

# HPLC Columns Made by AppliChrom GmbH

AppliChrom OTU TriKala AppliChrom OTU LipoMare AppliChrom OTU Amino AppliChrom OTU SiOH AppliChrom OTU Phenyl AppliChrom OTU HILIC AppliChrom ProteSep S-L / S-S AppliChrom VicoSil S-L





# Silica Based Chromatography Columns



AppliChrom GmbH Germendorfer Allee 20 D-16515 Oranienburg Germany





www.applichrom.com info@applichrom.com Phone: +49 3301 579293 Fax: +49 3301 209879



# AppliChrom OTU TriKala AppliChrom OTU LipoMare

## C<sub>8</sub> and C<sub>18</sub> RP-HPLC-Columns

- Universal high-performance RP-chromatography columns.
- Highly porous media with 105Å pore size, >300m<sup>2</sup>/g surface.
- High purity silica based material

(<10ppm Fe, Al, Mg - Warranty; approx. 1ppm Fe, Al, Mg current)

- For RP-chromatography investigations of molecules with up to 5 000Dalton.
- Spherical and high pressure stable particles for low backpressures during the chromatography combined with high long-term stability of the HPLC column.
- Conserve your budget and preserving its capacities through a combination of inexpensive columns with individual application support and service.



AppliChrom OTU LipoMare C<sub>18</sub>

For the analysis of neurotransmitters (dopamine,...)





Coffein (C8H10N4O2) MW: 194



Theophyllin (C7H8N4O2)

MW: 180



Theobromin (C7H8N4O2)

MW: 180



Xanthin (C5H4N4O2)

MW: 152

Paraxanthin (C7H8N4O2) MW: 180



ОТU TriKala C<sub>18</sub>, 105Å, 5µ, 250х4.6mm 1ml/min MeOH / H2O// 80 / 20 // vol./vol. Me, Et, Butybenzoat, 70bar, UV254nm

# AppliChrom GmbH☎+49 (0)3301 579293☑info@applichrom.com

AppliChrom silica-based columns AppliChrom OTU TriKala AppliChrom OTU LipoMare



# AppliChrom OTU TriKala AppliChrom OTU LipoMare C<sub>8</sub> and C<sub>18</sub> RP-HPLC-Columns



AppliChrom® Trikala C<sub>18</sub> 5µm, 105Å, 250x4.6mm

Flow rate: 1ml / min Eluent: 80/20 MeOH / H<sub>2</sub>O

Peaks in the order of elution: Uracil Ethylbenzen Propylbenzene Butylbenzene Amylbenzene

	OTU TriKala series (C <sub>8</sub> and C <sub>18</sub> )	<b>OTU LipoMare</b> series (C <sub>8</sub> and C <sub>18</sub> )	
	Lipophilic C8 and C18 media for the RP-chromatography in organic Solvents	Hydrophilic (polar) C8 and C18 media for the RP- chromatography in water or organic Solvents	
Particle size (µm)	5	5	
Surface (m³/g)	-300	-300	
Pore size (Å)	105	105	
USP classification	L7 / L1	L7/ L1	
Selected recommend	lations:		
Stereoids	$\checkmark\checkmark\checkmark\checkmark$	$\checkmark\checkmark\checkmark\checkmark$	
Flavonoids	$\checkmark\checkmark\checkmark\checkmark$	$\checkmark\checkmark\checkmark\checkmark$	
Gluco-Flavonoides	$\checkmark \checkmark \checkmark$	$\checkmark\checkmark\checkmark\checkmark$	
Alkaloides water soluble substances (Sugar, hydrophilic derivatives of alkaloids,)		$\checkmark \checkmark \checkmark \checkmark$	
Peptides	$\checkmark\checkmark\checkmark\checkmark$	$\checkmark\checkmark\checkmark\checkmark$	
$\checkmark \checkmark \checkmark \checkmark =$ very good, $\checkmark \checkmark \checkmark =$ good, = not recommended			



AppliChrom silica-based columns

AppliChrom OTU SiOH AppliChrom OTU Phenyl AppliChrom OTU DiO HILIC



## AppliChrom OTU SiOH

Unmodified, stable spherical silica phase for NP HPLC

- particularly suitable for polar applications e.g. HPLC of steroids and fatty acids



SOS105525046 AppliChrom OTU SiOH, 250	Price available upon request
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Precolumns or other dimensions also available - please ask for your personal quotation

## AppliChrom OTU Phenyl

Highly pure, spherical high-performance HPLC phase based on silica gel with 105Å pore size as a special selectivity using  $\Pi$  interactions.

- particularly suitable for aromatics, fatty acids, fatty acid derivatives, medium polar compounds

SOP105525046AppliChrom OTU Phenyl, 250mm x 4,6mmPrice available upon request	.t
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Precolumns or other dimensions also available – please ask for your personal quotation

## AppliChrom OTU DiO HILIC

with 105Å pore size for high resolution separations - as a selectivity alternative for the investigation of very polar analytes.

- particularly suitable for purines, sugars, ganidines, peptides, highly water-soluble vitamins (reverse order of peaks to RP-HPLC)

SOH105525046	AppliChrom OTU DiO HILIC, 250mm x 4,6mm	Price available upon request
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Precolumns or other dimensions also available – please ask for your personal quotation

**AppliChrom OTU Amino** 



# AppliChrom OTU Amino

- HPLC of mono- and disaccharides

- HPLC of sugar alcohols
- HPLC oligomeric sugars
- USP-L8
- 5µm
- High pressure stability 600bar
- pH stable 2-8 at 22  $^\circ$  C
- High purity Silica Base, 99.999%, <10ppm metal
- Increased long-term stability thanks to new surface treatment technology
- Can be used as standard phases HPLC (eluent: hexane, dichloromethane, ethyl acetate, mixtures with Isopropanol, ...).
- Can be used very well as a HPLC column for the separation of sugars (eluent: ACN / H2O)



AppliChrom OTU Amino 5µ, 250x4.6mm Eluent: ACN/H<sub>2</sub>O 85/15 Temperature: 27°C Flow: 1ml/min Detection: RI (alternative ELSD)

Peaks in elution order: 1) Meso-Erythritol 2) Xylitol 3) Sorbitol



AppliChrom OTU Amino 5µ, 250x4.6mm Eluent: ACN/H<sub>2</sub>O 55/45 Temperature: 27°C Flow: 1ml/min Detection: RI (alternative ELSD)

Peaks in elution order:

- 1) Maltose
- 2) Maltotriose
- 3) Maltotetraose
- 4) Maltopentose
- 5) Maltohexose
- 6) Maltoheptose



AppliChrom OTU Amino



# Gradient Separation Malto-Oligosaccharid



SOA105525046	HPLC-column AppliChrom® OTU Amino, 5µ, 250mm x 4,6mm	Price available upon request
SOA105515046	HPLC-column AppliChrom® OTU Amino, 5µ, 150mm x 4,6mm	Price available upon request
KOA10551046	Precolumn set AppliChrom® OTU Amino, 5µ, 10mm x 4,6mm	Price available upon request

AppliChrom (ABOA) ProteSep S-L

Appliculu Excellent results are our aim

# AppliChrom ProteSep S-L

AppliChrom ProteSep S-L HPLC columns are designed for the fast and accurate SEC/GPC separation of proteins, immunoglobulins and polymeric sugars in the range of 5.000 to 1.000.000D.

The special designed 5µ particles with their proprietary pore openings and structure secure good pore accessibility for the analytes from smaller biopolymers (e.g. vitamin B12) up to very large biopolymers (e.g. oligomers and complexes of immunoglobulins). This leads to a really good chromatographic kinetic resulting in fast analytical separations combined with a really high resolution of analytes that are separated according to the SEC resp. GPC mechanism.

The columns are designed in a way, that they fit to standard HPLC Systems (connected with the standard 10/32er fittings on 1/16<sup>---</sup> tubes).

High pressure stability of more than 150bar allow relatively high linear flowrates resulting in short analysis times of e.g. 10-20min for a 300x8mm column at 1.0 - 0.5ml/min. The chromatographic base material is a rigid inorganic special designed network that does not swell or shrink on changing the eluent from water to organics (e.g. DMSO or THF).

Recommended solvents are aqueous, buffered between pH 3 and 7.5.

Depending on molecules that must be separated it can be recommended to increase the ionic strength or to add organic modifiers like MeOH or ACN (10-30%).

The advantageous combination properties of the **AppliChrom**<sup>®</sup>**ProteSep S-L** packing material (high pressure stability, no swellability/shrinkability with organic solvents) allows the user to clean the column after long time use from adsorbed impurities by using organic solvents like DMSO or THF. By this the livetime can be extra-increased saving again money for the user.



SAP-S-L 2508	AppliChrom ProteSep S-L, 250mm x 8mm	Price available upon request
SAP-S-L 3008	AppliChrom ProteSep S-L, 300mm x 8mm	Price available upon request

Precolumns or other dimensions also available – please ask for your personal quotation





# AppliChrom ProteSep S-S

**AppliChrom ProteSep S-S** HPLC columns are designed for the fast and accurate SEC/GPC separation of proteins, immunoglobulins and polymeric sugars in the range of 100 to 100.000D.

The special designed 5µ particles with their proprietary pore openings and structure secure good pore accessibility for the analytes from smaller biopolymers (e.g. vitamin B12) up to very large biopolymers (e.g. oligomers and complexes of immunoglobulins). This leads to a really good chromatographic kinetic resulting in fast analytical separations combined with a really high resolution of analytes that are separated according to the SEC resp. GPC mechanism.

The columns are designed in a way, that they fit to standard HPLC Systems (connected with the standard 10/32er fittings on 1/16'' tubes).

High pressure stability of more than 150bar allow relatively high linear flowrates resulting in short analysis times of e.g. 10-20min for a 300x8mm column at 1.0 - 0.5ml/min. The chromatographic base material is a rigid inorganic special designed network that does not swell or shrink on changing the eluent from water to organics (e.g. DMSO or THF).

Recommended solvents are aqueous, buffered between pH 3 and 7.5.

Depending on molecules that must be separated it can be recommended to increase the ionic strength or to add organic modifiers like MeOH or ACN (10-30%).

The advantageous combination properties of the **AppliChrom**<sup>®</sup> **ProteSep S-S** packing material (high pressure stability, no swellability/shrinkability with organic solvents) allows the user to clean the column after long time use from adsorbed impurities by using organic solvents like DMSO or THF. By this the livetime can be extra-increased saving again money for the user.



AppliChrom ProteSep S-S 5µ, 300mmx8mm Eluent: 0,05M Na<sub>2</sub>HPO<sub>4</sub> + 0,15M NaCl, pH 7,0, Flow: 1ml/min Temperature: 25°C, Detection: UV 215nm, Injectionsvolume: 10µl,

Peaks in elution order: 1) Thyroglobulin (670kDa) 2) BSA (67kDa) 3) Ribonuclease (13kDa) 4) Uracil (0,112kD)

SAP-S-S 2508	AppliChrom ProteSep S-S, 250mm x 8mm	Price available upon request
SAP-S-S 3008	AppliChrom ProteSep S-S, 300mm x 8mm	Price available upon request

Precolumns or other dimensions also available - please ask for your personal quotation

AppliChrom VicuSil S-L



# AppliChrom VicuSil S-L

AppliChrom VicuSil, 10 - 950Å poresize,  $5\mu$ , **silylated silica based GPC column packings** (100-1Mio Da).

The AppliChrom VicuSil columns contain silica-based packings and are ideally suited for high performance SEC. The rigid, siliceous packings of the AppliChrom VicuSil columns have high mechanical strength and are not swelled or dissolved by any common organic or aqueous mobile phases.

# AppliChrom silica based columns / Fields of application

Analyte	Separation mechanism	HPLC-Material	AppliChrom <sup>®</sup> Product	Fields of application
		C <sub>18</sub>	AppliChrom® OTU LipoMare C18!	Universal for polar and non-polar molecules up to 4,500Da (peptides, sugars, flavonoids, glycoflavonoids, alkaloids, purines, neurotransmitters, antibiotics, highly polar and ionic compounds, carboxylic acids, nucleosides, toxins, wide range of vitamins)
	Reversed Phase (RP)	C <sub>8</sub>	AppliChrom <sup>®</sup> OTU LipoMare C <sub>8</sub> OTU Trikala C <sub>8</sub> !	Especially for polar separations up to 6.00Da (low molecular weight carboxylic acids, sugars, purines,). Polar selectivity alternative to the OTU LipoMare C18 for molecules up to 5,500Da - for peptides, proteins, flavonoids, glycoflavonoids, alkaloids, neurotransmitters, antibiotics,).
	Size exclusion	GPC/ SEC	AppliChrom <sup>®</sup> Protesep S-L / S-S	Proteins, immunoglobulins, polymeric sugars, polymeric derivatives, dextrans
water soluble	lon pair	C <sub>8</sub> /C <sub>18</sub>	AppliChrom <sup>®</sup> OTU LipoMare C <sub>18</sub> OTU LipoMare C <sub>8</sub>	Organic acids, organic amines, ionic compounds with ion pair reagent
	HILIC	HILIC	AppliChrom® OTU HILIC	Purines, sugars, ganidines, peptides, highly water- soluble vitamins (reverse order of peaks to RP-HPLC)
		C <sub>18</sub>	AppliChrom <sup>®</sup> OTU Trikala C <sub>18</sub>	Universal for non-polar molecules up to 4,500Da (derivatized amino acids, fatty acid derivatives, steroids, flavonoids, lipophilic vitamins, toccopherols).
Organical soluble	Reversed Phase (RP)	C <sub>8</sub>	AppliChrom <sup>®</sup> !Otu Trikala C <sub>8</sub> !	Polar selectivity alternative to the OTU LipoMare C18 for molecules up to 5,500Da - for peptides, proteins, flavonoids, glycoflavonoids, alkaloids, neurotransmitters, antibiotics,).
		Phenyl	AppliChrom <sup>®</sup> OTU Phenyl	Aromatics, fatty acids, fatty acid derivatives, medium polar compoundsn
	Normal Phase (NP)	SiOH	AppliChrom <sup>®</sup> OTU SiOH	Steroids, fatty acid derivatives
		$NH_2$	AppliChrom <sup>®</sup> OTU Amino	Steroids, fatty acid derivatives
	Size exclusion	GPC/ SEC	AppliChrom <sup>®</sup> VicuSil	For most of synthetic and natural polymers

**Tool Box** 



# Washing and regeneration steps recommended for AppliChrom silica based HPLC columns.

#### Impurities of HPLC columns:

Impurities of the samples for HPLC undergo enrichment in the HPLC column under certain circumstances. The user notices this in most cases by an increase in the back pressure or by changing the selectivity of the HPLC column. Typical is an accumulation of impurities in the column entrance.

#### Combating the causes:

Possible causes:

#### - smallest particles from the samples

The use of suitable syringe filters prevents blockage of the column due to the smallest particles coming from the sample and at the same time ensures a high recovery of the analyte. AppliChrom syringe filters create decisive time and cost advantages for the user.

#### - insufficiently dissolved components

Make sure the sample is completely dissolved Sample preparation - especially for samples with a high matrix load - can be decisive. AppliChrom SPE products create decisive advantages for the user during sample preparation. Good, complete dissolution of the sample.

#### - bacteria, algae or particles in the eluent

Algae or bacteria in the eluent (especially water) often cause problems. Fresh HPLC water, boiling water, storing water in amber glass bottles and/or the use of suitable suction filters for HPLC systems can be crucial. The user can choose between numerous suitable and at the same time cost-effective AppliChrom HPLC intake filters.

#### - septa cut out and injected into the flow system

Vials and septa should fit optimally to the autosampler. This reduces the risk of blockages of the column due to septa components that have been punched out and introduced into the flow path. AppliChrom offers suitable and cost-effective AppliChrom vials and septa for a range of autosamplers.

#### - Precipitation of agglomerates or polymers on the column

Make sure that the appropriate analysis conditions are available, pay attention to the (known) solution parameters of the analyte

**Tool Box** 



#### Impurities of HPLC columns:

#### - Reactive impurities of the analyte can react with the surface of the column material.

When choosing the column material, pay attention to any analyte matrices that may react with the column material. Also choose the appropriate pre-cleaning steps, if possible

#### - Undesirable accompanying substances reduce the pore availability of the column material.

Impurities of column material occur in particular at the column entrance. In order to extend the service life of HPLC columns, it is recommended to use pre-columns packed with the same material as in the main column. AppliChrom also offers the appropriate AppliChrom pre-columns for its HPLC columns. The list does not claim to be complete.

How to clean a silica based column:

# SiOH based Reversed Phase (RP) high performance HPLC materials (Applichrom Otu C<sub>4</sub>, C<sub>8</sub>, C<sub>18</sub>, Phenyl phases)

AppliChrom 4.6x250mm RP-HPLC High performance HPLC columns are normally operated at 1ml/min.

- For the cleaning, flow rates of 0.1-0.3ml / min are used, the flow is opposite to the "normal" flow direction.
- Heating the column to 40 ° C during the cleaning procedure can be favorable.
- The eluent that leaves the column during the washing procedure should be directed directly (bypassing the detector) into the waste.

# RP, HILIC and SEC applications on polar bonded AppliChrom high performance HPLC phases

#### Removal of lipophilic impurities:

- 1) Rinsing with 10 column volumes of water
- 2) Rinsing with 10 column volumes of isopropanol
- 3) Rinsing with 10 column volumes of water\*\*)
- 4) Rinsing with 10 column volumes of 7M urea\*\*)
- 5) Rinsing with 20 column volumes of water
- 6) Equilibration with 10 column volumes of mobile phase
- \*\*) Steps 3 and 4 should only be used if protein deposits are suspected (cause e.g. In the HPLC water quality).

**Users** guide



### How to clean a silica based column:

#### Another possibility to clean an RP-HPLC Column

- Rinsing the AppliChrom RP-HPLC column with 10 column volumes of isopropanol
- Rinsing with 10 column volumes of THF (only if the HPLC system is designed for the use of THF, otherwise with THF!!!!).
- Rinsing with 10 column volumes of hexane or heptane
- Rinsing with 10 column volumes of isopropanol
- Equilibrating with 10 column volumes of mobile phase.

#### Normal-phase (NP) applications on polar bonded AppliChrom high-performance HPLC phases

#### Removal of lipophilic impurities:

- Rinsing with 10 column volumes of CHCl3 or dichloromethane
- Rinsing with 10 column volumes of heptane or hexane
- Rinsing with 10 column volumes of isopropanol
- Rinsing with 20 column volumes eluent.

In individual cases, the recommendation for the column rinsing program may differ - depending on the specific contamination that is suspected on the HPLC column.

#### Polar, bound AppliChrom high-performance HPLC phases (Applichrom OTU Diol, OTU Silica, OTU Amino, OTU DiO-HILIC; ABOA ProteSep-S-S, ABOA ProteSep S-M, ABOA ProteSep S-L, SEC columns)

- Removal of polar impurities:
- Rinsing with 3 column volumes of isopropanol\*)
- Rinsing with 10 column volumes of water
- Rinsing with 10 column volumes of methanol
- Rinsing with 10 column volumes of water
- Rinsing with 3 column volumes of isopropanol\*)
- Equilibration with 10 column volumes of mobile phase

Steps 1 and 5 are necessary if the polar bound AppliChrom high-performance HPLC columns are used in normalphase applications (hexane / ethyl acetate, etc.). If the polar bound AppliChrom high-performance HPLC columns can be used as RP (reversed phase), HILIC or aqueous SEC columns eliminates steps 1 and 5.

#### **AppliChrom GmbH** +49 (0)3301 579293

**Users** guide



#### **Removal of polar impurities:**

- Rinsing the AppliChrom RP-HPLC column with 10 column volumes of water

For proteins (e.g. algae / bacteria from the eluent reservoir):

- a) repeated injection of DMSO or
- b) Rinsing of the column with 7M urea (back-piston rinsing!!!)
- c) then with min. 10 Column volumes Rinse with water (to remove DMSO or urea from to remove the pores)
- Rinsing with 10 column volumes of methanol
- Rinsing with 10 column volumes of THF or isopropanol (for THF: only if the HPLC system is designed for the use of THF, otherwise omit THF!!!!)
- Rinsing with 10 column volumes of methanol
- Oscillating with 10 column volumes of mobile phase



## Flavonoides

AppliChrom® Trikala C<sub>8</sub> 5µm, 105Å, 250 x 3mm

Mobile Phase:	
Eluent A:	H <sub>2</sub> O/Formic acid
	99.9:0.1 V/V
Eluent B:	Acetonitril/MeOH
	50:50 V/V
Flow:	0.4ml/min
Injection volume:	50µl
Temperature:	40C
Detection:	PDA

Chromatogram courtesy of Dennis Thurack, Diplomingenieur für Lebensmitteltechnologie Universität Potsdam

Arthur-Scheunert-Allee 114-116 14558 Bergholz-Rehbrücke



# Inquiry Form For Forwarding to AppliChrom GmbH

To place an order please fill in this form and send it by mail or fax to:

AppliChrom GmbH Germendorfer Allee 20 D-16515 Oranienburg Germany

Telephone:
Fax:
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Web:

+49 (0)3301 579293 +49 (0)3301 209879 info@applichrom.co www.applichrom.com

Catalog	Description	Dimension

bulk quantities on request

adress

name	
company / institute	
adress	
country	
telephone number	
e-mail	
VAT-number	
comment	

#### **AppliChrom GmbH**

☎ +49 (0)3301 579293☑ info@applichrom.com

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AppliChrom GmbH Germendorfer Allee 20 D-16515 Oranienburg Germany www.applichrom.com info@applichrom.com Phone: +49 (0) 3301 579293 Fax: +49 (0) 3301 209879