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(54) Title: EXTRACTION AND PURIFICATION OF CANNABINOID COMPOUNDS

(57) Abstract: Disclosed are effective methods for activating, washing, specifically extracting and purifying cannabinoids from *Cannabis* plant tissues using heat activation, washing impurities away with a polar solvent, optionally modified with an organic acid, base, surfactant or inorganic salt, extracting the activated non-polar cannabinoids with a potable selective solvent such as ethanol. The extracted active ingredients may be purified by chromatography and detected and quantified by mass spectrometry with external or isotopic or otherwise labelled standards.

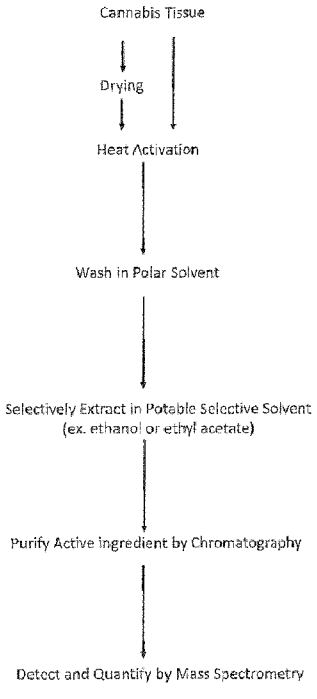

Figure 1

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EXTRACTION AND PURIFICATION OF CANNABINOID COMPOUNDS

Field of the Invention

[0001] The present invention relates to methods for the extraction of cannabinoid compounds from plant tissue.

Background

[0002] It may be desirable to extract cannabinoid compounds from plant tissue, particularly the flower or leaves of hemp or marijuana plants such as *Cannabis sativa, C. indica or C. ruderalis*. The most common cannabinoids of interest are cannabidiol (CBD) and tetrahydrocannabinol (THC). THC and CBD are non-polar isoprenoid compounds that are frequently found modified with carboxylic acid groups that renders them both polar and inactive (THCA and CBDA). THCA and CBDA can be decarboxylated by heat, causing the compounds to be both activated and rendered non-polar (lipophilic). The activation, i.e. decarboxylation, of CBDA or THCA results in the active ingredients CBD or THC. However, the activation of THC and CBD limits the solubility of THC and CBD in water, while rendering them soluble in organic solvents.

[0003] It is well known that activated cannabinoids may be extracted from plant tissue using organic solvents such as chloroform, acetonitrile, butane, hexane, isopropanol, butanol, methanol and others. However, these non-potable solvents may be harmful themselves or be contaminated with trace amounts of harmful solvents.

[0004] It is also known to use supercritical carbon dioxide (CO_2) to extract cannabinoids, and while supercritical CO_2 extraction leaves no residual solvents that might be toxic or

harmful, it is a difficult and slow process. A CO_2 extraction can take 8 to 24 hours and require precise parameters to obtain acceptable selectivity. It also needs extremely high pressures to be useful, leading to high equipment costs.

[0005] There remains a need in the art for effective and economic methods of extracting cannabinoids from plant tissue, without the use of toxic or non-potable solvents.

Summary of the Invention

[0006] In one aspect, the invention comprises a method of extracting a cannabinoid from a plant tissue, comprising the steps of:

(a) heating the plant tissue to convert carboxylated cannabinoid to a non-polar active form;

(b) washing the plant tissue with a polar solvent; and

(c) extracting the non-polar cannabinoid with a selective solvent.

Optionally, the plant tissue may be dried and/or ground before the heat activation step. Preferably, the polar solvent comprises water, a water/alcohol mixture, an organic acid, or a salt solution, and may optionally include a surfactant. The selective solvent is one which selectively extracts cannabinoid compounds. In some embodiments, the heated, activated plant tissue may be extracted with the selective solvent to produce an intermediate resin before washing with a polar solvent in step (b). Preferably, the selective solvent comprises ethanol or ethyl acetate.

[0007] Conversion of non-active, carboxylated forms of cannabinoids into their non-polar active ingredients allows for the subsequent, preferential removal of components which are soluble in a polar solvent. The cannabinoids may then be extracted using a selective

solvent, such as ethanol, which is preferred due to its potability. Non-polar activated cannabinoids such as THC and CBD are not soluble in polar solvents, therefore polar contaminants may be washed away using the polar solvent. The polar solvent may be modified with salts, buffers or inorganic or organic acids or bases. The non-polar cannabinoids may then be extracted in a potable selective solvent, such as ethanol, that contains no harmful residues.

[0008] Optionally, the extracted cannabinoids may be purified by chromatography and/or quantified by spectroscopic methods.

[0009] In some embodiments, cannabis plant tissue is heated using an oven or a water bath to cause the conversion of the native acid forms of the cannabinoids, such as tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), to the non-polar active ingredients THC and CBD. In some embodiments, the plant tissue is heated at a temperature between about 100° to about 140° C, for between about 30 minutes to about 2 hours, preferably at about 120°C for about 1 hour.

[0010] In some embodiments, the washing step uses a polar solvent comprising water, a water/alcohol mixture, water modified by salt, buffers, inorganic or organic acids or bases, potable organic solvents, and/or combinations thereof. In some embodiments, the tissue or intermediate resin is washed in a polar solvent comprising a potable surfactant or emulsifying agent, such as deoxycholate or n-octylglucoside or other amphipathic detergents. Preferably, the washing step is done at a refrigerated temperature, such as below about 5° C, more preferably below about 0° C, but obviously above the freezing temperature of the polar solvent.

[0011] In some embodiments, the heated and washed cannabis tissue is subsequently extracted with a potable organic solvent, such as >40% ethanol and preferably 80% ethanol (v:v). Preferably, the extraction step occurs at a refrigerated temperature, such as between about -80° C to about 5° C, and more preferably between about -20° C and about 0° C.

[0012] In an embodiment, the active cannabinoids extracted from washed tissue by the selective solvent may be subsequently purified or separated from other extracted components by chromatography, the separation may be performed by liquid chromatography (LC), optionally DEAE, CMC, QA, PS, normal phase, or reversed phase chromatography. The chromatography may be isocratic, step gradient or a linear gradient.

[0013] In yet another embodiment, the purity of the purified active ingredient may be quantified by liquid chromatography such as high-performance liquid chromatography (HPLC). In an embodiment, the HPLC is nanoflow liquid chromatography. In another embodiment, the HPLC can be reverse phase HPLC, ion exchange HPLC or normal phase HPLC. The chromatography mobile phase can for example be isopropyl alcohol (IPA), methanol, ethanol, propanol, or acetonitrile. The stationary phase can for example be silica based or polymer based, for example silica particles modified with octadecyl carbon chain (C18).

[0014] In an embodiment, the step of detecting one or more ionizable products using mass spectrometry (MS) comprises ionizing the one or more ionizable products, optionally by electrospray ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI) to produce one or more product ions with a selected signal-to-noise ratio, and subjecting the one or more product ions to MS, optionally tandem MS (MS/MS). In another embodiment,

the ionizing is positive ionization (e.g. using an acidic buffer in the mobile phase). In another embodiment, the ionizing is negative ionization (e.g using a basic buffer in the mobile phase). In an embodiment, the step of ionizing the one or more ionizable products comprises Matrix-assisted laser desorption/ionization (MALDI).

[0015] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

DBrief Description of the Drawings

[0016] Figure 1 is an illustration of a scheme to activate, wash, selectively extract, purify, identify and quantify the cannabinoid compounds.

[0017] Figure 2. The separation and detection of CBD, CBDa, THC and THCa by isocratic HPLC in 70% AcN 0.1% formic acid of 100% Ethanol extract prior by 300 Angstrom 5 micron C18 porous resin to LC-ESI-MS (n=1). Note the porous resin does not resolve CBD from THC.

[0018] Figure 3. The separation and detection of CBD in hemp A on a 300 Angstrom 5 micron C18 porous resin by LC-ESI-MS with a isocratic HPLC in 70% AcN 0.1% formic acid of HA1, HE8, and HE1 extract (n=5). Spectra depicts (A) 3x blank (B) external CBD standard curve from 0-200uM (C) detection of CBD (m/z 315) in hemp A HA1 extract, (D) HE8 extract (E) HE1 extract, (F) mix of HE8 and HE1 extract, (C1) HA1 extract

spiked with CBD-D3 (m/z 318), (D1) HE8 extract spiked with CBD-D3, (E1) HE1 extract spiked with CBD-D3 and (F1) mix of HE8 and HE1 extract spiked with CBD-D3. (HA1: sampled heated extracted in 100% can; HE8: sample heated extracted in 80% EtOH; HE1: sample heated extracted in 100% EtOH).

[0019] Figure 4. The separation and detection of CBD, CBDa, THC and THCa by isocratic HPLC in 70% AcN 0.1% formic acid and LC-ESI-MS (n=1). Figure 4A - CBD alone; 4B - THC alone; 4C - CBD and THC separation on a 6 cm column; 4D, CBD and THC separation on a 15 cm column over Kinetex™ coreshell resin.

[0020] Figure 5. The separation and detection of CBD, CBDa, THC and THCa by gradient HPLC and LC-ESI-MS (n=1). Panels: A, Sample 1 spiked with CBD-D3; B, Sample 2 spiked with CBD-D3; C and D, CBD and THC reference standards. Gradients - Samples were diluted in B buffer (65% AcN, 5%FA) with gradient 0 min at 70%, 10 min linear gradient to 80%, held for 5 min at 80% and equilibrate at 70%(base peak).

[0021] Figure 6. The separation and detection of CBD, CBDa, THC and THCa by isocratic HPLC in 70% AcN 0.1% formic acid and LC-ESI-MS (n=1) on a 15 cm column over Kinetex™ coreshell resin. Figure 6A, CBD and THC reference standard; 6B, CBD alone; 6C, THC alone; 6D, Sample A spiked with CBD-D3; 6E, Sample B spiked with THC-D3.

[0022] Figure 7. Decarboxylation of CBDa at different temperatures. A, 0.1g of hemp sample 1 was heated to the appropriate temperature for 1 hour and extracted 3x with 100% Ethanol prior to LC-ESI-MS (n=3); B, 0.1g of hemp sample 2 was heated to the appropriate temperature for 1 hour and extracted with 100% Ethanol prior to LC-ESI-MS

(n=1); C, , 0.1g of hemp sample 2 was heated to the appropriate temperature for 1 hour and extracted with 100% Ethanol prior to LC-ESI-MS (n=3)

[0023] Figure 8. CBD internal vs external standard curve (n=2) from 50-5000 nM. A, CBD external standard curve. B, Internal versus external CBD curve. C, Illustration spectra of the detection of CBD (m/z 315) in a hemp sample heated to 120°C spiked with CBD-D3 (m/z 318) measured by isocratic separation over C18 resin.

[0024] Figure 9. EtOH gradient extraction of CBD. 0.1g of hemp was heated to 120^0C for 1 hour, extracted in a sequential step gradient of increasing Ethanol and analyzed by LC-ESI-MS (n=3). A, CBD extracted in ethanol gradient from a hemp sample heated to 120°C vs no heating control (0C); B, CBD and CBDa extracted in an ethanol gradient from the no heating control sample (0C); C, CBD and CBDa extracted in an ethanol gradient from a hemp sample heated to 120°C for 1 hour.

[0025] Figure 10. Effect of pH on the extraction of CBD from hemp in 80% Ethanol (n=3). 0.1g of hemp was heated to 120°C for 1 hour, washed 3x with H2O, 3x with 40% Ethanol followed by extraction in 80% Ethanol at various pH levels prior to analysis by LC-ESI-MS. Sample 1, 0.1% TFA in 80% EtOH pH>2 (not adjusted); Sample 2, 0.1% FA in 80% EtOH pH2 (not adjusted); Sample 3, 0.1% Acetic acid in 80% EtOH pH3 (not adjusted); Sample 4, 10mM Citric acid in 80% EtOH pH4; Sample 5, 10mM Citrate in 80% EtOH pH5; Sample 6, 10mM Citrate in 80% EtOH pH6; Sample 7, 10mM Tris in 80% EtOH pH7; Sample 8, 10mM Tricine in 80% EtOH pH8; Sample 9, 0.1% Ethanolamine in 80% EtOH pH9; Sample 10, 0.1% Ethanolamine in 80% EtOH pH10; Sample 11, 0.1% Ammonia in 80% EtOH pH<10 (not adjusted); Sample 12, 80% EtOH in H2O (pH not adjusted).

[0026] Figure 11. Mass recovery after extraction with different solvents (n=3). A, 0.1g Hemp was heated to 120°C for 1 hour prior to extraction with various solvents. Mass yields after extraction with different solvents (n=3). A, 0.1g Hemp was heated to 120°C for 1 hour prior to extraction with various solvents (3 sequential extracts were pooled). Samples were dried to determine the mass extracted and re-dissolved for mass spectrometry analysis to measure CBD content.

[0027] Figure 12. CBD yields from 5 sequential extractions with and without pre-washing with H2O (n=3). 0.1g hemp was heated to 120°C for 1 hour and extracted in A, 5x H2O; B, 5x Acetonitrile; C, 5x Ethanol; D, 5x Acetonitrile pre-washed with 5x H2O; E, 5x Ethanol pre-washed with 5x H2O

[0028] Figure 13. Optimizing binding conditions for CBD using ion exchange chromatography (n=3). 0.1 g of hemp was heated to 120°C and extracted 3x in 100% ethanol. Extracts of 0.5 g total hemp per replicate were pooled and diluted with H2O to the appropriate ethanol concentrations. Samples were loaded at 100% ethanol and decreasing to 10% ethanol onto the various resins. The Flow Throughs (FT) were collected and analyzed by LC-ESI-MS.

[0029] Figure 14. Elution curves in Ethanol or Acetonitrile for HiQ columns (n=3). Figure 14A, ~7.5 mg CBD was extracted from 0.5g hemp in 100 % Ethanol, diluted to 20% ethanol and loaded onto a ~100ul HiQ column. The column was washed with loading solvent prior to elution with increasing amounts of Ethanol (25, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 55 and 60%). Up to ~ 50%, the fractions are observed to be clear. Above 50%, the elutions become green. Fractions were collected and analyzed by LC-MS. Figure 14B, ~7.5 mg CBD was extracted from 0.5g hemp in 100 % Acetonitrile, diluted to

20% acetonitrile and loaded onto a ~100ul HiQ column. The column was washed with loading solvent prior to elution with increasing amounts of Acetonitrile (25, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 55 and 60%). Up to ~ 35%, the fractions are observed to be clear. Above 35%, the elutions become green (elutions separate in 2 distinct layers, one clear and one green). Fractions were collected and analyzed by LC-MS.

[0030] Figure 15. CBD external standard curve 50-50,000nM (n=2) measured by LC-ESI-MS/MS using isocratic separation over C18 resin.

[0031] Figure 16. The wash of intermediate resin from cannabis sample A. A 0.1 g aliquot of cannabis sample A was washed heated to 120°C for 1 hour, extracted in ethanol and dried under vacuum to make a intermediate resin. The water soluble components of the resin were washed in water or water modified with organic acid, ethanol, or salt.

[0032] Figure 17. The wash of intermediate resin from cannabis sample B. A 0.1 g aliquot of cannabis sample A was washed heated to 120°C for 1 hour, extracted in ethanol and dried under vacuum to make an intermediate resin. The water soluble components of the resin were washed in water, water with 0.5% acetic acid (v:v), ethanol, or salt.

[0033] Figure 18. The extraction of intermediate resin from cannabis sample A. A 0.1 g aliquot of cannabis sample A was washed heated to 120° C for 1 hour, extracted in ethanol and dried under vacuum to make a intermediate resin. The water soluble components of the resin were washed in water with 0.5% acetic acid (v:v) and extracted with 1 ml of the solvent shown.

[0034] Figure 19. The extraction of intermediate resin from cannabis sample B. A 0.1 g aliquot of cannabis sample B was washed heated to 120°C for 1 hour, extracted in ethanol

and dried under vacuum to make a intermediate resin. The water soluble components of the resin were washed in water with 0.5% acetic acid (v:v) and extracted with 1 ml of the solvent shown.

Detailed Description

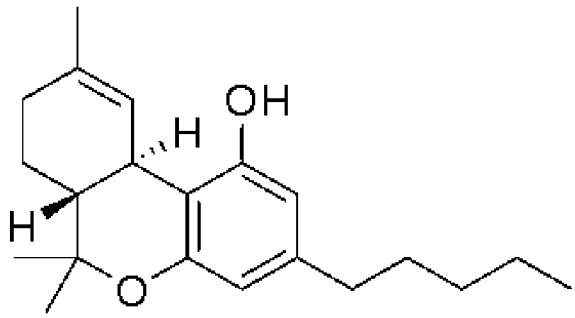
[0035] The present invention comprises methods of selectively extracting and purifying cannabinoids from plant tissue, such as cannabinoid plant tissue.

[0036] Cannabinoids are compounds which act on or modulate cannabinoid receptors in cells, which can alter neurotransmitter release in the brain. Cannabinoids were originally found in *Cannabis saliva L.*, the origin of marijuana and hashish. Marijuana or its components have been reported in the scientific literature to alleviate the symptoms of a broad range of conditions including multiple sclerosis and forms of muscular spasm, including uterine and bowel cramps; movement disorders; pain, including migraine headache; glaucoma, asthma, inflammation, insomnia, and high blood pressure. There may also be utility for cannabinoids as an oxytoxic, anxiolytic, anti-convulsive, anti-depressant and/or anti-psychotic agent, anti-cancer agent, or an appetite stimulant.

[0037] Many chemically related compounds, collectively classified as cannabinoids, have been isolated from *Cannabis* plants. The cannabinoids usually divided in the groups of classical cannabinoids, non-classical cannabinoids, aminoalkylindole derivatives and eicosanoids. Classical cannabinoids such as THC or CBD are isolated from *Cannabis sativa L.*, or they can comprise synthetic analogs of these compounds. Non-classical cannabinoids may comprise bi- or tricyclic analogs of tetrahydrocannabinol (THC), while aminoalkylindoles form a group which differs structurally substantially from classical and non-classical cannabinoids.

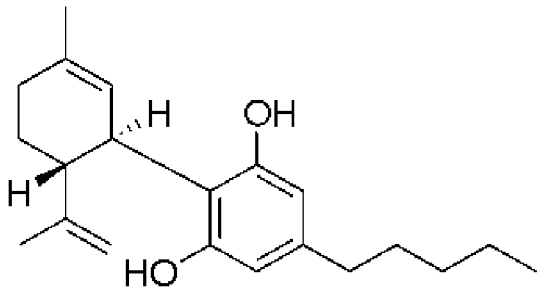
[0038] In various embodiments, cannabinoids can include, but are not limited to, cannabinoid compounds that may naturally occur in different combinations and relative quantities in the plant tissues of various species, subspecies, hybrids, strains, chemovars, and other genetic variants of the genus *Cannabis*, including material that may variously be classified as "marijuana" and "hemp" in accordance with various legal or technical definitions and standards.

[0039] An exemplary cannabinoid comprises THC, having the formula (I):



(I)

which includes delta-9-tetrahydrocannabinol (D9THC), acknowledged to be the main psychoactive compound in marijuana. Another exemplary cannabinoid is cannabidiol (CBD) IUPAC: 2-[(1R,6R)-6-isopropenyl-3-methylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol, having the formula (II):



(II)

Although CBD is not known to have the psychotropic effects of THC, it is still considered to have a wide scope of potential therapeutic applications. CBD may be derived from industrial hemp which has negligible amounts of THC, and may be legally grown and consumed in Canada and the United States.

[0040] Cannabinoid compounds may also include various other cannibinoids such as tetrahydrocannabinolic acid (THCA), delta-8-tetrahydrocannabinol (D8THC), cannabidiolic acid (CBDA), cannabinol (CBN), cannabinolic acid (CBNA), tetrahydrocannabinovarin (THCV), tetrahydrocannabinovarinic acid (THCVA), cannabidivarin (CBDV), cannabidivarin acid (CBDVA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabinodiol (CBND), and cannabinodiolic acid (CBNDA).

[0041] The term "selective" as used herein in reference to a solvent, a solid phase or chromatography system of a solvent or solid phase, is one that selectively extracts or purifies a target substance or compound, such as a cannabinoid, with greater specificity relative to another different substance or compound. In some embodiments, the selective system purifies the target substance or compound by at least 2 fold, 3 fold, or 5 fold.

[0042] Figure 1 is an illustration of a general scheme to activate, wash, selectively extract, purify, identify and quantify cannabinoid compounds from cannabis plant tissue. Accordingly, in one aspect, the invention may comprise a method of selectively extracting and purifying a cannabinoid from plant tissue comprising the steps of:

a. preparing plant tissue in fresh or dried form;

b. heating the tissue to decarboxylate cannabinoid compounds;

c. optionally, extracting the tissue with a selective solvent to produce an intermediate resin;

d. washing the tissue or resin with an polar solvent to selectively remove compounds soluble in the polar solvent while leaving decarboxylated cannabinoid compounds;

e. selectively extracting the cannabinoid from the plant tissue using a selective solvent to produce a cannabinoid extract.

[0043] Preferably, the cannabinoids from the extract can be purified by precipitation and/or partition chromatography drying. Finally, the cannabinoids can be detected by liquid chromatography electrospray or atmospheric pressure ionization and tandem mass spectrometry (MS/MS).

[0044] The methods disclosed herein may be performed on finely divided plant tissue, such as fresh or dried tissue which has been cut, chopped, ground, mashed or otherwise processed to reduce particle size. The method may also be performed in solution in the absence of a solid phase, wherein the target substance is not in the solid phase but in a colloidal suspension or fine powder in water or otherwise suspended or emulsified in a liquid phase.

[0045] Preferably, the polar solvent comprises purified water, or water mixed with alcohol such as ethanol, preferably less than about 40% ethanol (v:v), or acetic acid, preferably less than about 5% acetic acid (v:v). It is preferred that all components are potable. As used herein, a potable component is one that is classified as "Generally Regarded as Safe" or "GRAS" by the United States FDA.

[0046] Polar solvents have large dipole moments ("partial charges"); that is they contain bonds between atoms with very different electronegativities, such as oxygen and hydrogen.

Non polar solvents contain bonds between atoms with similar electronegativities, such as carbon and hydrogen. Bonds between atoms with similar electronegativities will lack partial charges. In one embodiment, the polar solvent is one with a dielectric constant greater than about 5.0 at 20° C, preferably greater than about 20, and more preferably greater than about 50.

[0047] In some embodiments, the washing polar solvent comprises a non-ionic, non-polymeric detergent or a bile acid detergent, such as sodium deoxycholate. In an embodiment, the wash solvent contains a potable buffer such as phosphate or carbonate buffer, such as Na_2CO_3 or $NaHCO_3$, an organic acid, such as acetic acid or formic acid, ammonia, ammonium hydroxide, methylamine trimethylamine or the like.

[0048] It is preferred that the polar solvent wash take place at a reduced temperature, preferably below about 5° C, and more preferably below about 0° C, but obviously above the freezing temperature of the solvent.

[0049] In some embodiments, multiple washes with different polar solvents is preferred. For example, a first wash in 0.5% acetic acid (v:v) may be repeated up to three times, followed by a second wash in 40% ethanol (v:v), repeated up to three times. Without restriction to a theory, it is believed that an initial wash in a weak organic acid may protonate water-soluble impurities, facilitating their dissolution in the aqueous phase. The subsequent washes in ethanol/water selectively removes additional polar impurities.

[0050] The selective solvent is one which selectively extracts the activated (decarboxylated) cannabinoid, and may be polar or non-polar. Preferably, the extract solvent comprises ethanol, or ethanol mixed in water, preferably greater than about 40%

ethanol (v:v), more preferably 80% ethanol. Potable ethanol is intended for human consumption and contains no unacceptable residues of harmful solvents.

[0051] In some embodiments, the cannabinoid is selectively extracted with about 80% ethanol at a refrigerated temperature, preferably between about -80° C and about 5° C, and more preferably between about -20° C and 0° C.

[0052] Extraction may be followed by precipitation or drying to recover the desired cannabinoid compounds.

[0053] The extracted compounds may be purified by liquid partition chromatography as monitored by electrospray ionization or atmospheric pressure chemical ionization and tandem mass spectrometry (LC-ESI-MS/MS) is more sensitive and definitive than colorimetric, fluorescent, flame ionization, or electron capture detection and permits standards labelled with isotopes or isobaric tags. Since mass spectrometers can separate and analyze many analytes simultaneously using the methods described herein, it can allow identification and quantification of many different cannabinoids at the same time to levels far below that which is possible by direct mass spectrometric analysis.

[0054] While the present application has been described with reference to what are presently considered to be preferred examples, it is to be understood that the application is not limited to the disclosed examples. To the contrary, the application is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

Examples:

[0055] The following examples are intended to illustrate specific embodiments of the claimed invention, and not be limiting in any way.

Mass Recovery

[0056] 0.1g Hemp was heated to 120°C for 1 hour prior to extraction with various solvents: ethanol, methanol, acetonitrile, isopropyl alcohol, ethyl acetate and acetone. 3 sequential extracts were pooled. Samples were dried to determine the mass extracted and redissolved for mass spectrometry analysis to measure CBD content. Total CBD was set to 1.7082mg/100 mg. The results are shown in Table 1 below

[0057] Table I. Mass yields after extraction with different solvents (n=3).

Extraction solvent	mass extracted (mg) from 100 mg hemp	CBD (mg) from 100 mg hemp	% mass recovery from total in tissue	% CBD of mass extracted
EtOH	7.004692478	0.7931933	46.43282818	11.32
MeOH	6.772186616	0.8139417	47.64742232	12.02
AcN	3.839389904	0.9036164	52.8968976	23.54
IPA	5.506566586	0.7875847	46.10450387	14.30
Ethyl Acetate	5.569882112	0.9563186	55.9820377	17.17
Acetone	4.758931245	0.7973442	46.6758185	16.75

- The first column - (((Tube+dried extract)-tube)*100mg hemp)/actual weight of hemp (mg).
- The second column - STD curve equation used to find concentration of CBD. Then, CBD (mg)= (Mw x V(L) x M) x 1000. Standardize to 100.00 mg hemp: ((CBD (mg)*100mg)/actual hemp (mg)
- Mass recovery (3rd column) = (CBD(mg)/CBD Total (mg))*100%
- 4th column = (CBD (mg)/mass extract (mg))*100%

[0058] The results are shown graphically in Figure 11 and appear to indicate that the solvents are substantially similar in their ability to extract CBD.

Effect of Drying and Pre-washing

[0059] 0.1g hemp was heated to 120° C for 1 hour and extracted in 3x 100% ethanol extraction with no pre-wash and without drying the extract (results in Table IIA); or in 80% ethanol extraction pre-washed with 3x water, 3x 40% ethanol and extracted once in 500ul 80% ethanol (results in Table IIB). Samples were detected on a LC-ESI-MS using a 300 Angstrom 5 micron C18 porous resin.

Table IIA. Effect of drying on the re-solubilization of CBD and THC from cannabis tissue (n=3).

	mass extracted (mg) from 100 mg tissue	**CBD (mg) /100 mg tissue** Extracted with 3x 100% Ethanol without pre-washing, without drying extract	**CBD (mg) /100 mg tissue** Extracted with 3x 100% Ethanol without pre-washing, extracts were dried to determine yield	**% recovery from total in tissue**	**% CBD of mass extracted**
rep1	6.858287815	1.058157929	1.122850101	65.7306445	16.3721636
rep2	6.960101951	1.113238091	1.176511817	68.87195354	16.90365781
rep3	7.001634043	1.106011877	1.187489191	69.51455927	16.96017221
AVG	6.940007936	1.092469299	1.162283703	68.03905244	16.74533121

Table IIB. Tissue extracted with 80% Ethanol was pre-washed with 3x water and 3x 40% ethanol and extracted 1x with 80% ethanol

	mass extracted (mg) from 100 mg tissue	CBD (mg) /100 mg tissue Extracted with 80% Ethanol with pre-washing in water and 40% ethanol, without drying extract	CBD (mg) /100 mg tissue Extracted with 80% Ethanol with pre-washing in water and 40% Ethanol, extracts were dried to determine yield	% recovery from total in tissue	% CBD of mass extracted
rep1	1.415841584	0.537632812	0.531468885	31.11171503	37.53731288
rep2	1.411530815	0.59309931	0.521653691	30.53714231	36.95659249
rep3	1.580516899	0.591777471	0.460355469	26.94879899	29.12689318
AVG	1.469296433	0.574169864	0.504492682	29.53255211	34.54026618

Multiple Extracts

[0060] CBD yields from 5 sequential extractions with and without pre-washing with water (n=3). 0.1g hemp was heated to 120°C for 1 hour and extracted in:

- A, 5x H2O without pre-washing;
- B, 5x Acetonitrile without pre-washing;
- C, 5x Ethanol without pre-washing;
- D, 5x Acetonitrile pre-washed with H2O;
- E, 5x Ethanol pre-washed with H2O.

The mass yields and yield CBD of five fractions combined are shown in Table IIIA. The mass yields and yield CBD of the first three fractions combined are shown in Table IIIB. Samples were detected on a LC-ESI-MS using a 300 Angstrom 5 micron C18 porous resin. Mass is defined as the dry tissue product extracted.

Table III. The effect of multiple extracts of the yield and purity of CBD and THC from Cannabis tissue.

A.

5 sequential extracts combined	Dried hemp (mg)	Mass extract (mg)	CBD (mg)	% recovery from total in tissue	% recovery from total in tissue	% of mass extracted	% of extractable
H2O	100	21.72245	0.00668	21.7224505	0.391161409	0.030761044	
AcN	100	18.81004	0.851712338	18.81004126	49.85848141	4.527966344	
EtOH	100	11.06295	0.88375334	11.06295119	51.73413315	7.988404947	
AcN pre-washed w 5x H2O	100	6.30919	0.758266369	6.309190786	44.38823766	12.01844095	89.028459
EtOH pre-washed w 5x H2O	100	4.33586	0.762428385	4.335861815	44.63187836	17.58424086	86.271627

B.

First 3 sequential extracts combined	Dried hemp (mg)	Mass extract (mg)	CBD (mg)	% recovery from total in tissue	% recovery from total in tissue	% of mass extracted	% of extractable
H2O	100	18.50383	0.00592	18.50383271	0.346333151	0.031973212	
AcN	100	15.95629	0.830843944	15.95629159	48.63686421	5.206999	
EtOH	100	8.31385	0.854943702	8.313853682	50.04764262	10.28336239	
AcN pre-washed w 5x H2O	100	4.96295	0.72285888	4.962954964	42.31551479	14.56509045	87.0029667
EtOH pre-washed w 5x H2O	100	3.85851	0.703263897	3.858510015	41.16844198	18.22630743	82.2585038

The results are shown graphically in Figures 12A-12E.

Effect of Pre-Wash with Salt, Acid or Base

[0061] 0.1 g of hemp was heated to 120° C and pre-washed 3x with PBS, PBS+600mM NaCl, 0.5% acetic acid (Hac), 0.5% ammonia or H_2O, followed by 3 washes of 40% Ethanol. CBD was then extracted with 3x 500ul of 80% Ethanol and the three fractions were pooled. The samples pre-washed in H_2O were extracted 1x with 1ml of Ethanol.

Table IV-A. Effect of pre-washing with salt, acid or base on the extraction efficiency of CBD from hemp (n=3).

Pre-wash	Mass extract (mg) from 0.100 g hemp	CBD (mg) from 0.100 g hemp	% recovery from total in tissue	% of mass extracted
PBS	3.9401649	0.76486	44.774197	19.40
PBS + 600mM NaCl	10.7597825	0.67671	39.6142859	6.92
0.5% HAc	3.52848406	0.81832	47.9036073	23.34
0.5% Ammonia	4.15815607	0.76476	44.7686614	19.51
H2O	2.14577484	0.65554	38.3749606	30.54

In a separate experiment, samples were pre-washed in 3x water followed by 3 washes in 10%, 20%, 30% or 40% ethanol and extracted in 3x500ul 80% ethanol. Fractions were pooled.

Table IV-B

Pre-wash	Mass extract (mg) from 0.100 g hemp	CBD (mg) from 0.100 g hemp	% recovery from total in tissue	% of mass extracted
10% EtOH	3.51704	0.55792	32.6601693	15.86335
20% EtOH	4.10537	0.5485614	32.1122937	13.36206
30% EtOH	4.10044	0.5997451	35.1085456	14.62637
40% EtOH	4.23101	0.5910847	34.6015711	13.97028

[0062] Figures 2A and 2B shows the separation and detection of CBD, CBDa (Fig. 2A) THC and THCa (Fig. 2B) by isocratic HPLC in 70% AcN 0.1% formic acid, of a 100% Ethanol extract by 300 Angstrom 5 micron C18 porous resin to LC-ESI-MS (n=1). Note the porous resin does not resolve CBD from THC.

[0063] Figure 3 shows the separation and detection of CBD in hemp sample A on a 300 Angstrom 5 micron C18 porous resin by LC-ESI-MS with a isocratic HPLC in 70% AcN 0.1% formic acid. , HA1 is a sample heated and extracted in 100% acetonitrile (AcN); HE8 is a sample heated and extracted in 80% EtOH; and HE1 is a sample heated and extracted in 100% EtOH. (n=5). Spectra depicts (A) 3x blank (B) external CBD standard curve from 0-200uM (C) detection of CBD (m/z 315) in hemp A HA1 extract, (D) HE8 extract (E) HE1 extract, (F) mix of HE8 and HE1 extract, (C1) HA1 extract spiked with CBD-D3 (m/z 318), (D1) HE8 extract spiked with CBD-D3, (E1) HE1 extract spiked with CBD-D3 and (F1) mix of HE8 and HE1 extract spiked with CBD-D3.

[0064] Figure 4 shows the separation and detection of CBD, CBDa, THC and THCa by isocratic HPLC in 70% AcN 0.1% formic acid and LC-ESI-MS (n=1), using a Kinetex™ coreshell resin.

- Figure 4A - CBD alone;
- Figure 4B - THC alone;
- Figure 4C - CBD and THC separation on a 6 cm column;
- Figure 4D, CBD and THC separation on a 15 cm column.

[0065] Figures 5A and 5B shows the separation and detection of CBD, CBDa, THC and THCa by gradient HPLC and LC-ESI-MS (n=1). Figure 5A shows the results for Sample 1 spiked with CBD-D3; and Figure 5B shows the results for Sample 2 spiked with CBD-D3; Figures 5C and 5D show CBD and THC reference standards respectively. Gradients - Samples were diluted in B buffer (65% AcN, 5%FA) with gradient 0 min at 70%, 10 min linear gradient to 80%, held for 5 min at 80% and equilibrate at 70% (base peak).

[0066] Figure 6 shows the separation and detection of CBD, CBDa, THC and THCa by isocratic HPLC in 70% AcN 0.1% formic acid and LC-ESI-MS (n=1) on a 15 cm column over Kinetex™ coreshell resin. Figure 6A shows the CBD and THC reference standard; 6B shows CBD alone; 6C shows THC alone; 6D shows sample A spiked with CBD-D3; 6E shows sample B spiked with THC-D3.

[0067] Figure 7 shows the decarboxylation of CBDa at different temperatures. Figure 7A, 0.1g of hemp sample 1 was heated to the appropriate temperature for 1 hour and extracted 3x with 100% Ethanol prior to LC-ESI-MS (n=3); Figure 7B, 0.1g of hemp sample 2 was heated to the appropriate temperature for 1 hour and extracted with 100% Ethanol prior to

LC-ESI-MS (n=1); Figure 7C, , 0.1g of hemp sample 2 was heated to the appropriate temperature for 1 hour and extracted with 100% Ethanol prior to LC-ESI-MS (n=3)

[0068] Figure 8 shows a CBD internal vs external standard (ES) curve (n=2) from 50-5000 nM. Figure 8A shows a CBD external standard curve. Figure 8B shows an internal versus external CBD curve. Figure 8C shows an illustration spectra of the detection of CBD (m/z 315) in a hemp sample heated to 120°C spiked with CBD-D3 (m/z 318) measured by isocratic separation over C18 resin.

[0069] Figure 9 shows an EtOH gradient extraction of CBD. 0.1g of hemp was heated to 120^0C for 1 hour, extracted in a sequential step gradient of increasing Ethanol and analyzed by LC-ESI-MS (n=3). Figure 9A shows CBD extracted in ethanol gradient from a hemp sample heated to 120°C vs no heating control (0C); Figure 9B shows CBD and CBDa extracted in an ethanol gradient (from 0% to 100% vol in water) from the no heating control sample (0C); Figure 9C shows CBD and CBDa extracted in an ethanol gradient from a hemp sample heated to 120°C for 1 hour.

[0070] Figure 10 shows the effect of pH on the extraction of CBD from hemp in 80% Ethanol (n=3). 0.1g of hemp was heated to 120°C for 1 hour, washed 3x with H2O, 3x with 40% Ethanol followed by extraction in 80% Ethanol at various pH levels prior to analysis by LC-ESI-MS. Sample 1, 0.1% TFA in 80% EtOH pH>2 (not adjusted); Sample 2, 0.1% FA in 80% EtOH pH2 (not adjusted); Sample 3, 0.1% Acetic acid in 80% EtOH pH3 (not adjusted); Sample 4, 10mM Citric acid in 80% EtOH pH4; Sample 5, 10mM Citrate in 80% EtOH pH5; Sample 6, 10mM Citrate in 80% EtOH pH6; Sample 7, 10mM Tris in 80% EtOH pH7; Sample 8, 10mM Tricine in 80% EtOH pH8; Sample 9, 0.1% Ethanolamine in 80% EtOH pH9; Sample 10, 0.1% Ethanolamine in 80% EtOH pH10;

Sample 11, 0.1% Ammonia in 80% EtOH pH<10 (not adjusted); Sample 12, 80% EtOH in H2O (pH not adjusted).

[0071] The results show that CBD recovery is not significantly affected by the pH of the selective solvent.

[0072] Figure 13 shows optimized binding conditions for CBD using ion exchange chromatography (n=3). 0.1 g of hemp was heated to 120°C and extracted 3x in 100% ethanol. Extracts of 0.5 g total hemp per replicate were pooled and diluted with H_2O to the appropriate ethanol concentrations. Samples were loaded at 100% ethanol and decreasing to 10% ethanol onto the various resins (DEAE – Figure 13A, CMS – Figure 13B, HiQ – Figure 13C, HiS – Figure 13D. The Flow Throughs (FT) were collected and analyzed by LC-ESI-MS.

[0073] Figure 14 shows elution curves in Ethanol or Acetonitrile for HiQ columns (n=3). Figure 14A, ~7.5 mg CBD was extracted from 0.5g hemp in 100 % Ethanol, diluted to 20% ethanol and loaded onto a ~100ul HiQ column. The column was washed with loading solvent prior to elution with increasing amounts of Ethanol (25, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 55 and 60%). Up to ~ 50%, the fractions are observed to be clear. Above 50%, the elutions become green. Fractions were collected and analyzed by LC-MS. Figure 14B, ~7.5 mg CBD was extracted from 0.5g hemp in 100 % Acetonitrile, diluted to 20% acetonitrile and loaded onto a ~100ul HiQ column. The column was washed with loading solvent prior to elution with increasing amounts of Acetonitrile (25, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 55 and 60%). Up to ~ 35%, the fractions are observed to be clear. Above 35%, the elutions become green (elutions separate in 2 distinct layers, one clear and one green). Fractions were collected and analyzed by LC-MS.

[0074] Figure 15 shows a CBD external standard curve 50-50,000nM (n=2) measured by LC-ESI-MS/MS using isocratic separation over C18 resin.

[0075] Figure 16 shows the effect of a wash of intermediate resin from cannabis sample A. A 0.1 g aliquot of cannabis sample A was washed in water and heated to 120°C for 1 hour, extracted in ethanol and dried under vacuum to make an intermediate resin. The water soluble components of the resin were washed in water or water modified with 0.5% acetic acid (v:v), 40% ethanol, salt/ethanol and saturated salt.

[0076] The results show that 40% ethanol was a better polar solvent in removing soluble compounds compared to the other solvents from the resin in sample A.

[0077] Figure 17 shows the results of a wash of intermediate resin from cannabis sample B. A 0.1 g aliquot of cannabis sample A was washed in water and heated to 120°C for 1 hour, extracted in ethanol and dried under vacuum to make an intermediate resin. The water soluble components of the resin were washed in water or water modified with 0.5% acetic acid, 40% ethanol, salt/ethanol and saturated salt. The results show that that 40% ethanol was a better polar solvent in removing soluble compounds compared to the other solvents from the resin in sample B.

[0078] Figure 18 shows the results of a selective extraction of intermediate resin from cannabis sample A. A 0.1 g aliquot of cannabis sample B was washed in water and heated to 120° C for 1 hour, extracted in ethanol and dried under vacuum to make an intermediate resin. The water soluble components of the resin were washed in water modified with 0.5% acetic acid and extracted with 1 ml of the solvent shown.

[0079] Figure 19 shows the results of extraction of intermediate resin from cannabis sample B. A 0.1 g aliquot of cannabis sample B was washed in water and heated to 120°C for 1 hour, extracted in ethanol and dried under vacuum to make a intermediate resin. The water soluble components of the resin were washed in water modified with 0.5% acetic acid and extracted with 1 ml of a solvent: acetone, acetonitrile, ether, ethyl acetate, ethanol, hexane, isopropyl alcohol, and methanol.

Definitions and Interpretation

[0080] The description of the present invention has been presented for purposes of illustration and description, but it is not intended to be exhaustive or limited to the invention in the form disclosed. Many modifications and variations will be apparent to those of ordinary skill in the art without departing from the scope and spirit of the invention. Embodiments were chosen and described in order to best explain the principles of the invention and the practical application, and to enable others of ordinary skill in the art to understand the invention for various embodiments with various modifications as are suited to the particular use contemplated. To the extent that the following description is of a specific embodiment or a particular use of the invention, it is intended to be illustrative only, and not limiting of the claimed invention.

[0081] References in the specification to "one embodiment", "an embodiment", etc., indicate that the embodiment described may include a particular aspect, feature, structure, or characteristic, but not every embodiment necessarily includes that aspect, feature, structure, or characteristic. Moreover, such phrases may, but do not necessarily, refer to the same embodiment referred to in other portions of the specification. Further, when a particular aspect, feature, structure, or characteristic is described in connection with an

embodiment, it is within the knowledge of one skilled in the art to combine, affect or connect such aspect, feature, structure, or characteristic with other embodiments, whether or not such connection or combination is explicitly described. In other words, any element or feature may be combined with any other element or feature in different embodiments, unless there is an obvious or inherent incompatibility between the two, or it is specifically excluded.

[0082] It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for the use of exclusive terminology, such as "solely," "only," and the like, in connection with the recitation of claim elements or use of a "negative" limitation. The terms “preferably,” “preferred,” “prefer,” “optionally,” “may,” and similar terms are used to indicate that an item, condition or step being referred to is an optional (not required) feature of the invention.

[0083] The singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise. The term "and/or" means any one of the items, any combination of the items, or all of the items with which this term is associated.

[0084] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges recited herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof, as well as the individual values making up the range, particularly integer values. A recited range (e.g., weight percents or carbon groups) includes each specific value, integer, decimal, or identity within the range. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves,

thirds, quarters, fifths, or tenths. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc.

[0085] As will also be understood by one skilled in the art, all language such as "up to", "at least", "greater than", "less than", "more than", "or more", and the like, include the number recited, and such terms refer to ranges that can be subsequently broken down into sub-ranges as discussed above. In the same manner, all ratios recited herein also include all sub-ratios falling within the broader ratio.

Claims:

1. A method of extracting a cannabinoid from a plant tissue comprising the steps:

a. heating the plant tissue to convert carboxylated forms of the cannabinoid to a decarboxylated active non-polar form;

b. washing the Cannabis tissue with a polar solvent to selectively remove components soluble in the polar solvent, leaving the activated cannabinoid; and

c. selectively extracting the activated cannabinoid using a potable selective solvent.

2. The method of claim 1, wherein the Cannabis tissue is solid or has been processed to a powder or suspension.

3. The method of claim 1 or 2, wherein the extracted cannabinoids are separated using chromatography including ion exchange or reverse phase.

4. The method of claim 3, wherein the separated cannabinoids are identified and/or quantified using mass spectrometry (MS).

5. The method of any one of claims 1-4 wherein the polar solvent comprises water, water modified by a salt, a non-ionic non-polymeric detergent or a bile salt such as sodium deoxycholate, a potable buffer such as a phosphate or carbonate buffer, an organic acid such as acetic acid or formic acid, ammonia, ammonium hydroxide, methylamine trimethylamine, or ethanol, provided the ethanol is less than 40% (v:v).

6. The method of claim 5 wherein the polar solvent comprises less than 5.0% acetic acid in water by volume, such as less than 1.0% acetic acid, such as 0.5% acetic acid.

7. The method of claim 5 wherein the polar solvent comprises 40% ethanol in water by volume.

8. The method of claim any one of claims 1-7 wherein the washing step is performed multiple times.

9. The method of claim 8 wherein the washing is performed multiple times with a first polar solvent, followed by multiple washes with a second polar solvent, different from the first.

10. The method of claim 9 wherein the first polar solvent comprises water modified by an organic acid such as acetic acid and the second polar solvent comprises ethanol, such as 40% ethanol in water.

11. The method of any one of claims 1-8 wherein the washing step is performed at a temperature less than about 5° C.

12. The method of any one of claims 1-11 wherein the selective solvent comprises ethanol or greater than 40% ethanol in water.

13. The method of claim 12 wherein the selective solvent comprises 80% ethanol in water.

14. The method of any one of claims 1-12 wherein the extraction step is performed at a temperature less than about 5° C.

15. The method of claim 14 wherein the extraction step is performed at a temperature less than about 0° C.

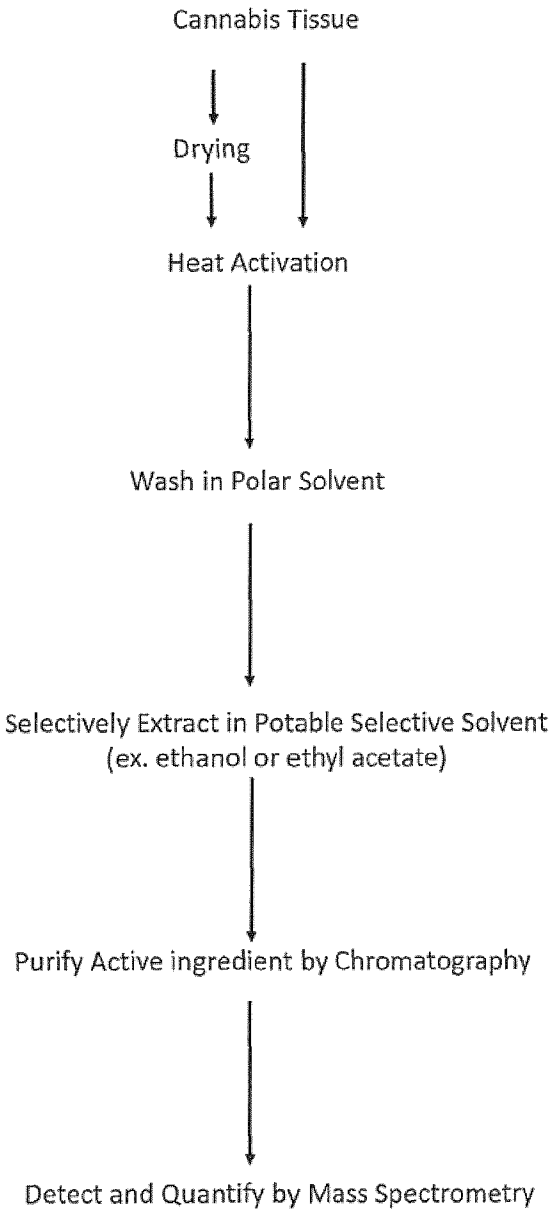

Cannabis Tissue
Drying
Heat Activation
Wash in Polar Solvent
Selectively Extract in Potable Selective Solvent
(ex. ethanol or ethyl acetate)
Purify Active ingredient by Chromatography
Detect and Quantify by Mass Spectrometry

Figure 1

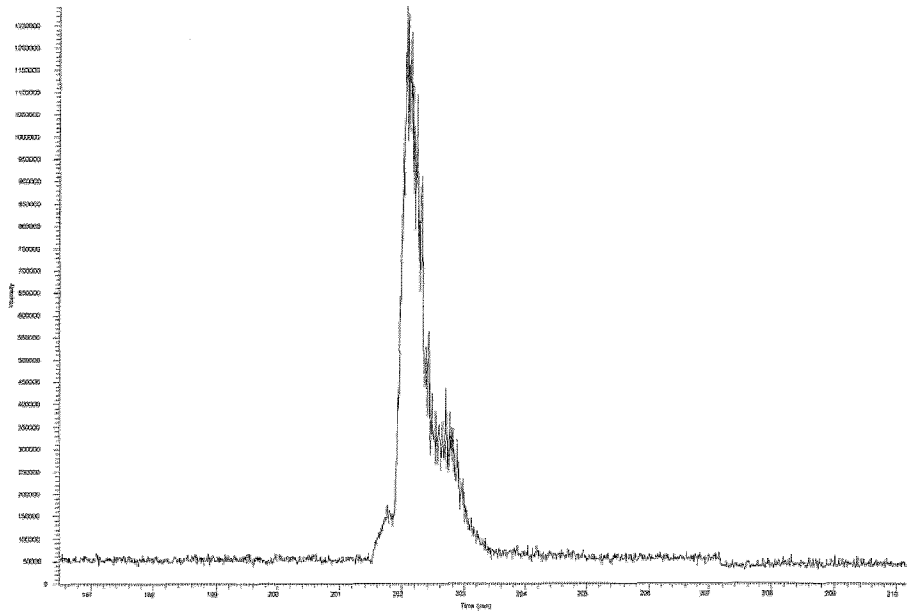


Figure 2A

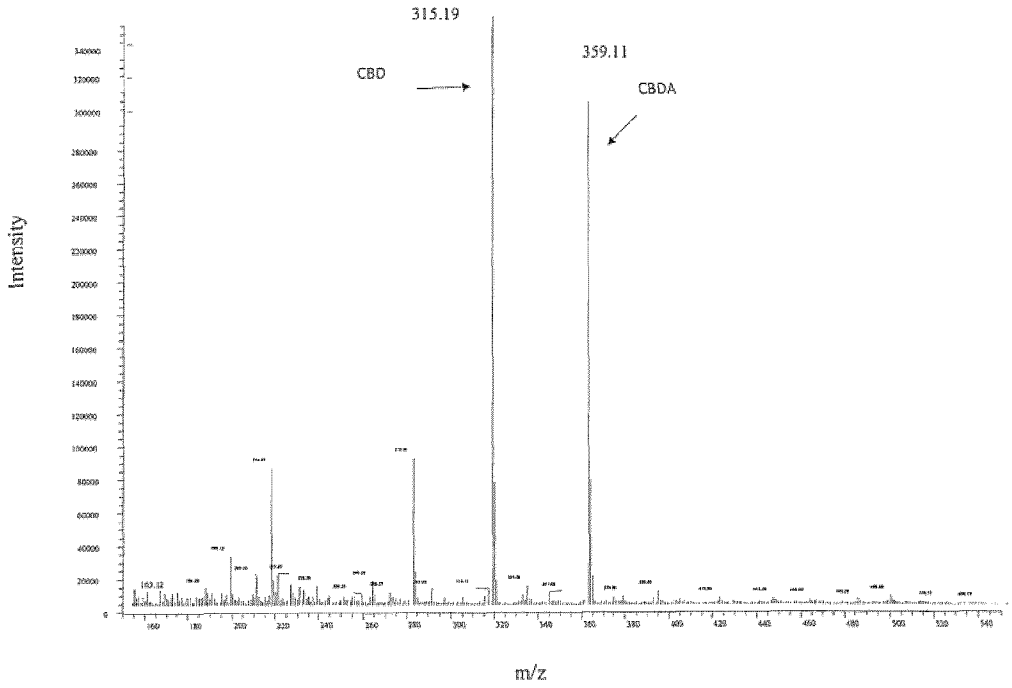


Figure 2B

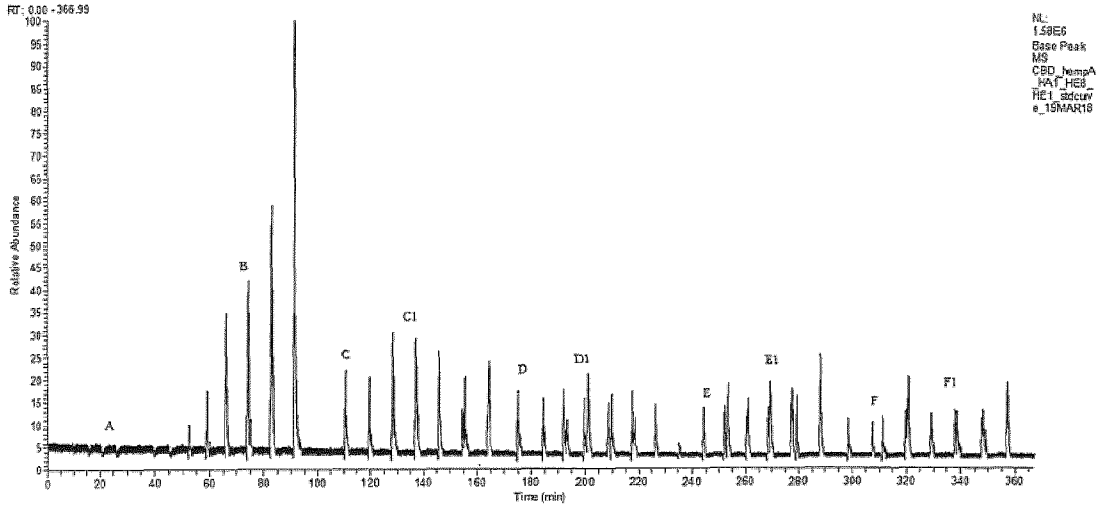

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Figure 3

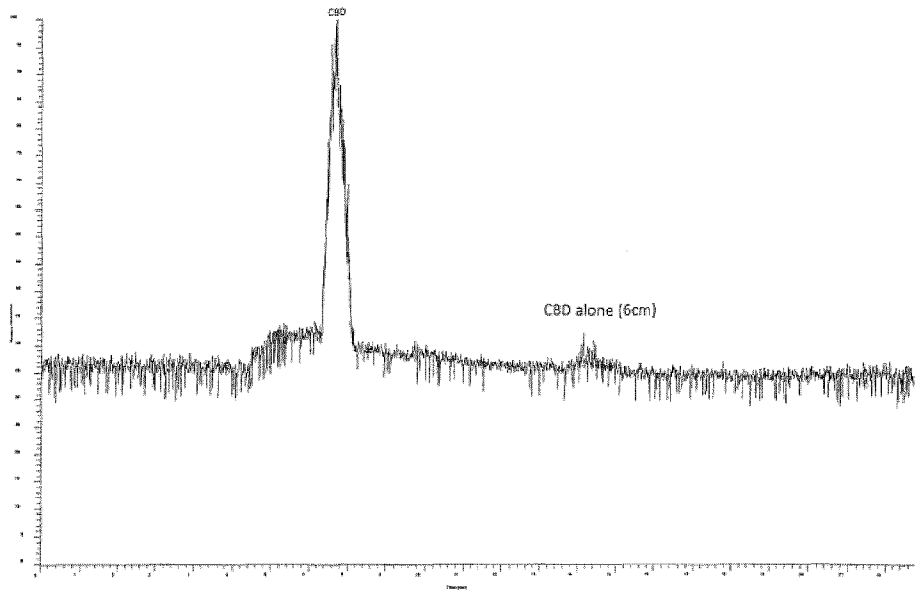


Figure 4A

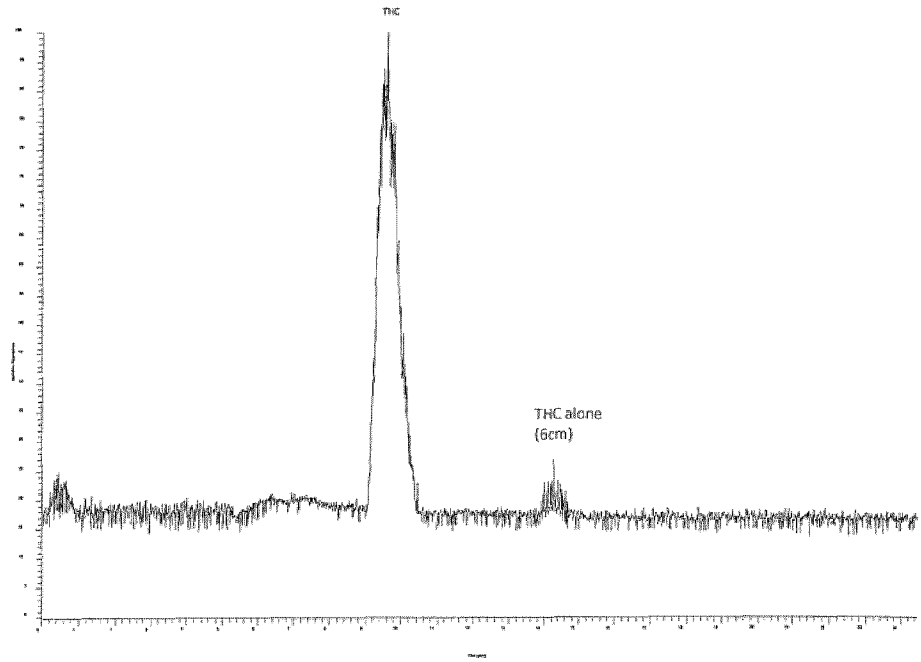
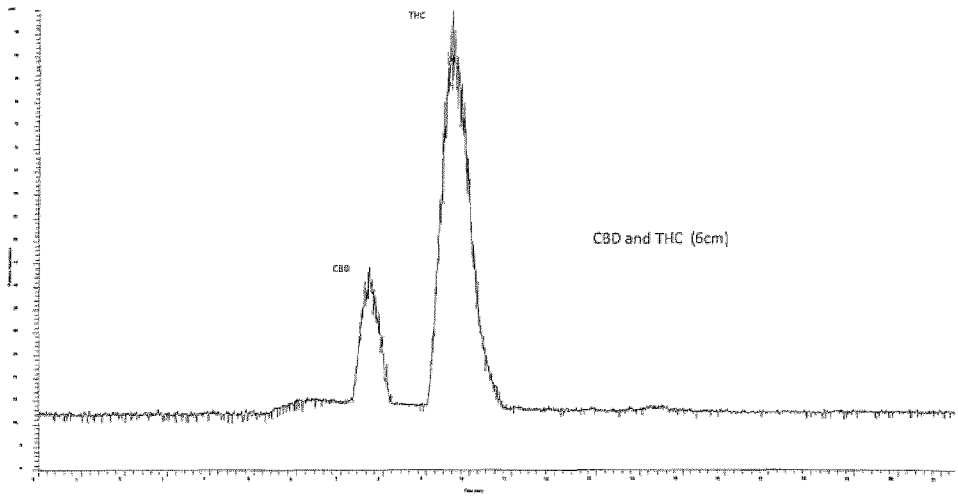
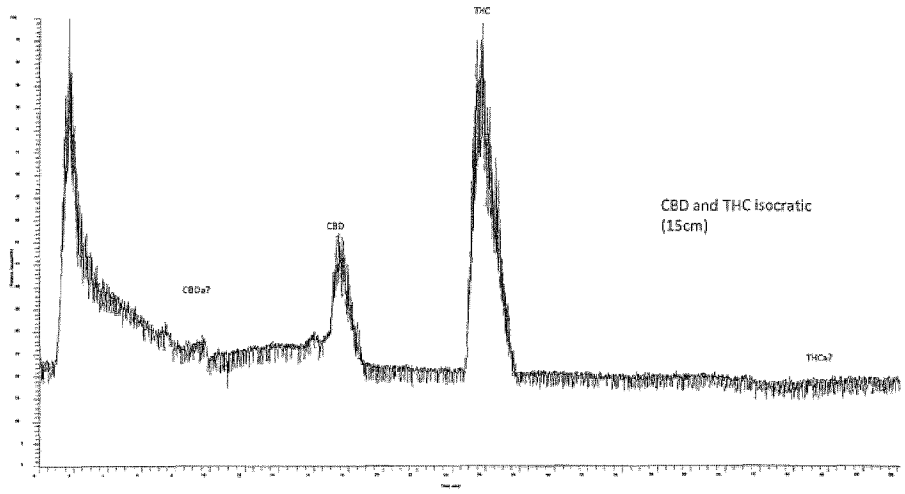
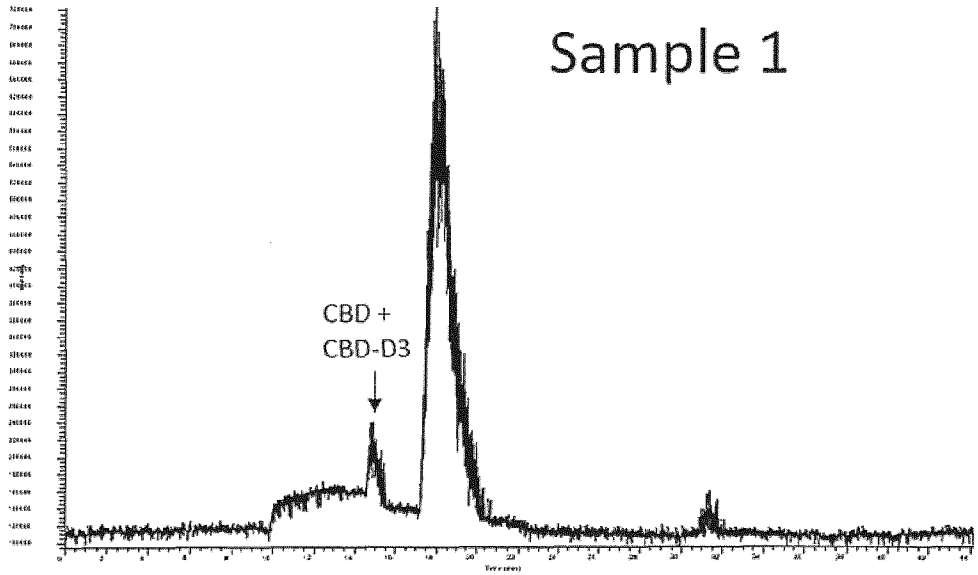


Figure 4B





Figures 4C and 4D



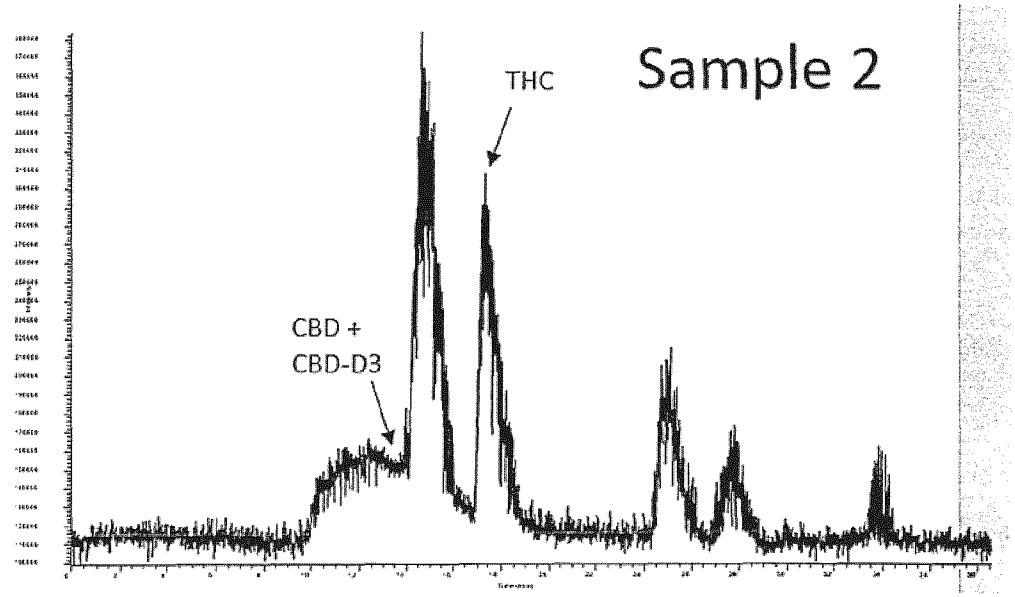


Figure 5A and 5B

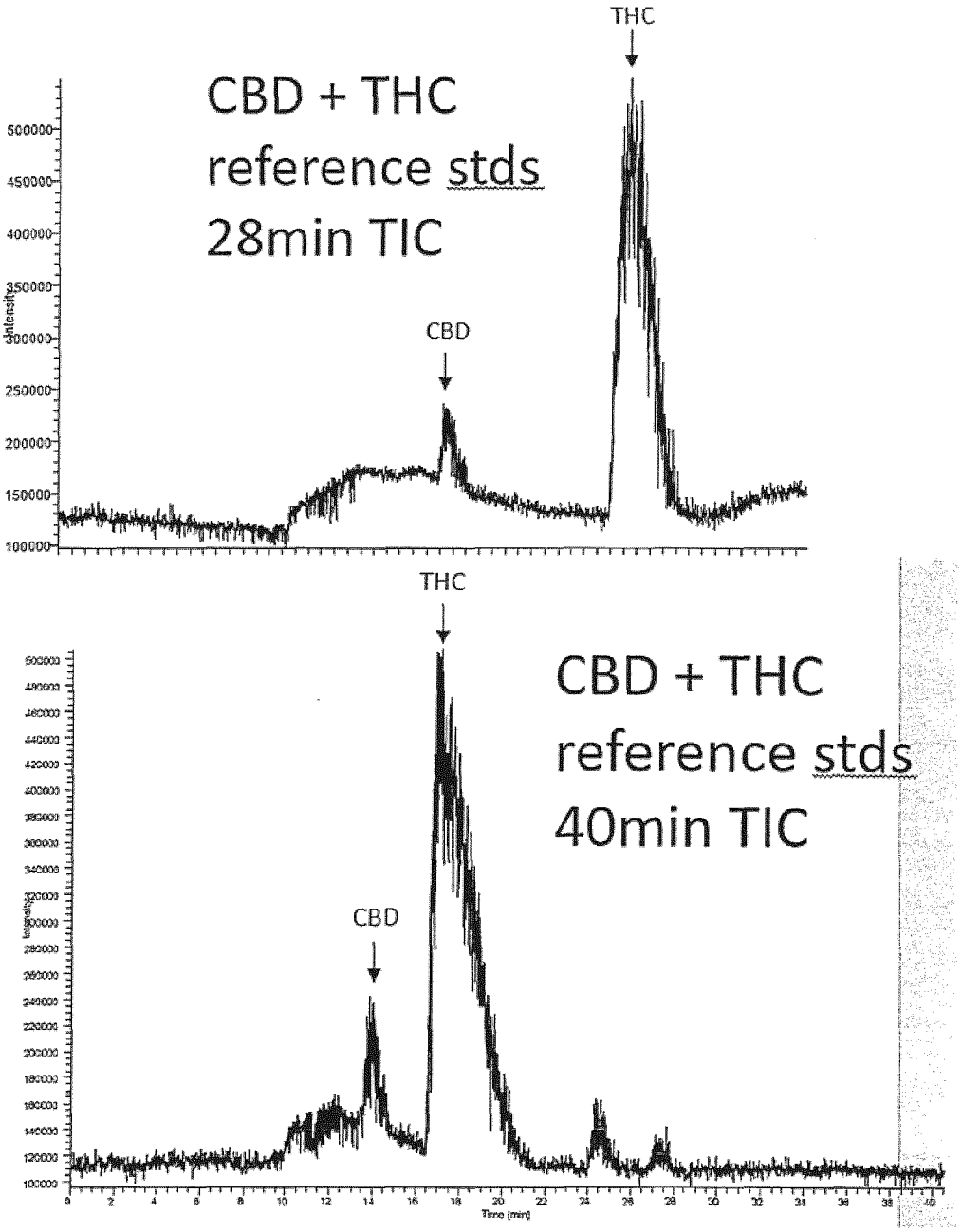


Figure 5C and 5D

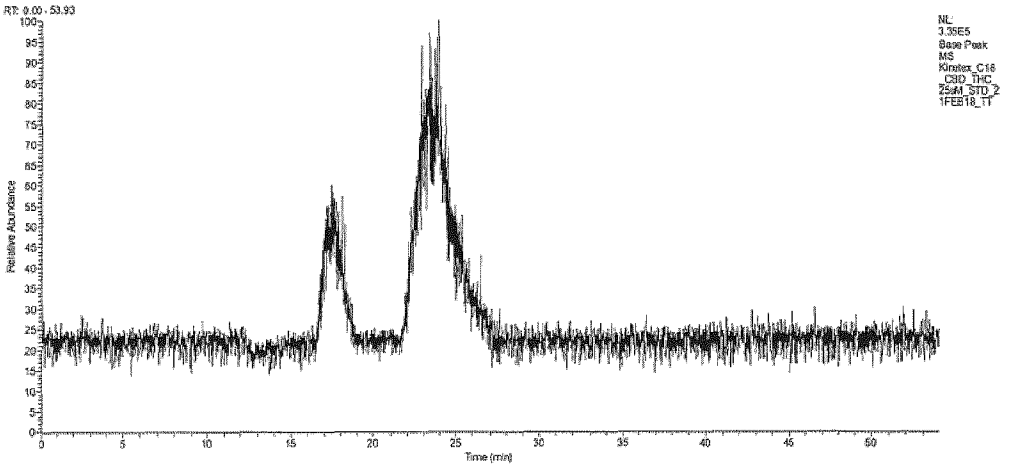


Figure 6A

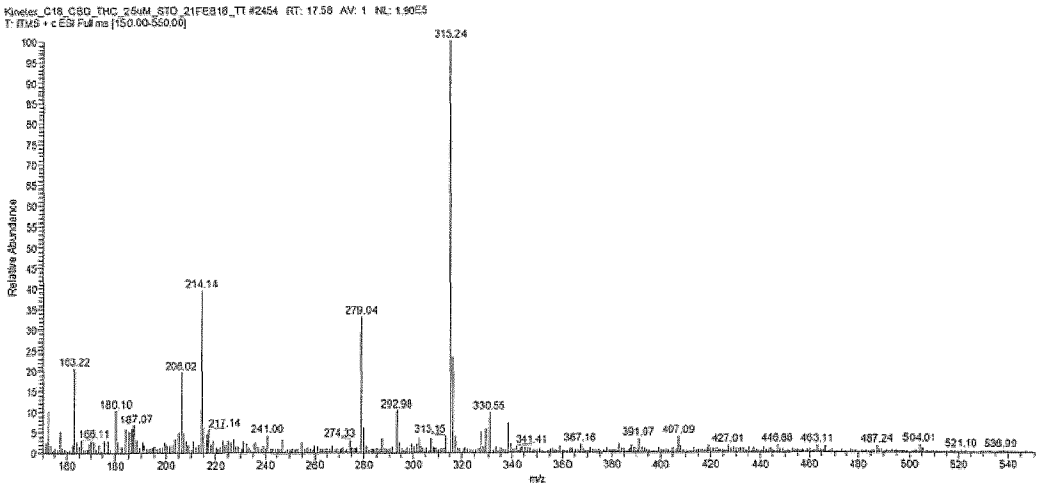


Figure 6B

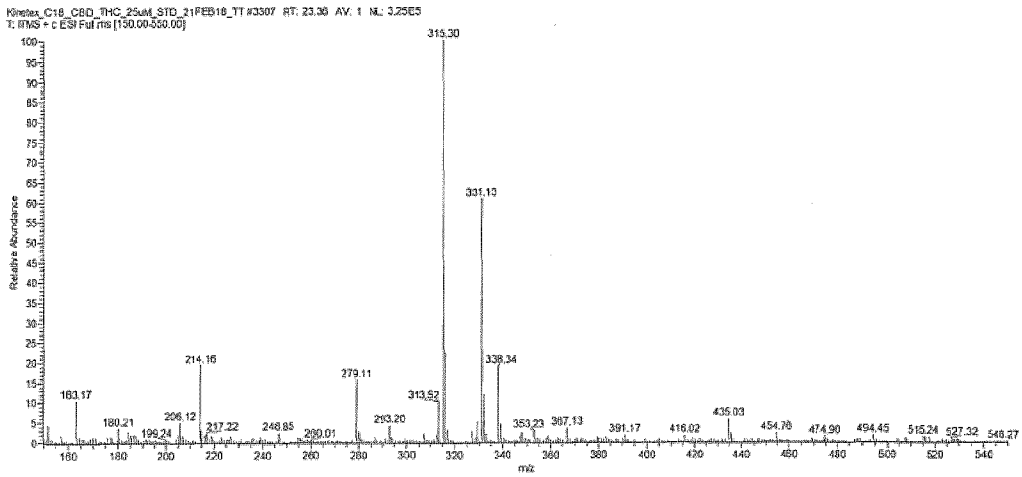


Figure 6C

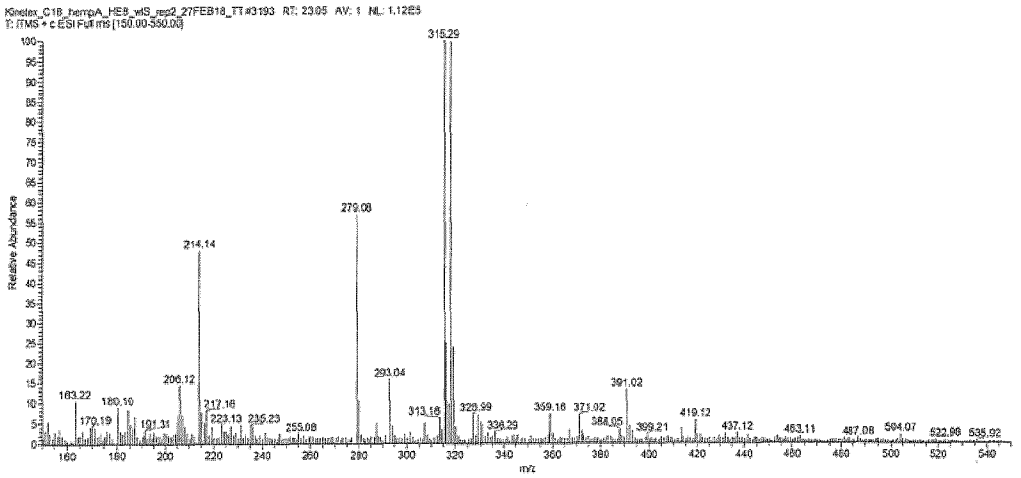
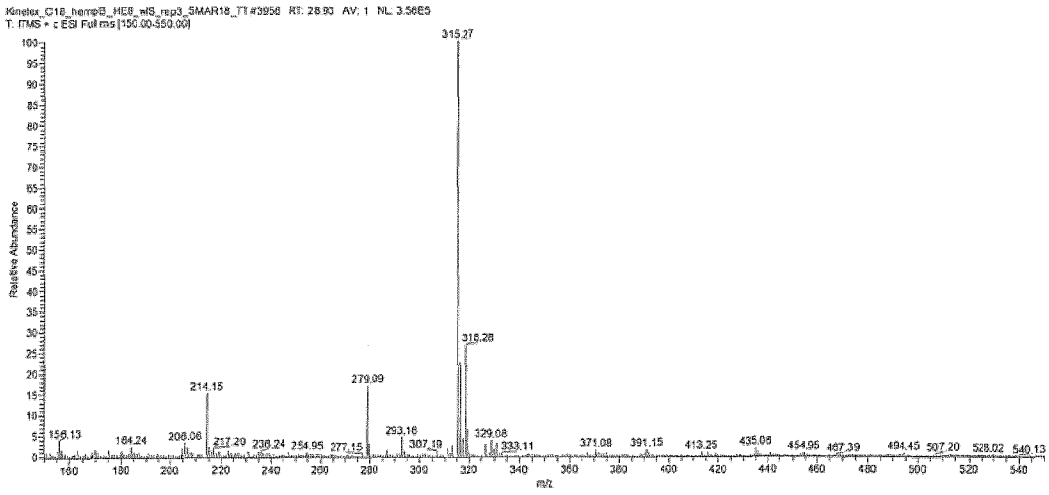


Figure 6D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208.06
217.20
236.24
254.95
277.15
293.16
307.19
329.08
333.11
371.08
391.15
413.25
435.06
454.95
467.39
494.45
507.20
528.02
540.13

Figure 6E

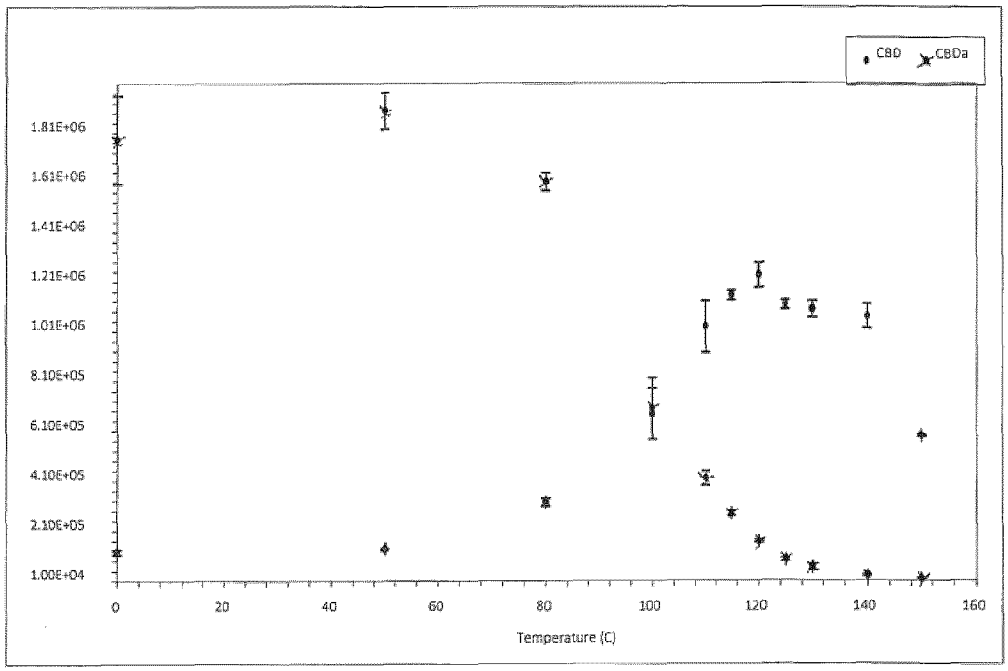
CBD
CBDa
1.81E+06
1.61E+06
1.41E+06
1.21E+06
1.01E+06
8.10E+05
6.10E+05
4.10E+05
2.10E+05
1.00E+04
0
20
40
60
80
100
120
140
160
Temperature (C)

Figure 7A

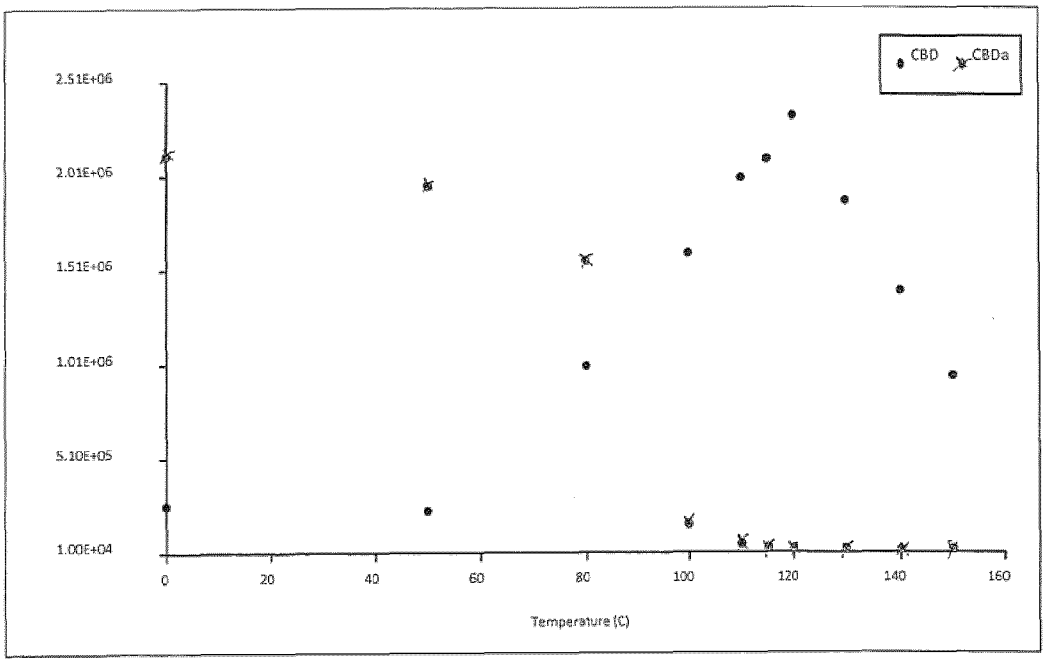
CBD
CBDa
2.51E+06
2.01E+06
1.51E+06
1.01E+06
5.10E+05
1.00E+04
0
20
40
60
80
100
120
140
160
Temperature (C)

Figure 7B

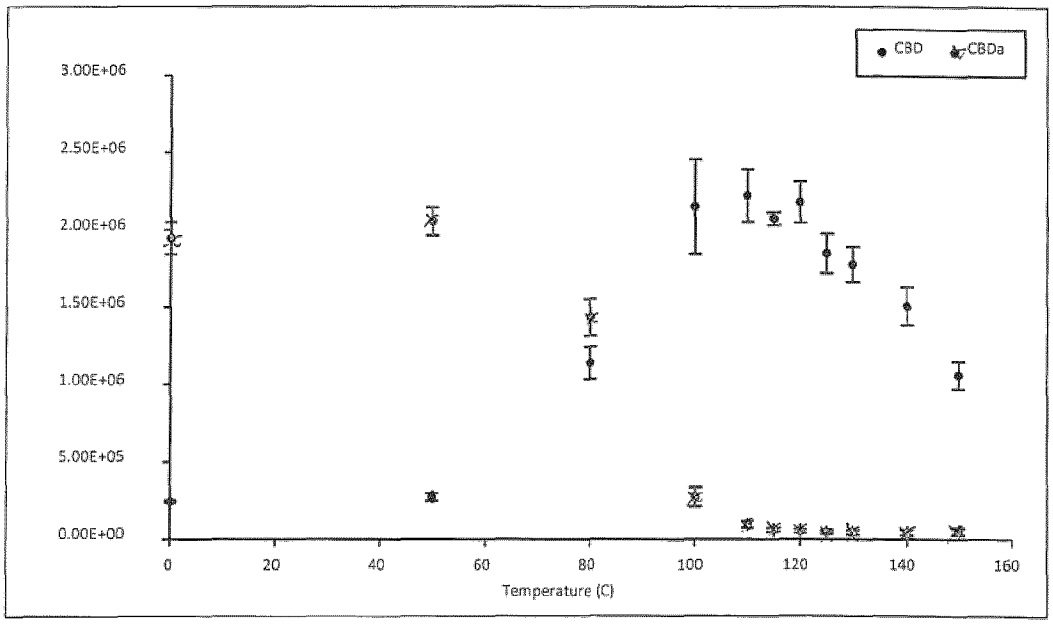
CBD
CBDa
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
0
20
40
60
80
100
120
140
160
Temperature (C)

Figure 7C

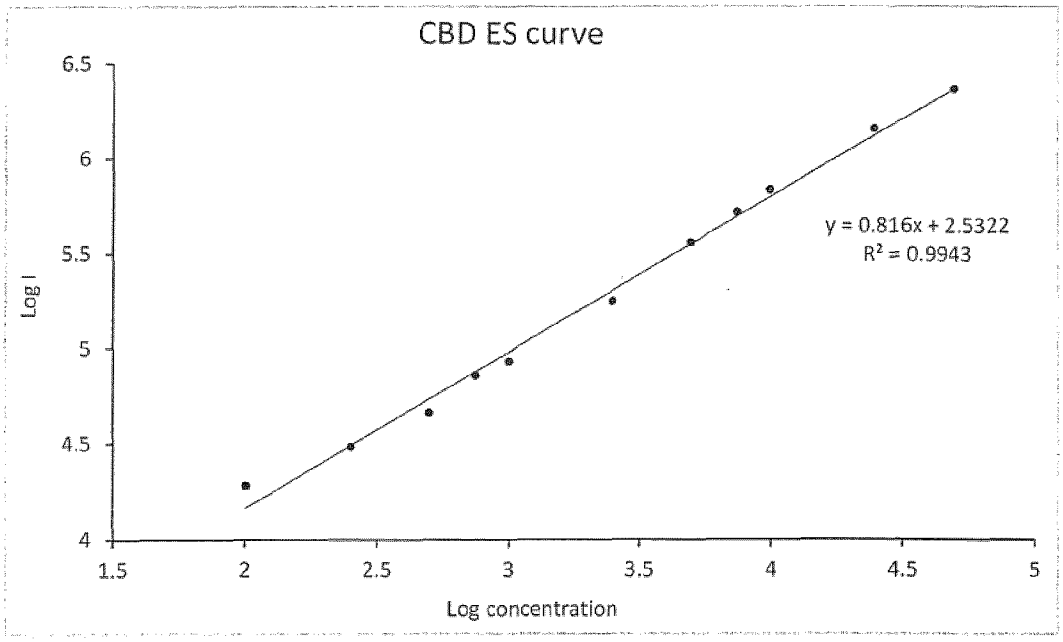
CBD ES curve
6.5
6
5.5
5
4.5
4
Log I
1.5
2
2.5
3
3.5
4
4.5
5
Log concentration
y = 0.816x + 2.5322
R² = 0.9943

Figure 8A

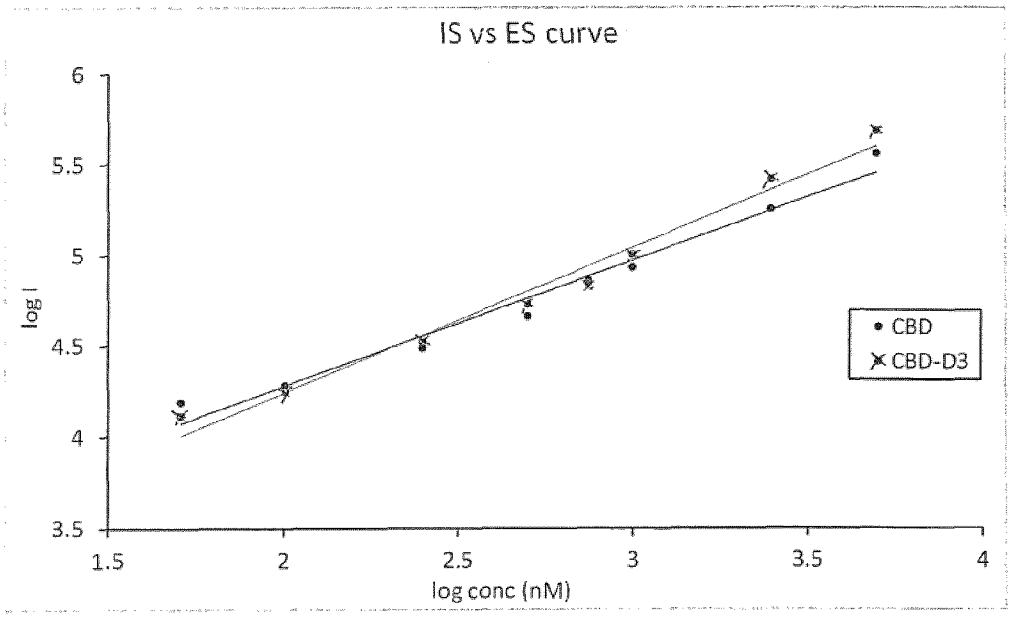
IS vs ES curve
6
5.5
5
4.5
4
3.5
log I
1.5
2
2.5
3
3.5
4
log conc (nM)
CBD
CBD-D3

Figure 8B

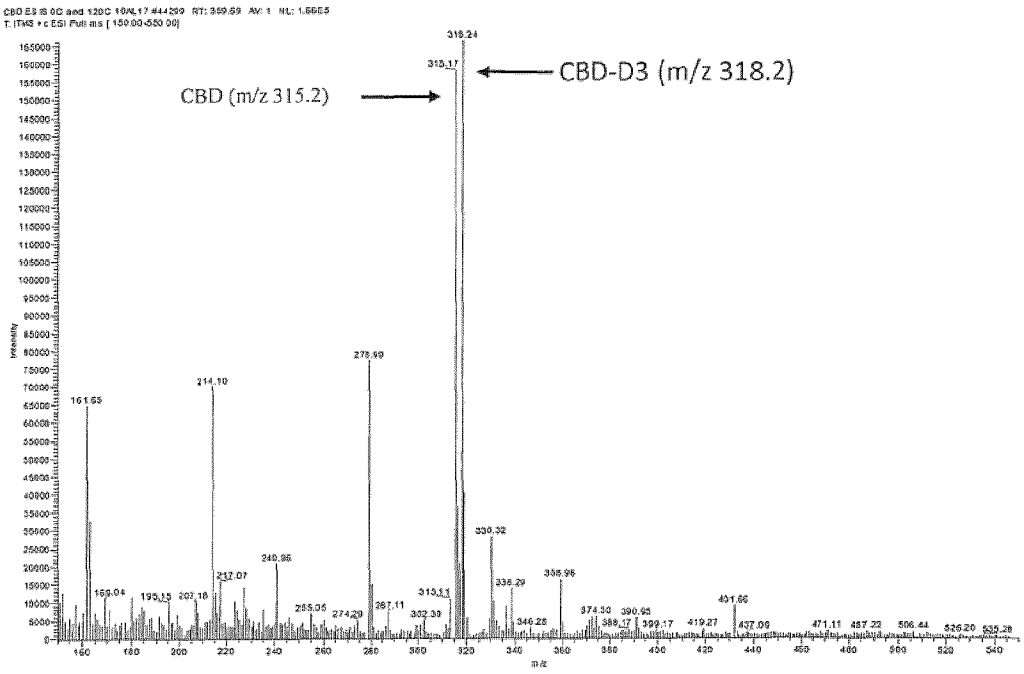
CBD (m/z 315.2)
CBD-D3 (m/z 318.2)
318.24
315.17
278.99
214.10
161.65
330.32
240.96
217.07
336.29
355.96
169.04
195.15
203.18
265.05
274.29
287.11
313.11
302.39
346.26
374.30
390.95
388.17
399.17
419.27
431.66
437.09
471.11
487.22
506.44
526.20
535.28
Intensity
m/z

Figure 8C

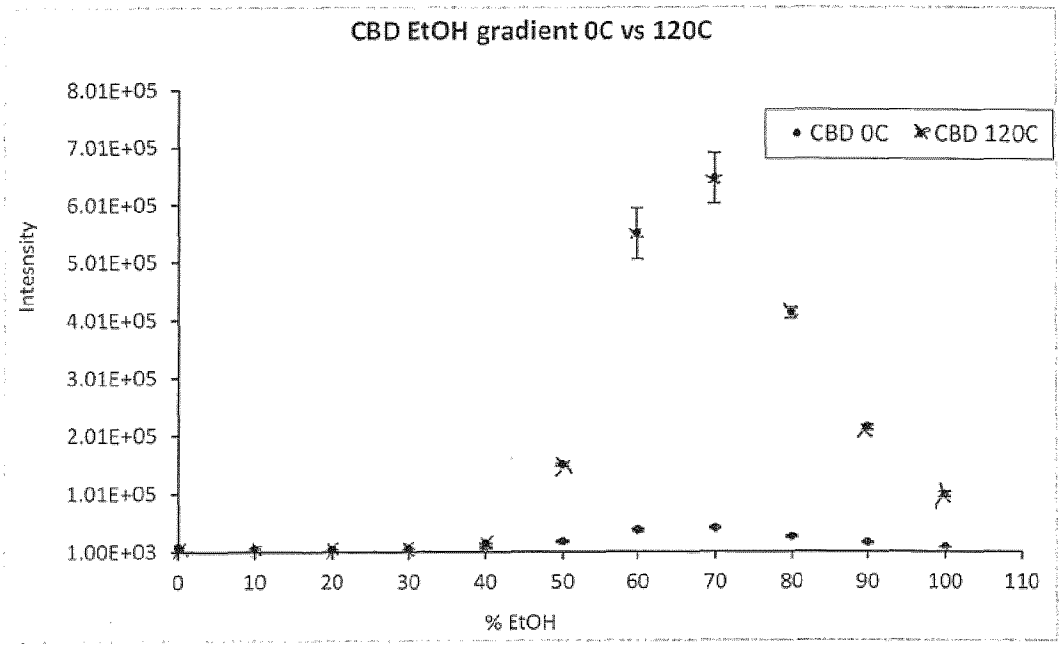
CBD EtOH gradient 0C vs 120C
8.01E+05
7.01E+05
6.01E+05
5.01E+05
4.01E+05
3.01E+05
2.01E+05
1.01E+05
1.00E+03
Intesnsity
0
10
20
30
40
50
60
70
80
90
100
110
% EtOH
CBD 0C
CBD 120C

Figure 9A

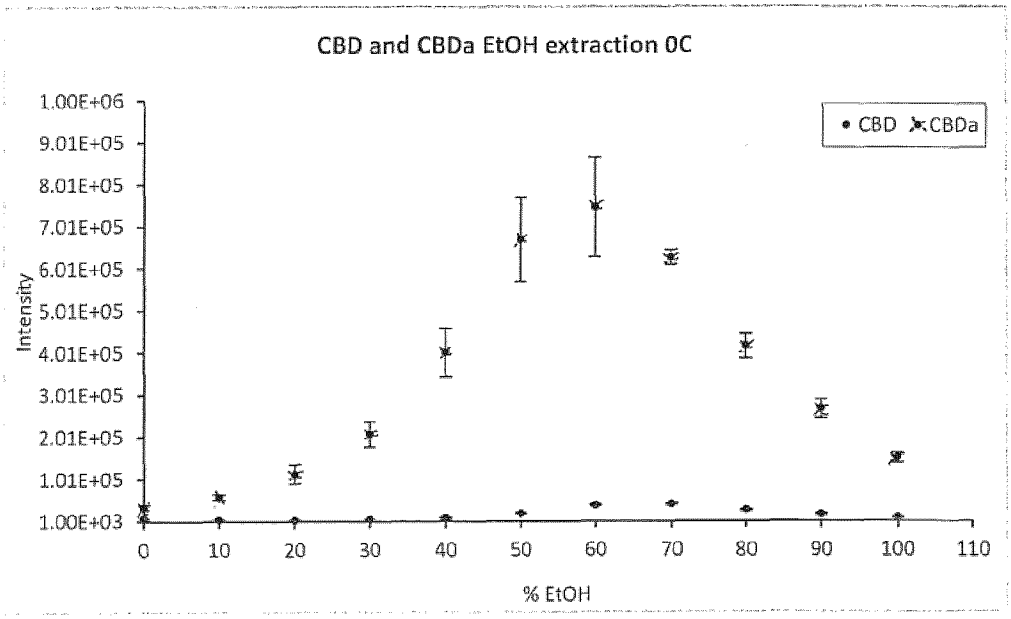
CBD and CBDa EtOH extraction 0C
1.00E+06
9.01E+05
8.01E+05
7.01E+05
6.01E+05
5.01E+05
4.01E+05
3.01E+05
2.01E+05
1.01E+05
1.00E+03
Intensity
0
10
20
30
40
50
60
70
80
90
100
110
% EtOH
CBD
CBDa

Figure 9B

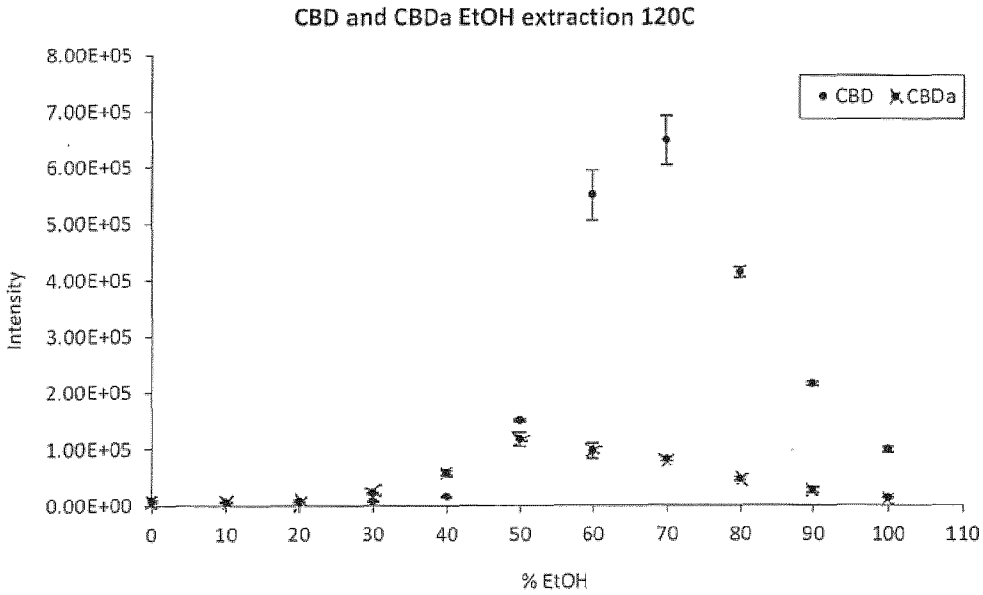
CBD and CBDa EtOH extraction 120C
8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Intensity
0
10
20
30
40
50
60
70
80
90
100
110
% EtOH
CBD
CBDa

Figure 9C

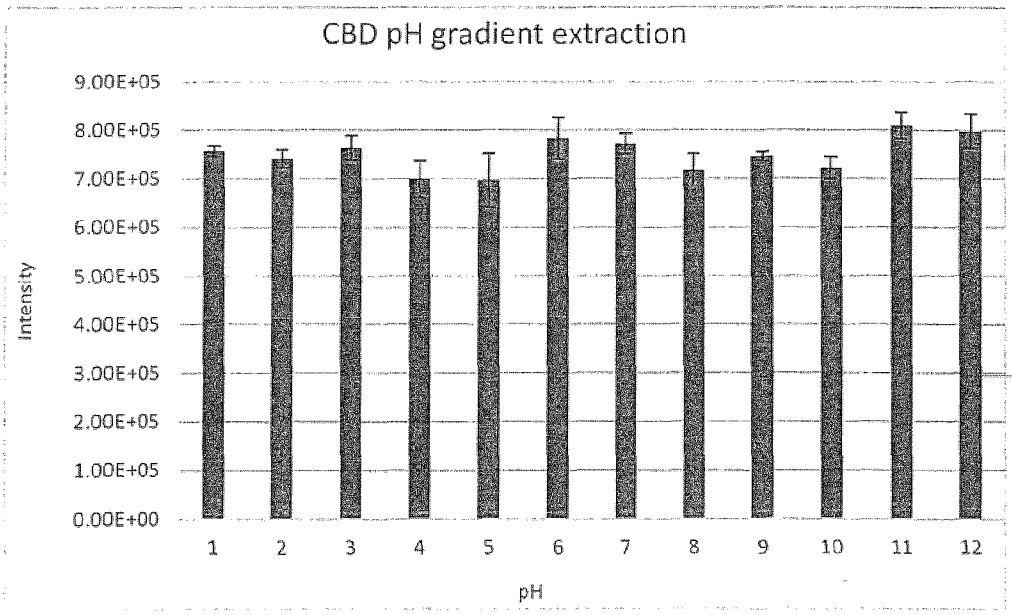
CBD pH gradient extraction
9.00E+05
8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Intensity
1
2
3
4
5
6
7
8
9
10
11
12
pH

Figure 10

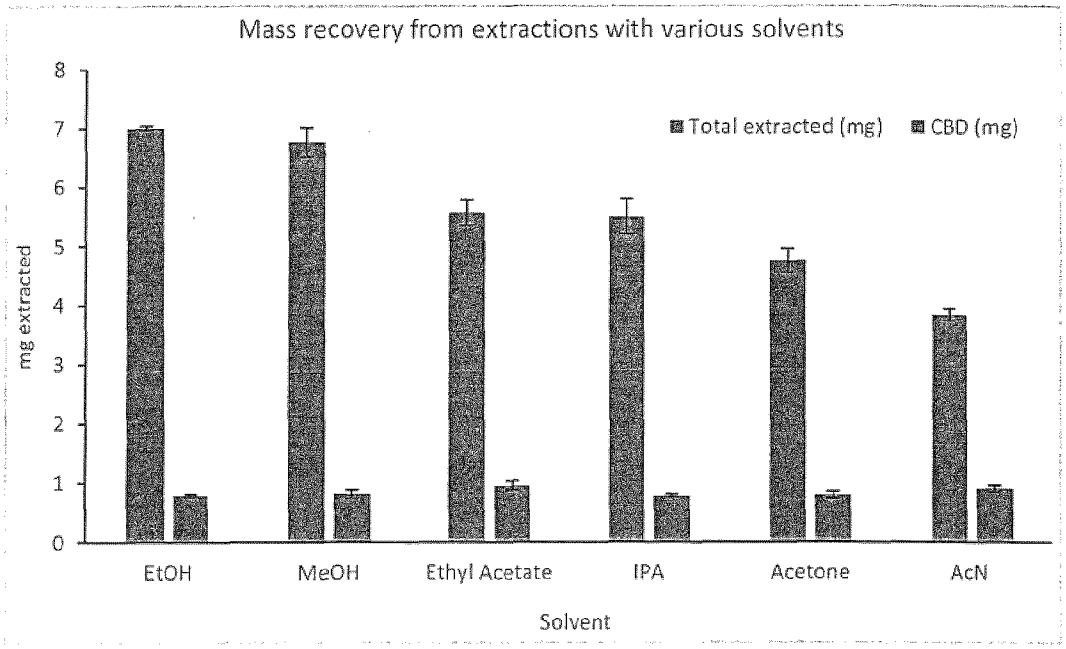
Mass recovery from extractions with various solvents
Total extracted (mg)
CBD (mg)
mg extracted
0
1
2
3
4
5
6
7
8
EtOH
MeOH
Ethyl Acetate
IPA
Acetone
AcN
Solvent

Figure 11

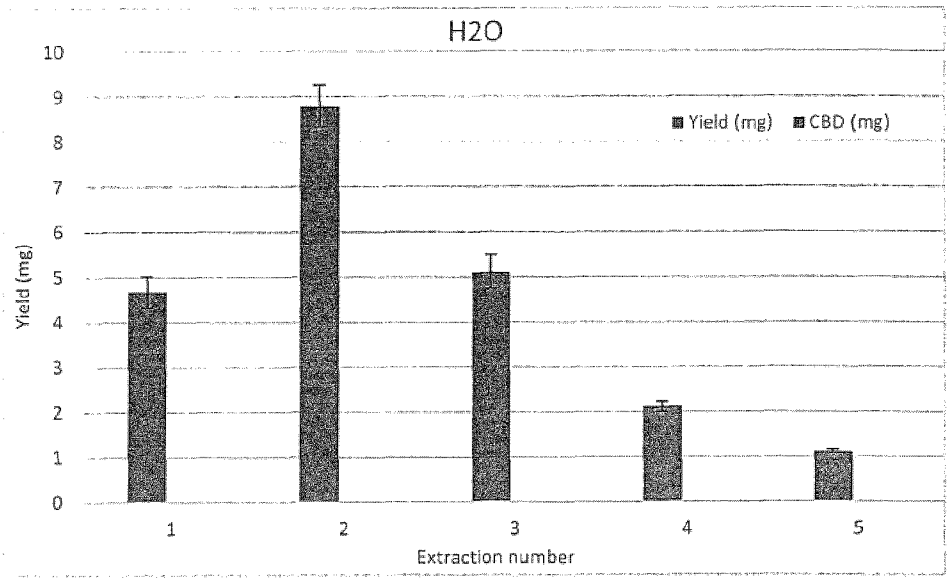

Figure 12A

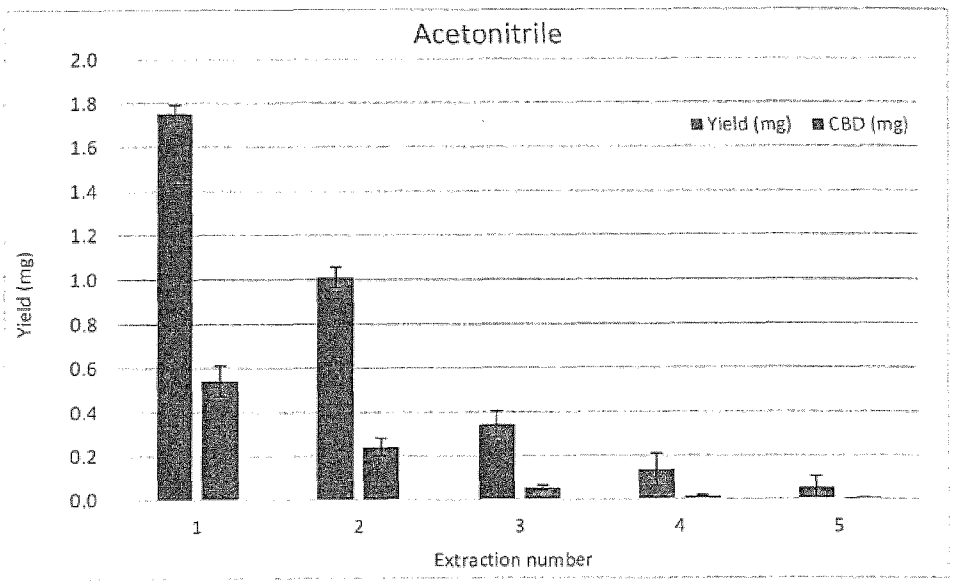

Figure 12B

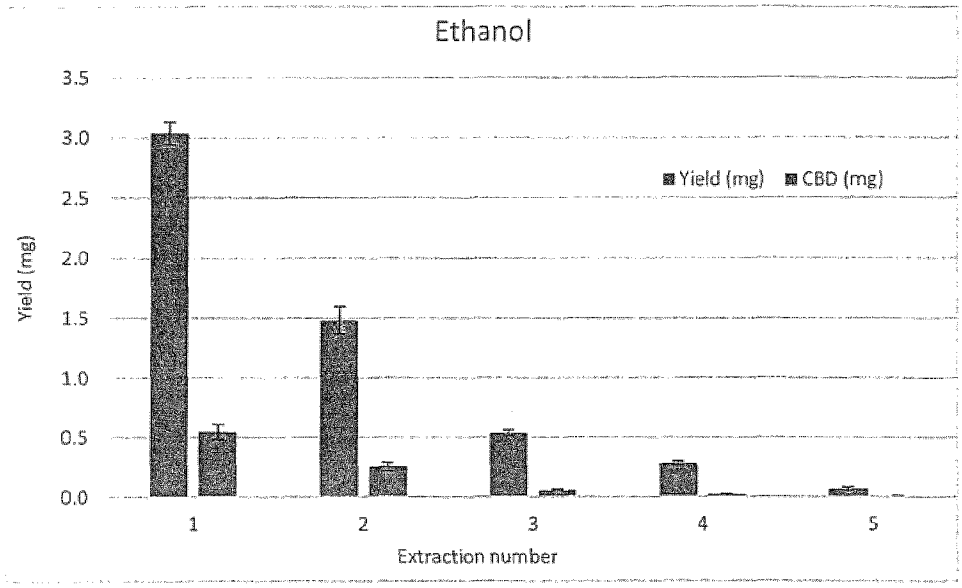

Figure 12C

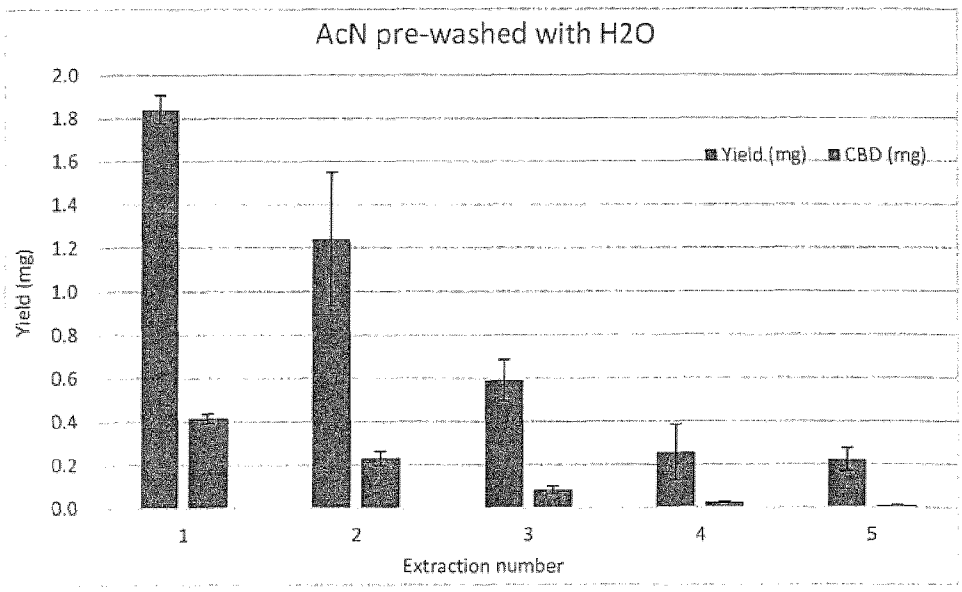

Figure 12D

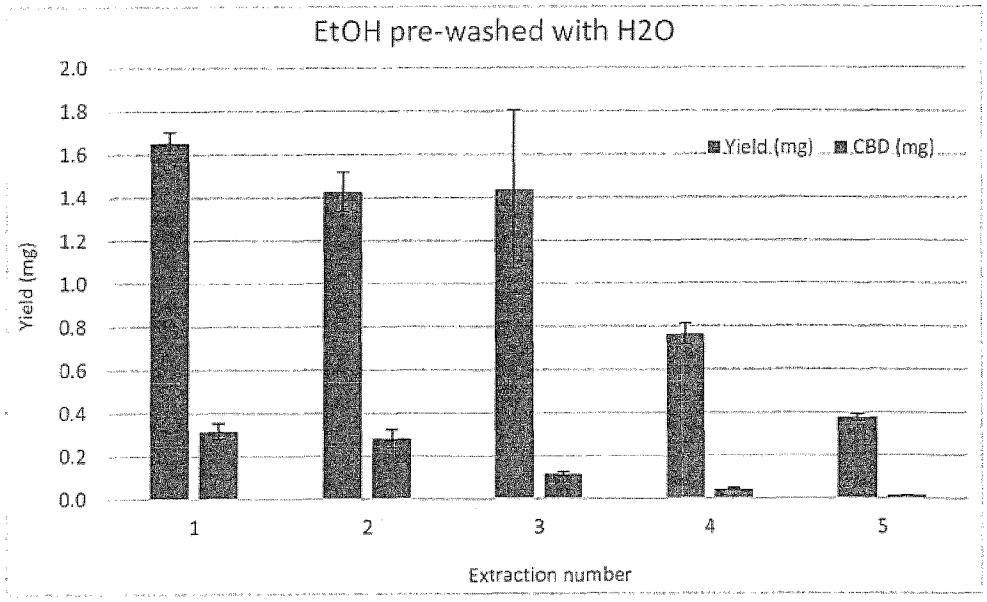
EtOH pre-washed with H2O
Yield (mg)
CBD (mg)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Yield (mg)
1
2
3
4
5
Extraction number

Figure 12E

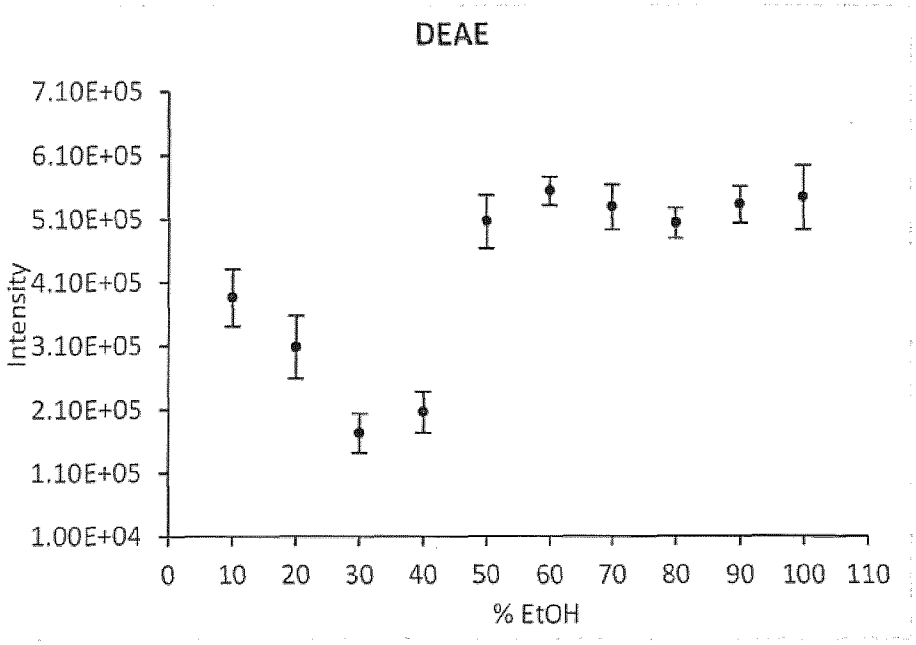

Figure 13A

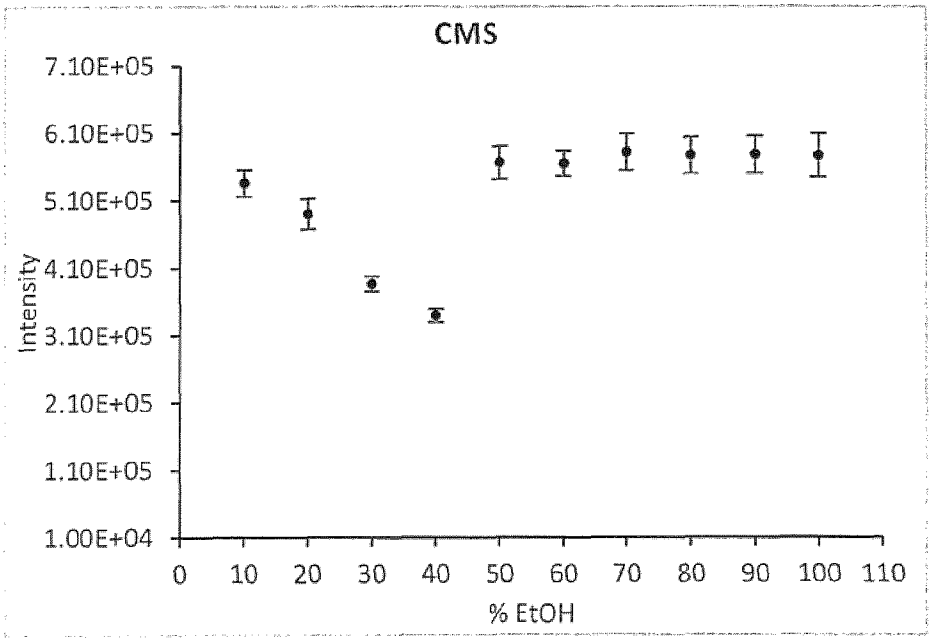

Figure 13B

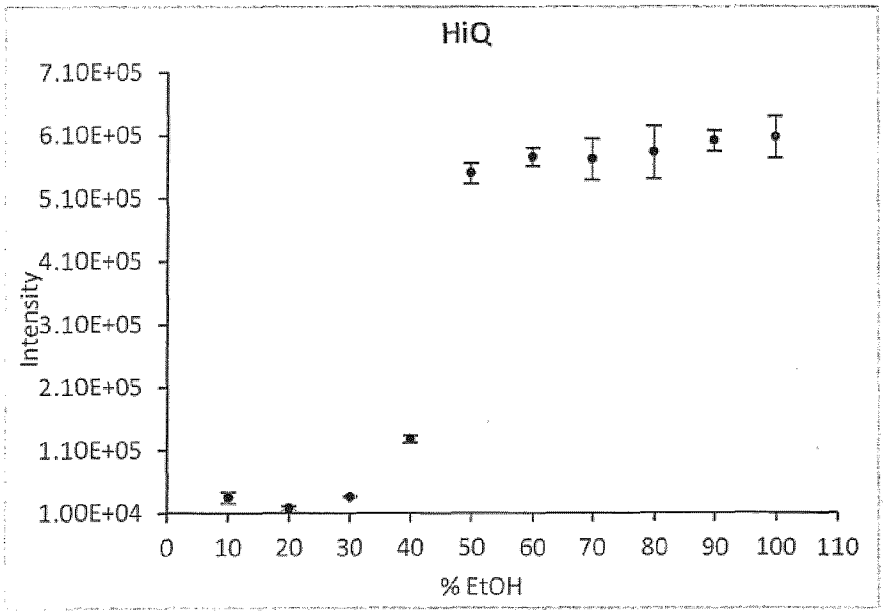

Figure 13C

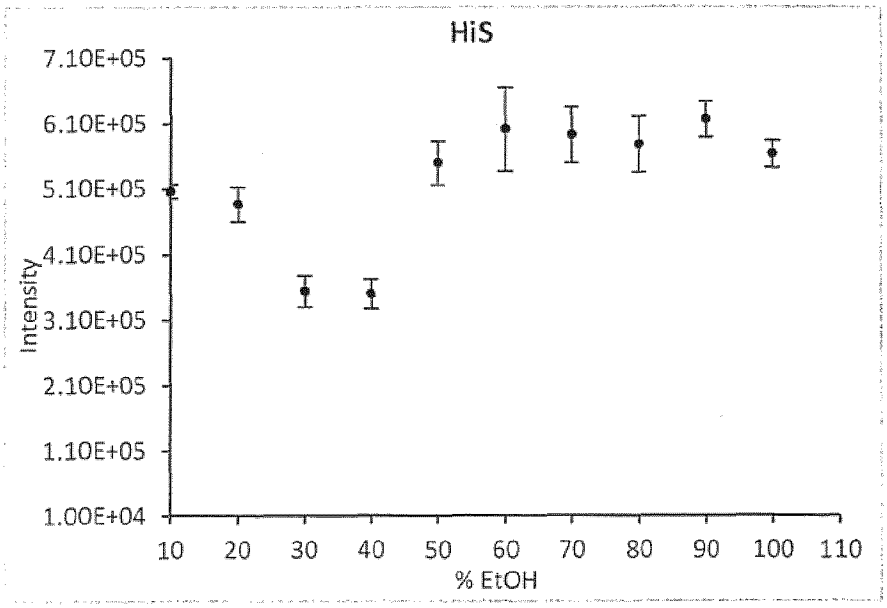

Figure 13D

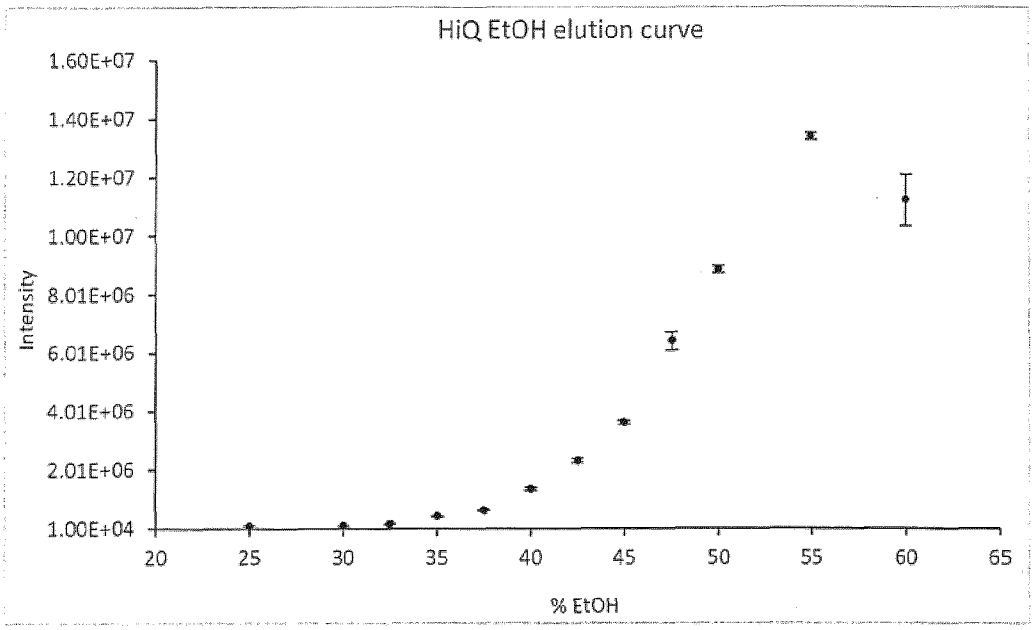
HiQ EtOH elution curve
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.01E+06
6.01E+06
4.01E+06
2.01E+06
1.00E+04
Intensity
20
25
30
35
40
45
50
55
60
65
% EtOH

Figure 14A

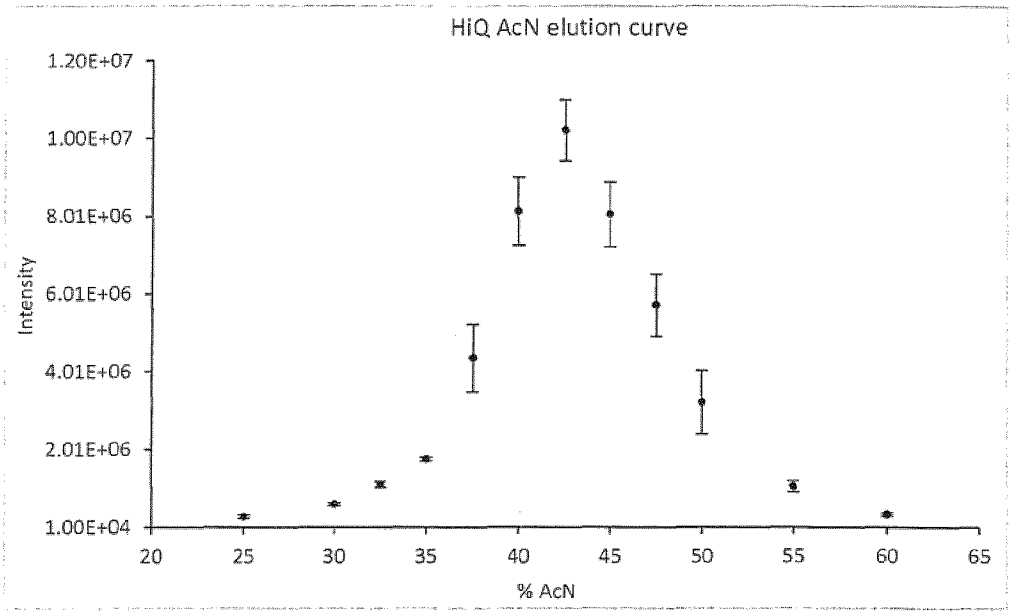
HiQ AcN elution curve
1.20E+07
1.00E+07
8.01E+06
6.01E+06
4.01E+06
2.01E+06
1.00E+04
Intensity
20
25
30
35
40
45
50
55
60
65
% AcN

Figure 14B

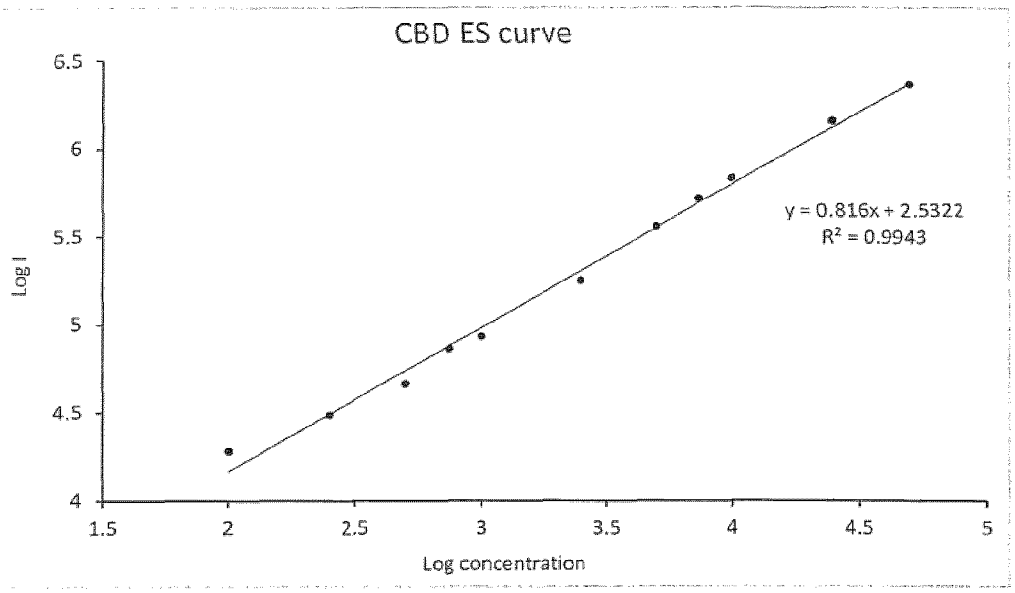
CBD ES curve
6.5
6
5.5
5
4.5
4
Log I
1.5
2
2.5
3
3.5
4
4.5
5
Log concentration
y = 0.816x + 2.5322
R² = 0.9943

Figure 15

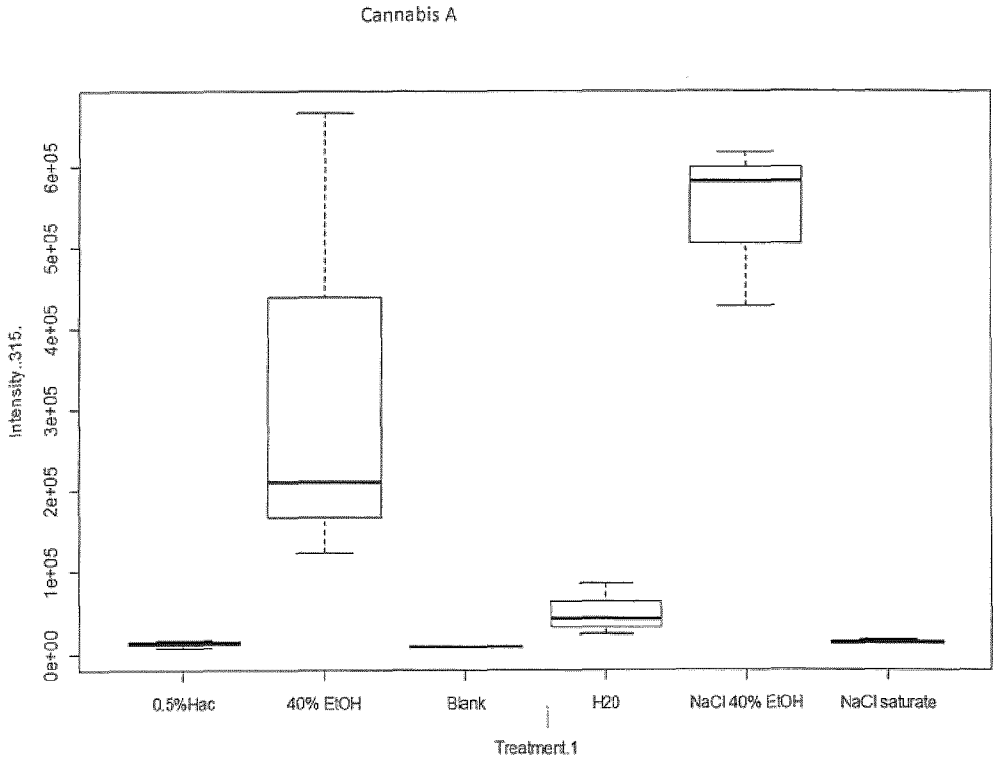
Cannabis A
Intensity..315.
0e+00
1e+05
2e+05
3e+05
4e+05
5e+05
6e+05
0.5%Hac
40% EtOH
Blank
H20
NaCl 40% EtOH
NaCl saturate
Treatment.1

Figure 16

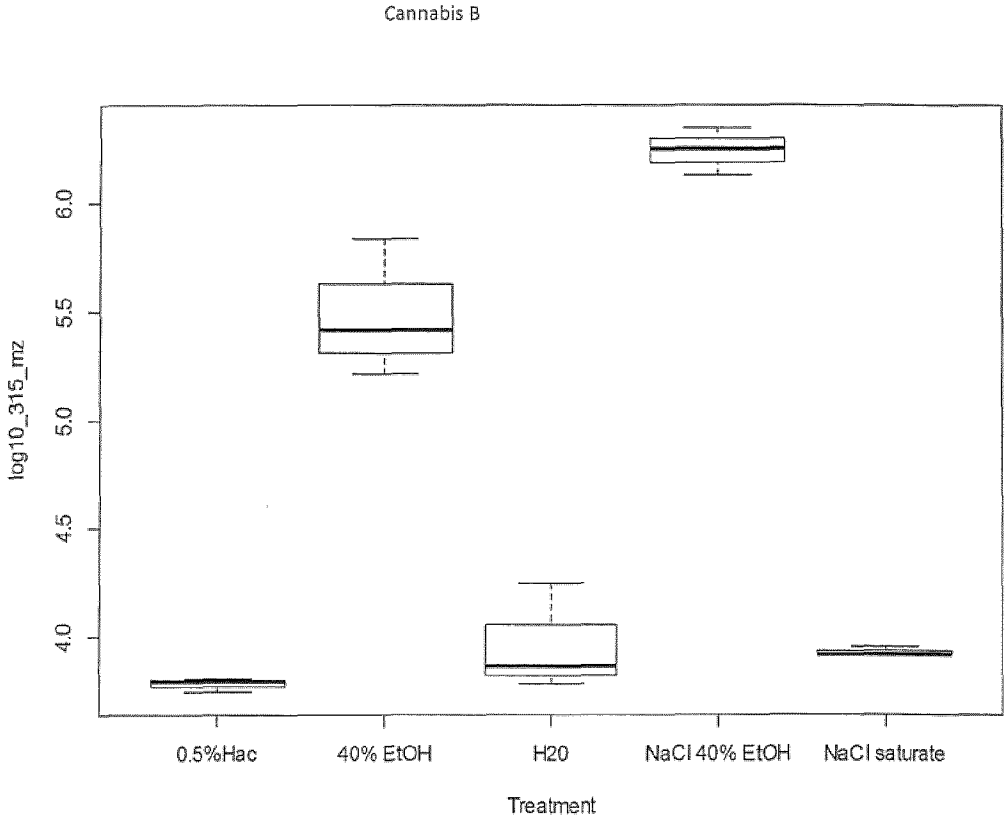
Cannabis B
log10_315_mz
4.0
4.5
5.0
5.5
6.0
0.5%Hac
40% EtOH
H20
NaCl 40% EtOH
NaCl saturate
Treatment

Figure 17

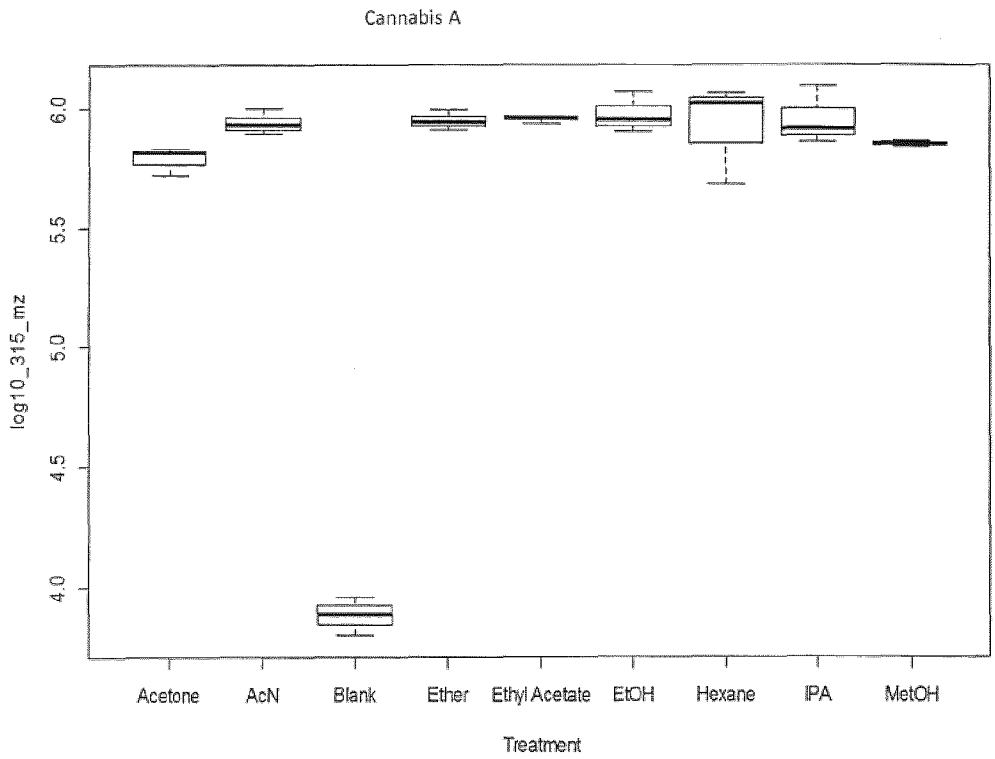
Cannabis A
log10_315_mz
4.0
4.5
5.0
5.5
6.0
Acetone
AcN
Blank
Ether
Ethyl Acetate
EtOH
Hexane
IPA
MetOH
Treatment

Figure 18

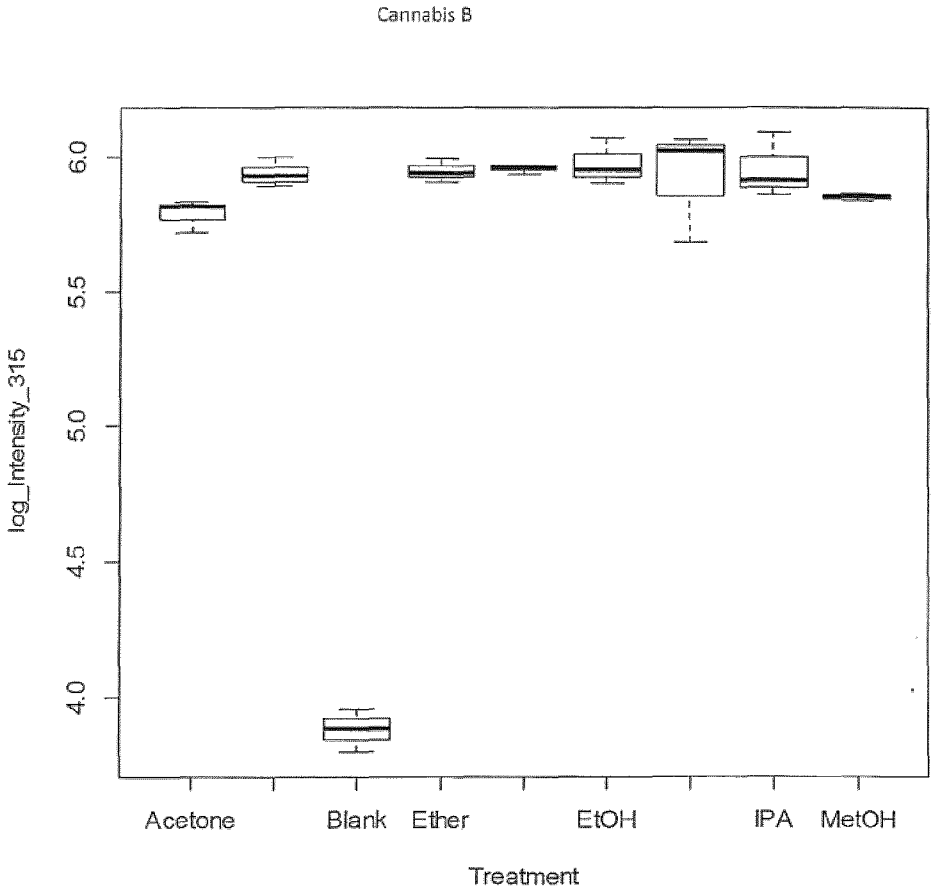
Cannabis B
log_Intensity_315
4.0
4.5
5.0
5.5
6.0
Acetone
Blank
Ether
EtOH
IPA
MetOH
Treatment

Figure 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2018/051508

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***B01D 11/02*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***B01D 11/02*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Orbit, Canadian Patents Database, World Wide Web
Keywords: cannabi+, decarboxylat+, polar solvent, non-polar solvent, extract+, ethanol, double extraction, polar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2,499,492 A1 (WHEATLEY, G. W. et al) 01 April 2004 (01-04-2004) ** the whole document **	1-15
X	WO 2017/051398A1 (EYAL, A.M.) 30 March 2017 (30-03-2017) ** the whole document **	1-2, 5, 7-9 and 12-13

☐ Further documents are listed in the continuation of Box C.

☑ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 12 February 2019 (12-02-2019)	Date of mailing of the international search report 21 February 2019 (21-02-2019)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Pierre Cuerrier (819) 635-5078

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2018/051508

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2499492A1	01 April 2004 (01-04-2004)	CA2499492A1	01 April 2004 (01-04-2004)
		CA2499492C	12 July 2011 (12-07-2011)
		AT450521T	15 December 2009 (15-12-2009)
		AU2003269167A1	08 April 2004 (08-04-2004)
		AU2003269167A8	08 April 2004 (08-04-2004)
		DE60330363D1	14 January 2010 (14-01-2010)
		EP1542984A2	22 June 2005 (22-06-2005)
		EP1542984B1	02 December 2009 (02-12-2009)
		EP2161262A1	10 March 2010 (10-03-2010)
		ES2337460T3	26 April 2010 (26-04-2010)
		GB0222077D0	30 October 2002 (30-10-2002)
		GB0322263D0	22 October 2003 (22-10-2003)
		GB2393721A	07 April 2004 (07-04-2004)
		GB2393721B	19 October 2005 (19-10-2005)
		GB0504777D0	13 April 2005 (13-04-2005)
		GB2408978A	15 June 2005 (15-06-2005)
		GB2408978B	05 April 2006 (05-04-2006)
		US2005266108A1	01 December 2005 (01-12-2005)
		US7700368B2	20 April 2010 (20-04-2010)
		US2010168448A1	01 July 2010 (01-07-2010)
		US8846409B2	30 September 2014 (30-09-2014)
		US2015203434A1	23 July 2015 (23-07-2015)
		WO2004026857A2	01 April 2004 (01-04-2004)
		WO2004026857A3	12 August 2004 (12-08-2004)
WO2017051398A1	30 March 2017 (30-03-2017)	WO2017051398A1	30 March 2017 (30-03-2017)
		IL258324D0	31 May 2018 (31-05-2018)
		US2018280459A1	04 October 2018 (04-10-2018)