ChromTech BioTrap 500

- **Direct injection of serum/plasma**
- **General methods for extraction**
- **Automated methods**
- **High recoveries of highly protein bound drugs (normally > 95%)**

In bioanalysis, the sample usually has to go through an isolation procedure prior to the chromatographic step. All isolation methods are time consuming and may introduce errors in the assay. The more manipulations the sample undergo prior to the quantification step, the lower the accuracy and precision obtained. It is therefore advantageous to inject the sample directly into a liquid chromatographic system without off-line isolation procedures. ChromTech has developed a series of online extraction columns to make the sample preparation easier and faster. The series is called BioTrap 500 and is available in three different phases, C18, C8 and now MS.

The principles behind the BioTrap 500 column

The BioTrap 500 column material has an external surface which is biocompatible due to the coverage with the extremely stable α_1-acid glycoprotein (AGP). The internal surface is very hydrophobic. The pore size of the particle is small enough to allow only low molecular weight drug molecules to penetrate and absorb to the inner surface. The macromolecules are excluded and directly flushed to waste.

Injection volume on BioTrap 500

Up to 1000µL plasma/serum can be injected directly onto the BioTrap 500 columns (20 x 4.0mm I.D.). By using optimal conditions, a total amount of more than 100mL of plasma/serum may be injected onto the BioTrap 500 column.

How to use BioTrap 500

Figure 1 demonstrates how to connect one BioTrap 500 online-extraction column together with an analytical column using a 6-port valve. Two pumps are used, one for the extraction mobile phase (pump A) and the other for the analytical mobile phase (pump B).

In the extraction position the analyte is trapped on the BioTrap 500 column, while the plasma proteins are transported to waste. When all the proteins are removed, the valve is switched to the elution position. In that position, the trapped analyte is back flushed onto the analytical column with the analytical mobile phase.

When the analyte has been transferred to the analytical column, the valve can be switched back to the extraction position for re-equilibration of BioTrap 500 with the extraction mobile phase, prior to the next injection.

Double the speed of analysis using a 10-port valve

By using a 10-port valve faster bioanlayses can be achieved. By connecting two BioTrap 500 columns in a 10-port valve (2-position), it is possible to handle two samples simultaneously. **Figure 2** shows the connection of two BioTrap 500 columns and one analytical column in one HPLC system.

Electrically Actuated Valves

Cat. No.	Description
MX7900	6-port electrically actuated valve
MX7960	10-port electrically actuated valve

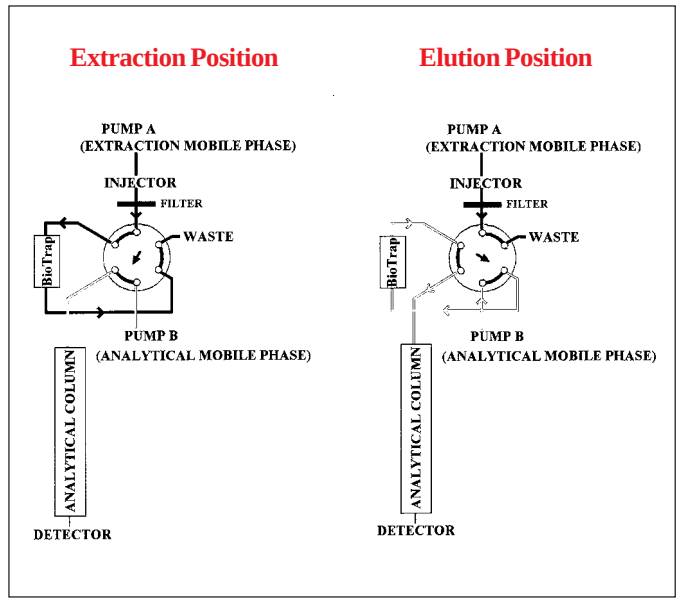


Fig. 1: How to Use BioTrap 500

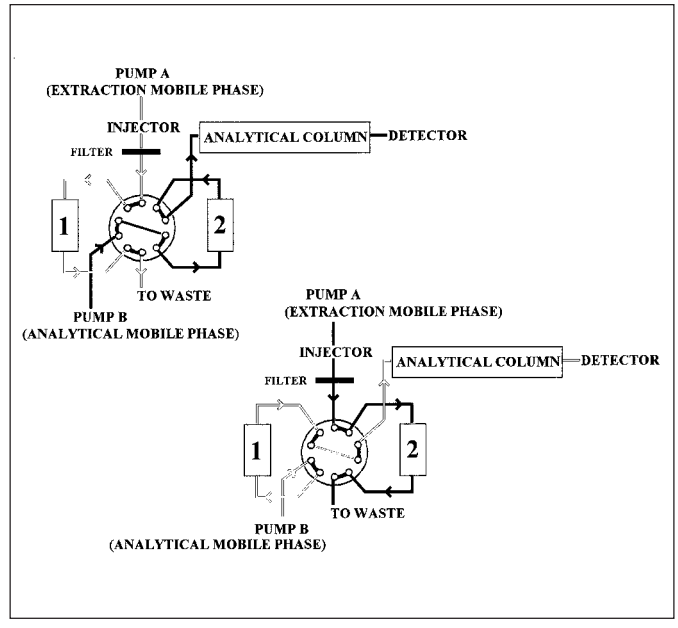


Fig. 2: Double the Speed Using a 10-Port Valve

In the first position, the sample is extracted on BioTrap 500 column **1**, while the previous sample is back-flushed with the analytical mobile phase from BioTrap 500 column **2** onto the analytical column. In the second valve position, the sample extracted on BioTrap 500 column **1** is back-flushed onto the analytical column, while the next sample is injected onto BioTrap 500 column **2** for extraction.

General bioanalytical methods with BioTrap 500 MS when combined with mass spectrometry

BioTrap 500 MS is a new online extraction column designed for MS detection. The new column is not silica based and can be used in a wider pH range from 2 to 10, however, avoid use between pH 3 to 5 for the extraction of serum/plasma. Plasma proteins are more easily precipitated in this pH range. It has nothing to do with the stability of the column. This wider pH range makes possible the extraction of basic compounds at high pH and acidic compounds at low pH. Under those conditions the compounds can be extracted in the uncharged form. This means that no ion-pair agent is needed in order to obtain high recovery and efficiency, which makes BioTrap 500 MS a very suitable online extraction column when using MS detection. By combining the new extraction column and MS detection more or less general bioanalytical methods are obtained.

BioTrap 500 MS can also be used together with fluorescence, UV and EC-detection.

When to use BioTrap C18 or C8

The BioTrap C8/C18 columns can be used between pH 2.3 to 7.5, however, as stated above, avoid use between pH 3 to 5 due to the risk of precipitation of plasma proteins during extraction. The BioTrap 500 C8 material gives different retention characteristics than the BioTrap C18 material. The C8 material should be used for the most hydrophobic analytes that give high enough recovery on this material. This means that higher selectivity can be generated in the extraction step for the type of analytes.

BioTrap Extraction Method

- **Economical and time saving**
- **No manual sample preparation**
- **Very high accuracy and precision**
- **Automated bioanalysis**

The method enables direct injection of plasma/serum without any time consuming clean-up procedures.

The extraction method with BioTrap 500 is easy to use in a conventional HPLC equipment. Fully automated methods can be developed very fast using the general extraction procedures.

Offline sample preparation method	**BioTrap 500 (Online sample preparation method)**
Serum/plasma	Serum/plasma
↓	↓
Conditioning of extraction column	Centrifugation of sample
↓	↓
Application of sample	Injection onto the BioTrap 500 column in the HPLC system
↓	
Elution of sample matrix	
↓	
Concentration of extract	
↓	
Injection onto an analytical HPLC column for separation	

We recommend the BioTrap MS column as the first choice. This columns gives very good chromatographic performance and a very good recovery. The BioTrap MS column uses general extraction methods for basic and acidic analytes.

See page 86 for BioTrap applications.

BioTrap 500 MS

Cat. No.	Description
BMS134C	BioTrap 500 MS, 13x4mm 2 cartridges
BMS134K	BioTrap 500 MS, 13x4mm w/holder
BMS204C	BioTrap 500 MS, 20x4mm 2 cartridges
BMS204K	BioTrap 500 MS, 20x4mm w/holder
BMS202C	BioTrap 500 MS, 20x2mm 2 cartridges
BMS202K	BioTrap 500 MS, 20x2mm w/holder

BioTrap 500 MS Kits include:

BT-KIT10	13x4.0mm (MS), 20x4.0mm (MS), holder
BT-KIT13	13x4.0mm (MS), 20x4.0mm (MS), holder, filter holder, 5 filters
BT-KIT11	13x4.0mm (MS), holder, filter holder, 5 filters
BT-KIT12	20x4.0mm (MS), holder, filter holder, 5 filters

BioTrap 500 C18

B18134C	BioTrap 500 C18, 13x4mm 2 cartridges
B18134K	BioTrap 500 C18, 13x4mm w/holder
B18204C	BioTrap 500 C18, 20x4mm 2 cartridges
B18204K	BioTrap 500 C18, 20x4mm w/holder
B18202C	BioTrap 500 C18, 20x2mm 2 cartridges
B18202K	BioTrap 500 C18, 20x2mm w/holder

BioTrap 500 C18 Kits include:

BT-KIT1	13x4.0mm (C18), 20x4.0mm (C18), holder
BT-KIT4	13x4.0mm (C18), 20x4.0mm (C18), holder, filter holder, 5 filters
BT-KIT2	13x4.0mm (C18), holder, filter holder, 5 filters
BT-KIT3	20x4.0mm (C18), holder, filter holder, 5 filters

BioTrap 500 C8

B8134C	BioTrap 500 C8, 13x4mm 2 cartridges
B8134K	BioTrap 500 C8, 13x4mm w/holder
B8204C	BioTrap 500 C8, 20x4mm 2 cartridges
B8204K	BioTrap 500 C8, 20x4mm w/holder
B8202C	BioTrap 500 C8, 20x2mm 2 cartridges
B8202K	BioTrap 500 C8, 20x2mm w/holder

BioTrap 500 C8 Kits include:

BT-KIT5	13x4.0mm (C8), 20x4.0mm (C8), holder
BT-KIT8	13x4.0mm (C8), 20x4.0mm (C8), holder filter holder, 5 filters
BT-KIT6	13x4.0mm (C8), holder, filter holder, 5 filters
BT-KIT7	20x4.0mm (C8), holder, filter holder, 5 filters

Methods Development Kits

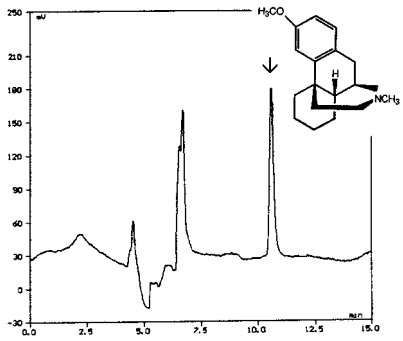
BT-KIT14	20x4.0mm (MS), 20x4.0mm (C18), holder, filter holder, 5 filters
BT-KIT15	20x4.0mm (MS), 20x4.0mm (C8), holder, filter holder, 5 filters
BT-KIT16	20x4.0mm(MS), 20x4.0mm (C18), 20x4.0mm (C8), holder, filter holder, 5 filters
BT-KIT9	20x4.0mm (C18), 20x4.0mm (C8), holder, filter holder, 5 filters

Accessories

BT-CH2	Holder with endfittings 2mm, 2/pk
BT-CH4	Holder with endfittings 4mm, 2/pk
A-430	Biocompatible filter assembly, 2µm
A-429X	PEEK filter end fittings, 2µm, 10/pk

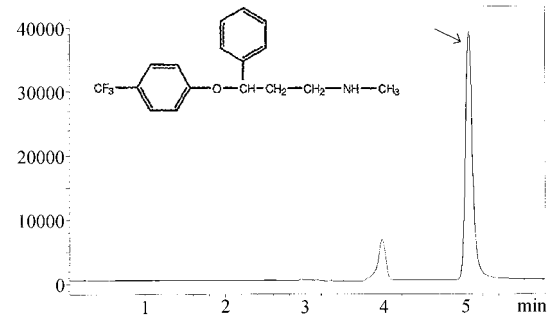
ChromTech BioTrap 500 Applications

Dextromethorphan



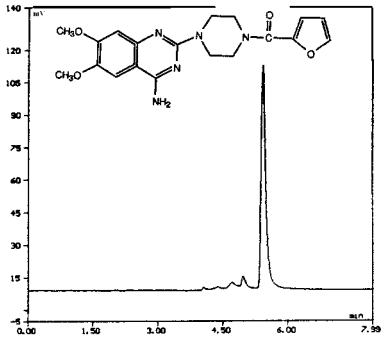
Sample: Dextromethorphan (55ng/mL)
Inj.vol.: 200µL serum
Extraction column: BioTrap 500 C8, 20x4.0mm
Mobile phase: 4% 2-propanol and 5mM sod.octanesulfonic acid in 20mM sod. ph.b. pH 7.0
Flow: 1.6mL/min.
Analytical column: Zorbax XDB-C8, 5µm, 150x4.6mm + guard
Mobile phase: 30% acetonitrile and 2mM sod. octanesulfonic acid in 116mM sod. ph.b. pH 2.8
Flow: 1.0mL/min.
Detection: Ex = 220nm, Em = 305nm
Analysis Program
Extraction time: 3 min.
Elution time: 5 min.
Equilibration time: 7 min.

Extraction of Amines
Fluoxentine – MS Detection



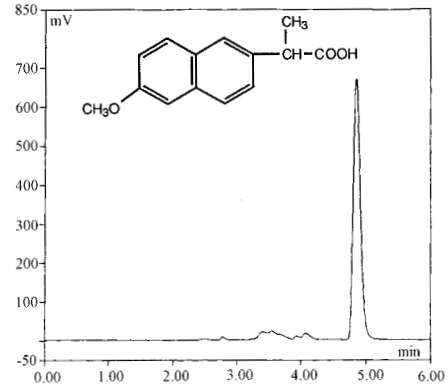
Sample: Fluoxetine 50 ng/mL
Injection volume: 50µL serum
Extraction column: BioTrap 500 MS, 20x4.0mm
Extraction conditions: 4% 2-propanol in 10mM ammonium acetate buffer, pH 10 (adjusted w/amonium hydroxide)
Extraction flow: 3.2mL/min.
Analytical column: Zorbax SB-CN, 150x4.6mm, 5µm + guard 12.5x4.6mm, 5µm
Mobile phase: 35% acetonitrile in 50mM formic acid
Flow: 1mL/min.
Detection: HP 1100 LC/MSD, API-ES positive at 310.1
Analysis Program
Extraction time: 1 min.
Elution time: 3 min.
Equilibration time: 2 min.

Prazosin



Sample: Prazosin (23ng/mL)
Inj.vol.: 50µL serum
Extraction column: BioTrap 500 C18, 20x4.0mm
Mobile phase (ext.): 4% 2-propanol and 5mM sod.octanesulfonic acid in 30mM sod. ph.b. pH 7.0
Flow: 1.6mL/min.
Analytical column: Zorbax SB-CN, 150x4.6mm, 5µm + guard
Mobile phase: 35% acetonitrile and 2mM sod. octanesulfonic acid in 116mM sod. ph.b. pH 2.8
Flow: 1.0mL/min.
Detection: Ex = 340nm, Em = 385nm
Analysis Program
Extraction time: 2 min.
Elution time: 4 min.
Equilibration time: 2 min.

Extraction of Acids
Naproxin



Sample: Naproxen 3.4 µg/mL
Injection volume: 10µL serum
Extraction column: BioTrap 500 MS, 20x4.0mm
Extraction conditions: 4% 2-propanol in 100mM formic acid
Extraction flow: 3.2mL/min.
Analytical column: Zorbax XBD-C8, 150x4.6mm, 5µm + guard 12.5x4.6mm, 5µm
Mobile phase: 30% acetonitrile in 25mM ammonium acetate
Flow: 1mL/min.
Detection: Fluorescence: ex=230nm, em=350nm
Analysis Program
Extraction time: 1 min.
Elution time: 3 min.
Equilibration time: 2 min.

ChromTech RePeat Extraction Cartridges

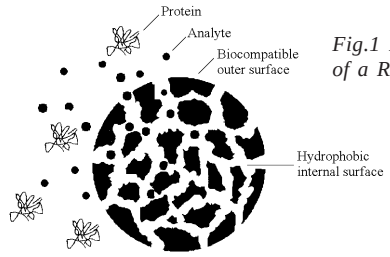


Fig.1 Illustration of a RePeat particle

- **A large number of samples on the same cartridge – Repeated use, reduced cost per sample**
- **Easy to use**
- **High recoveries of highly protein bound drugs**

RePeat is a unique off-line extraction cartridge designed for **repeated extractions** of drugs from complex matrices such as plasma, serum, milk, supernatants of cell cultures and fermentation broth. In contrast to ordinary disposable solid phase extraction columns each RePeat cartridge can be used for a large number of samples. This will highly **reduce the cost per sample.**

RePeat is based on polymeric particles with a hydrophobic internal surface and a biocompatible external surface. The biocompatibility has been obtained by attachment of the plasma protein α_1- acid glycoprotein (AGP) on the external surface of the particles. Immobilized AGP is an extremely stable protein which tolerates the organic solvents used in off-line solid phase extractions. The pores of the particles are small enough to exclude the plasma proteins and other macromolecular compounds whereas drug molecules and other low molecular mass compounds can penetrate the pores and be adsorbed to the hydrophobic inner surface (see Fig.1).

Since RePeat is polymer based, the cartridge can be used between pH 2 to 13. This property will give possibilities of extracting ionized analytes in their uncharged form. The uncharged analyte has higher affinity to the hydrophobic inner surface of the RePeat particle giving an improved recovery.

RePeat is available in 1.5mL (25 mg) cartridges.

Off-Line Extraction with RePeat

The wide pH limit of RePeat enables the extraction of basic compounds at high pH where they are present in their uncharged form. The elution step can be performed in two different ways:

- by using an organic solvent mixed with triethylamine (for basic compounds) or with acetic acid (acidic compounds)
- by using the mobile phase used for HPLC-analysis (a mix of buffer and organic solvent).

If the analysis requires a concentration step, the former procedure is preferred. However, when eluting the analyte with a buffer/organic solvent mixture, it is possible to inject the sample directly without evaporation and reconstitution. This is a very time saving and convenient procedure when applicable. Examples of different extraction procedures used for the analysis of the amines are available upon request.

Repeat Extraction Cartridges Ordering Information

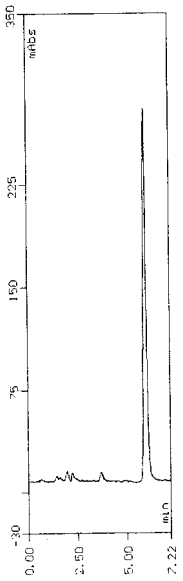
Cat. No.	Description
RE252	RePeat 25mg, 2 pcs
RE256	RePeat 25mg, 6 pcs
RE2512	RePeat 25mg, 12 pcs
RE2516	RePeat 25mg, 16 pcs
RE2550	RePeat 25mg, 50 pcs

Extraction procedure for ibuprofen in serum

RePeat 25 mg cartridge

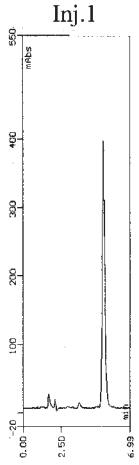
Conditioning: 1-2mL 1% acetic acid in methanol
1mL distilled water
Application: 100µL sample (serum mixed 1:1 with 4% 2-propanol in 100mM formic acid)
Washing: 1mL 4% 2-propanol in 100mM formic acid 0.5mL distilled water
Elution: 1mL 1% acetic acid in methanol. Evaporate and reconstitute.

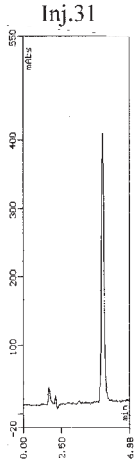
Sample: Ibuprofen, 6.9 µg/mL, in serum
Inj. vol.: 100µL (the eluate evaporated and reconstituted in 300µL 35% acetonitrile in 25mM formic acid)
Column: Zorbax SB-CN, 150x4.6mm, 5µm + guard, 12.5x4.6mm, 5µm
Mobile phase: 30% acetonitrile in 50mM ammonium acetate pH 6.0
Flow: 1mL/min.
Detection: Fluorescence: ex=225nm, em= 555nm



Extraction of Propranolol in serum

Same RePeat 25 mg cartridge





*The chromatograms show an example of the reusability of **RePeat**. 133 samples of **500 ml serum** was injected onto the same **RePeat** 25 mg cartridge.*
The sample is propranolol, 48 ng/ml.