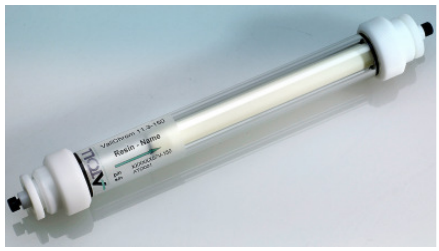
MediaScout® ValiChrom

Columns for parameter evaluation, validation and small scale separation



Introduction

MediaScout® ValiChrom glass columns are available customized to different inner geometries and come professionally packed with any resin or chromatography media. These are individually flow-packed, either according to resin manufacturers' recommendations or customized, in order to precisely mimic the conditions in corresponding full scale columns. Thus, the ValiChrom represent an exact scale-down model of the respective full-scale column and are ideally suited for parameter evaluation and optimisation, as well as for process validation purposes, both utilizing the original bed height and resin compression at the smallest feasible inner diameters.
ValiChrom columns are available in inner diameters of 5, 8, 11.3, 16 and 25 mm and with **any fixed bed heights** ranging **from 2 up to 60 cm**.
The ValiChrom range is designed for qualitative and quantitative resin screening experiments, small scale methods development work, virus validations, small scale separation (e.g. by size exclusion chromatography), and more.
They are unique in their ability to provide a reliably packed and valid evaluation system for comparisons of dissimilar resin types.
The simple fixed bed height design of the ValiChrom columns allows to avoid complicated adaptor constructions. Therefore, these columns are available at comparatively moderate pricing.
Materials and production procedures are available that minimize the risk of BSE/TSE contamination (Certificate).

Product Description

MediaScout® ValiChrom columns are delivered ready to use, packed with chromatography media from a large collection of commercially available resins or gel products for bioseparation. Unless otherwise stated, packed media are equilibrated with 20% ethanol or a mixture of 20% ethanol and 150 mM sodium chloride (ion exchangers, IMAC and HIC resins, size exclusion resins). Columns are equipped with synthetics fittings (M6, UNF 10/32 depending on column inner diameter).

Components of MediaScout® ValiChrom columns are made from precision borosilicate glass (column tube), bio-compatible polymers, mainly polyoxymethylene (end piece, filter adapter, USP Class VI material available), polyethylene (distribution net, certified) and from porous polypropylene/polyethylene material (filter plates, certified). The protective outer tube is made from acrylic glass.

Columns may be connected by standard HPLC/ÄKTA male connectors directly to most liquid chromatography systems or workstations (Connection to earlier FPLC® systems is simple with standard 10-32 male / M6 female adapters or M6 male adapters).

MediaScout® ValiChrom columns are primarily designed for use with chromatography systems. They may be reused as long as the resin remains in acceptable condition, which will depend largely upon the care taken, the cleaning performed and the tolerance of the resin to user conditions. ValiChrom columns

can be refilled. Storage of the columns is usually at room temperature (note: Protein A or other sensitive resins may require storage at 5±3°C).
The protective outer tube can be equipped with barb connectors to thermostat the column e.g. by a circulating cooling fluid (please inquire).

The most important properties of MediaScout® ValiChrom columns are summarized in table 1.

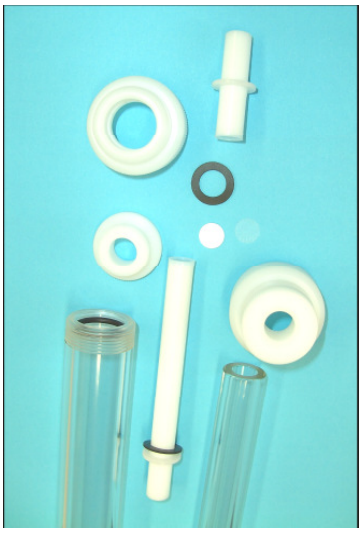


Fig. 1 Parts of the MediaScout® ValiChrom columns.

- Column tube (precision borosilicate 3.3 glass)
- PMMA (acrylic glass) protective outer tube, with o-ring
- Sealing ring (Viton)
- End pieces
- Adapters (top and bottom) with female M10-32 UNF or M6
- Filter plates
- Distributor net
- End plug (M10-32 UNF or M6, not shown)

Table 1 Properties of MediaScout® ValiChrom columns

Column Type	Inner Diameter × Packed Bed Height	Column Cross Section	Column Volume	Flowrate[1]	Max. Flowrate[1]	Connector (male)
	(mm × mm)	cm^2	ml	ml/min / cm/h	ml/min / cm/h	
ValiChrom 5-X[1]	5.0 × 20-600	0.2	0.4 - 12	0.5 / 150	< 5 / 1500	10-32 UNF
ValiChrom 8-X	8.0 × 20-600	0.5	1.0 - 30	1.25 / 150	< 5 / 600	10-32 UNF
ValiChrom 11.3[2]-X	11.3 × 20-600	1.0	2.0 - 60	2.5 / 150	< 25 / 1500	M6
ValiChrom 16-X	16.0 × 20-600	2.0	4.0 - 120	5.0 / 150	< 25 / 750	M6
ValiChrom 25-X	25.0 × 20-600	4.9	10 - 295	12.3 / 150	< 49 / 600	M6

Maximum Operating Pressure[3]	30 bar
Chemical Stability[4]	Columns are tolerant to aqueous buffers and salt solutions, 1 M alkaline solutions, 0.1 M phosphoric acid, 8 M urea, 6 M guanidine hydrochloride, non-halogenated organic solvents and detergents. They are not compatible with strong oxidants and mineralic acids and halogenated solvents.

[1]X represents the packed bed height

[2]The precise ID of the glass tube is 11.28 mm, corresponding to 1.00 cm^2 cross section

[3]This depends on the chromatography material as well!

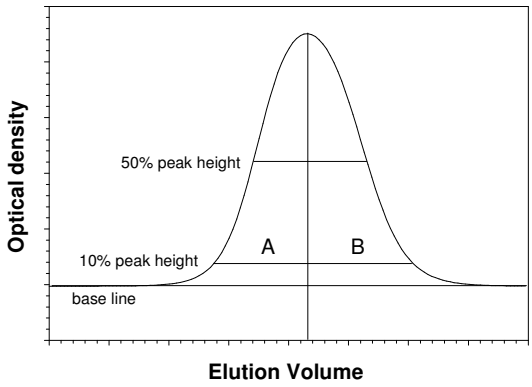
[4]The chemical stability refers to the column hardware parts only. The individual packed chromatography media may have different stability. To avoid damage to the chromatography media, please refer to the relevant manufacturer's data.

Performance Testing

Theoretical plate number and Asymmetry factor ('Tailing')

The chromatographic performance of ValiChrom columns should be checked at regular intervals by determination of plate number and peak asymmetry.

The evaluation of the elution profile of a suitable sample should be carried out as follows, with parameters as shown in the schematic drawing:



The theoretical plate number N (unit: 1/m) and the asymmetry factor (A_s) are calculated according to equations (1) and (2):

$$N = 5.54 \times \left(\frac{V_e}{W_{1/2}} \right)^2 \times \frac{100}{BH} \qquad (1)$$

$$A_s = \frac{B}{A} \qquad (2)$$

with

V_e = elution volume, $W_{1/2}$ = width at 50% peak height, BH = bed height in cm, B and A = left and right section of a line, drawn parallel to the base line at 10% peak height (IUPAC), drawn vertically from peak maximum to base line.

In **Fig. 2** the results of two real example performance tests are shown for columns of 10 and 20 cm bed height.

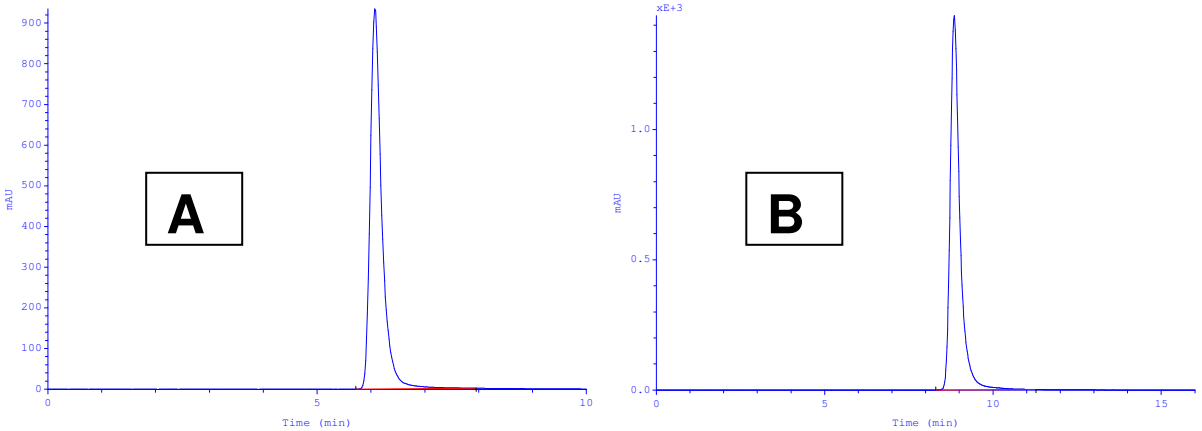


Fig. 2 Column Performance Test
Elution profile of a sample of Vitamin B_{12}, 0.8 mg/ml, with a MediaScout® ValiChrom 11.3-100 (A) and 11.3-200 (B) column, packed with SOURCE™ 15Q (2.5% compression compared to gravity settled resin). Sample injection was at 3 min.

Eluant:	50 mM Tris-HCl, 0.9% NaCl, pH 8.0
Flow rate:	2.5 ml/min (150 cm/h)
Detection:	UV at λ=280 nm

Theoretical plate numbers were 11700/m (A) and 12000/m (B) (Total plate numbers: 2340 (A), 2400 (B). The asymmetry factors were both calculated as 1.5.

The functional test for ValiChrom columns packed with chromatography media incompatible with acetone, e.g. immobilized Protein A resins, should be performed with an alternative low molecular weight analyte.
1 M sodium chloride solution may be detected by conductivity, but the eluant should be of low ionic strength. Potassium nitrate (10 mg/ml) may be used as a UV-detectable alternative to sodium chloride. Furthermore, a solution of vitamin B_{12} (0.1 mg/ml) in water and visible light detection at λ=540 nm may be used, in order to visualize sample migration on the column. The injection volume should be 1-2% of the column bed volume.

Pressure drop

The back pressure of ValiChrom columns largely depends on the bed height, resin features (particle size, porosity, support material, etc.), packing density and operational parameters (linear flow rate, solvent viscosity, piping, etc.).

The bore diameter in the top and bottom adapters is regularly 1.5 mm, resulting in a low contribution of column hardware to back pressure. **Figure 3** shows typical pressure versus flowrate profiles for two column sizes, packed with a 15 µm rigid polymer resin and run with an aqueous buffer system.

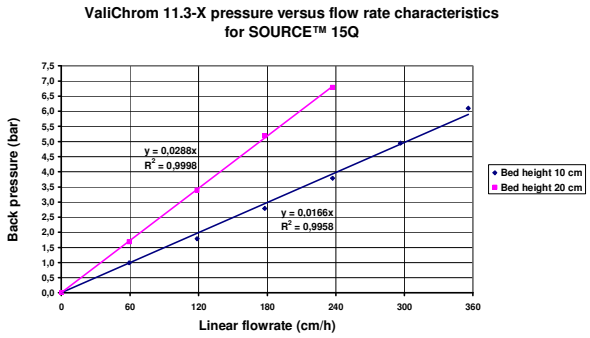


Fig. 3 Pressure versus flow rate profiles of two ValiChrom columns, packed with SOURCE™ 15Q, to 10 and 20 cm bed height, respectively.

Ordering information

MediaScout® ValiChrom columns are packed to order with any commercially available particulate separation material, most of which are available ex-stock.

Atoll GmbH will be pleased to pack any resin that you wish to send in for packing into any of the MediaScout® system formats.

For details, including the current resin selection list, please visit the Atoll website shop within

http://www.atoll-bio.com

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