VarioPrep columns

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column.
VarioPrep columns are quality products. They are especially developed for HPLC analysis. If carefully and properly used excellent chromatographic results and long column lifetime can be achieved. Depending on the specific separation potential of the packed stationary phases, this product can be used for preparative separation of numerous mixtures. It must exclusively be used in accordance with universally accepted laboratory regulations and HPLC working methods. Before running the column the entire chromatography system (column and equipment) must be carefully checked by the operator. Chromatographic conditions (mobile phase, flow, temperature etc.) must be adapted to the preparative application. MACHEREY-NAGEL does not give any warranty and is not liable for the success of a separation or application. If you have any questions after reading this manual, please call our service or technical support.

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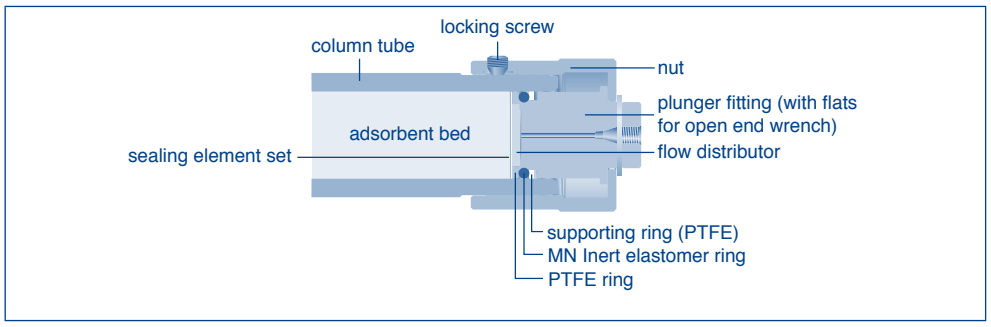
Safety indication
Follow the general safety instructions for handling of HPLC solvents used as mobile phases (e.g., acetonitrile, methanol) and take precautions against any kind of injuries or damage to health (e.g., skin and eye protection in case of broken capillaries). Disposal of used HPLC columns must follow international, national and local environmental protection regulations. The use of HPLC columns is only permitted to staff members, who are qualified in their field. Keep HPLC columns away from children. MACHEREY-NAGEL disclaims and excludes all warranties of any kind or nature whatsoever and MN shall not be liable for any damages (whether direct, indirect, foreseeable, incidental, compensatory, consequential or special), whether based upon warranty, contract, tort or strict liability, if damages and / or losses occur caused by improper use, maintenance, neglect or improper treatment (especially exposure of the column bed by not inadequate opening of the column).

Description of VarioPrep column system
VarioPrep columns are packed with modified or unmodified, spherical silica gels (NUCLEODUR®, NUCLEOSIL® etc.). Detailed information about these stationary phases and about their use can be found on ***www.mn-net.com***, in the MN chromatography catalog or in the instruction leaflets of the respective analytical columns.
The system of VarioPrep columns features the possibility to axially adjust both end parts (end fittings). This allows the easy and fast compensation of a dead volume, which could result at the column inlet after some time of operation at demanding conditions, without need for opening the column.

Design of the adjustable end fitting:



When designing these columns special care was taken to use only highest quality and completely inert materials. The sealing system is built up of an MN Inert elastomer ring and sealing rings from PTFE. Distribution of the liquid stream is effected by the flow distributor. The plunger fitting is fixed on the adsorbent bed by the nut, which is locked against torsion with the locking screw. Sealing of the column is achieved by prestretching the elastomer ring; consequently the force, with which the nut is tightened, does not have any effect on the tightness of the system. However, this force has a direct influence on the pressure, with which the packing material is compressed in the column. Thus the nuts are only used for retaining the HPLC material between the VP plunger fittings. If it is tightened too strongly, the HPLC packing can be damaged!

Installation
The VarioPrep column should be installed in the flow direction indicated on the column label. The adjustability of both endfittings (compensation of a possible dead volume) allows a frequent use of back-flushing techniques. The VP column is connected with 1/16" capillaries and fittings, typical for HPLC instruments. Due to the design of the end parts the plunger fitting has to be held with an open end wrench when connecting the capillary fittings in order to prevent turning.

Guard columns
For protection and an extension of column lifetime the column should always be used with guard columns. The filter elements and the adsorbent in the guard column retain contaminants from the sample or the eluent. Connection of the guard column with the separation column is made by a suitable guard column holder (see ***www.mn-net.com*** or MN chromatography catalog). Cartridge replacement is required when increased column pressure and / or loss of performance is observed.

Sample
Sample solutions, generally solved in eluent should be passed through a syringe filter (e.g., CHROMAFIL® Xtra PET, 0.45 µm, 25 mm, REF 729220) before entering the column. If injected sample solutions are still turbid even after filtration, the lifetime of the column may be significantly reduced. The sample amount to be injected can be increased by overloading of the preparative column. Thus, a high yield is possible. Typical sample masses for RP columns are shown in the up-scaling table below:

Up-scaling								
ID x length [mm]	4 x 250	8 x 250	10 x 250	16 x 250	21 x 250	32 x 250	40 x 250	50 x 250
Column volume [mL]	3	13	20	50	87	201	314	491
Linear scale-up factor	1	4	6.25	16	27.6	64	100	156.3
Typical sample mass* [mg]	0.02–2	0.08–8	0.13–13	0.3–35	0.6–60	1.3–130	2–210	3–350
Typical flow rate [mL/min]	0.5–1.5	2–6	3–9	8–24	14–40	32–96	50–150	80–250

* For RP material; the maximum amounts given here always depend on the separation problem and on the sample composition. In some cases half of the amount given can cause drastic overload, in other cases the maximum amounts can be even higher still giving acceptable separations.

Separation conditions (eluent, flow rate, pressure, temperature)
VP columns are supplied with an eluent, matched with the stationary phase (see column certificate). For the column in hand only suitable eluents should be used as mobile phase. The selection and composition of the eluent should be ideally determined by a prior analytical method development, if no preparative application note is available. (Information about the selection of eluent can be found in the instruction leaflets of the respective analytical columns; see ***www.mn-net.com***.)
The typical flow rate is given inter alia by the inner diameter of the column (see up-scaling table). It influences the time needed for separation, the resolution and the column lifetime. Furthermore it is limited by the maximum column back pressure, which should not exceed the limits listed in the table below.

		Maximum pressure [bar]						
Silica	**Inner diameter [mm]:**	8	10	16	21	32	40	50
NUCLEODUR®, NUCLEOSIL® up to 120 Å, NUCLEODEX		400	400	400	400	400	400	400
NUCLEOSIL® 300 Å		300	300	300	300	300	300	300
NUCLEOSIL® 500 Å		250	250	250	250	250	250	250
NUCLEOSIL® 1000 Å, 4000 Å		200	200	200	200	200	200	200
NUCLEOGEN®			200					
NUCLEOCEL		150	150	150				

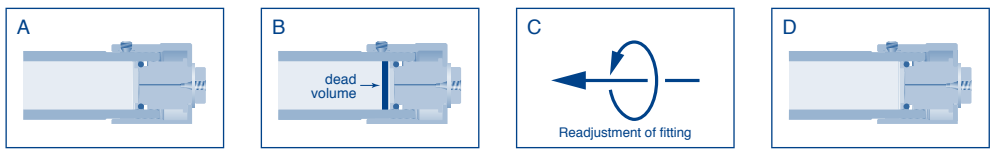
We recommend to control the back pressure regularly. If a high pressure results from the use of the column at nominal flow rates, this usually indicates that some contaminants have become deposited on the packing material, which must be removed (see troubleshooting).
The maximum column temperatures are also determined by the stationary phase. Variation of the temperature influences retention times and especially the peak shape. Optimum temperatures for successful separations should be determined empirically.

Detection
Spectrophotometers, refractometers and electrochemical detectors can be used with the preparative columns.

Equilibration
Prior to measurement of samples the column must be rinsed with the eluent at the same flow rate and temperature as the method to be applied. Column equilibration is finished, when the baseline of the detector no longer shows a drift.

Column storage
The original eluent (see column certificate) is recommended for storage. For long-term storage mobile phases containing inorganic salts are not recommended. Methanol is also not recommended for a longer storage, because of a possible impurity with metal ions (e.g, iron(III)). For column storage be sure the end fittings are tightly sealed using column end plugs, because storage without these seals can result in drying of the packing material. Under these circumstances rinse the column with ca. 10 column volumes of the eluent of storage.

Repair of a possible dead volume (VarioPrep principle)
Based on a special packing procedure VarioPrep columns are produced with highest packing quality and bed density (A). Due to intensive chemical and / or mechanical strain of the column adsorbent, shrinking of the column bed could occur (B). In this case readjustment of the VarioPrep column fitting (C; turning the nut at column inlet clockwise) will eliminate a possible dead volume (D).



Procedure for readjusting a plunger fitting in order to remove a dead volume in the column (Fig. C):
1. Test the column with a standard test mixture (e.g., the so-called aromatics test).
2. Turn off the HPLC pump → the column has to be pressure-free.
3. Remove the capillaries.
4. Loosen the locking screw of the nut.
5. Turn the nut clockwise by hand, until you feel a slight resistance. Please, take care not to turn too far!
6. Tighten the locking screw and connect the capillary again. Hold the plunger fitting with an open end wrench in order to prevent turning.
7. Check the quality of the column with the above-mentioned standard test mixture.

If you are satisfied, you can proceed with your work. If you are not satiesfied, repeat the above procedure, until you get the required results or until you reach the end of the adjustment length of your column. (Take care – do not tighten the nut too strongly!)

Troubleshooting (column regeneration)
All columns are subject to the strict regulation and control of our quality assurance system. VP columns, especially those based on silica, are robust and hold their separation efficiency for long periods by correct maintenance and treatment. Additionally, the lifetime can be extended by the VarioPrep principle (see above). According to experience, column failures are mostly a result of injection of contaminants to the sorbent bed. The usage of a guard column, as well as an appropriate sample pretreatment will help to minimize these risks. However, if a performance loss is observed, the phase specific instruction leaflets of the analytical columns give important instructions for troubleshooting and column regeneration (see ***www.mn-net.com***).

Important instructions for opening the VarioPrep columns
If a replacement of the sealing elements (e.g., due to impurities) and / or of the sealing rings (e.g., due to leaking) should be necessary, please note the instructions below for proper opening of the VP columns:
1. Test your column with a standard test mixture prior to opening.
2. Remove the capillaries.
3. Move the plunger fitting only carefully. If it is stuck, fill the space between the plunger fitting and the column wall with, e.g., methanol. Pull the plunger fitting, while turning it at the same time (this will result in a screw-shaped movement).
4. If you wish to replace the phase sealing elements (REF 718931 for 10 mm ID columns, REF 718853 for 21 mm ID columns, for columns with other IDs on request), please take care not to damage the packing.
5. If you have problems with the tightness of the column, you need to replace the MN Inert sealing combination (718848 for 10 mm ID columns, 718870 for 21 mm ID columns, for columns with other IDs on request). It should be wetted with methanol before. It is important to take care, that the MN Inert elastomer ring is not damaged, when the plunger fitting is introduced! Please take care, that the surface of the column packing is completely level, before you insert the new phase sealing elements. Of course, the surface has to be perpendicular to the column axis.
6. Tighten the nut by hand, until you feel a clear resistance.
7. After these procedures, test the column under the same conditions as above.

If after this replacement and the readjustment of the plunger fittings, the VarioPrep column is not yet tight, please contact MACHEREY-NAGEL for support.

Abstract
To extend column lifetime, please keep in mind the following:
1. As eluents, phase specific eluent systems are recommendable. (If necessary, please inform yourself with the instruction leaflet of the respective analytical column, ***www.mn-net.com***.) Eluents should be filtered through a 0.45 µm membrane and degassed.
2. Filter samples through a 0.45 µm CHROMAFIL® Xtra PET syringe filter before injection.
3. Use a guard column for protection against impurities.
4. Typical flow rates are given in the up-scaling table.
5. Adjust flow rate to keep column pressure below the maximum value of your column.
6. Store the column in the original eluent (after removal of buffer salts).
7. Use analytical grade reagents and HPLC grade solvents for all work.
8. Note the instructions for the VarioPrep principle and for opening the VarioPrep columns.

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