Highly pure Cannabidiol (CBD) by twin-column chromatography (MCSGP)

Cannabidiol (CBD) is under investigation as therapeutic for several indications including pain relief. Recently, the FDA has approved a highly pure CBD formulation (Epidiolex®) for epilepsy. Crude CBD extracts from hemp plants contain a large number of natural products, including tetrahydrocannabinol (THC), a psychoactive component. Since THC is a controlled substance in most countries, CBD purification must ensure removal of THC down to 100-1000 ppm levels. Traditional preparative purification methods for CBD, like single-column chromatography, can reach such low THC contents only at the expense of CBD yield, wasting a large amount of valuable product. This can be prevented by using a continuous twin-column chromatography process: Multi-column Counter-current Solvent Gradient Purification (MCSGP) is capable of isolating target compounds with high purity, yield and throughput, significantly outperforming single column chromatography. This application note shows how highly pure CBD with a THC content below 100 ppm was obtained from pre-treated hemp plant extract. The process provided a yield of 95%, an increase of 72% relative to single column batch chromatography.

Introduction

In Cannabidiol (CBD) production, THC and also other plant-based impurities such as waxes and pigments can be difficult to remove. Obtaining highly pure CBD from hemp for medicinal applications involves a number of pre-treatment and purification steps aiming to obtain highly pure product CBD with low THC content.

The purification of CBD from hemp plant extracts comprises a number of pre-treatment steps, including the shredding of plant blossoms, drying, CO_2 extraction, decarboxylation and distillation, chromatography and crystallization. Pretreatment is necessary to remove waxes and other impurities which would foul the resins in the subsequent chromatography purification. The chromatography step is important to remove cannabinoid-related impurities, mostly THC, to below 100 ppm. It is run using reverse phase stationary phases and mobile phases with a high organic solvent content due to the poor water solubility of cannabinoids. The separation is challenging as some impurities overlap with the CBD product, requiring a high-resolution separation. Single-column batch chromatography always operates as a tradeoff between yield and purity. Therefore, highly pure CBD is only obtained by a significant loss of yield.

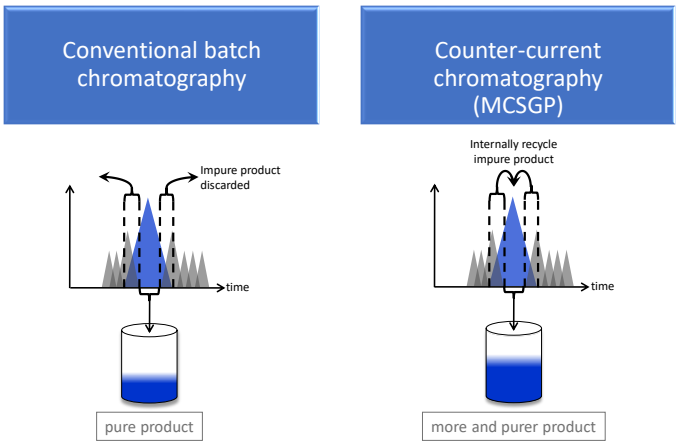


Fig. 1. Traditional single column chromatography (left): side fractions are discarded or kept for re-chromatography thereby accumulating impurities. MCSGP (right) re-processes side fractions internally continuously removing impurities and collecting pure product.

Multi-column Counter-current Solvent Gradient Purification (MCSGP) is a continuous chromatography process overcoming the yield-purity trade-off of single-column chromatography. MCSGP is operated fully automated with a Contichrom HPLC system. This application note shows the benefits of applying MCSGP versus single column batch chromatography for the purification of CBD.

Principle of MCSGP

MCSGP is a chromatographic method operated in continuous mode, as opposed to the batch mode of single-column chromatography. Much of the basic principle, however, remains the same (see Fig. 1). In a chromatographic process, the impure feed material mixture is separated, giving fractions with pure product, fractions with a mixture of impurities and product, and fractions containing only impurities. Pure product fractions are collected and product-containing impurity fractions are discarded or re-processed. As single-

column batch chromatography discards the product containing side fractions, valuable product is lost. Re-processing those side fractions accumulates impurities and can be only applied to a limited extent. MCSGP can isolate most of the product included in the product-impurity mixture by internally recycling product-containing impure fractions and repeatedly removing impurities and pure product. The recycling is done automatically and does not require off-line purity analysis. The impure side fractions are transferred from one chromatographic column to another. During this transfer, fresh feed material is added. This ensures continuous operation and leads to a product with high yield without compromising purity.

MCSGP does not accumulate impurities achieving simultaneously higher purity and yield. Thus, MCSGP overcomes the yield-purity tradeoff typically seen in single-column bath chromatography and can often operate with an up to 10-fold higher productivity and 70% lower solvent consumption. MCSGP has been shown to increase yield at target purity by up to 90% compared to single-column batch chromatography in a scalable manner.

Materials and methods

Pre-treatment steps: Hemp was grown in-door under controlled environment. The hemp buds were harvested, dried and subjected to several pre-treatment and purification steps (Fig. 2).

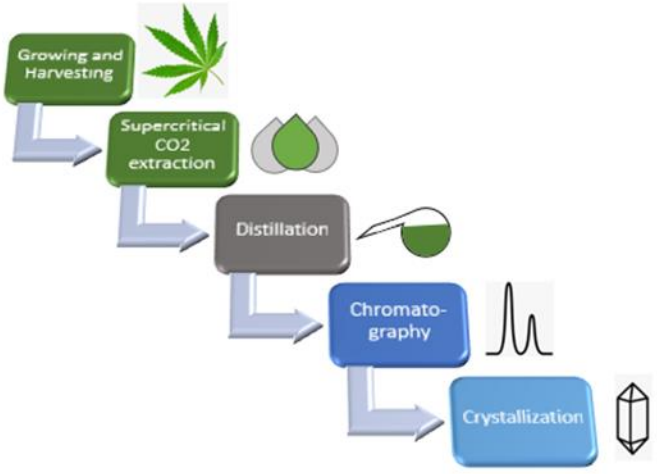


Fig. 2. Treatment of hemp for obtaining highly pure CBD.

Feed: The starting material for chromatography was a mixture of 50% distillate and 50% methanol. The CBD purity was 85.2% and the concentration of the starting material was 250 g/L CBD, as determined by analytical reverse phase HPLC with a C8 reverse phase column (Nucleosil 120-3) on an Agilent 1100 HPLC instrument with UV absorbance measurement at 230 nm. The standard injection volume was 10 µL. The THC content in the starting material was determined to be 4.8% (48,000 ppm). An analytical chromatogram of a typical feed mixture is shown in Fig. 3.

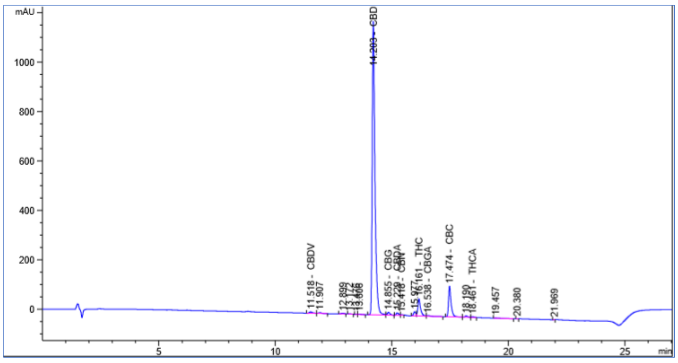


Fig. 3. Analytical chromatogram of the feed material.

Preparative chromatography: CBD was purified in single-column and twin-column MCSGP mode. For both modes, a Contichrom® HPLC 30 system was used (flow rate range 0.1-36 mL/min), including external variable single wavelength UV detectors. UV absorbance was measured and recorded at 280 nm. The columns had an inner diameter of 10 mm and a bed height of 150 mm. Total column volume was 11.9 mL. The columns were packed with Reprosil 100 C18 with 15 µm particle size. The solvents for preparative chromatography were de-ionized water (solvent A) and 96% ethanol in water (solvent B). The load volume per injection was 0.37 mL. The elution was run under isocratic conditions at 70% B for 90 min at a linear flow rate of 305 cm/h (4 mL/min). For column cleaning, acetone was used. All runs were performed at room temperature (25 °C ± 3 °C). The column backpressure under these conditions was 50 bar.

Single column chromatography results

A chromatogram of a preparative purification run is shown in Fig. 4 and Fig. 5 below. The elution was fractionated and the fractions were analyzed by HPLC (Fig. 5). Pure fractions were pooled. Impure fractions were stored for later re-purification. Fractions containing no product were discarded.

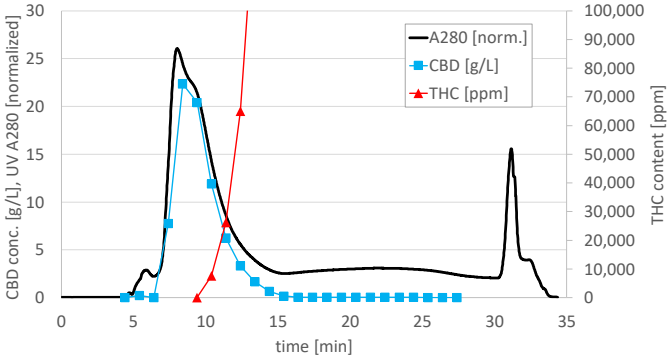


Fig. 4. Preparative chromatogram of CBD purification on C18 material showing UV A280 signal, CBD concentration and THC content. THC content starts to rise sharply at around 10 min.

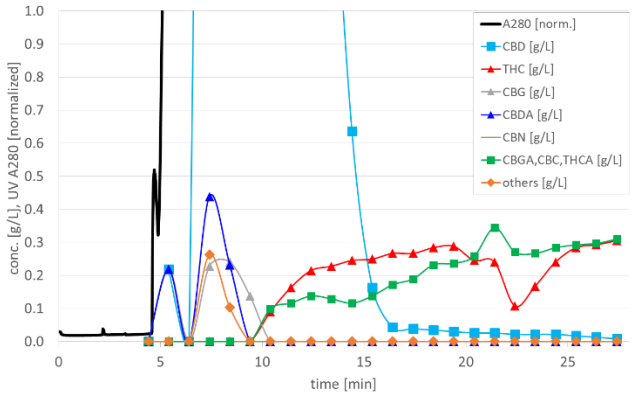


Fig. 5. Zoom of preparative chromatogram showing product (CBD) and concentrations of main impurities.

Designing an MCSGP run

The MCSGP Wizard in the ChromIQ operating software helps to design a twin-column MCSGP process based on a single-column chromatography process (see Fig. 6). The MCSGP process uses the same columns, solvents and same washing and cleaning protocol as the single column preparative process.

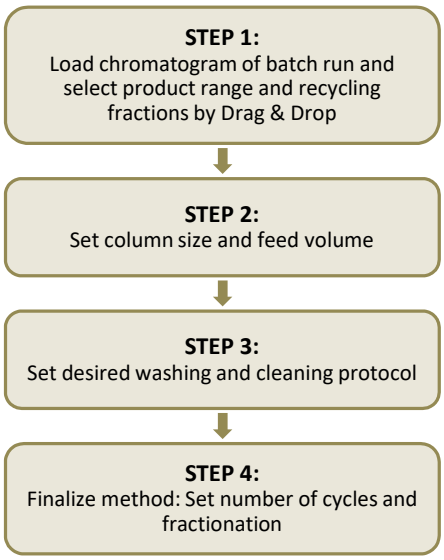


Fig. 6. Schematic of the guided MCSGP process design procedure provided by the MCSGP Wizard. Starting from a single column batch chromatogram, the overall design procedure was completed within 15 min.

In step 1 of the design procedure, the single column batch chromatogram was divided into zones corresponding to fractions with pure product, impure product or impurities (see above and Fig. 7). Three zones are highlighted, corresponding to pure product (red) and impure product (blue and green). For MCSGP operation, these zones correspond to tasks of recycling of impure product fractions in front of the peak (from A to B in Fig. 7), to product elution (from B to C in Fig. 7), and to recycling of impure product fractions in the peak tail (from C to D in Fig. 7). In summary, the zone borders A-D were defined and assigned to tasks as follows:

- A: start recycling at a UV value corresponding to 2.7 g/L CBD concentration (2,000 mAU)
- B: start product collection at a UV value corresponding to 13.3 g/L CBD concentration (10,000 mAU)
- C: stop product collection 10% below peak maximum (based on UV signal)
- D: 6 min after C

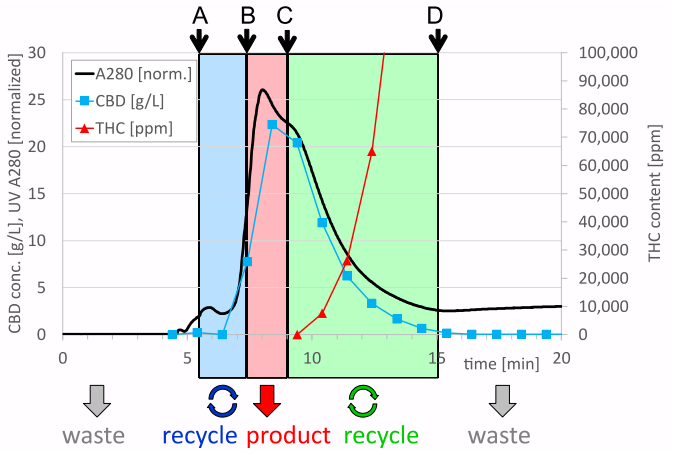


Fig. 7. Schematic of step 1 of the design procedure of to the MCSGP Wizard, which encompasses the definition of the zones of the chromatogram selected for internal recycling (A-B and C-D), and the product elution window (B-C).

In step 2 of the design procedure (Fig. 6), the column dimensions and feed volume were defined. Two columns of the same type and dimensions as in the single-column batch run were used for MCSGP. The Wizard recommends a feed volume based on the batch chromatogram and the zone definitions. The default value was confirmed.

In step 3 of the design procedure (Fig. 6), the washing and cleaning protocols for MCSGP were entered. The same protocol as in batch chromatography was used.

In step 4 of the design procedure (Fig. 6), the number of operating cycles was entered according to the desired total processed feed volume. The MCSGP Wizard displays the expected consumption of starting material, allowing adjustment of the number of cycles. Furthermore, the automatic generation of start-up and shutdown methods and the dynamic process control (MControl) was activated. MControl automatically compensates for variations in environmental conditions such as temperature. More information on MControl can be found in a dedicated Application Note.

The MCSGP Wizard calculates suitable in-line dilution steps for the recycling phases. Finally, the total volume of required feed material, solvents and time are displayed. The complete design procedure was completed within 15 min.

MCSGP operation

To optimize the MCSGP process, several runs were carried out with varied operating conditions. The zones of product elution and recycling were slightly shifted in order to investigate the impact on product purity. The optimal conditions were then used to perform a representative MCSGP run. Each MCSGP trial run was operated continuously for several cycles. A chromatogram of eight sequential cycles of a MCSGP run for CBD purification is shown in Fig. 8. The chromatogram shows the repetitive pattern of product elutions from either column.

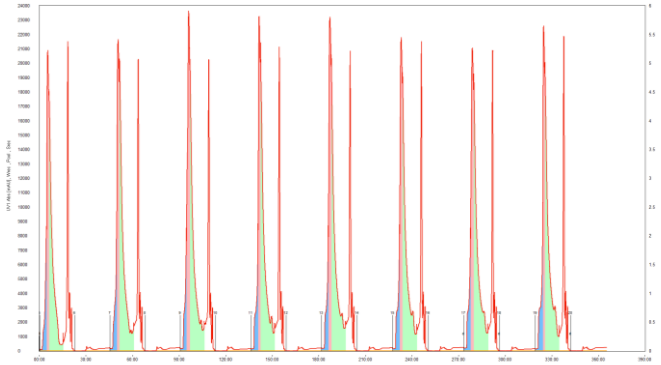


Fig. 8. Chromatograms of an MCSGP run with 8 cycles. Only the UV signal of Column 1 is shown. Zones for internal recycling are colored blue and green, flanking the very narrow product elution zone (red).

The Contichrom® operating software allows superimposing consecutive cycles of cyclic processes such as MCSGP. Fig. 9 shows the chromatogram with superimposed cycles from the representative MCSGP run. The good overlay fit of the chromatograms indicates that cyclic steady state has been reached and that the eluted product amount, concentration and purity is constant from cycle to cycle.

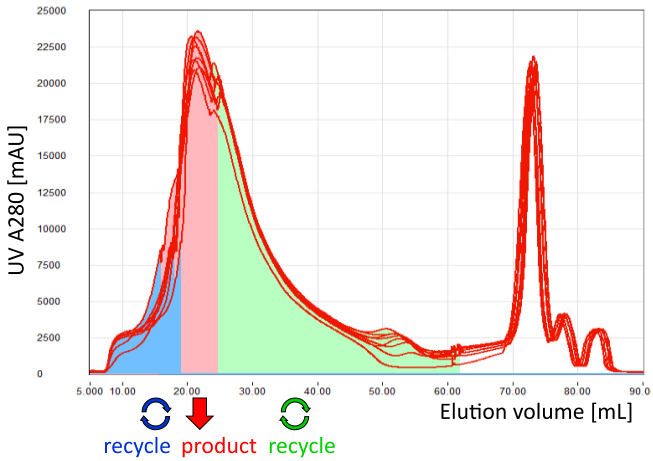


Fig. 9. Superposition of 8 cycles of the MCSGP run. The signal UV1 recorded behind column 1 is displayed. It can be seen that the elution profiles change only slightly due to accumulation of the recycling fractions and that the product peaks are very similar. Variations in retention time and peak shape are automatically compensated by the MControl algorithm, which starts CBD collection based on a UV threshold and stops collection when the UV signal is 10% lower than the peak maximum.

The eluted product fractions of the MCSGP runs for each cycle (containing two product elutions, i.e. one per column) were analyzed for purity and product concentration using analytical HPLC. A representative analytical chromatogram is shown in Fig. 10. The purity of the CBD product fraction was > 99.5%.

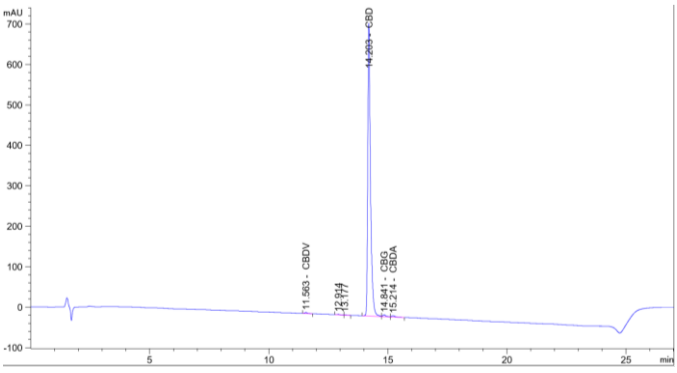


Fig. 10. Analytical chromatogram of a representative CBD product fraction obtained by MCSGP.

Process comparison of single-column and MCSGP operation

The process performance for single-column batch and twin-column MCSGP processes was calculated and compared in terms of yield, purity, productivity, product concentration and solvent consumption. MCSGP displays significant advantages over batch chromatography as shown in the following.

Fig. 11 shows analytical results from preparative runs comparing single column batch chromatography and MCSGP. The maximum THC content has been specified to be 100 ppm. At this level, the single column batch process provides a CBD yield of only 52%, whereas the MCSGP provides a CBD yield of 94%, an increase by 80%.

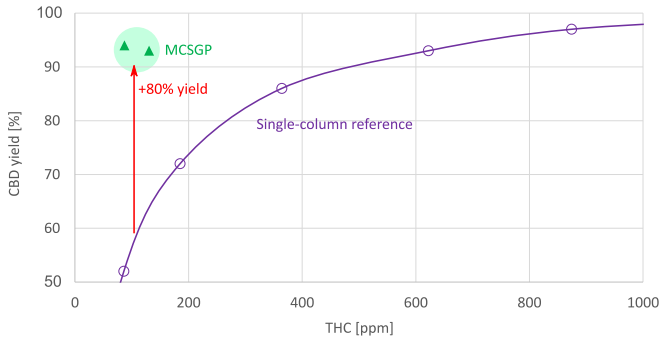


Fig. 11. Purity-yield chart showing experimental results of the MCSGP and the single-column batch reference run. The batch performance is shown in open circles and as a curve (purple). The individual data points represent different options for pooling product containing fractions. The MCSGP performance is shown as green triangles. At 100 ppm THC, the defined maximum limit, the batch yield is only 52%, whereas the MCSGP yield is 94%.

MCSGP can be operated in different ways. If the target product purity can be lower, the productivity can be further increased, meaning more product per time period can be purified. Fig. 12 shows the consequences for a scenario, with an upper THC content limit of 1000 ppm and a minimum yield level of 85%. By using MCSGP instead of single-column batch, the productivity of the purification process can be improved about 7.5-fold, from below 8 g/L/h to above 60 g/L/h.

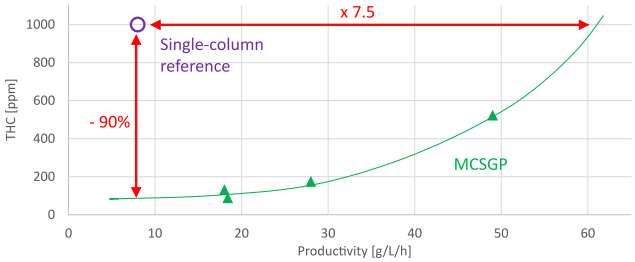


Fig. 12. Purity-productivity chart showing experimental results of MCSGP and the single-column batch reference run. Results from a single-column batch reference run are shown as open purple circle and MCSGP results as green triangles. At the THC content limit of 1000 ppm, the productivity with MCSGP is 7.5-fold higher with single-column chromatography. Alternatively, keeping productivity constant when switching from batch to MCSGP, a 10-fold reduction in THC content could be obtained, from 1000 ppm to 100 ppm.

The solvent consumption of single-column batch and MCSGP chromatography was compared (see Fig. 13). Under the boundary condition of a maximum THC content of 1000 ppm, the solvent consumption can be reduced by about 30%. The consumption of solvent with single-column chromatography is 95 mL solvent per mL of feed, while with MCSGP it is only 65 mL solvent per mL of feed.

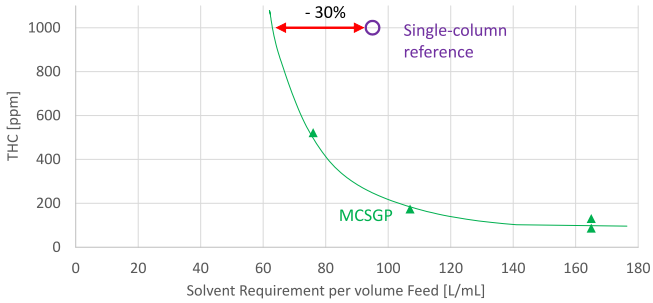


Fig. 13. Purity-solvent consumption chart showing experimental results of MCSGP and single-column batch reference run. At comparable THC content of 1000 ppm, the solvent consumption is 30% lower with MCSGP.

Summary

MCSGP offers significant advantages over batch chromatography in the purification of Cannabidiol (CBD) and the removal of THC, a key impurity. The advantages of MCSGP compared to single-column batch chromatography include:

- CBD yield is improved by around 80% at 100 ppm THC target impurity specifications
- The productivity is increased by a factor of 7.5 at a THC content of 1000 ppm
- The solvent consumption is reduced by 30% at a THC content of 1000 ppm.

The reduction in solvent consumption significantly lowers solvent purchase costs, solvent regeneration/disposal costs and reduces the footprint required to prepare and store solvents.

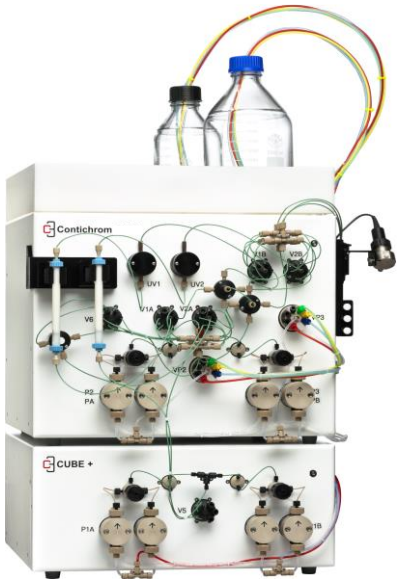
The reduced number of samples in MCSGP (only one sample is generated per MCSGP cycle, while multiple fractions are generated in single-column chromatography) leads to a significant reduction of requirements for off-line HPLC analysis.

The increase in productivity by MCSGP allows multiple manufacturing options:

- The same target amount can be produced within the same time with smaller columns
- A specified target amount can be produced in a shorter period of time with columns of the same size
- More target compound can be produced per total column volume in the same time.

Contichrom® HPLC for reverse phase purifications

The MCSGP process can be operated by all Contichrom HPLC systems. The Contichrom® HPLC is a versatile preparative laboratory-scale chromatography system with a number of single- and two-column processes. ChromIQ®, the operating software of Contichrom® systems, contains a wizard for operating the MCSGP process.

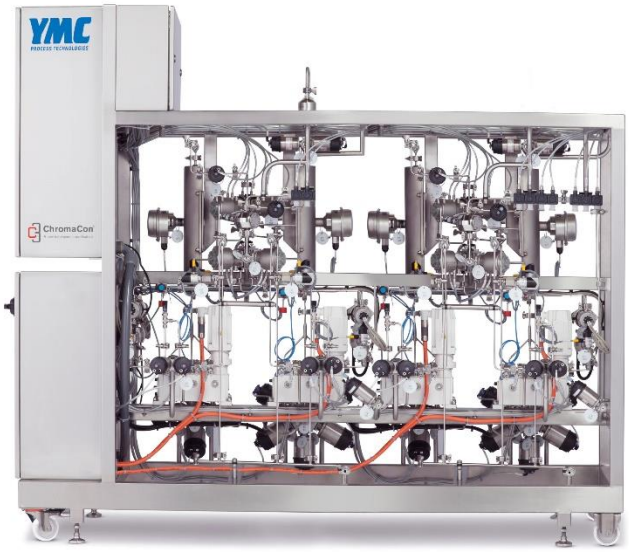


Contichrom HPLC 30/100 System Specifications	
Flow rate range	0.1 – 36 / 0.1 – 100 mL/min
Pressure rating	100 bar
Number of columns	1-2
Number of buffers / solvents	Up to 18
Fractionation	3 fractions (valve), optional fraction collector
UV Detectors	Fixed wavelength A280, A255, detection behind each column Optional external variable wavelength detectors with 190-500 nm wavelength
Conductivity / pH detectors	1 each included

For enquiries regarding the Contichrom systems, please visit www.chromacon.com or contact sales@chromacon.com.

EcoPrime® Twin HPLC scale-up systems

With the EcoPrime Twin HPLC series from YMC (formerly LEWA Process Technologies), MCSGP is available for manufacturing under GMP conditions. The twin-column scale-up systems have been co-developed by YMC and ChromaCon to ensure transferability through the scales.



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