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(54) Benævnelse: **CPC FORDELINGSKROMATOGRAFI AF CANNABINOIDER**

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ARNO HAZEKAMP ET AL: "Preparative Isolation of Cannabinoids from Cannabis sativa by Centrifugal Partition Chromatography", JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES, vol. 27, no. 15, 11 January 2004 (2004-01-11), pages 2421 - 2439, XP055202081, ISSN: 1082-6076, DOI: 10.1081/JLC-200028170

CPC PARTITION CHROMATOGRAPHY OF CANNABINOIDS

Description

The invention relates to cannabinoids and their isolation and purification as well as extraction through *centrifugal partition chromatography* (in short: CPC).

The CPC is used for the extraction and enrichment of plant constituents from plant extracts on an analytical, semi-preparative and preparative scale. The CPC is a liquid-liquid chromatography process that predominantly uses a two-phase solvent system.

It enables virtually loss-free separation of highly complex substance mixtures from crude extracts. Manufacturers of such centrifugal partition chromatographs for performing a CPC include Kromaton S.a.r.l (Annonay, FR) and Armen Instrument Sas (Saint-Avé, FR).

Compared to liquid chromatography (HPLC), the CPC is simpler and also more cost-effective, since the matrix effects and irreversible adsorption on solid phases do not occur.

As in case of conventional liquid-liquid chromatographic methods, such as High Speed Countercurrent Chromatography (HSCCC), a 2-phase solvent mixture is used in the CPC method. Either the upper or the lower phase can be used as the stationary phase. Unlike the HSCCC, however, the CPC does not use a capillary coil, but a rotor with several hundred separation chambers. The substances included in the plant extract are distributed between the mobile and stationary phases in these chambers, which are connected directly in series.

The system is set into rapid rotation during the separation process (up to 2,500 rpm). Depending on the direction of flow, the desired phase is retained in the rotor of the CPC and the separation of the two phases is accelerated by the centrifugal force. This enables the use of high flow rates and consequently the throughput of large quantities of substance in a short time, so that a preparative application of this separation technique is possible.

The partition coefficient K of the desired substance between the two phases should be in the range between 0.7 and 4.5. At lower K, the substance elutes too quickly and hence separation cannot take place. At higher K, on the other hand, the retention time is too long for the rapid purification of large quantities of a plant extract.

The latest technological advancements describe that cannabinoids can be obtained from cannabis extracts using CO_2 extraction (DE10051427C1). However, cannabinoids such as THC (A, “dronabinol”) or CBD (B, cannabidiol) are not obtained with absolute purity.

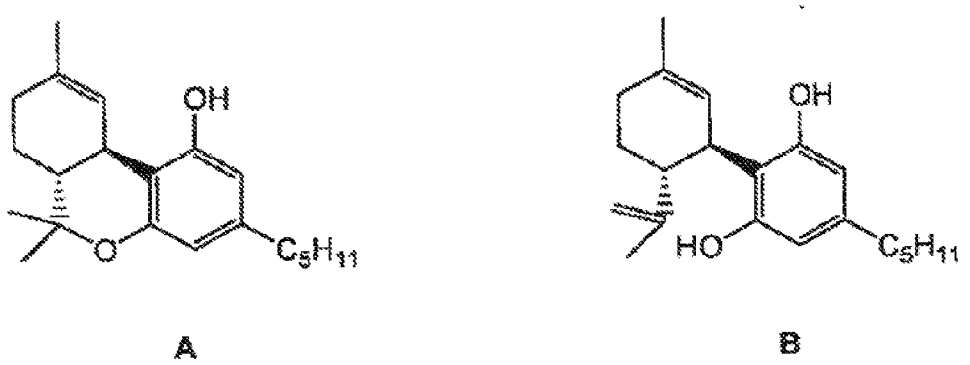


Fig. 1: Dronabinol (A) ((6aR-trans)-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol, $\triangle^9$-Tetrahydrocannabinol ($\triangle^9$THC)), Cannabidiol (CBD) (B) ((2-[1R-3-Methyl-6-(1-methylethenyl]-2-cyclohexen-1-yl]-5-pentyl-1,3-benzoldiol))

The chromatographic separation and preparative purification of cannabinoids is still a challenge, in particular the extraction of cannabinoids, preferably $\triangle^9$THC and CBD in high purity of more than 95%.

In the latest technological advancements, Hazekamp, A., 2007, Doctoral thesis, Leiden University and Arno Hazekamp, Ruud Simons, Anja Peltenburg-Looman, Melvin Sengers, Rianne van Zweden, Robert Verpoorte, Preparative isolation of cannabinoids from Cannabis sativa by centrifugal partition chromatography, J. Liq. Chrom. Rel. Technol. 2004, 27(15): 2421-2439, describe the preparative extraction of cannabinoids using the CPC. However, the latest technological advancements use a two-phase system based on hexane and achieve a maximum purity of 93.1%. In addition, low yields are achieved. Furthermore, hexane is a neurotoxin of solvent class 2 (ICH Guidelines).

Therefore, the task is to provide an improved process for the separation and/or purification of cannabinoids from cannabis plant extracts, which at least partially eliminates the existing disadvantages and in particular can provide a higher yield and purity of cannabinoids, in particular provision of preparative CBD and/or THC, in many applications.

The task is solved by a process according to one of the patent claims.

The density as well as the viscosity of cyclohexane, n-heptane, iso-octane are greater than the density/viscosity of n-hexane, so that, a more stable two-phase system is produced compared to the second mobile phase such as acetonitrile, so that a better retention of the stationary phase is made possible and consequently an increased separation performance is achieved, so that a higher purity and yield is always achieved compared to n-hexane; with purity of over 95%, even 99%.

The process according to the invention and/or the use according to the invention advantageously exhibit low eluent consumption in addition to no deposits on a stationary

phase, so that no regeneration of the stationary phase or preparation or removal of interfering components is necessary. Product recovery is almost quantitative, since the stationary and mobile phases can simply be interchanged. In addition, the stationary phase is completely renewed with each run and can be easily purified or renewed by distillation. The purity of the isolated cannabinoids is often so high that further chromatographic purification steps can be omitted. The process can also be easily carried out on an industrial scale. The yield of cannabinoids is often greatly increased compared to the latest technological advancements as explained in more detail below. The number of process stages required can usually be significantly reduced compared to alternative techniques.

The extraction of cannabinoids from any cannabis plants and their extracts can be particularly advantageous. This comprises such cannabis extracts from cannabis plants (*Cannabis sativa, Cannabis indica, Cannabis ruderalis*), such as hemp, industrial hemp, industrial hemp, drug hemp, fiber hemp and similar.

For example (and not according to the invention), the advantageous use of the two immiscible solvents acetonitrile and heptane at a flow of 50 to 600 mL per minute is intended for cannabinoids, preferably at a flow of 200 to 300 mL per minute during separation and maximum flow during rinsing, at a rotational speed of 50 to 1,500 rpm, preferably a rotational speed of 900 to 1,100 rpm during separation. Furthermore, the use of the upper phase as a stationary phase is preferred.

In a further preferred embodiment, t-butyl methyl ether (TBME) can be added to the respective solvent, namely 1 to 15 % (v/v), preferably 9 to 13 % (v/v).

The second immiscible liquid phase may also comprise such solvents as methanol (according to the invention), ethyl acetate (not according to the invention) or water (not according to the invention), which may be added with 1-15% t-butyl methyl ether (TBME).

Examples of solvent systems are not exhaustive (stationary phase/mobile phase):

n-heptane/acetonitrile 1:1 (without t-butyl methyl ether) (not according to the invention),

n-heptane/ethyl acetate/acetonitrile (not according to the invention),

n-heptane/ethyl acetate/t-butyl methyl ether/acetonitrile (not according to the invention),

n-heptane/ethyl acetate/methanol/water,

n-heptane/methanol/water,

n-heptane/ethanol/water (not according to the invention),

n-heptane/acetone/water (not according to the invention),

n-heptane/methanol/acetonitrile,

n-heptane/ethanol/acetonitrile (not according to the invention),

n-heptane/acetone/acetonitrile (not according to the invention),
n-heptane/chloroform/acetonitrile (not according to the invention),
n-heptane/chloroform/methanol,
n-heptane/methanol,
n-heptane/ethanol/methanol,
n-heptane/n-butanol/acetonitrile (not according to the invention),
n-heptane/2-propanol/water (not according to the invention),
n-heptane/n-propanol/water (not according to the invention),
n-heptane/2-propanol/acetonitrile (not according to the invention),
n-heptane/n-propanol/acetonitrile (not according to the invention),
n-heptane/dichloromethane/acetonitrile (not according to the invention),
n-heptane/dichloromethane/methanol,
n-heptane/tetrahydrofuran/acetonitrile (not according to the invention),
n-heptane/benzotrifluoride/acetonitrile (not according to the invention),
cyclohexane/methanol/water (not according to the invention),
cyclohexane/methanol/acetonitrile (not according to the invention),
cyclohexane/t-butyl methyl ether/water (not according to the invention),
cyclohexane/acetonitrile/water (not according to the invention),
isooctane/methanol (not according to the invention),
isooctane/methanol/water (not according to the invention),
isooctane/ethyl acetate/methanol/water (not according to the invention)
However, acetonitrile, possibly added with 1-15% t-butyl methyl ether (TBME), is particularly preferred.
Furthermore, it may be intended that the process according to the invention is carried out once or several times (see e.g. example 1).
The selection of these solvents allows extraction of cannabinoids, preferably $\triangle^9$THC and CBD with a high purity of more than 95%.
Therefore, a cannabis extract that can be obtained or is obtained by the process according to the invention is also disclosed, wherein the dronabinol purity is more than 95%, in particular 99.6%.
Therefore, a cannabis extract that can be obtained or is obtained by the process according to the invention is also disclosed, wherein the cannabidiol (CBD) purity is more than 95%, in particular 99.3%.
In the context of this invention, cannabinoids are understood to mean, in particular the following materials:

Cannabigerol-like (CBG): Cannabigerol ((E)-CBG-C_5), cannabigerol monomethyl ether ((E)-CBGM-C_5 A), cannabinerolic acid A ((Z)-CBGA-C_5 A), cannabigerovarin ((E)-CBGV-C_3), Cannabigerolic acid A ((E)-CBGA-C_5 A), cannabigerolic acid A monomethyl ether ((E)-CBGAM-C_5 A), cannabigerovarinic acid A ((E)-CBGVA-C_3 A);

Cannabichromene-like (CBC): Cannabichromene (CBC-C_5), Cannabichromenic acid A (CBCA-C_5 A), cannabichromevarin (CBCV-C_3), cannabichromevaric acid A (CBCVA-C3 A);

Cannabidiol-like (CBD): cannabidiol (CBD-C_5), cannabidiol monomethyl ether (CBDM-C_5), cannabidiol-C4 (CBD-C_4), Cannabidivarin (CBDV-C_3), cannabidiorcol (CBD-C_1), Cannabidiolic acid (CBDA-C_5), cannabidivarinic acid (CBDVA-C_3);

Cannabinodiol-like (CBND): cannabinodiol (CBND-C_5), Cannabinodivarin (CBND-C_3);

Tetrahydrocannabinol-like (THC): △9-tetrahydrocannabinol (△9-THC-C_5), △9-tetrahydrocannabinol-C4 (△9-THC-C_4), △9-tetrahydrocannabivarin (△9-THCV-C_3), △9-tetrahydrocannabiorcol (△9-THCO-C_1), △9-tetrahydrocannabinolic acid (△9-THCA-C_5 A), △9-tetrahydrocannabinolic acid B (△9-THCA-C_5 B), △9-tetrahydrocannabinolic acid-C4 (△9-THCA-C_4 A and/or B), △9-tetrahydrocannabivarinic acid A (△9-THCVA-C_3 A), △9-tetrahydrocannabiorcolic acid (△9-THCOA-C_1 A and/or B), (-)-△8-trans-(6aR,10aR)-△8-tetrahydrocannabinol (△8-THC-C_5), (-)-△8-trans-(6aR,10aR)-tetrahydrocannabinolic acid A (△8-THCA-C_5 A);

(-)-(6aS,10aR)- △9-tetrahydrocannabinol ((-)-cis-△9-THC-C_5);

Cannabinol-like (CBN): cannabinol CBN-C_5, cannabinol-C4 (CBN-C_4), cannabivarin (CBN-C_3), cannabinol-C2 (CBN-C_2), cannabiorcol (CBN-C_1), Cannabinolic acid A (CBNA-C_5 A), Cannabinol methyl ether (CBNM-C_5)

Cannabitriol-like (CBT): (-)-(9R,10R)-trans-cannabitriol ((-)-trans-CBT-C_5), (+)-(9S,10S)-cannabitriol ((+)-trans-CBT-C_5), (±)-(9R,10S/9S,10R)-cannabitriol ((±)-cis-CBT-C_5), (-)-(9R, 10R) -trans [10-O-ethyl-cannabitriol] ((-) -trans-CBT-OEt-C_5), (±) - (9R, 10R/9S,10S)-cannabitriol-C_3 ((±)-trans-CBT-C_3), 8,9-dihydroxy-△6a(10a) tetrahydrocannabinol (8,9-Di-OH-CBT-C_5), cannabidiolic acid A (CBDA-C_5 9-OH-CBT-C5 ester), (-)-(6aR,9S,10S,10aR)-9,10-dihydroxy-hexahydrocannabinol, Cannabiripsol Cannabiripsol-C5, (-)-6a,7,10a-trihydroxy-△9-tetrahydrocannabinol ((-)-Cannabitetrol), 10-Oxo-△6a(10a) tetrahydrocannabinol (OTHC);

Cannabielsoin-like (CBE): (5aS,6S,9R,9aR)-C_5-cannabielsoin (CBE-C_5), (5aS,6S,9R,9aR)-C_3-cannabielsoin (CBE-C_3), (5aS,6S,9R,9aR)-cannabielsoinic acid A (CBEA-C_5 A), (5aS,6S,9R,9aR)-cannabielsoic acid B (CBEA-C_5 B), (5aS,6S,9R,9aR)-

C3-cannabielsoic acid B (CBEA-C_3 B), Cannabiglendol-C3 (OH-iso-HHCV-C_3), dehydrocannabifuran (DCBF-C_5), cannabifuran (CBF-C_5);

Isocannabinoids: (-)-△7-trans-(1R,3R,6R)-isotetrahydrocannabinol, (±)-△7-1,2-cis-(1R,3R,6S/1S,3S,6R)-isotetrahydrocannabivarin, (-)-△7-trans-(1R,3R,6R)-isotetrahydrocannabivarin;

Cannabicyclol-like (CBL): (±)-(1aS,3aR,8bR,8cR)-cannabicyclol (CBL-C_5), (±)-(1aS,3aR,8bR,8cR)-cannabicyclolic acid A (CBLA-C_5 A), (±)-(1aS,3aR,8bR,8cR)-cannabicyclovarin (CBLV-C_3);

Cannabicitran-like (CBT): cannabicitran (CBT-C_5);

Cannabichromanone-like (CBCN): cannabichromanone (CBCN-C_5), Cannabichromanone-C3 (CBCN-C_3), cannabicoumaronone (CBCON-C_5).

However, cannabidiol (CBD-C_5) and △9-tetrahydrocannabinol (△9-THC-C_5) are particularly preferred.

The term “liquid-liquid partition chromatography step” in the sense of this invention is understood in particular to mean a chromatography in which the procedure is as follows: A certain quantity of a substance mixture is passed with a liquid mobile phase through a stationary phase, wherein the latter being kept stationary using a centrifugal force. In another preferred embodiment, liquid-liquid partition chromatography can be carried out continuously. For this purpose, the two phases are passed in countercurrent and a continuous separation is achieved instead of a time-delayed (discontinuous) discharge (see e.g. Yin, Lianhong; Li, Yingnan; Lu, Binan; Jia, Yujie; Peng, Jinyong, Trends in Counter-Current Chromatography: Applications to Natural Products Purification Separation and Purification Reviews (2010), 39 (1-2), 33-62).

Depending on their varying degrees of interaction with the stationary phase, the substances emerge continuously or discontinuously and can be separated. However, whereas in liquid chromatography the stationary phase consists of a packed fixed bed in a column, in liquid-liquid partition chromatography it is a second immiscible liquid phase which is kept stationary by centrifugal force using suitable devices, such as a rotor, in particular by means of a corresponding centrifugal partition chromatograph (supra).

“Cannabis extract” according to this invention means any processed extract from a cannabis plant or hemp plant including cannabinoids. The extract may be a primary extract, or partially processed extract. The production of cannabis extracts is adequately described in the latest technological advancements. Suitable cannabis plants or (fiber) hemp plants are those such as drug hemp, fiber hemp.

In a further preferred embodiment, at least one preparative column (solid phase, such as silica gel) may be upstream or downstream (example 2).

Examples and figures:

These examples and figures are provided solely to illustrate the invention, without limiting the invention to these examples.

Example 1: cannabinoids from hemp

Extraction of THC (dronabinol) (Figures 1-7), CBD (Figure 8) or CBC (Figure 9) from drug hemp or industrial or fiber hemp: extraction of cannabis flos using heptane, decarboxylation at 120 °C in a vacuum, dissolution in heptane, purification in the CPC.

1. CPC

1st run: quantitative separation CBN, proportional separation CBC:

Flow agent: acetonitrile TBME approximately 12% (not according to the invention)

Stationary phase: heptane TBME approximately 10%

from dronabinol 84.1% with approximately 15.9% impurities, wherein 5.4% are CBN and 2.2% CBC.

Color: deep brown to blackish.

Figure 1 (not according to the invention)

The result is:

Dronabinol 97.7% with approximately 2.3% impurities, wherein 0.4% are CBN and 0.9% are CBC.

Color: yellow.

Figure 2 (not according to the invention)

Optional 2nd run: quantitative cleaning of CBC

Flow agent: acetonitrile TBME approximately 12%

Stationary phase: heptane TBME approximately 10%

from dronabinol 97.7% with approximately 2.3% impurities, wherein 0.8% are CBN and 0.9% are CBC.

Color: yellow.

Figure 3 (not according to the invention)

The result is:

Dronabinol 99.6% with approximately 0.4% impurities, wherein 0.04% are CBN and 0.05% are CBC.

Color: yellowish (further color lightening)

Figure 4 (not according to the invention)

Example 2: CPC with downstream run over silica gel column

Run: quantitative separation of CBN

no separation of CBC

Flow agent: acetonitrile purum

Stationary phase: heptane purum

from dronabinol 81% with approximately 19% impurities, wherein 4.2% are CBN and 1.6% are CBC.

Color: blackish to deep brown

Figure 5 (not according to the invention)

The result is:

Dronabinol 94.5% with approximately 5.5% impurities, wherein 0.16% are CBN and 1.4% are CBC.

Color: yellow.

Figure 6 (not according to the invention)

Followed by preparative chromatography over silica gel for quantitative separation of CBC:

Flow agent: heptane 3% TBME

from dronabinol 96.4% with approximately 3.6% impurities, wherein 0.36% are CBN and 1.8% are CBC.

Color: yellow.

Figure 7 (not according to the invention)

The result is:

Dronabinol 98.6% with approximately 1.4% impurities, wherein 0.4% are CBN and 0.0% are CBC.

Color: colorless.

Figure 8 (not according to the invention):

The result is:

Cannabidiol 99.3% with approximately 0.7% impurities, wherein 0.3% are CBN.

Color: colorless, can be crystallized

Figure 9 (not according to the invention)

The result is:

Cannabichromene 94.1% with approximately 5.9% impurities, wherein dronabinol is 3.39% and △8-THC is 0.19%.

CPC FORDELINGSKROMATOGRAFI AF CANNABINOIDER

Patentkrav

1. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt, hvor fremgangsmåden indeholder mindst et flydende-flydende-fordelingskromatografitrin bestående af en første opløsningsmiddelfase og en anden, ikke-blandbar flydende fase, hvorved den første opløsningsmiddelfase er n-heptan, der holdes stationær via centrifugalkraft, og den anden, ikke-blandbare flydende fase er metanol.

2. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt ifølge krav 1, hvorved 1 % til 15 % (v/v) t-butylmethylether tilsættes til den første opløsningsmiddelfase og/eller den anden, ikke-blandbare flydende fase.

3. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt ifølge krav 2, hvorved den første opløsningsmiddelfase og/eller den anden, ikke-blandbare flydende fase er forskudt med 9 % til 13 % (v/v) t-butylmethylether.

4. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt ifølge et af kravene 1 til 3, med et flow fra 50 mL til 600 mL pr. minut og/eller et omdrejningstal fra 50 omdr/min til 1.500 omdr/min.

5. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt ifølge et af kravene 1 til 4, hvorved den første opløsningsmiddelfase og den anden, ikke-blandbare flydende fase ledes i modstrøm, og en kontinuerlig separation finder sted.

6. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt ifølge et af kravene 1 til 5, hvorved mindst en præparativ søjle er for- eller efterkoblet.

7. Fremgangsmåde til separation og/eller rensning af cannabinoider fra et cannabisekstrakt ifølge et af kravene 1 til 6, hvorved der anvendes en centrifugal-fordelingskromatograf til separation og/eller rensning.

Figures

Figure 1:

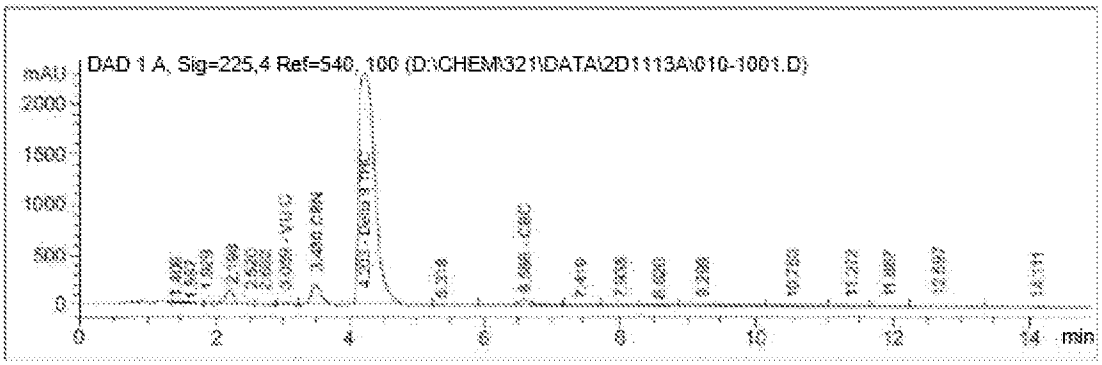


Figure 2:

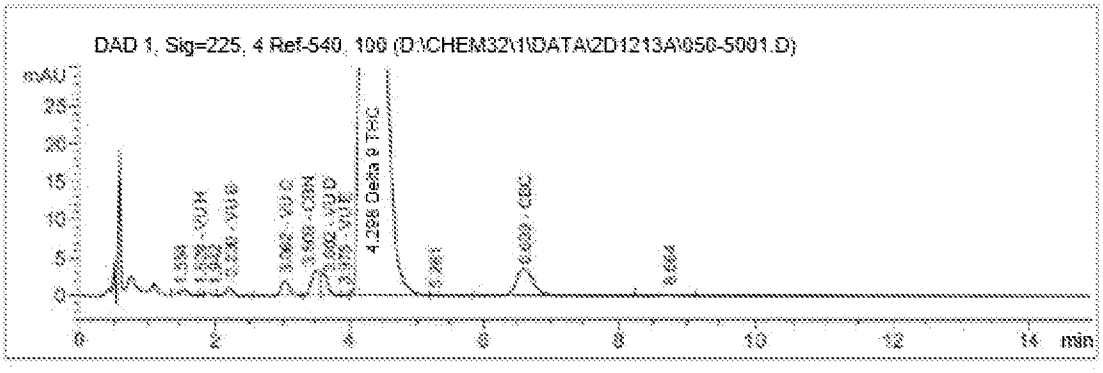
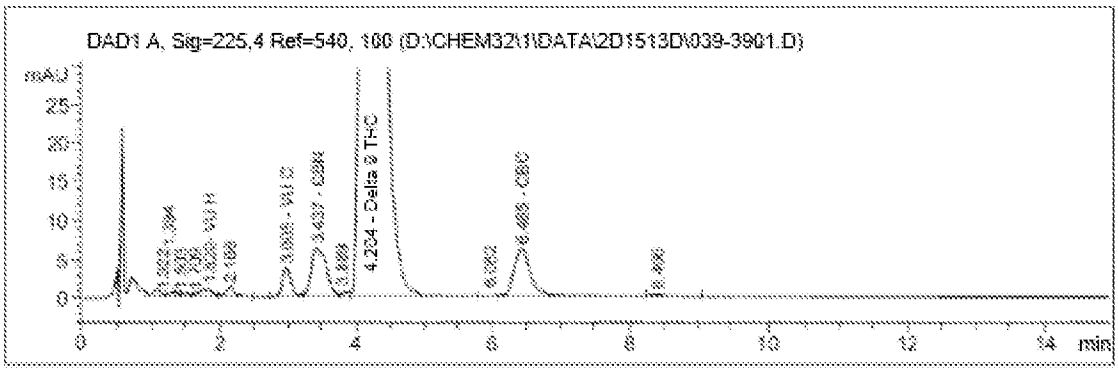


Figure 3:



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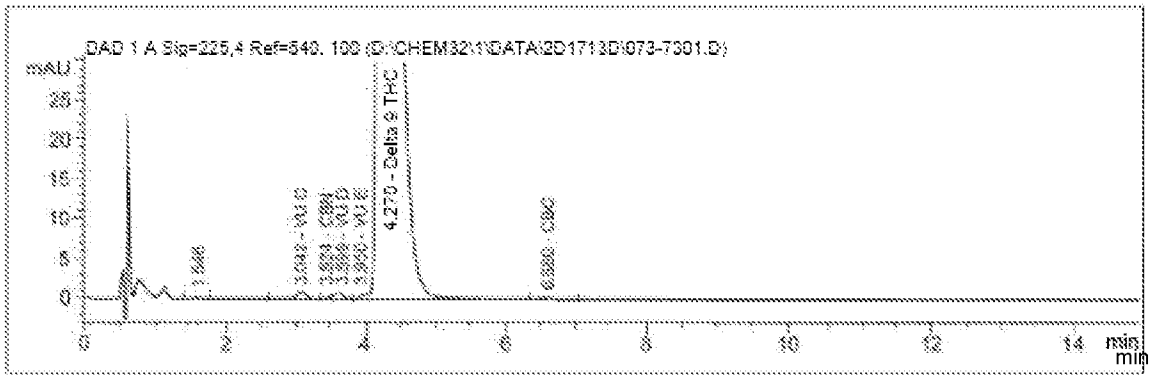

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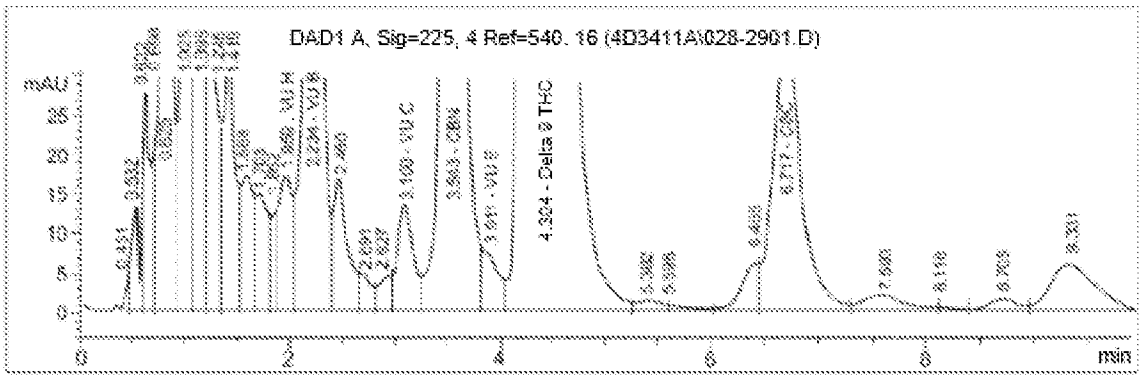

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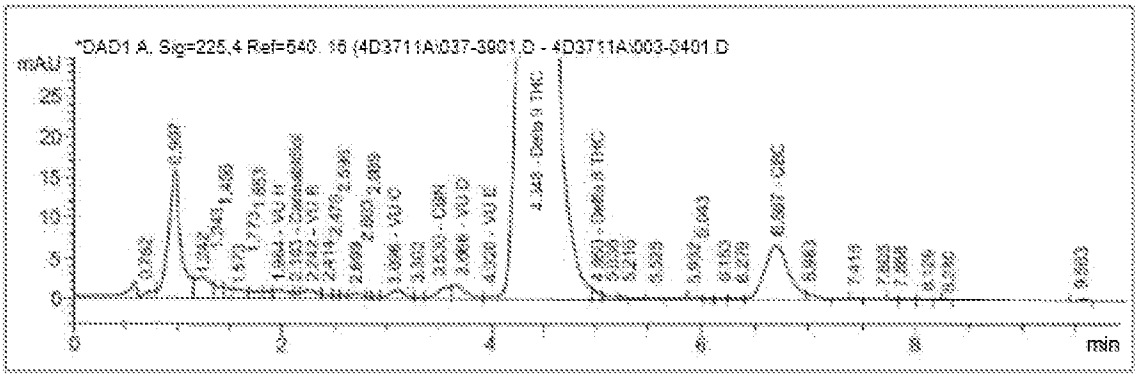

Figure 7:

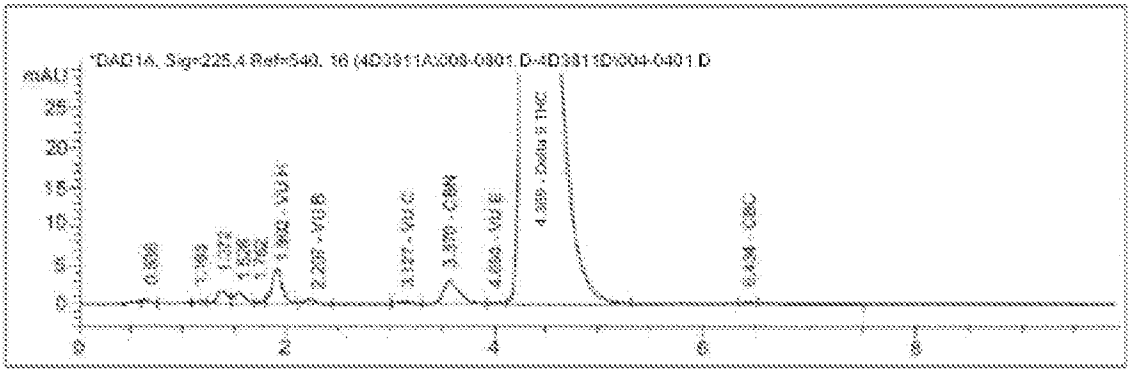

Figure 8:

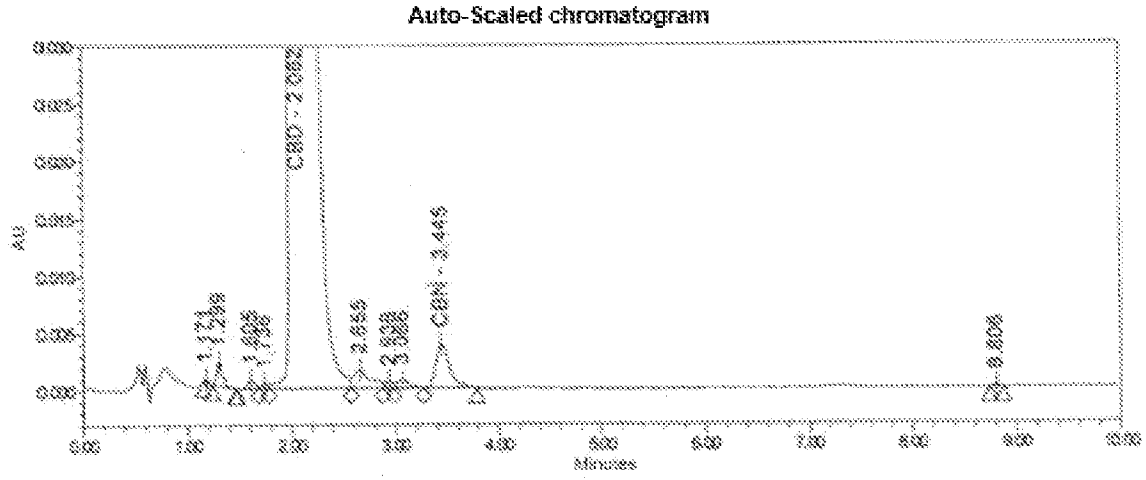

Figure 9: