separlab

PREPARATIVE COLUMN FOR LIQUID CHROMATOGRAPHY
PC 01 150/1000
HYDRAULIC DEVICE
HPC 1150/350

user manual

1. Desription and use

PC 01 columns are designed for high pressure, high performance preparative liquid chromatography. They are equipped by moving pistons on both ends. All part in connection with mobile phase are made of 316 (316L on the request) stainless steel according AISI.
*PC 01*l columns are used for high performance liquid chromatography separations in instances where small rigid particles rigid are used as column filling. Only high quality stainless steel and polytetrafluorethylene (PTFE) are in contact with mobile phase. Columns are resisting to all common chromatographic solvents.
PC 01 150 columns with inner diameter 153 mm are designed for medium scale process chromatography and typically are working with flow rate 300 ml/min – 1000 ml/min depending on sorbent type and separation mode. They are mostly used with manual high pressure hydraulic system. Such combination has no electric systems and can be used in space where ATEX explosion rules has to be kept.

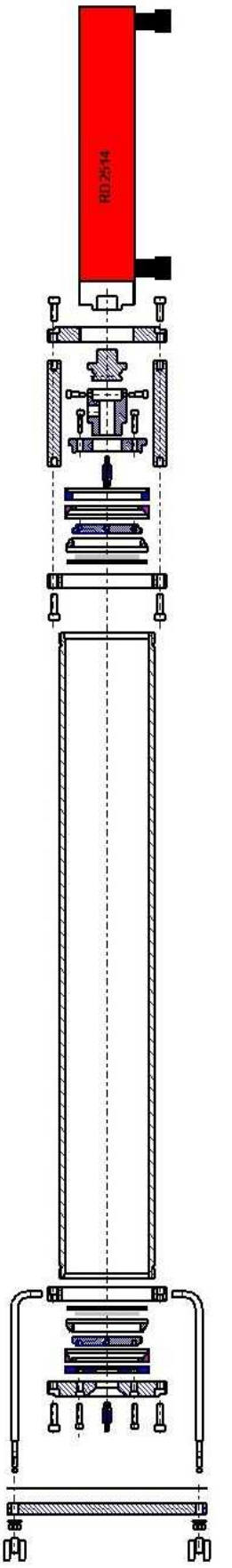

Fig. 1 Column design

2. Column design

Typically the *PC 01 150* (full column schema on Fig 1) column consists of tube, I.D. 153 mm made of 316 stainless steel. The internal surface of the column is mechanically polished to attain high smoothness (Ra better than 0.3 um). The tube is provided with two stainless steel column flanges, each with holes for the clamping screws. Bottom flange is equipped with M12 threads.
The upper and bottom parts of the column are closed by stainless steel pistons with PTFE and polypropylene (PP) made seal. Each piston unit consist of five parts (see Fig. 2, 3, 4):

- porous disc consisting of screen multilayer have important function to distribute the liquid and form a piston flow through the column is fixed in frit ring with a large thread for piston connecting
- own piston unit with liquid input or output and outer thread is screwed to a frit ring
- a set of stainless-steel nets with channels for liquid distribution are pressed from one side by a frit and from opposite side by a piston
- PTFE conical seal with polypropylene support part which seals both outer tube contact and inner piston thread
- support plate which is connected to a stainless steel tube which second end is attached to the hydraulic piston on bottom side is used a flange instead of a support plate.

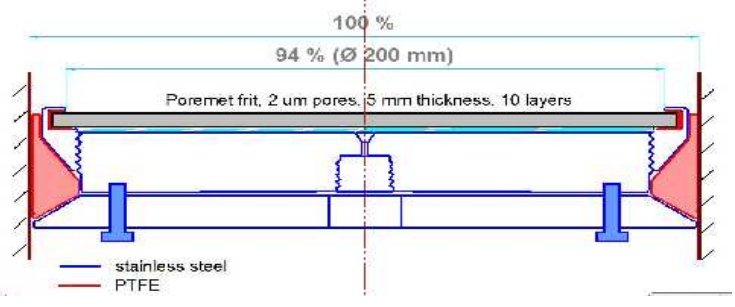

Fig. 2 Column piston principle

PTFE and PP piston seals are attached to the inner conical part of the piston unit and acts as pressure transducer. Its tightness increases when pressure is increased.
Porous disc covers nearly all tube cross section. This eliminates sorbent areas without full liquid flow. Net layers support the frit and make impossible a deformation under sorbent pressure. The liquid can flow not only through channels in net layers but also inside layers. It forms perfect piston flow over whole column cross section.

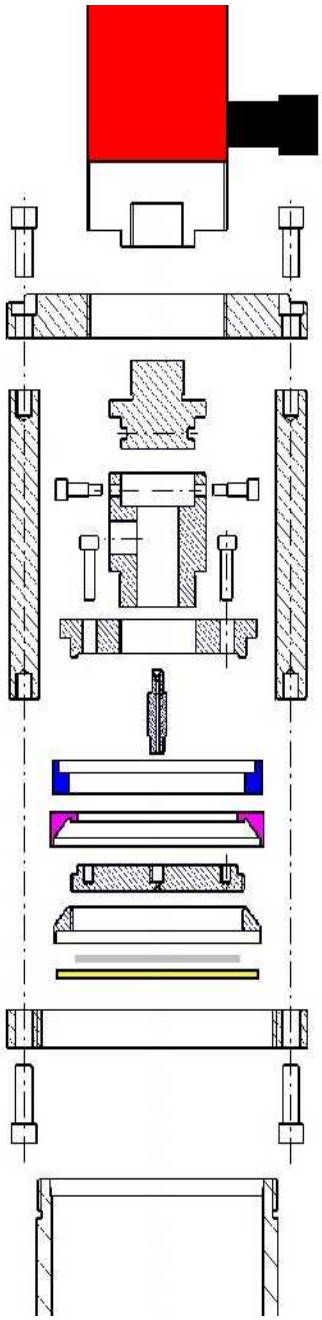

Fig. 3 Upper column part

To the upper column flange is connected a hydraulic cylinder. The cylinder is designed for much higher pressure then is used inside the column (700 bar against 150 bar). It allows to use hydraulic cylinders with much smaller diameter than column has.
Bottom column flange has side threads for stand legs. They are hooked in 90 ° angle and connected to a circular polypropylene plate covered by a stainless steel sheet. Under the plate are legs ended by a plastic wheels.
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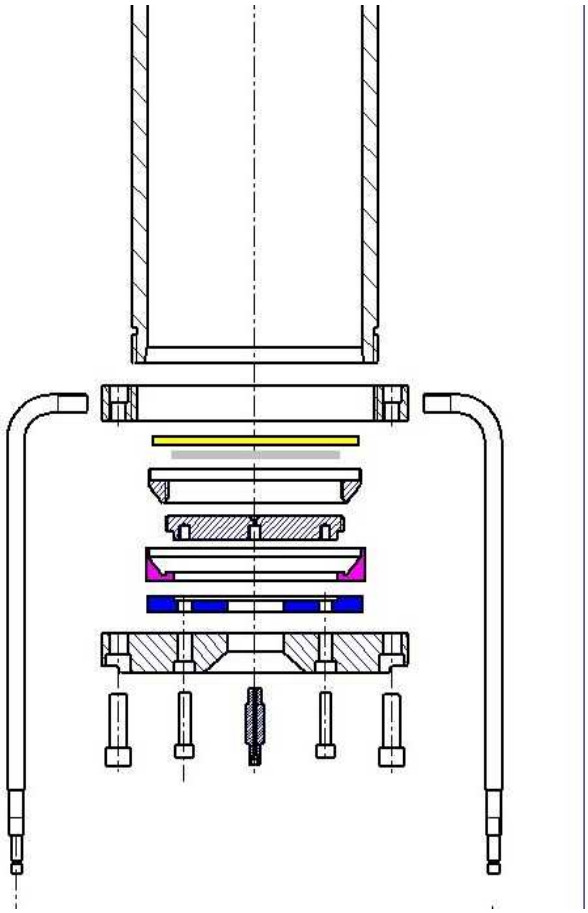
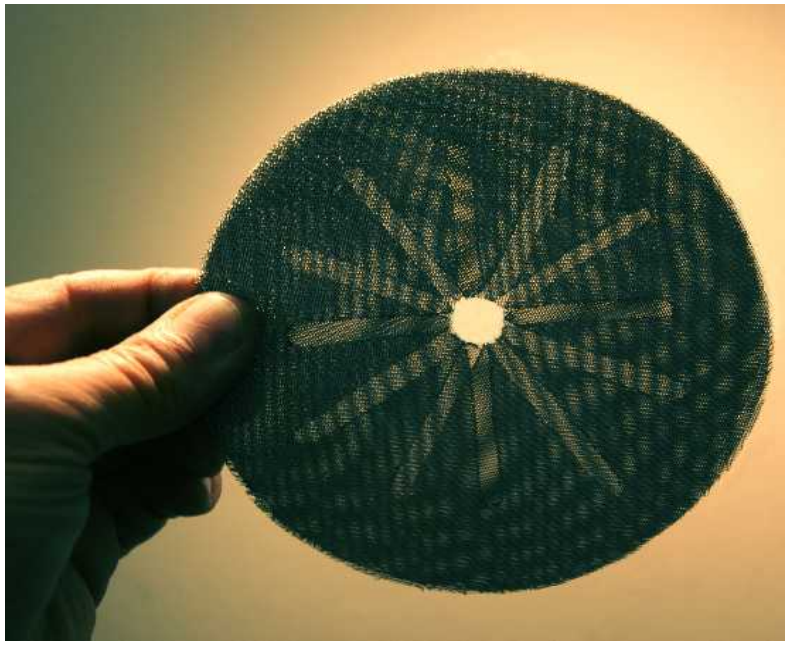


Fig. 4 Bottom column part

3. Column assembling

PC 01 150/1000 columns are usually delivered partially assembled, but here is described full assembling process to allow to the user to replace parts are repair column when necessary.
Piston assembling starts with distributor nets inserting into the frit ring (see next Figures). The net without channels is firstly inserted and nets with channels follow. It is necessary to take care on symmetrical spacing of channels. Finally the piston thread covered by thin layer of PTFE tape is screwed to the ring and tighten.



The piston is – when the unit is assembled – complemented with sealing rings and supprt plate (bottom flange) with screws. Both piston has to be connected to input and output fittings with a sealing PTFE tape before. There is a central thread in upper supporting plate to connect the piston tube and an hydraulic piston. Input pipeline (cca 500 mm in length, 3,3 mm O.D.) is to be installed before. It has to be fixed to the input fitting by a nut with ferrule and conducted to a side hole in connecting tube. Finally 8 bolts M8 has to be used to connect support plate to the piston unit

Output piston is assembled with its flange (see last Figure) and is connected by eight bolts too. Both pistons are identical and can be replaced.
Bottom piston with flange is now inserted to the column tube using special long bolts delivered with column. These bolts are after finishing the operation replaced by regular short bolts M12.
Column is connected to stand legs and stand plate. Wheels are added as well as an output tube O.D. 3,3 mm (1/8") with proper length. Column is now ready for packing.

4. Column packing

Column packing procedure has to be accomplished different way. There is either dynamic slurry packing method or a sedimentation method. Both methods are working with sorbent which is mixed with proper solvent for so called „slurry". Sedimentation method is described here.
Generally speaking sedimentation method uses nearly whole length of column for sorbent bed, but dynamic slurry method needs to use part of column for full volume of sorbent slurry. I case of sedimentation method the part slury is added to a simple reservoir which is attached to the own column. Sedimentation can be realized on single column with an adapter and upper piston which is pressed into column by means of a hydraulic device.
Prior to packing the column by sedimentation the packing adapter is to be assembled to the column. It consist of a polypropylene (stainless steel) tube with flange of the same dimension as the column flange has is then connected to the inlet side of the column by means of stainless steel bolts using expanded PTFE sealing tape (10x3 mm, 2 layers). Bolts are to be tightened only slightly to prevent deformation of polypropylene flange (in case it is used).
A suspension of the sorbent in proper solvent (follow manufacturer´s recommendation) is poured into the adapter - column assembly and allowed to flow through the outlet opening to a container. A suitable (not air tight) cover is placed on the upper opening. Sedimentation is allowed to proceed for 12 hours (overnight). The remaining solvent, if any, is then removed, the packing adapter is screwed off and an upper sorbent layer (some 25 mm thick) is scraped off.
The hydraulic cylinder screwed to the upper flange is connected to the column upper flange using only four (2+2 nearly each to other) connecting rods. The cylinder is connected to manual oil pump and its piston equipped with small connecting nut is pressed to the most upper position. The piston

unit with connecting rod is inserted opon the tube and fixed to oil piston using three side bolts on the connecting tube. The piston is carefully pushed to the column tube by oil pump.
Once the liquid begins to flow out from the inlet, it is closed and only outlet remains open. The piston is pushed in until the resistance is increased and there is visible a pressure on oil manometer. When is impossible to push the piston inside more (pressure on manometer increases rapidly when dosing oil), inlet is connected to the chromatograph by means of stainless steel capillaries.
The column is then washed with a solvent which was used for packing (about 10 column volumes) and tightness of the whole system is checked. The flow rate is increased gradually until at least 1.5 times the nominal pressure is reached. Pressure of oil in hydraulic cylinder is increases paralelly with increasing of mobile phase pressure in order to keep total power from side of hydraulic cylinder even higher then is a power generated on column piston side by mobile phase. It is necessary to take in the account that power does not means pressure. Usually hydraulic piston crossection is smaller then a crossection of column piston. Oil pressure has to be calculated from the ratio of crossections:

oil pressure= (mobile phase pressure) x (column piston crossection) / (oil piston crossection)

for 153 mm I.D. column and RD 2514 oil cylinder (25 ton by 700 bar) are used the ration between oil pressure and mobile phase inside the column pressure is 4,6.

The column nuts and bolts are checked and then tightened if necessary. The process is repeated with methanol or acetonitrile (for reverse-phase chromatography) or another suitable solvent similar to that subsequently used as the mobile phase. The oil pressure is again set to such value which is a bit higher than mobile phase pressure. Note: prior this recommendation the sorbent manufacturer instruction is followed during packing process.

5. Column unpacking

Semiautomatic column unpacking procedure is used having larger stroke hydraulic cylinder and elongation elements to upper column piston unit. Then all quantity of sorbent can be removed when piston is pressed down the column and sorbent is coming out on opposite side. The column output flange is released and oil piston is pressed up to the output piston is moved out of the column. A proper vessel is the inserted among column legs and sorbent is collected. When maximal stroke of hydraulic piston is reached, piston is moved back, an elongation rod is added and piston is moved inside the column again.

6 . Manufacture and servicing:

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