &\le m_I \\ K_a < m_{II} &\le K_b \\ K_a \le m_{III} &\le K_b \\ m_{IV} &\le K_a \end{aligned} \tag{7}$$

The triangular region, relative to the complete separation (Separation Region, S. R., Fig. 2), is delimited by the equilibrium constants K_a and K_b. The highest separation performance, in terms of purity and productivity, is achieved when $m_{II} = K_a$ and $m_{III} = K_b$. Outside the triangle, only one pure outlet or both impure outlets can be achieved. For a more detailed description of the triangle theory, the reader is referred to [22,30,32,34].

2.1. Performance parameters

The performance of a purification process is usually evaluated through some fundamental parameters: purity, recovery and productivity [22,35].

The purities of the raffinate (P_R) and extract (P_E) fractions at cyclic steady state can be defined both as the ratio of the concentration of the target (C_i^R and C_i^E) and the concentration of all the species or as the ratio of the target peak area (A_i^R or A_i^E) and the total area (A_{total}) of the chromatogram:

$$P_R(\%) = \frac{\int_{t_{start}}^{t_{final}} C_a^R\, dt}{\sum_i^n \int_{t_{start}}^{t_{final}} C_i^R\, dt} \cdot 100 = \frac{A_a^R}{A_{total}} \cdot 100 \tag{8a}$$

$$P_E(\%) = \frac{\int_{t_{start}}^{t_{final}} C_b^E\, dt}{\sum_i^n \int_{t_{start}}^{t_{final}} C_i^E\, dt} \cdot 100 = \frac{A_b^E}{A_{total}} \cdot 100 \quad (8b)$$

These equations can also be applied to the case of a n components system. For a binary mixture n=2, i.e. a and b components.

Recovery or yield of the target product has to be evaluated especially for high-valuable compounds:

$$R_R(\%) = \frac{Q_R \int_{t_{start}}^{t_{final}} C_a^R\, dt}{Q_F \int_{t_{start}}^{t_{final}} C_a^F\, dt} \cdot 100 \quad (9a)$$

$$R_E(\%) = \frac{Q_E \int_{t_{start}}^{t_{final}} C_b^E\, dt}{Q_F \int_{t_{start}}^{t_{final}} C_b^F\, dt} \cdot 100 \quad (9b)$$

Finally, the productivity is defined as the mass of the target compound collected per total volume of stationary phase and per time:

$$Prod_R(g/L/h) = \frac{mass_R}{V_{col} \cdot 8 \cdot time} \quad (10a)$$

$$Prod_E(g/L/h) = \frac{mass_E}{V_{col} \cdot 8 \cdot time} \quad (10b)$$

3. Experimental section

3.1. Chemicals and solvents

Cannabinoids standard solutions, namely cannabidivarin (CBDV), cannabinol (CBN), cannabidiol (CBD), cannabicyclol (CBL), cannabichromene (CBC), cannabigerol (CBG) and (−) − Δ^9-tetrahydrocannabinol (THC) were purchased from Cerilliant (Round Rock, Texas, USA). Orthophosphoric acid (85%), HPLC-grade solvents, including acetonitrile (ACN), heptane (Hept) and ethanol (EtOH) were from VWR (Radnor, PA, USA).

3.2. Sample preparation

Dried cannabis leaves, flowers and seeds of *Cannabis Sativa* L. (variety Santhica 27) were kindly donated by Radice Cubica S.r.l. (via delle Bombarde 14, 73100, Lecce, Italy). In order to convert acidic cannabinoids into neutral ones, a decarboxylation step was performed (oven at 140 °C for 1 h). Then cannabinoids have been extracted from the plant material with ethanol (25 °C for 15 min). The solvent was then completely evaporated through rotational evaporation to form a solid extract. A defined amount of decarboxylated concentrated extract was dissolved in pure ACN and filtered with 0.20 μm PTFE filters for the analytical quantification analysis under RP conditions (see Section 3.5). The initial purity of CBG was evaluated through the ratio of the single cannabinoid area and the total peak area (Eq. (8)). Once the optimal composition of apolar and polar solvents has been found (see Section 3.3), a defined amount of concentrated extract has been dissolved in the mobile phase of choice to get the desired feed concentration and placed at -20 °C for 24 h for the winterization step in order to remove waxes from the sample. The sample was then filtered with 0.2 μm PTFE filters prior to injection.

3.3. Normal phase conditions and scale-up

The effect of the composition of the mobile phase on the separation of CBG-rich extract was evaluated in analytical scale using an Eurospher II 150 × 4.0 mm CN column packed with 5 μm fully porous particles (KNAUER, Berlin, Germany) on a stainless steel AZURA ® HPLC system (KNAUER, Berlin, Germany) equipped with a quaternary low pressure gradient pump (max pressure: 862 bars), a column thermostat, an autosampler and a photodiode array detector.

Based on the results obtained in [18], a mobile phase made of heptane and ethanol (94:6%) was selected. This choice was driven by greenness requirements, since heptane is classified as a less problematic solvent than hexane [37], and by retention characteristics, since when applied to the real cannabis extract a slightly better resolution was obtained with ethanol compared to isopropanol (data not shown). Afterwards, the linearity of the adsorption isotherm of CBG was evaluated by verifying that retention time was constant independently from the injected amount (up to 50 μL at 25 g/L).

The process was then scaled-up to a 150 × 8.0 mm CN column packed with 10 μm fully porous particles from the same manufacturer. The flow rate was 4 mL/min and the detection wavelength was set at 228 nm. This I.D. 8 mm column was used to obtain SMB starting parameters, through the injection of the mixture to be separated (see Fig. 3) and toluene was employed as dead volume marker.

3.4. Simulated moving bed process

The SMB purification has been performed on a stainless steel AZURA Lab SMB System (KNAUER, Berlin, Germany) equipped with seven 8-multiposition valves, four pumps and two mass flow controllers (Mini CORI-Flow). The system was controlled by PurityChrom® MCC software. Eight 150 × 8.0 mm CN columns were used with a 2:2:2:2 configuration, as schematically reported in Fig. 1. The flow rate in zone I was set at 4 mL/min (i.e. the same flow rate used with the single I.D. 8 mm column in Section 3.3) and feed flow rate was 0.1 mL/min. Other flow rates were modified according to SMB simulations.

Each purification was performed for 20 cycles of continuous operation.

3.5. Offline fraction analysis

The offline analysis of raffinate and extract fractions from the SMB process has been performed under both NP and RP elution conditions. In the NP analytical method the I.D. 4.0 mm CN column was used. The mobile phase composition was the same as in the SMB separation (94:6% heptane/ethanol) and the flow rate was set to 1 mL/min. This

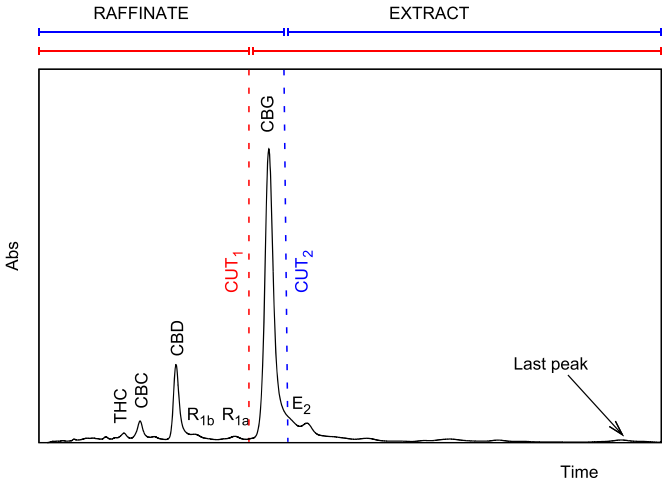

Fig. 3. Chromatogram representing the separation cut between raffinate and extract fractions measured on I.D. 8.0 CN column. Red lines (CUT_1) indicate the first purification, where R_{1a} is the raffinate retention reference and CBG is the extract retention reference. Blue lines (CUT_2) indicate the second purification, where CBG is the raffinate retention reference and E_2 is the extract retention reference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

method allows to check the goodness of the SMB separation by measuring the extract and raffinate outlets in less than 10 min, permitting to perform a rapid change of the SMB parameters, if needed. This fast analytical method is particularly suitable in the first stage of the SMB setup, where parameters need to be accordingly modified in order to find the best experimental conditions for the separation problem. On the other hand, a longer but more precise RP analytical method (described in Ref. [18,36]) has been used for the purity control of the two outlets.

4. Results and discussion

Figure 3 shows the feed chromatogram of the CBG-rich extract obtained with I.D. 8.0 mm column, as described in Section 3.3. The two main species present in the feed mixture are CBD and CBG. A 5 g/L hemp extract contains roughly 1100 μg/mL CBG, 200 μg/mL CBD and other minor species, among which 6 μg/mL THC, 14 μg/mL CBC and other unknown impurities. The initial purity of CBG is 58%.

Once the optimal experimental conditions have been chosen in analytical chromatography, the linearity of the adsorption isotherms of the target compound has been verified and the scale-up to preparative scale has been performed (Section 3.3), the initial set of parameters to model the SMB process can be identified. Based on the feed chromatogram, two subsequent purification steps have been simulated and performed to reach a CBG final purity >90%. The first purification will ensure the separation between early eluting compounds and the main cannabinoid, CBG, indicated as "CUT_1" in Fig. 3 (red lines). In this case, retention parameters are accordingly chosen to set a separation region able to ensure the maximum achievable purity of CBG (Run 2, see below). This has been accomplished by imposing CBG as extract ($K_b = K_{CBG}$) and R_{1a} peak as raffinate ($K_a = K_{R_{1a}}$), conversely to common approaches which suggest to use the most abundant compounds as reference peaks, in this case CBD and CBG (Run 1, see below). With the second purification a highly pure CBG fraction will be obtained through the separation pointed out as "CUT_2" in Fig. 3 (blue lines). This will be achieved by setting CBG peak as raffinate ($K_a = K_{CBG}$) and the adjacent stronger eluting impurity peak, indicated as E_2, as extract ($K_b = K_{E_2}$). Data reported in Table 1 refers to SMB starting parameters, i.e. column volume, void volume and retention data related to the raffinate and extract peaks for the two purifications.

A well-established simulation software, based on the Chromatography Analysis and Design Toolkit (CADET-SMB) (http://github.com/modsim/cadet, http://github.com/modsim/cadet-smb), has been used to calculate and solve the *n*-components mass balance equations. For a more detailed description, the reader is reffered to Ref. [24] and to SI. All simulations have been carried out at constant feed concentration (CBG = 1 g/L), feed flow rate (Q_{feed}= 0.1 mL/min) and zone I flow rate (Q_I= 4 mL/min), considering linear adsorption isotherms. All the species having an influence on CBG purity present in the real sample were considered in the simulations. Outputs of the simulation are purity and recovery for each component in extract and raffinate fractions.

Experimentally, raffinate and extract outlet fractions were collected and analysed for each cycle to check concentration and purity variations. This has allowed to determine that, when the columns are initially empty, the steady state was reached just after 5 cycles. Moreover, the continuous sample collection from the first cycle permits not to waste any target molecule, reaching a final recovery close to 100%.

Table 1
SMB parameters.

Parameter	value
Column diameter [mm]	ID = 8
Column Volume [mL]	V = 7.54
Particle size [μm]	d_p= 10
Number of columns [-]	n= 8
Zone configuration [-]	2-2-2-2
Total porosity [-]	ϵ= 0.71
Q_I [mL/min]	4.0
Q_{feed} [mL/min]	0.1
t^* [min]	6.04
K_{CBG} [-]	3.52
K_{CBD} [-]	1.96
$K_{R_{1a}}$ [-]	2.92
K_{E_2} [-]	4.14

4.1. First purification: THC depletion

The optimal SMB parameters for the first purification step were chosen based on the results of simulations. In this case, a 5-components system composed by CBG (1 g/L), CBD (0.2 g/L), R_{1a} (0.075 g/L), R_{1b} (0.075 g/L) and E_2 (0.25 g/L) peaks was considered (Fig. 3). Concentrations of CBG and CBD derived from analytical analysis, concentrations of unknown peaks (R_{1a}, R_{1b}, E_2) were calculated by area normalization. It is worth noting that E_2 peak considered in the simulation encloses all species eluting after CBG peak. In this way, all the species having an influence on CBG purity present in the real sample have been taken into account in the simulations.

A first run has been simulated by choosing m_{II} and m_{III} values to ensure the separation between CBD and CBG peaks ($K_b = K_{CBG}$ and $K_a = K_{CBD}$). Simulation results indicate that these parameters lead to the absence of CBD but ~100% recovery (essentially quantitative) of both R_{1a} and E_2 peaks and ~10% recovery of R_{1b} peak in the extract fraction, leading to roughly 75% purity of CBG, as shown in Table 2 (Run 1). In order to obtain the highest possible CBG purity with the first purification, raffinate retention time has been moved from CBD to R_{1a} peak ($K_a = K_{R_{1a}}$). In this case, the separation region obtained with the triangle theory by using K_{CBG} and $K_{R_{1a}}$ values is very limited (Fig. 4, red triangle). Indeed, a difference of only 0.6 points ($K_b - K_a$=0.6) is available to satisfy the inequalities of Eq. (7) for m_{II} and m_{III}. Despite this limitation, the correct choice of zone II flow rate may lead to the best results, in terms of CBG purity and recovery. Q_{II} was then increased from 1.75 to 2.00 mL/min, bringing to a shift to the right of the previous separation region, permitting to reach the separation "CUT_1" shown in

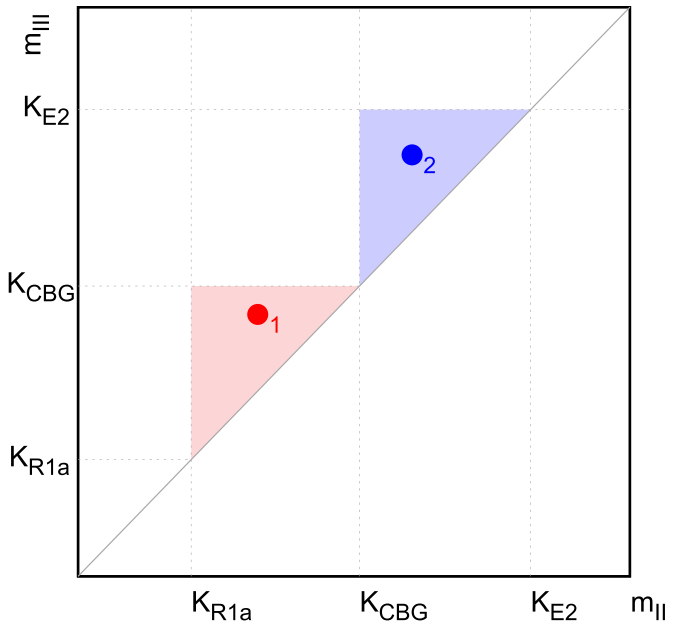

Fig. 4. Triangle theory for the first purification, with K_{R1a} and K_{CBG} Henry constants (red separation region) and for the second purification, with K_{CBG} and K_{E2} Henry constants (blue separation region), with indicated experimental points (see Tables 2 and 3 for details). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Theoretical and experimental SMB output parameters related to the first purification: R_E recovery of extract fraction; P_E purity of extract fraction; $Prod_{CBG}$ productivity values related to CBG, expressed as g/L/h; P_{CBG} purity of CBG in extract fraction. The initial purity of CBG in the feed mixture was 58%.

Run #	Q_{II}	Q_{III}	Simulated R_E (%)					Simulated P_E (%)					$Prod_{CBG}$(g/L/h)		P_{CBG}(%)
	[mL/min]	[mL/min]	CBG	R_{1b}	R_{1a}	CBD	E_2	CBG	R_{1b}	R_{1a}	CBD	E_2	Sim.	Real	Real
1	1.75	1.85	99.7	10.1	96.7	0.9	99.4	75.1	0.6	5.5	0.1	18.7	0.105	0.104	74.1
2	2.00	2.10	96.7	0.0	8.1	0.6	98.5	79.5	0.0	0.5	0.0	20.1	0.099	0.104	80.1

Table 3
Theoretical and experimental SMB output parameters related to the second purification: R_R recovery of raffinate fraction; P_R purity of raffinate fraction; $Prod_{CBG}$ productivity values related to CBG, expressed as g/L/h; P_{CBG} purity of CBG in raffinate fraction. The purity of CBG in the feed mixture was 80%.

Run #	Q_{II}	Q_{III}	Simulated R_R (%)		Simulated P_R (%)		$Prod_{CBG}$(g/L/h)		P_{CBG}(%)
	[mL/min]	[mL/min]	CBG	E_2	CBG	E_2	Sim.	Real	Real
3	2.2	2.3	94.2	12.9	96.7	3.3	0.103	0.110	97.1

Fig. 3. This practically allows for the inclusion of R_{1a} and R_{1b} peaks into the raffinate fraction, contributing to an increase of the CBG purity in the extract fraction to 80%, as clearly shown by data in Table 2 (Run 2) and by chromatograms of raffinate and extract fractions in Fig. 5 (red traces).

The use of Run 2 parameters for the SMB purification of the crude mixture permits to reach a +38% increase in CBG purity, achieving 80% pure CBG in the extract fraction, with 0% CBD and 0% THC, at ~100% CBG recovery. Moreover, a very good agreement between productivity and purity output data obtained with simulations and experimental purification is achieved for each run, as it can be seen from Table 2.

From a practical viewpoint, this SMB method, using only one chromatographic step, may be useful for industrial and commercial purposes, since it permits the rapid and easy THC removal from the sample with a ~100% CBG recovery. Indeed, as reported in the chromatograms from Fig. 5, THC is eluted in the raffinate fraction, leading, in this case, to a THC-free CBG extract. It is well known that substances, based on their therapeutic potential and the risk of resulting in abuse and addiction, are classified into five schedules under the Controlled Substances Act (CSA), and THC and marijuana are categorized as hallucinogens in schedule I. Non-psychoactive cannabinoids can be obtained from drug-type varieties of cannabis but they require purification step(s) in order to remove some or all the THC present in the final extract [39]. In this context, the method here proposed represents a boost in the preparation of (legal) cannabis extracts and products. Indeed, the methods currently employed in industry, mainly based on flash chromatography and other multiple steps, are unable to fully remove all the THC from the sample [14,40].

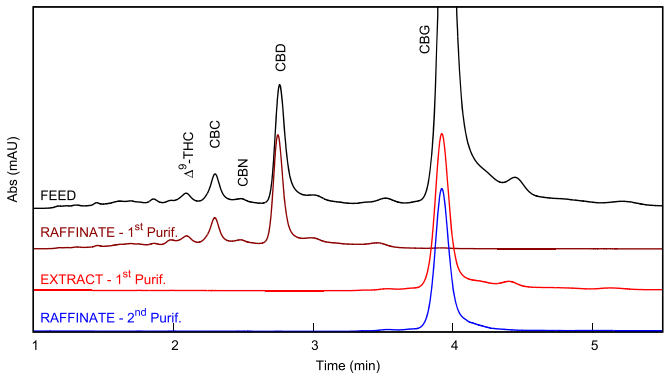

Fig. 5. Analytical chromatograms measured on I.D. 4.0 CN column. Black line: feed mixture; red lines: raffinate and extract of the first purification; blue line: raffinate of the second purification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Second purification: High purity CBG

A second purification has been performed in order to provide a final product purity comparable to the one proposed by other available methods.

The extract fraction from the first purification (Section 4.1), with a CBG purity of 80%, was reprocessed as new feed for the second purification. The solvent was removed to maintain a constant CBG concentration in the feed (= 1 g/L).

In this second step, CBG peak is eluted in the raffinate fraction ($K_a = K_{CBG}$), that is the opposite situation encountered in the first purification (Section 4.1), and all the other stronger eluting impurities are in the extract fraction, with E_2 taken as extract reference retention time ($K_b = K_{E_2}$). The separation region of the second purification is displayed in Fig. 3 as "CUT_2" and graphically shown in Fig. 4, blue triangle.

Results are listed in Table 3 and raffinate chromatogram is reported in Fig. 5 (blue line). Data show that a +20% increase of purity has been achieved between the first and the second purification, obtaining a 97% pure CBG fraction. This indicate an overall gain in purity of +67% from the initial crude hemp extract to the final product. Moreover, also this time, simulation and experimental results are in optimal agreement.

The overall purification process may be considered as a green and environmentally friendly alternative to traditional methods for cannabinoids purification that make use of multiple steps. Indeed, the target product is isolated at a comparable purity, while the purification and the solvent removal have been accomplished in only two-step each.

4.3. Optimization of productivity

The productivity is defined as the amount of target cannabinoid recovered per unit time and column volume (Eq. (10)). It can be enhanced in two ways: i) by increasing feed flow rate keeping feed concentration constant; or ii) by increasing feed concentration maintaining feed flow rate constant. Other options, such as decreasing cycle time and/or column volume, will not be taken into account since they affect the goodness of the separation process. Nevertheless, an increase in the feed flow rate (Q_{feed}) will alter zone III flow rate, since $Q_{III} = Q_{II} + Q_{feed}$, resulting in an increase in m_{III} value. As a consequence, in this case, since adsorption isotherms are linear, productivity can be enhanced by keeping Q_{feed} constant and increasing feed concentration. As an example, a CBG feed concentration corresponding to 6 g/L results in a simulated productivity of 0.6 g/L/h of purified CBG, that corresponds to roughly 30 mg of CBG per cycle, with a 6 fold increase in productivity with respect to Runs 1–3 (see Tables 1 and 2). Theoretical results are in optimal agreement with experimental data, as reported in Table 1 of the SI. These results indicate that, specifically for this combination of system and sample, the typical trade-off between purity and productivity, often encountered in preparative chromatography, can be

overcome.

5. Conclusions

This work shows that the SMB purification of real cannabis extract containing mainly CBG is feasible by using solvents that are considered to be green (ethanol) or that are greener than traditional organic solvents employed in chromatography (heptane). Results are very promising. Through the proposed methodology, using CN stationary phases under normal phase conditions, a THC-free CBG extract with 100% recovery and a final purity of 97% was achieved. Furthermore, SMB purification process can be performed for days or weeks, depending on the total feed volume, without the need of human intervention, thanks to continuous feeding and operation automation.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.greeac.2023.100066.

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