Chromatography

MACHEREY-NAGEL

CHROMABOND® PL

Professional phospholipid removal by SPE

For more reproducible results in the analysis of bioanalytical samples

CHROMABOND® PL

What is a phospholipid?

Phospholipids are major building blocks of all cell membranes. Due to their chemical structure (amphiphilic character) they can form lipid bilayers which shield the inside of the cell. The most common class of phospholipids are the phosphoglycerides. Their hydrophilic "head" consists of a phosphate group which is linked via glycerol to the hydrophobic "tail" consisting of two fatty acid chains. The phosphate group can be modified with an additional organic molecule, e.g., ethanolamine or choline.

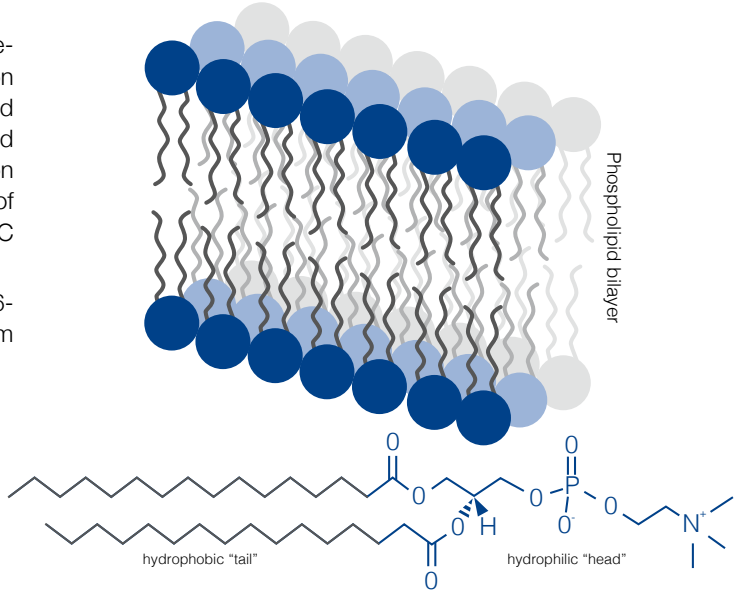
Why is it necessary to remove phospholipids by SPE?

Problem: If phospholipids in analytical samples are not removed prior to chromatographic analyses they can accumulate on (U)HPLC columns, which causes higher backpressures and even blocking. Phospholipids can bleed from columns and co-elute with small molecules, which leads to ion suppression and retention time shifts as well as different peak shapes of your analytes. Thus, irreproducible analytical results and HPLC system downtimes occur.

Solution: By using CHROMABOND® PL SPE cartridges and 96-well plates you selectively remove interfering phospholipids from bioanalytical samples.

How do you benefit from CHROMABOND® PL

- Less maintenance of HPLC systems and columns
- Reproducible results
- High recovery rates for acidic, basic and neutral analytes in one method
- Superior handling in comparison with liquid-liquid extraction or simple protein precipitation
- Performs well with different biological samples
- Simple, fast and rugged procedure
- Cost-efficient



An example of a phospholipid: 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine

Extraction procedure

Sample matrix: plasma or serum

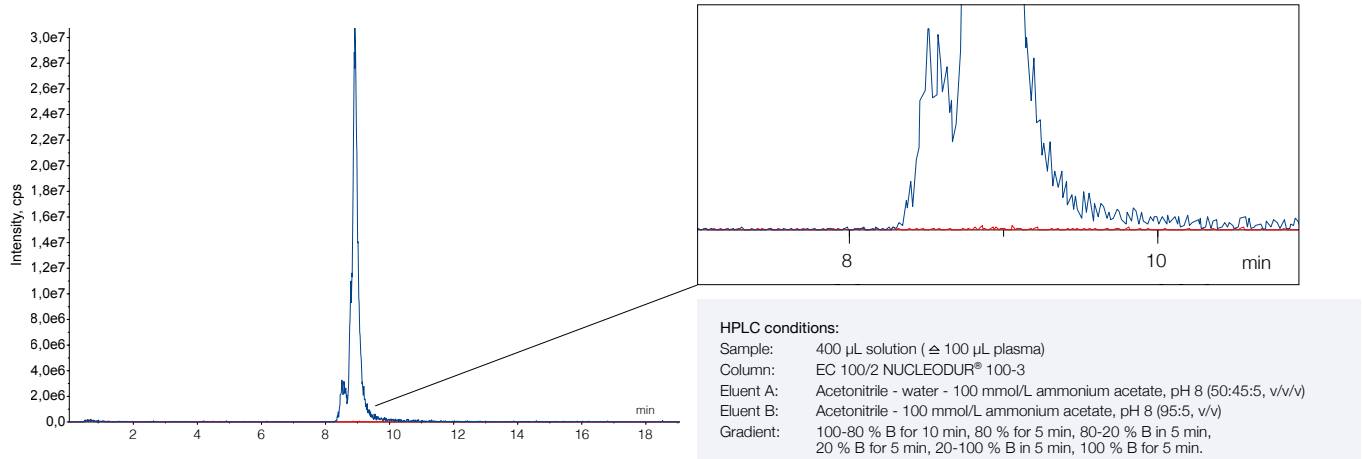
Method A internal protein precipitation	Method B external protein precipitation
add up to 100 µL sample into well/onto column	add up to 100 µL sample into centrifuge tube
add protein precipitation reagent a final ratio of 3:1 to 4:1 organic solvent:sample is recommended	
mix thoroughly, avoiding cross contamination	
	centrifuge
	transfer supernatant into well/onto column
Slowly elute using vacuum or positive pressure	

CHROMABOND® PL products are designed for internal protein precipitation (method A). External protein precipitation (method B) could be necessary in order to prevent upper frit and adsorbent bed clogging due to the nature of precipitated proteins. The choice of the protein precipitation reagent depends on the application.

Phospholipid removal

MN Appl. No. 306090

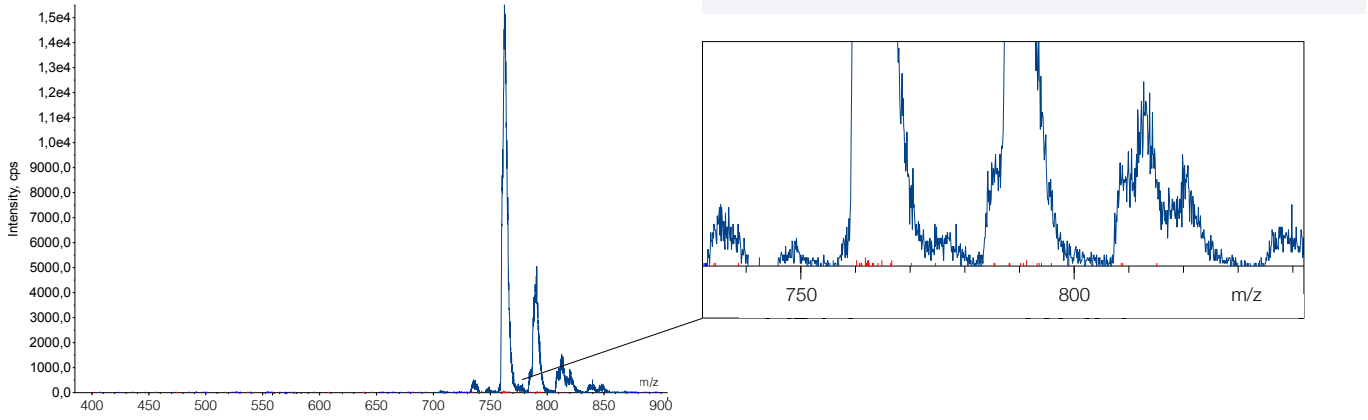
As an example to prove the effectiveness of CHROMABOND® PL in the removal of phospholipids, a lyophilized bovine plasma sample was chosen. Proteins were precipitated using 1 % formic acid in acetonitrile. An eluate sample was directly analyzed by LC-MS/MS (blue curve), another sample was prepared using a 1 mL CHROMABOND® PL cartridge and was then analyzed accordingly (red curve). The following mass spectrum shows that 95 % of the phosphatidylcholines were successfully removed.



Overlay of phosphatidylcholine without SPE (blue) and with CHROMABOND® PL (red) TIC.

HPLC conditions:

Sample:	400 µL solution (≙ 100 µL plasma)
Column:	EC 100/2 NUCLEODUR® 100-3
Eluent A:	Acetonitrile - water - 100 mmol/L ammonium acetate, pH 8 (50:45:5, v/v/v)
Eluent B:	Acetonitrile - 100 mmol/L ammonium acetate, pH 8 (95:5, v/v)
Gradient:	100-80 % B for 10 min, 80 % for 5 min, 80-20 % B in 5 min, 20 % B for 5 min, 20-100 % B in 5 min, 100 % B for 5 min.
Flow rate:	0.5 mL/min
Temperature:	35 °C
Injection:	5 µL
Detection:	MS

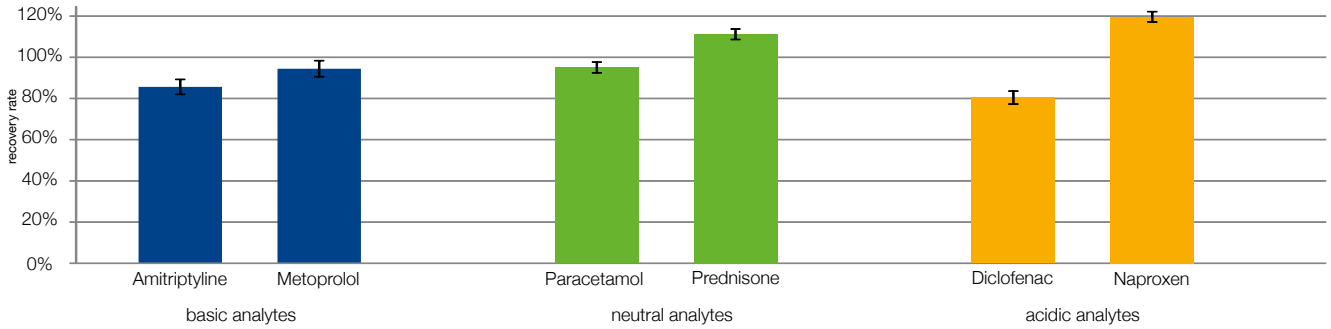


Overlay of phosphatidylcholine without SPE (blue) and with CHROMABOND® PL (red) + Prec (184) mass spectrum.

Recovery rates of different analytes

MN Appl. No. 306100/127520

CHROMABOND® PL is selective towards phospholipids. In order to determine the recovery rates of active agents, a plasma sample was spiked with six analytes with different properties. Subsequently, the sample was prepared using a CHROMABOND® PL 1 mL cartridge and the obtained eluate was then analyzed by LC-MS/MS.



Ordering information

CHROMABOND® PL products

Description	Quantity	REF
1 mL cartridge, 30 mg	100	730703
MULTI 96-well plate, 96 x 30 mg	1	738702.030M

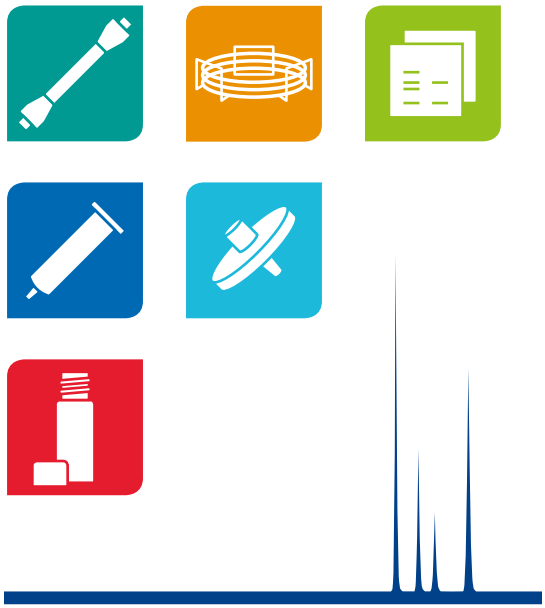
CHROMABOND® accessories

Description	Quantity	REF
Vacuum manifold 12 positions*	1	730150
Vacuum manifold 16 positions*	1	730360
Vacuum manifold 24 positions*	1	730151
MULTI 96 vacuum manifold with reservoir tank, vacuum gauge, and control valve	1	738630.M
MULTI 96 Collection rack for PP-Vessels (twelve 8-well strips), 96 x 1.0 mL	5	738637
Polypropylene tube strips (twelve 8-well strips), 96 x 1.0 mL	10	738652
8-well strip sealing caps for PP tube strips (REF 738652)	30	738638
Butyl rubber pad, PTFE covered for sealing of individual rows of the 96-well plate, 125 x 85 mm	1	738645

* consists of: glass cabinet with lid and lid gasket, removable needles on lower side of lid, vacuum gauge, control valve, valves and caps, variable rack; PP-tank (only REF 730150).

More CHROMABOND® accessories are available. For details see our website or contact us directly.

Do you need products for subsequent analyses? MACHEREY-NAGEL provides everything from one source for your competitive advantage.



... we Meet your Needs

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