# CBD Purification:
# Reverse Phase Chromatography Resins

Resin technology can maximize your yield to separate CBD in high purity from the cannabis extract and can help to ensure compliance with industry regulations while increasing profits.

Purolite®

Purolite®

## About Purolite

Purolite is a leading manufacturer of ion exchange, catalyst, adsorbent and specialty resins. With global headquarters in the United States, Purolite is the only company that focuses 100% of its resources on the development and production of resin technology.

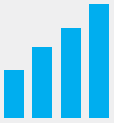
Responding to our customers' needs, Purolite has the widest variety of products and the industry's largest technical sales force. Globally, we have five strategically located research and development centers and eight application laboratories. Our ISO 9001 certified manufacturing facilities in the United States of America, United Kingdom, Romania and China combined with more than 40 sales offices in 30 countries ensure complete worldwide coverage.

### PREMIER PRODUCTS

The quality and consistency of our products are fundamental to our performance. Throughout all Purolite plants, production is carefully controlled to ensure that our products meet the most stringent criteria, regardless of where they are produced.

### RELIABLE SERVICE

We are technical experts and problem solvers. Reliable and well-trained, we understand the urgency required to keep businesses operating smoothly. Purolite employs the largest technical sales team in the industry.



### INNOVATIVE SOLUTIONS

Our continued investment in research and development means we are always perfecting and discovering innovative uses for ion exchange resins and adsorbents. We strive to make the impossible possible.

# CBD Purification: Reverse Phase Chromatography Resins

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# Maximize Your Yield and Increase Your CBD Purity

Cannabidiol (CBD) is one of at least 110 active compounds (cannabinoids) that can be found in hemp or marijuana cannabis plants. A highly purified preparation of cannabidiol (CBD) has recently been approved as a treatment for epilepsy under the name of Epidiolex, and further cannabidiol-based treatments are being researched for a wide variety of illnesses, such as Parkinsons, schizophrenia and many more. Commercial-grade CBD has benefits that can help with everyday living, including addressing anxiety, insomnia and chronic pain.

To utilize CBD, it must be compliant with industry regulations. Purolite can help to accomplished with purification using reverse-phase chromatography. When considering your purification options, it is important to ensure that your solution will provide:

- THC ($\Delta^9$-THV, $\Delta^9$-tetrahydrocannabinol) levels that comply with regulations
- Avoidance of high temperatures that can transform or degrade minor cannabinoids
- Cost effective processing for high demand

# Complexity of CBD Separations

CBD and THC have identical molecular weight (314 g/mol) and similar water solubility (4.0 ppm for CBD and 2.8 ppm for THC at 20 °C). These similarities also result in similar boiling points, making distillation less effective or requiring multiple distillation stages. One difference the two compounds do have is within the ring structure of the compounds, which makes THC more hydrophobic than CBD. This slight difference in hydrophobic nature is ideal for a reverse phase chromatographic step to separate the two molecules efficiently.

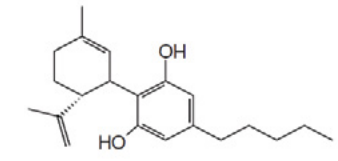
Chromalite resins are the ideal choice to support cannabinoid separation, either on analytical scale for quality control purposes either for production scope. From Chromalite AD2, specifically designed for HPLC separation to PCG resins, design for large scale separation, the separation profile will remain the same. Chromalite AD2 resins and Chromalite PCG resins are both fully divinylbenzene resins so the interaction with cannabinoid compounds remains unaltered from 5 micron up to 200 micron bead.

On analytical scale (HPLC) it is possible to efficiently separate cannabinoids related compounds using isocratic conditions of water/organic solvent mixtures (Figure 1). The chromatogram below, kindly provided by KNAUER Wissenschaftliche Geraete GmbH, highlights the separation of CBD and THC compounds within a cannabinoid extract using Chromalite AD2 polymeric chromatography media.

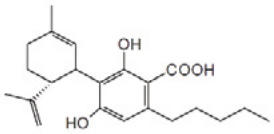
Chromalite AD2 resins are ideal for analytical separation of cannabinoid compounds and are offered in pre-packed HPLC columns (150 x 4.6 mm or 250 x 4.6 mm).

## FIGURE 1

### Difference in Hydrophobicity of CBD and THC

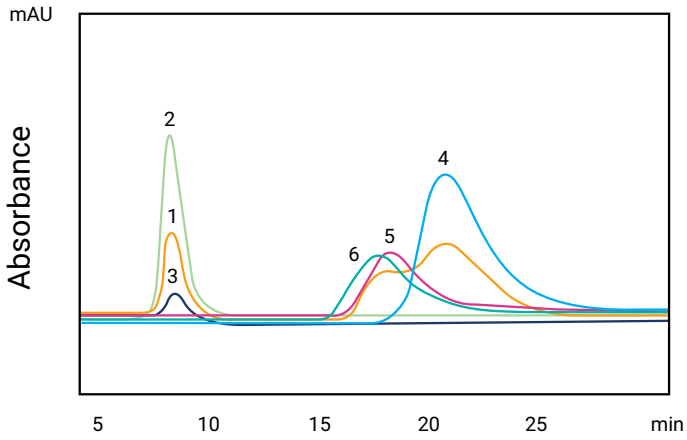



Cannabidiol
**CBD**




Δ⁹-Tetrahydrocannabinol
**Δ⁹-THC**

Chromatogram kindly provided by
KNAUER Wissenschaftliche Geraete GmbH



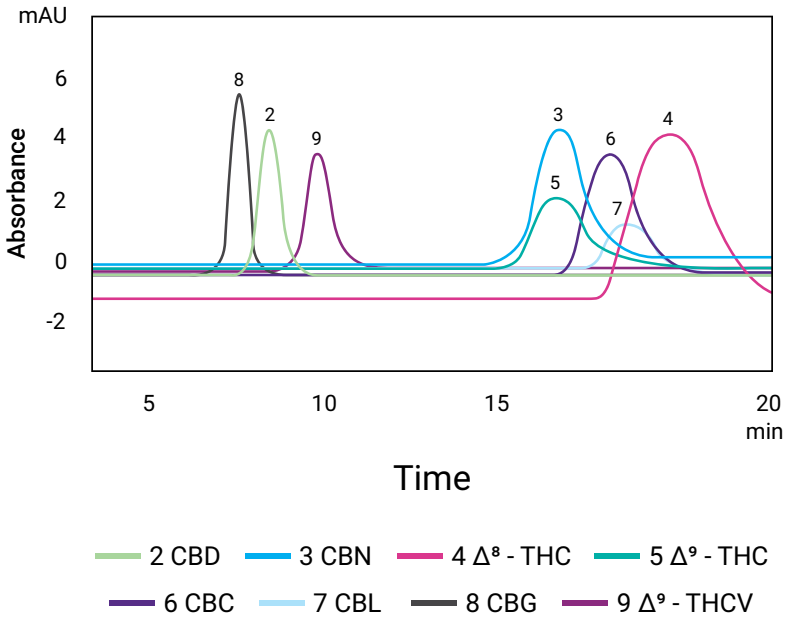

Chromatogram shows HPLC Elution (15/85% Water/EtOH)
on Hydrophobic Chromalite 10AD2 in 150 x 4.6 mm column

1 Mix 2 CBD 3 CBDA
4 CBN 5 Δ⁸ - THC 6 Δ⁹ - THC

It is possible to separate other cannabinoids with change of procedure and solvent. Figure 2 shows that using acetonitrile as organic solvent it is possible to get full separation of CBD from THC but also separate completely species as $\Delta^9$-THCV, CBD and CBG (see Figure 7 for chemical structures).

## FIGURE 2

**Isocratic Run, Chromalite 10AD2 in 150 x 4.6 mm, 25/75 vol% $H_2O$/ACN Loading 10 µg/ml, 10 µL Injection Volume, 1mL/min, 25 °C**

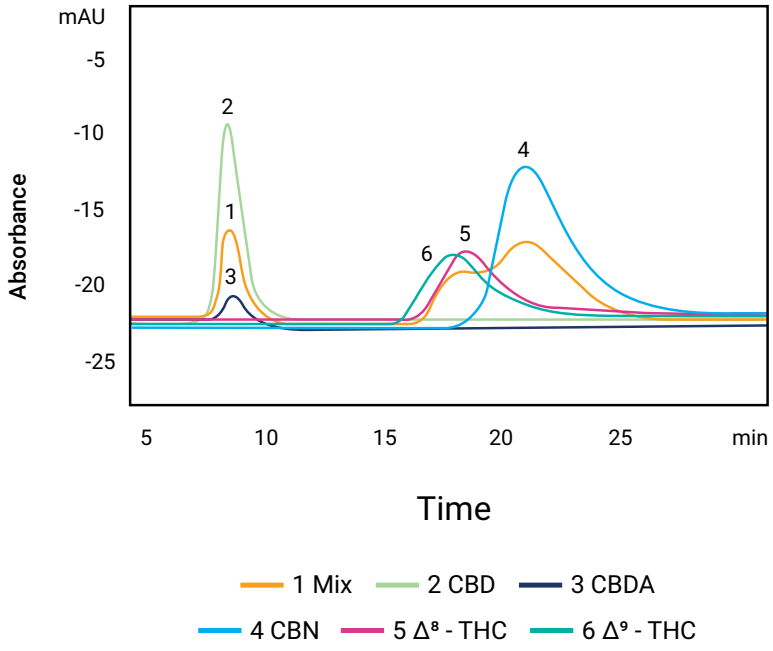



Chromatograms kindly provided by
KNAUER Wissenschaftliche Geraete GmbH

Also using ethanol as organic cosolvent it is possible to get a very good separation on analytical scale of CBD and THC (Figure 5). It is also possible to run complete separation of CBD and THC using a 100% ethanol as organic solvent (data not shown). This is a plus for all processes (see Figure 2. CBD Purification Processing.) that uses full organic solvents with out water. After solvent extraction the ethanol mixture can be efficiently purified using chromatography in neat organic solvent.

## FIGURE 3

**Isocratic Run, Chromalite 10AD2 in 150 x 4.6 mm, $H_2O$/ EtOH 15/85 Loading 10 µg/ml, 10 µL Injection Volume, 1mL/min, 25 °C**



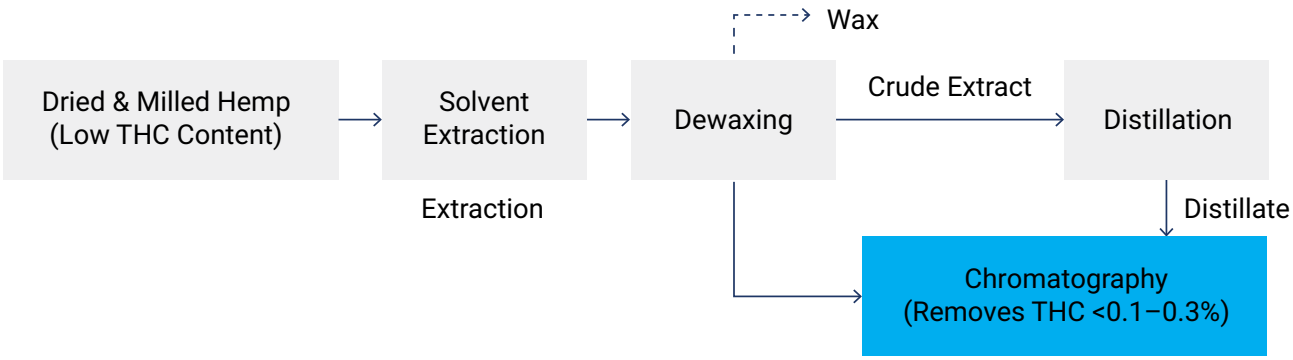

Chromatograms kindly provided by
KNAUER Wissenschaftliche Geraete GmbH

# Why Reverse-Phase Chromatography

In a typical CBD purification process using chromatography (Figure 4) hemp is the first subjected to solvent extraction. In this step all the hydrophobic compounds are extracted, including waxes. A further step of dewaxing, named winterization, is applied to remove waxes and lipids, before moving to the chromatographic step for THC reduction. Purolite provides high-quality resins that can eliminate the risk of product contamination and provides a solution to achieve consistency for CBD extraction to reach desired THC removal requirements.

## FIGURE 4

### CBD Purification Processing




Purolite offers Chromalite AD2, PCG600, PCG900 and PCG1200 (Table 1) for chromatographic purification of CBD extracts. Chromalite AD2 is designed for HPLC applications and it is offered in 5, 10, 15 and 30 micron particle sizes. Chromalite PCG resins are designed for low pressure chromatography and offered with three particle sizes and different porosities, each offering advantages in specific processes (Table 2).

For CBD purification on bulk scale, Chromalite PCG in particle size 50–100 micron (M grade) or 100–200 micron (C grade) are the ideal solution due to the low backpressure generated by these resins.

## TABLE 1 Purolite Chromatographic Resins for CBD Purification

| Resin | Porosity (Å) | Matrix | pH Stability | Particle Size Available (Micron) | System |
|---|---|---|---|---|---|
| AD2 | 200–300 | Polystyrene/DVB | 1–14 | 5, 10, 15 and 30 | HPLC (P > 1500psi) |
| PCG600 | 75–200 | DVB | 1–14 | F = 20–50<br>M = 50–100<br>C = 100–200 | Low pressure < 3 bar (< 45 psi) |
| PCG900 | 125–200 | | | | |
| PCG1200 | 300–500 | | | | |

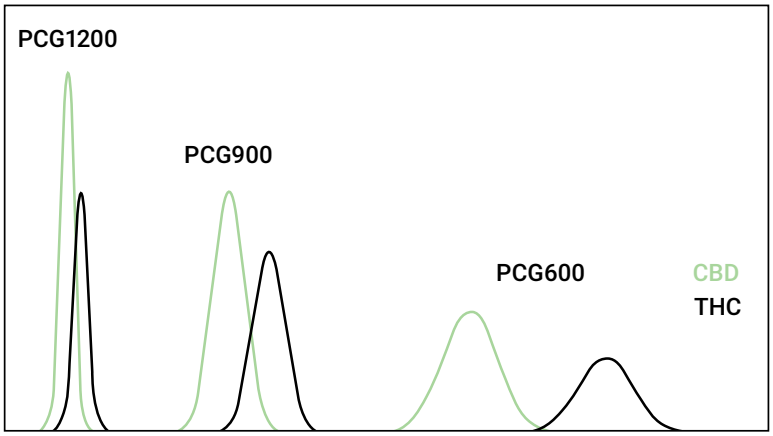
## TABLE 2 Key Properties of Chromalite PCG for CBD Purification

| Resin | Properties | Separation |
|---|---|---|
| PCG600 | Low porosity | High selectivity leading to higher purity due to more pore/surface interaction. Great for CB isolate or individual cannabinoid isolation. |
| PCG900 | Medium porosity | Good compromise between yield and purity. |
| PCG1200 | High porosity | Faster diffusion leading to less dilution and higher yield. Provides superior THC extraction with other terpenes retained in the effluent. |

During a chromatographic separation of CBD from THC the expected elution profiles are as shown in Figure 1, 2 and 3. This indicates the effect of the varying diffusion of the two compounds, controlled by resin porosity, on the separation.

## FIGURE 5

**Typical Expected Profile of CBD and THC Separation Using Chromalite PCG600, PCG900 and PCG1200**




It is important to note that the application of the Chromalite PCG resins is limited to low pressure chromatographic systems. Most applications that currently use C18 silica as the mode of purification will require the use of HPLC systems due to the rigidity and small size of the C18 silica, which provides a better performance in such systems. If using HPLC systems, Purolite offers the Chromalite AD2 reverse phase chromatographic media in small particle sizes such as 5, 10, 15 and 30 microns.

# Comparing CBD Purification Options

For some applications it is required to obtain completely pure CBD, whereas for the majority it is important to reduce the THC but still maintaining the balance and presence of the other cannabinoids. Tetrahydrocannabinol (THC) is among the most important cannabinoid impurities to remove. There are regulatory mandates by region and consumer driven desires for low THC. Therefore, the reduction of THC from the final CBD product is a key step in production.

There are three main ways to purify CBD:

1. Reverse-phase chromatography using very hydrophobic media
2. Solvent extraction
3. Distillation

Table 3 shows the advantage and disadvantages of the different methodologies.

**TABLE 3 Methods for Purification of CBD**

| | Chromalite Reverse-Phase Chromatography | Reverse-Phase Chromatography Silca C18 | Solvent Extraction | Distillation |
|---|---|---|---|---|
| **Mechanism** | Hydrophobic interaction; (Solid phase solvent extraction) | | Difference of solubility toward two solvents | Difference in boiling temperatures |
| **Advantages** | Easy to scale up<br>Chromatographic separation enable to separate multicomponent<br>High recovery | | Availability of abundant basic data<br>Low cost of equipment | Availability of abundant basic data<br>Easy to give high purity |
| **Disadvantages** | Specialized equipment<br>Large amount of eluent might be required to get eluent usages without solvent recovery | High capital cost (equipment), shorter media life, higher energy costs to operate | Heavy use of organic solvent<br>Multi-step extraction is required for multi-component separation | Potential thermal degradation of cannabinoids<br>High energy cost |

Your purification technology decision will affect both product yield and quality of purification to achieve low levels of CBD:

- High temperatures are necessary during distillation since the removal of THC happens at a temperature above 155 °C (b.p THC 155–157 °C).
- Since C18 silica can degrade at high pH (pH > 7), CBD purification using C18 silica requires acidic pH to ensure longer life of the media.
- Solvent extraction requires significant amounts of solvent since it requires multiple cycles for complete extraction.

In addition to purification using Chromalite, Purolite provides specialized processing methods to include metal/smoke extraction, water treatment, decolorization, water-soluble CBD compound processing and extraction of minor cannabinoids.

For a cost-effective solution that produces high yield and reliable remediation, reverse-phase chromatography outperforms other purification methods. The chromatographic separation offers advantages in terms of temperature, since it operates in mild conditions, pH range (polymeric media are fully stable from pH 1–14) and significant reduction of solvent consumption.

Figure 1 shows some THC-related degradation products formed from CBD under specific conditions, such as prolonged high temperature, acidity and exposure to certain solvents.

# Chromalite Purification Outcomes

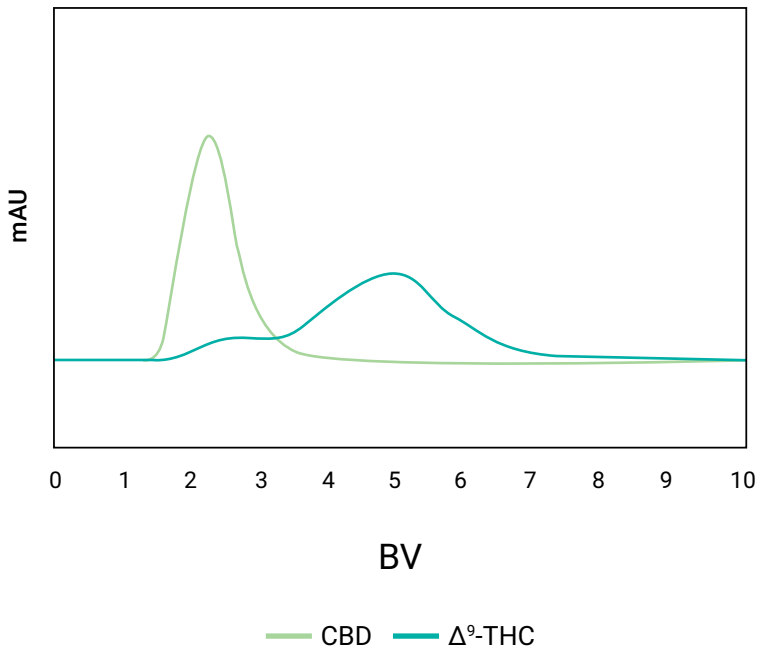
On large scale, PCG600 has shown efficient remediation of THC in cannabis oils, being able to obtain a final extract having THC < 0.3% in reverse phase mode (Figure 6). PCG600M has shown efficient purification using different organic solvents as ethanol, pentane, etc.

An equipment provider and remediation company in the hemp space, required a solution for their cannabinoid processing to satisfy the market need for a THC-free CBD oil to be used in consumer products.  The large-scale process was run in isocratic mode and Figure 6 shows the good separation of CBD from THC obtained in a 5Kg column using ethanol as organic solvent.

Chromalite PCG600M was successfully implemented within their simulated moving bed design to maximize yield and product margin. This evaluation produced 8.0 kg/day in a demonstration plant with a planned capacity expectation of 15 kg/day has already begun.

## FIGURE 6

**Isocratic Run, Chromalite PCG600M Packed in 5Kg Resin Columns, in Pedix/EtOH**




(Recreated from Patent US 10,799,546 B1, 2020)

# Chromalite PCG Packing Guidelines

For Chromalite AD2 packing, we recommend using pre-packed columns made of stainless steel. Packing is done by experts in handling such kind of materials.

## Pre-Packing

We recommend to pack Chromalite PCG resins at a pressure between 0.5 and 3 bar (7 to 45 psi) across the bed length. Recommended columns for packing are glass, acrylic or stainless-steel columns. Chromalite PCG resins can be packed by simple gravitational settling, although it is preferable to use pressure for bed consistency. For optimal performance, pack the resins at a high flow rate and pressure.

Chromalite PCG resins are provided with strict control of fines, and removal of fines is not required, the resins are ready-to-use as provided. Magnetic or manual stirring of settled resin or a packed bed may damage the resin and generate fines. Packed column beds can be removed by gently pumping liquid into the column with the opposite end open.

Chromalite PCG resins are supplied wet (M and C grade) or as a slurry in a 20% ethanol solution (F grade) and ready-to-use; the only necessary pre-treatment is resuspension in packing buffer. Selection of packing buffer will depend on the specific application; however, it is advisable to pack in the highest ionic strength mobile phase (including cleaning/sanitisation) that is intended for use.

## Example Packing Instructions

1. If required, attach packing reservoir to the top of the column. Ensure combined volume of reservoir and column is sufficient to contain the whole quantity of slurry.
2. Make sure column is level, wet bottom frit/screen with buffer and allow to drain to remove air bubbles. Attach outlet and add 1–2 cm of buffer to column.
3. Mix the resin slurry well, making sure that it is fully homogenous.
4. Avoiding the entrapment of air, gradually pour the slurry into the column along the inside wall.
5. Rinse the column walls with packing buffer.
6. Attach the column flow adaptor to the reservoir/column, lowered to the resin slurry height, avoiding any trapped air.
7. Open the outlet and slowly begin to pump packing buffer into the column.
8. Gradually increase the flow rate to the target maximum. A rapid increase may result in hydraulic shock to the incompletely packed bed and result in uneven packing.
9. Once the volume of the bed has reached a consistent size, stop the pump and close the outlet.
10. Empty and remove the reservoir from the column, then reattach the flow adaptor lowered to 2–3 cm above the packed bed. Ensure no air is forced into the column.
11. Open the outlet and start the pump, again resulting in bed compression. Once the new bed height is settled, stop the pump and close the outlet.
12. Lower the flow adaptor closer to the packed bed and repeat steps 11–12 until no further compression is observed.
13. Lower the adaptor 1–5 mm into the bed, the column is now ready for use.

## TABLE 4 Purolite Chromatographic Resins for CBD Purification

| | |
|---|---|
| **Feed Solvent** | 80–100% methanol or 75–100% ethanol |
| **Feed Load as % of Column Volume** | 10–20% |
| **Elution Method** | Isocratic at feed solvent concentration. |
| **Elution Flow Rate/Volume** | It takes a few minutes for the CBD and THC to appear (less than 30 minutes at the high concentration). Process is ran at 2 bed volumes per hour in most cases. |
| **Residence Time of CBD/THC** | Dependent on the resin used (Chromalite PCG600M takes longer), concentration of the solvent (higher concentration elutes faster), type of solvent used (ethanol generally elutes faster than methanol but methanol gives better separation). CBD typically elutes approximately 1 minute before THC. |

In case of fouling of the resin due fats and waxes, if elution with full organic solvent is not sufficient steam or caustic wash can be applied. The strength of the stripping effect is as following:

## TABLE 5 Regulatory Compliance for Chromalite Resins

| | Compliant with | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Product Name** | **21CFR173. 25[a]** | **21CFR173. 65[b]** | **21CFR177. 2710[c]** | **ResAP(2004) 3[d]** | **Halal[e]** | **Kosher[f]** | **GMO Free[g]** | **TSE/ BSE Free[h]** |
| **AD2** | | ● | | ● | ● | ● | ● | ● |
| **PCG600** | | ● | | ● | ● | ● | ● | ● |
| **PCG900** | | ● | | ● | ● | ● | ● | ● |
| **PCG1200** | | ● | | ● | ● | ● | ● | ● |

a. Secondary direct food additives permitted in food for human consumption/ion exchangers.
b. Secondary direct food additives permitted in food for human consumption/divinylbenzene co-polymer.
c. Substances for use only as components of articles intended for repeated use/styrene-divinylbenzene, cross-linked resins.
d. EU Resolution on ion exchange and adsorbents resins used in the processing of food stuffs.
e. Products certified, manufactured plant and raw materials audited by Ifanca. Raw materials free of porcine, alcohol, blood, etc.
f. Products certified, manufactured plant and raw materials certified and audited for orthodox compliance. Raw materials free of porcine, alcohol, blood, etc.
g. Product does not contain any GMOs or modified genetic material.
h. TSE/BSE free — The product has been manufactured without the use or inclusion of any animal products which carry a TSE/BSE risk.

## TABLE 6 Pre-Packed Columns

| | |
|---|---|
| **Internal Volume** | 200ml |
| **Material** | Polypropylene |
| **Column Dimension (Internal)** | 36mm D. x 202mm H. |
| **Recommended Flow Rate** | 60 ml/min (max 150) |
| **Max. Operating Pressure** | 1.3 MPa (200 psi) |

Available for:
PCG600M and PCG600C
PCG900M and PCG900C
PCG1200M and PCG1200C


Sample pre-packed 200 mL columns

## TABLE 7 Pre-Packed Columns

| | | |
|---|---|---|
| **Material** | Stainless steel | Available for:<br>5AD2<br>10AD2<br>15AD2<br>30AD2 |
| **Column Dimension (Internal)** | 150 x 4.6 mm<br>250 x 4.6 mm | |
| **Recommended Flow Rate** | 1–2 ml/min | |
| **Max. Operating Pressure** | 3.4 MPa (1500 psi / 34 bar) | |

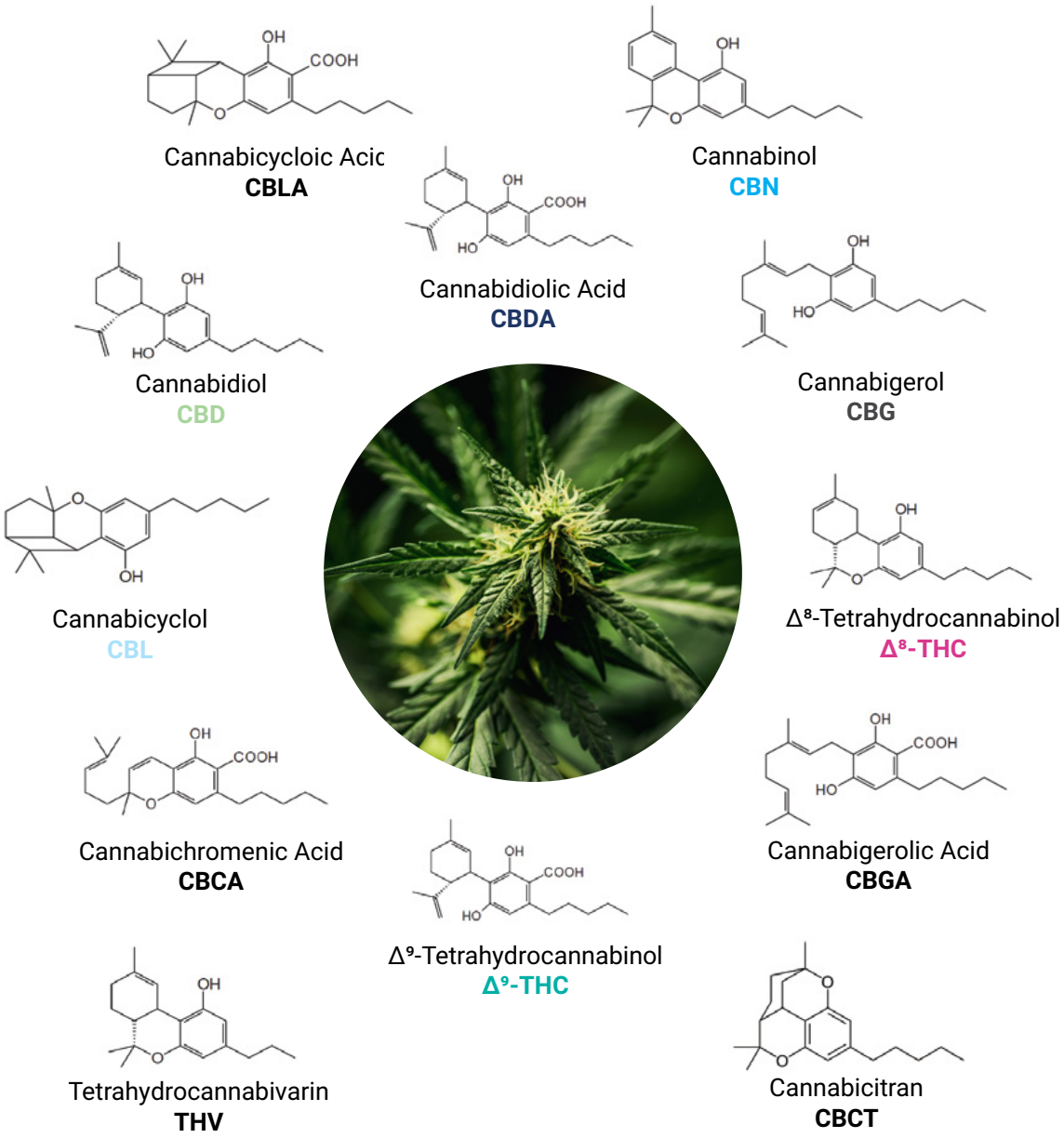

Sample stainless steel pre-packed columns (preferred for Chromalite AD2)

## TABLE 8 Ordering Information

| Product | Order Number |
|---|---|
| Chromalite 5AD2 4.6 mm x 150 mm HPLC Column | LS06950-601 |
| Chromalite 5AD2 4.6 mm x 250 mm HPLC Column | LS06950-602 |
| Chromalite 10AD2 4.6 mm x 150 mm HPLC Column | LS06840-601 |
| Chromalite 10AD2 4.6 mm x 250 mm HPLC Column | LS06840-602 |
| Chromalite 15AD2 4.6 mm x 150 mm HPLC Column | LS06890-601 |
| Chromalite 15AD2 4.6 mm x 250 mm HPLC Column | LS06890-602 |
| Chromalite 30AD2 4.6 mm x 150 mm HPLC Column | LS06870-601 |
| Chromalite 30AD2 4.6 mm x 250 mm HPLC Column | LS06870-602 |
| Chromalite PCG600F | LS04740 |
| Chromalite PCG600M | LS04750 |
| Chromalite PCG600C | LS04760 |
| Chromalite PCG900F | LS04770 |
| Chromalite PCG900M | LS04780 |
| Chromalite PCG900C | LS04790 |
| Chromalite PCG1200F | LS06720 |
| Chromalite PCG1200M | LS06725 |
| Chromalite PCG1200C | LS06730 |
| Chromalite PCG600M 200mL Column | LS04750-615 |
| Chromalite PCG600C 200mL Column | LS04760-615 |
| Chromalite PCG900M 200mL Column | LS04780-615 |
| Chromalite PCG900C 200mL Column | LS04790-615 |
| Chromalite PCG1200M 200mL Column | LS06725-615 |
| Chromalite PCG1200C 200mL Column | LS06730-615 |
| Chromalite PCG 200 mL Column KIT<br>Contains: PCG600M 200mL Column, PCG900M 200mL Column, PCG1200M 200mL Column, PCG950M 200mL Column | LS04610-KIT |

## FIGURE 7

### Cannabinoid Chemical Structures



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