PREPARATIVE COLUMN FOR LIQUID CHROMATOGRAPHY
SEPARCHROM PC02 600/1200 MLP

HYDRAULIC DEVICE
SEPARPRESS D30 EE 600

User manual

Column SEPARCHROM PC 02 600/1200 DC is made of stainless steel and designed for medium pressure preparative liquid chromatography. It is used together with hydraulic axial compression device SEPARPRESS D30 EE 600, which is pressing column piston to the sorbent bed, eventually allows column packing by dynamic slurry method.

1. Description

Column design is apparent from Fig. 1. Column consists of a stainless steel made tube (316 L grade) with inner diameter 597 mm, length 1200 mm and wall thickness 10 mm (Fig. 2). Column has on both sides flanges with 24 holes each with M20 threads. In the column are from both sides inserted identical pistons made of circular plates of 50 mm thick antistatic UHMWPE (ultra high molecular weight polyethylene). Pistons (Fig 3) are provided with integral sealing rings (Fig. 4). There is a flow distributor inside each piston made of UHMWPE plates with groves and holes for nearly pressure less liquid distribution (Fig 5 and Fig. 6). There are Poremet 5 316 L stainless steel frits (7 layers, 3 mm thickness) with pore size 5 um between the distributor plates and sorbent bed (Fig. 7) fixed by steel rings.

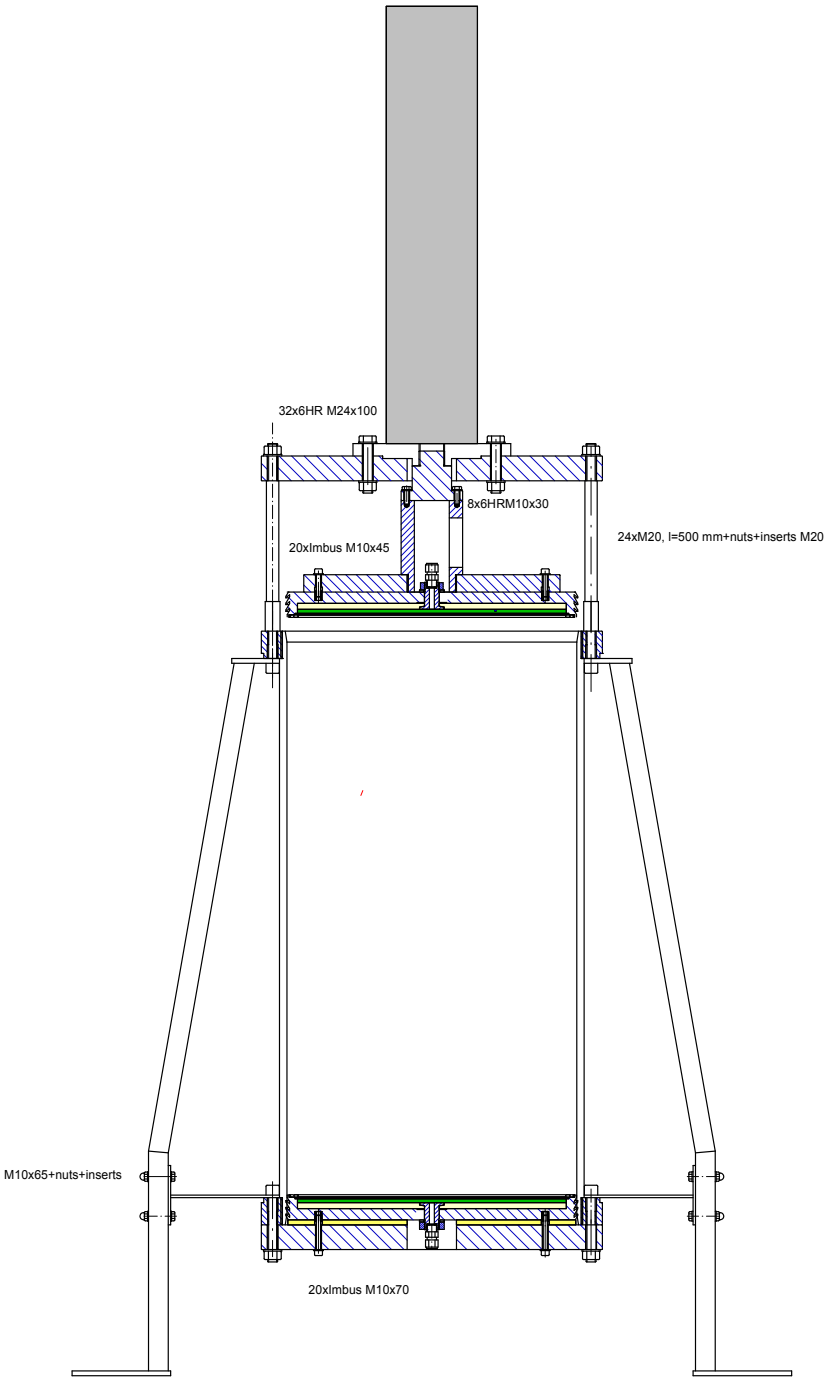

Fig. 1: Column cross section

Fig. 2: Column tube

Column output piston is connected to outlet tube by end fitting designed for a tube 1/2" (12,7 mm) O.D.. On the input piston is screwed 1/2" stainless steel tube, the elbow piece and armed PTFE tube 1/2" (12,7 mm).

Bottom piston is inserted to the column tube and fixed to bottom stainless steel flange (Fig 8). Between the piston and the flange is an additional polypropylene support inserted. To the piston body are screwed bolts which are going through the bottom flange and fix the piston with support to the flange.

Upper piston side being in contact with a liquid is the same as in the case of bottom piston. The whole piston is attached to the

stainless steel piston support with connecting tube and connecting flange that is fixed to the piston of hydraulic cylinder.

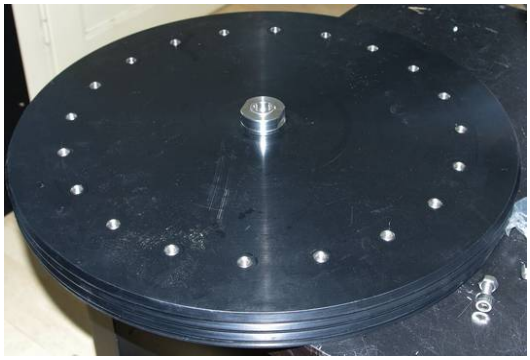

Fig. 3: Column piston

Hydraulic dual action cylinder 160 mm I.D. with maximal oil pressure 200 bar is situated beyond the column. Cylinder flange is connected to the upper column flange (Fig 8) by 8 bolts M24. Hydraulic piston is provided by a connecting flange and it is jointed to piston tube (8 bolts M10x30). The flange is screwed into hydraulic piston (see Fig 9).

The upper column flange with hydraulic cylinder is connected to the upper tube flange by 24 thread rods M20 (see Fig. on front page). Eight of them are provided with distance tubes to fix the distance of upper column flange. Four of them are shorter and have additional long nut, as they are connected to bolts (INBUS, M20x80) which are used for legs fixing.

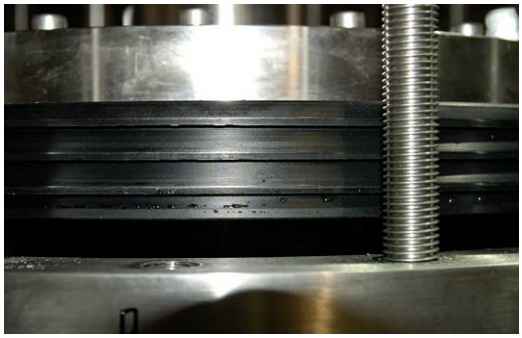

Fig. 4: Integral piston sealing

Bottom column flange with piston is connected to the tube flange by 20 bolts M20x110 with inserts. Four holes in tube flange are used for connection of leg supports (Fig 10) . They are connected by INBUS type 140 mm long bolts M20 with narrow head (OB 27,5 mm). Part of the bolt going through the flange is used for fixing of the column flange using 4 nuts and inserts.

Fig. 6: Flow distributor detail

Fig. 5: Flow distributor

Four legs are connected to the upper tube flange from its bottom side in an angle 90 °using mentioned M20 INBUS bolts with special narrow (O.D. 27,5 mm) head (Fig. 11). Each leg has an support connected to the bottom tube flange as explained. They are connected to the legs by two M10x65 bolts with nuts and and inserts. To the bottom tube flanges are supports connected by mentioned INBUS bolts M20.

Hydraulic cylinder is connected by two high pressure armed hoses to the electric motor driven oil aggregate (Fig. 12). Tubes have fast connection fittings on the side of the cylinder and are connected to the aggregate outlets which are on the front of its stainless steel box.

The oil aggregate with an electronic manometer has 10 l reservoir and a solenoid switching valves for movement of hydraulic piston up and down. The aggregate is delivered as a block where own pump, oil reservoir, valve system and pressure sensor are integrated. The pump is driven by a 3 phase motor with output 1,1 kW. Switchning valved are driven by 24 V (DC) and have output 9,5 W. Installed pressure sensor (0-250 bar) has output 4-20 mA.

Fig. 7: Frit in the piston

Hydraulic device SEPARPRESS D30 EE 600 EX is composed of four parts. Instead of mentioned hydraulic cylinder and hydraulic aggregate (placed in the stainless steel box), there is a control box (Fig. 13), connected to the oil aggregate by a cable. The control box provides the movement of the cylinder up and down and can switch the system to the position HOLD, where preset pressure is hold. Remaining parts of the system (electronic processor board, keyboard and display) are situated

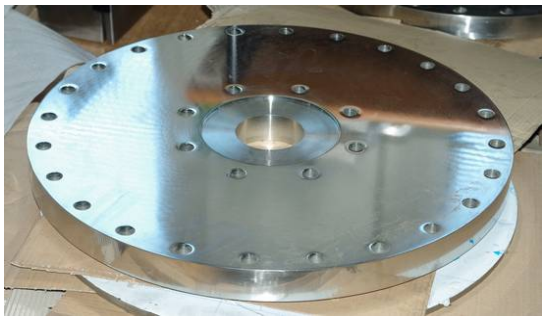

Fig. 8: Upper column flange

in remote switchboard.

Control box for hydraulic system has buttons DOWN, UP, STOP, switch LOC-REM and two control lights RUN + MAINS.

On the display and keyboard in remote switchboard (Fig. 14) is possibbe to set oil system parameters as described in Chapter 3.

2. Column assembling

Column tube is symmetrical. Column pistons are identical, made of antistatic UHMWPE, but upper piston has a stainless steel support and bottom a PP one.

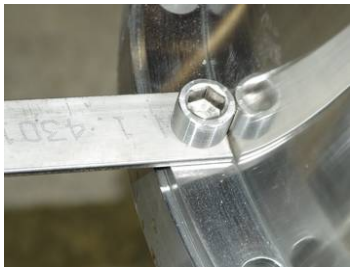

Fig. 10: Leg support

Fig. 11: Leg on the upper flange

Pistons are delivered partially assembled and following description of their mounting (in italics) only informative.

Each piston is composed of plastic piston plate inside which is inserted a flow distributor, connected to piston plate by set of bolts. The frit is laying on the flow distributor plate and is fixed by by a stainless steel ring connected to the piston plate by set of bolts. When frit is to be changed, these bolts have to be removed, old frit is removed too. New frit is covered on edges by few layers of PTFE tape (20 mm width, 0,2 mm thick) and pressed to the grove in piston plate is fixed by the ring and bolts again. Frit has to be oriented such way, that fine frit screens are on the sorbent side. Frit ring is added and carefully fixed to the plate by bolts. It has to be oriented (with regard to the piston plate) in same way as before deassembling.

Fig. 9: Hydraulic piston with connecting flange and tube

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Fig. 13: Congtrol box

Piston units with frits are assembled with their support – upper with steel support plate and connecting tube, bottom with polypropylene support and bottom column flange. Care can be taken on asembling of input tubes for upper piston. First of all is assembled piston plates with end fitting and first part of tubing with elbow. Then piston support is added and tubing has to go through connecting tube and its end is on the level of side hole in connecting tube. Support is fixed by bolts to the piston and last part of input tubing is connected. (see Fig. 15).

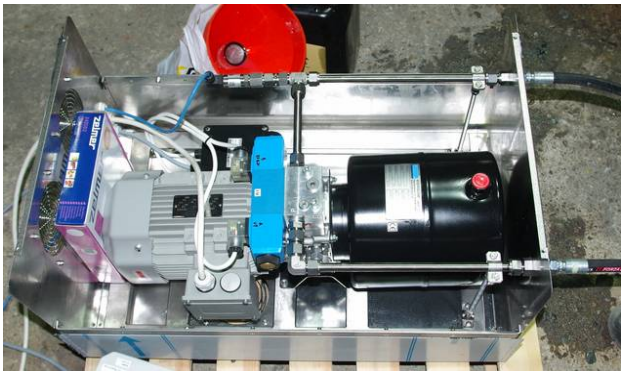


Fig. 12: Oil aggregate

Column tube is equipped with 4 legs which are connected from bottom side to upper tube flange. Legs are fixed by leg supports connected to the bottom tube flange from its upper side. The bottom piston with flange is evevated and pressed to column tube by tightening of special long (140 mm) assembling bolts. When piston is pressed inside and only cca 5 mm remains in flange opening, the flange is

connected by regular 20 bolts M22x110 with inserts.

Upper column flange is elevated than and connected to the columm by five thread rods with distance tubes. These rods have to be situated near to each other on one side of the flange to keep the flange, but to allow the piston to move inside the frame upon the column tube. The hydraulic cylinder is elevated and assembled to the top of column flange by 12 bolts M24x110. A connecting plate is than screwed on the hydraulic piston.

Caution:

For next operations is necessary to complete automatic hydraulic system and connected it to the electrical net.

A thin sheet of metal (cca 3 mm thick) is to be laying on the tube flange and the piston unit with support and connecting tube is (better by aid of a crane) put on and moved inside rods frame under the hydraulic piston. The hydraulic piston is moved slightly down near to the connecting tube. The column piston is set such way that connecting tube is just under connecting plate mounted on the hydraulic piston (common vertical axis) and holes for bolts on both parts are against each other. Parts are connected by bolts M10x30 than. The piston is moved a bit up and a metal sheet is released. Column is ready for packing and use.

3. Use of hydraulic system

Fig. 14: Oil control display and keyboard

Hydraulic instalation is composed of hydraulic cylinder, pump unit, control box with buttons and remote control box. Hydraulic cylinder is filled with hydraulic oil and equipped with fast connector for armed oil tube. The pump unit is in a stainless steel box, equipped with 10 l oil tank, system of solenoid valves and pressure gauge. The pump unit is connected by cables to the control box situated in switchboard. The control box is equipped with PLC unit for system control and by a display with keyboard. It is used for setting parameters.

The small control box with buttons is used to operate with the system on the place (to move the hydraulic piston up and down and to start automatic regime) as well to switch the system off by emergency button.

Description of Keypad drawing

F1:	used to move items between displays down
F2	key: used to move items between displays up
Key "arrow down":	used for deleting the setpoint
Key "arrow up":	used for adding setpoint
ENTER key:	used to confirm the setpoint a
Key START / STOP:	is used for starting and stopping the oil pump

Note: The last key is functional on any item at any time even if the system is controlled from an external source and the whole keyboard is off.

The order of display items:
Flow (%)
Pressure (bar)
Flow Settings

Pressure Settings
Hysteresis Settings
Time T1 and T2 Settings (for sorbents which can change volume during use)
Password Settings (the following items are accessible only after entering a password):
Zero pressure settings
Max pressure settings

Example of operation

After switching on the unit the first screen is displayed. In the upper right corner shows the status (at this moment, STOP). The display shows the current flow and the current pressure. After pressing the F1 key to get to the second operating item display, where it is displayed as the current primary pressure and secondary current flow rate of oil.

Pressing the F1 key gradually check set flow rate, maximal pressure and hysteresis (sensitivity of pressure regulation) and end up in the Password entry the pressure sensor calibration is possible.

After checking the set of values is possible by pressing F2 to return to the default item and the unit can be started by pressing the START / STOP on the keyboard. After pressing the change in the upper right corner is visible (to RUN) and the pump starts to pump. If not, it is possible that the pump is blocked by one of the following reasons.

a) pressure exceeded the set limit (in the bottom row shows the actual pressure)

b) autonomic control is disabled with the command on the serial line

c) drive motor is not ready or is in an error state, then RUN flashes for a while and just starts STOP.

Then is possible to press the START / STOP again to stop the pump. The pump motor starts stops rotation stepwise during approx. 4 s.

Pressure limit control function stops and starts the pump depending on the current pressure which

was set. To avoid fast on and off switching, an interval in which pump stops and starts again is to be set. This interval is called hysteresis and can be set between 1 and 15 bar. It is recommended to set hysteresis between 5 and 10 bar. Pump stops when the real pressure excess set pressure limit + hysteresis and starts again when pressure is going down set pressure value - hysteresis.

Two time values Time 1 a Time 2. (Time 1 in the range 1 ... 120s and Time 2 in the range 1 ... 120min) are used to set an additional function – periodical release of sorbent bed presurizing in HOLD regime. Some sorbents (ion exchange polymenr resigns) can change volume during use when mobile phase is changed. To avoid overpressurizing and sorbent particles deformation, the position of upper piston can be changed. After time T2 is pressure in oil released on the time T1 (oil aggregate valves position is changed and oil is delivered under the hydraulic cylinder piston to move column piston up). After T1 time the position of valves is again changed to the regime of pressurizing for time T2.

Only in case of sample injecting (STOP button released) is this process deactivated for 30 minutes (setting pressure is used) to allow sample injection, because during injection has to be the piston fixed on upper level of sorbent bed to avoid free space between sorbent and frit. After mentioned periode starts the process again. In case values of T1 and T2 are set on 0, normal use of HOLD function is possible (continuous preset pressure on the piston)

Calibration of the pressure

Performed after entering the service password on the left keyboard. Attention: in these settings change the items set important parameters pumps!

The three items relate to the calibration gauge. The first is the "Settings Zero pressure". To execute it, the pump has to be in pressure less state. When figures on the display stabilize, press ENTER. The transducer value for pressure 0 bar is recorded. Numerical data are raw, unadjusted data A / D converter, thus they are constantly changing a bit. The second is "Max pressure Settings" Here enter the value of the pressure at which is to calibrate the gauge. It is recommended to use at least half of the maximum pump pressure. The third alows to set "Max pressure". Here pressurized to a pressure pump from the previous item and after stabilization figure press ENTER. A value of converter for a given pressure is recorded. Once calibrated repeatedly press the F2 key to leave the screen of calibration.

Control box near the column

The control box situated on the flexible cable near to the column and is used to operate hydraulic system. On the bottom side of the box is

a switch allowing to set local or remote (in the electronics box) control. When local is chosen a down or up movement of hydraulic piston can be selected or an automatic function of pressure hold can be selected by pressing STOP button (RUN light is on). This function is mainly used for column packing and working with.

4. Column packing

A. Preparation of suspension:

Column has inner diameter 600 mm and length cca 1100 mm. It means that column volume is cca 310 L. In case column has to be packed in the active length 700 mm, proper amount of sorbent has to mixed with packing liquid (mixture of water and ethanol ?) to form a suspension 300 L in volume.

Necessary equipment: vessel cca 400 L in volume equipped with fast mixing device

B. Transport od suspension preparation

Column output has to be opened to a vaste. Column input has to be connected the same way. Both tubes must allow a closing by valve.

Suspension vessel has to be equipped with proper tubing (inner diameter not less than 10 mm) for suspension sucking, a pump for transport of suspension and its output tubing to column neck.

Column hydraulic system including oil pressure measurement has to be in function.

Necessary equipment: inert flexible tubing, pump for suspension

C. Own column packing

Sorbent is mixed with packing liquid not less than 15 minutes to eliminate air bubbles. Column output is open. Sorbent suspension is transported into the column. When the liquid starts to flow from column output, the output is closed. Transport of the suspension has to be accomplished in 10 minutes to eliminate sorbent sedimentation (sedimentation velocity is 12 mm/min in water with 20 % ethanol).

When sorbent is transported to the column, upper column piston is moved down by oil pressure (output from upper piston is open, output from bottom piston is closed). When liquid without bubbles is going out of the upper output, the output is closed and bottom output is opened. Piston is pressed to the column to keep output liquid flow rate on cca 2 L/min. Oil pressure is monitored.

When column piston comes to the sorbent bed, piston movement is retarded and oil pressure is increased. It is important not to overpressurize sorbent in the column. According our experience pressure 2 bar (i.e. 28 bar in oil) on the sorbent has no influence on the bed quality, so limit pressure 30 bar has to be set on oil pump.

When oil reachs the pressure limit, oil pump is stopped and packing is finished. Sorbent nevertheless will be pressurize a bit in next time interval and oil pump will start its run times to time to compensate it.

5. Piston level measurement

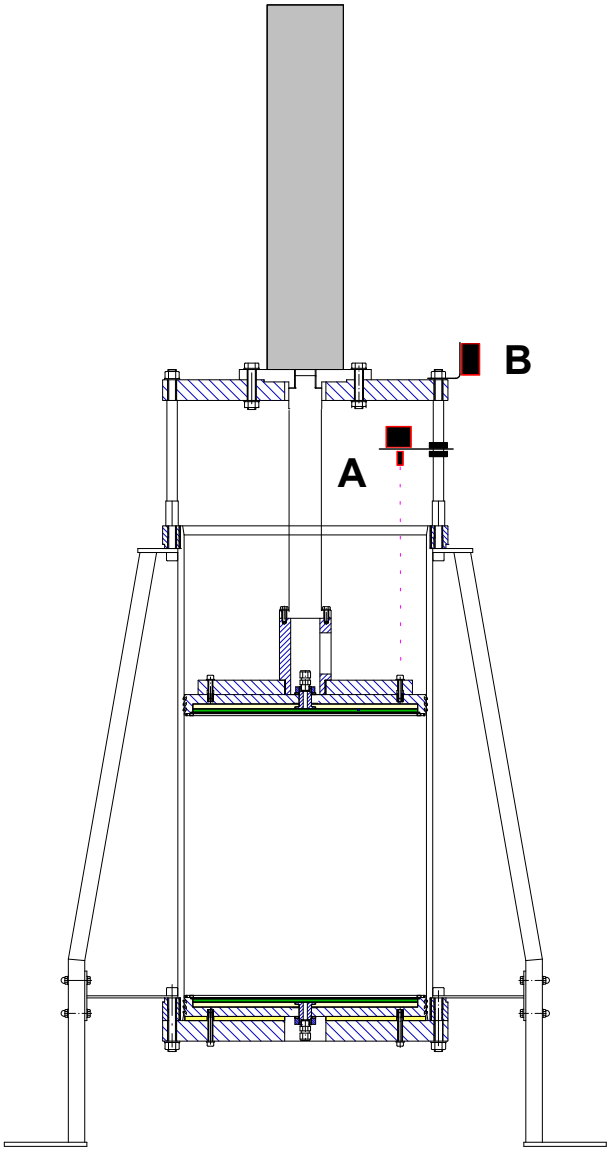
Column according this Instruction manual is equipped with electronic measurement of upper piston position. Information about piston position is useful in case when sorbent in the column is nor rigid, can swell and change its volume.

Measuring system for piston position is composed from an ultrasonic measuring device ULM 53 and a display that informs user abot actual position in user selected unit. Position both the probe (A) and display (B) are demonstrated on Fig. 16.

Position of the piston has to be calibrated before use according the Manual for ULM 53 sensor (ULX-

53.pdf) and PA 440 display unit (DIS_PA440.pdf). Both manuals are delivered as belongings of this Manual.

Fig. 16



6. Table of technical parameters

Parameter specification	**Value**
Column inner diameter (mm)	597
Column outer diameter of flanges (mm)	692
Column tube total length (mm)	1200
Maximal active length od sorbent bed (mm)	1060
Minimal active length of sorbent bed (mm)	630

Column wall thickness (mm)	14,5
Maximal distance of legs (mm	1460
Total height of the system with hydraulic cylinder (mm)	2780
Column inner surface quality Ra	<0,3
Column inner surface quality Ra	<0,8
Maximal column (as pressure vessel) working pressure (bar)	30
Maximal system working pressure with hydraulic cylinder (bar)	11
Piston friction in the column tube = inner pressure to start piston movement (bar)	<2
Ratio between column and hydraulic piston cross sections	14
Hydraulic cylinder inner diameter (mm)	160
Hydraulic cylinder stroke (mm)	600
Hydraulic cylinder power (kg) by 200 bar oil pressure	40000
Hydraulic aggregate flow rate (l/min)	2
Hydraulic aggregate pressure (bar)	200
Hydraulic aggregate tank volume	10

7. Column pressure stability

Preparative column for liquid chromatography SEPARCHROM PC02 600/1200 MLP with hydraulic device SEPARPRESS D30 EE 600 is made according Directive 97/23/EC (PED), annex III, module H, that is transposed by Czech Government Order No. 26/2003 Coll., as amended, according to Act No. 22/1997 Coll., as amended using Czech ČSN EN 13445 production norm and Czech ČSN-EN 69 0010 production norm.

Maximal working pressure for the described system is 11 bar (testing pressure is 15 bar). Preparative column for liquid chromatography SEPARCHROM PC02 600/1200 MLP itself is designed for the working pressure up to 30 bar (testing pressure 42 bar). Test protocols are part of technical documentation.

8. Column safety in the environment

The column SEPARCHROM PC02 600/1200 MLP with hydraulic cylinder is nonelectric equipment, which is designed for the use in environment II2G IIB T4 according Directive 94/9/ES, appendix 1. Conformity evaluation was done according §12 par.. 4 subparagraph a) CZ law No. 22/1997 Coll. in amended - evaluation of conformity under specified conditions by manufacturer In case of column packing is necessary to keep instructions in User manual. Maximal pressure in the column with hydraulic cylinder can not be higher than 11 bar. Maximal temperature of the column and cylinder can not exceed 60 °C. Hydraulic aggregate is equipped with independently tested motor and solenoid valves. These parts are allowed for the use in the environment II 2 G cT4 according European Directive 94/9/ES. Hydraulic system control box is certified for the use in the environment II 2 G T4 according European Directive 94/9/ES

10. Manufacturing by

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