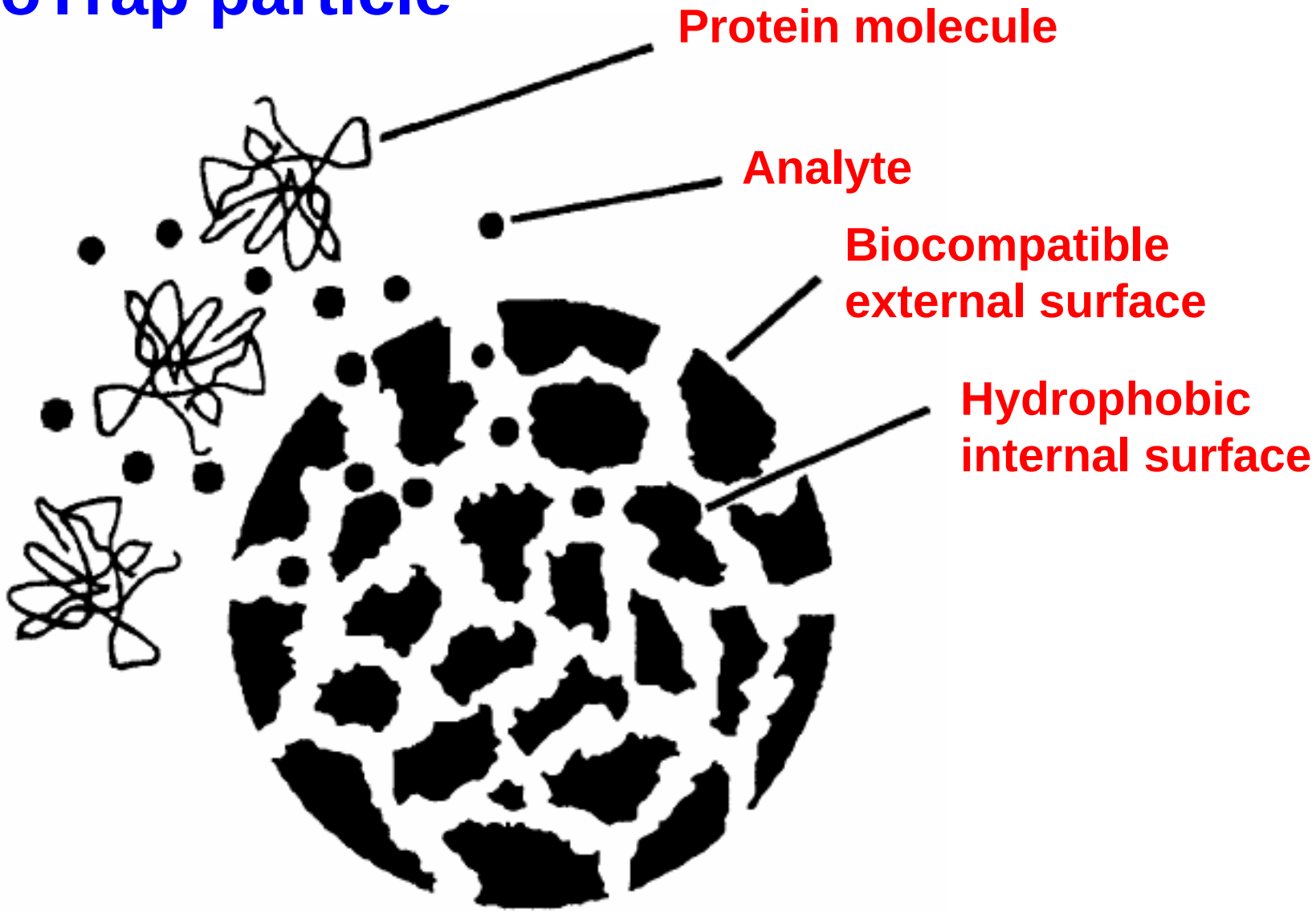
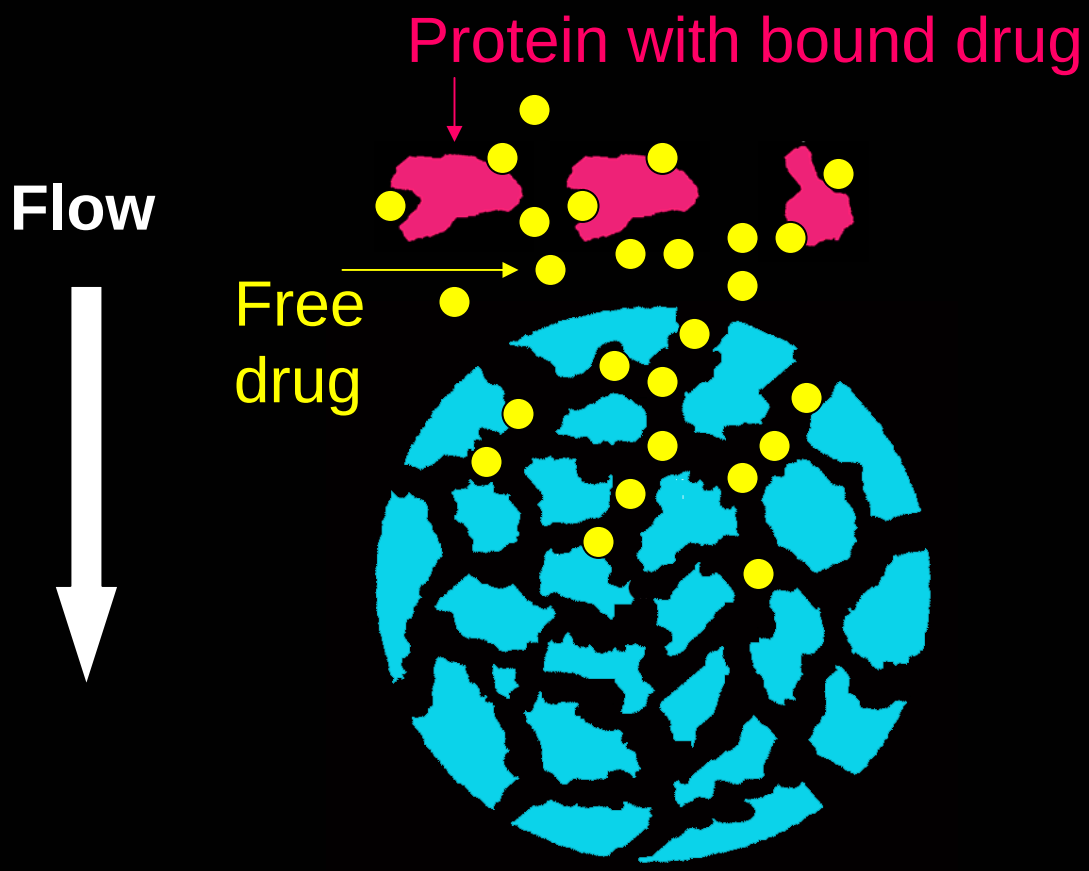
BioTrap

ChromTech

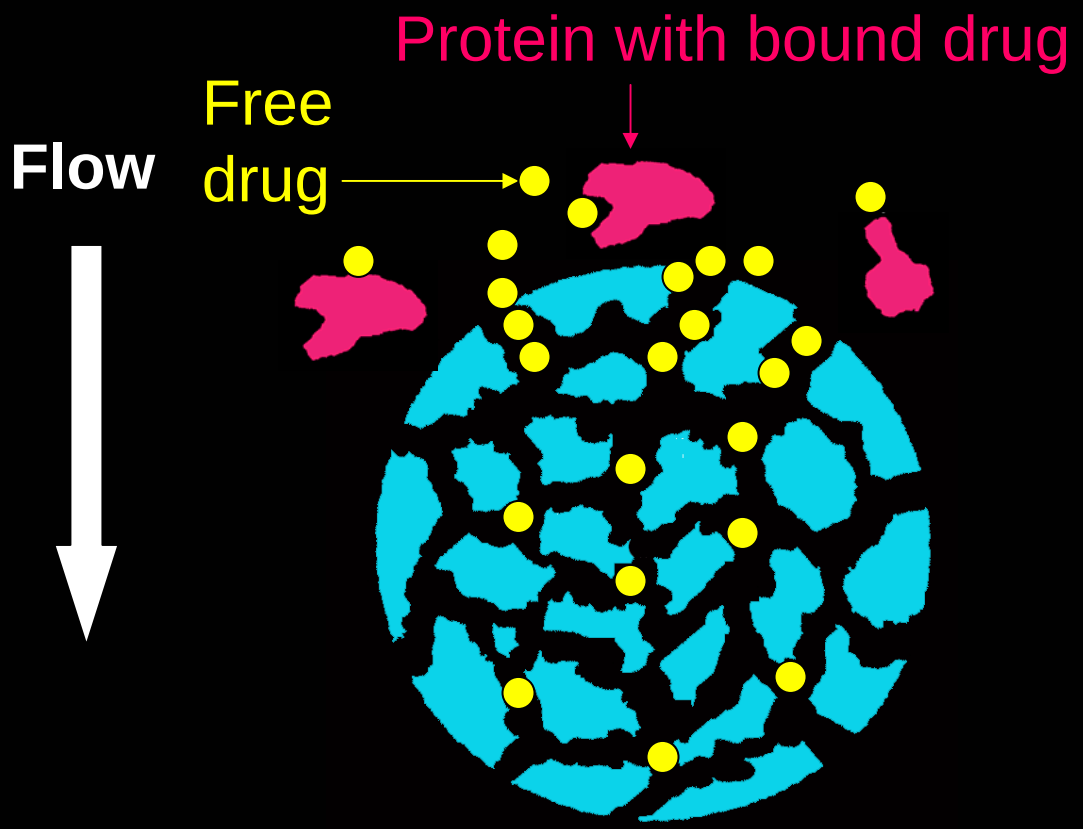
BioTrap particle



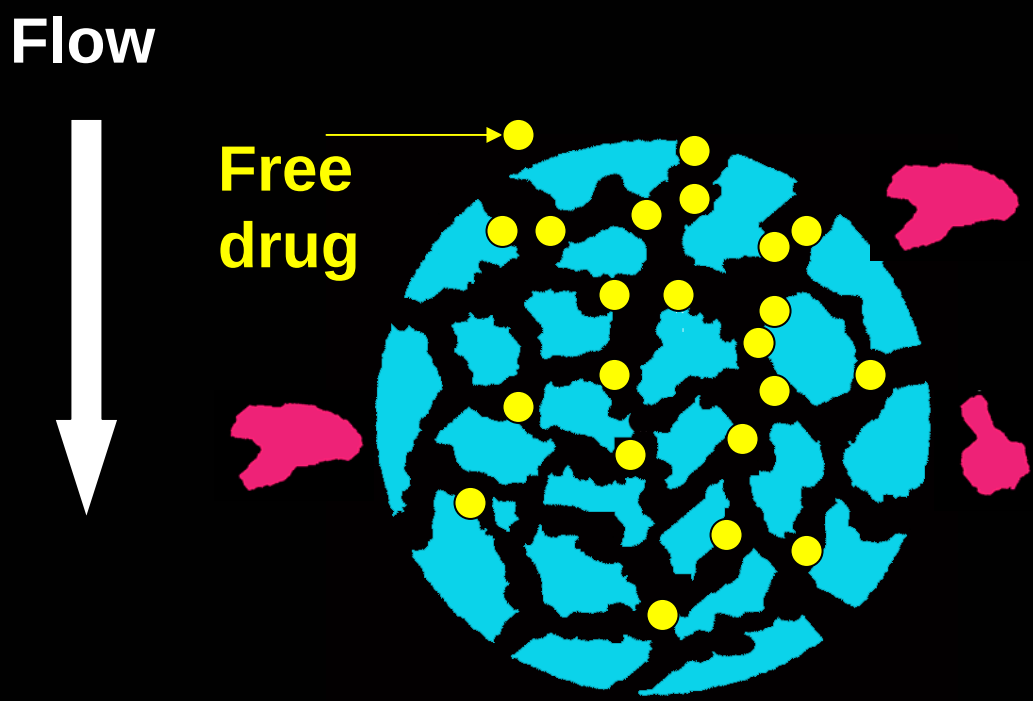
BioTrap extraction process



BioTrap extraction process



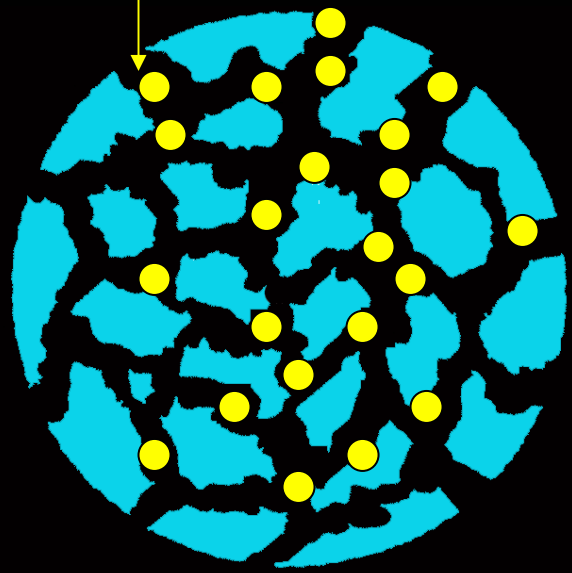
BioTrap extraction process



BioTrap extraction process

Flow

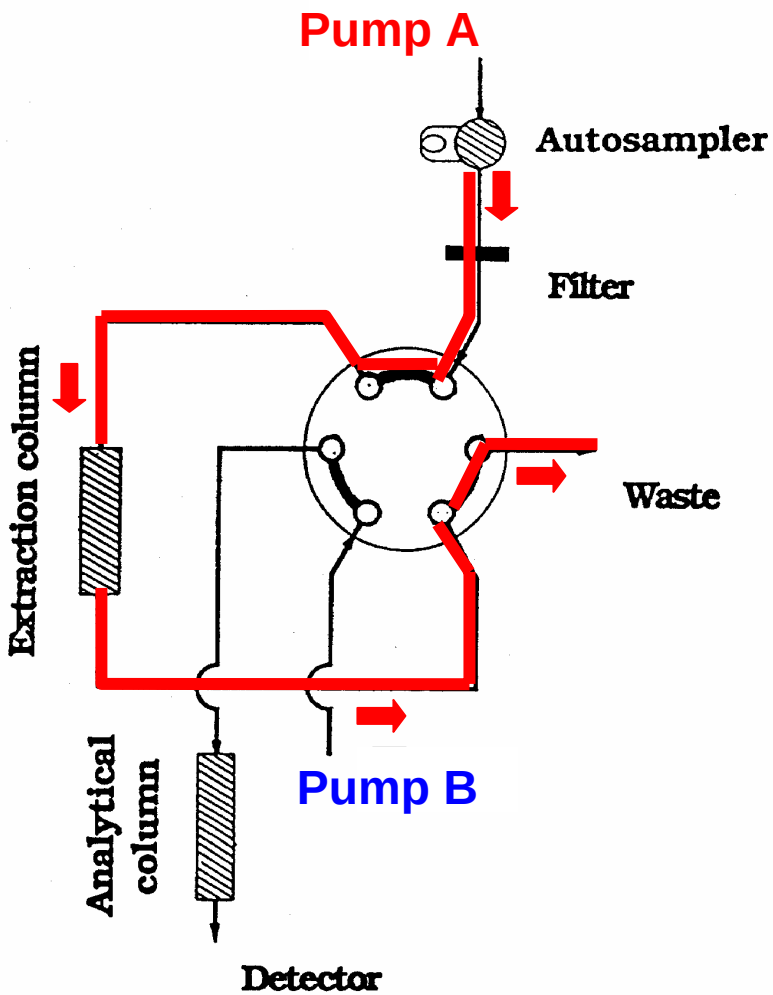
Drug extracted into the pores of the particle



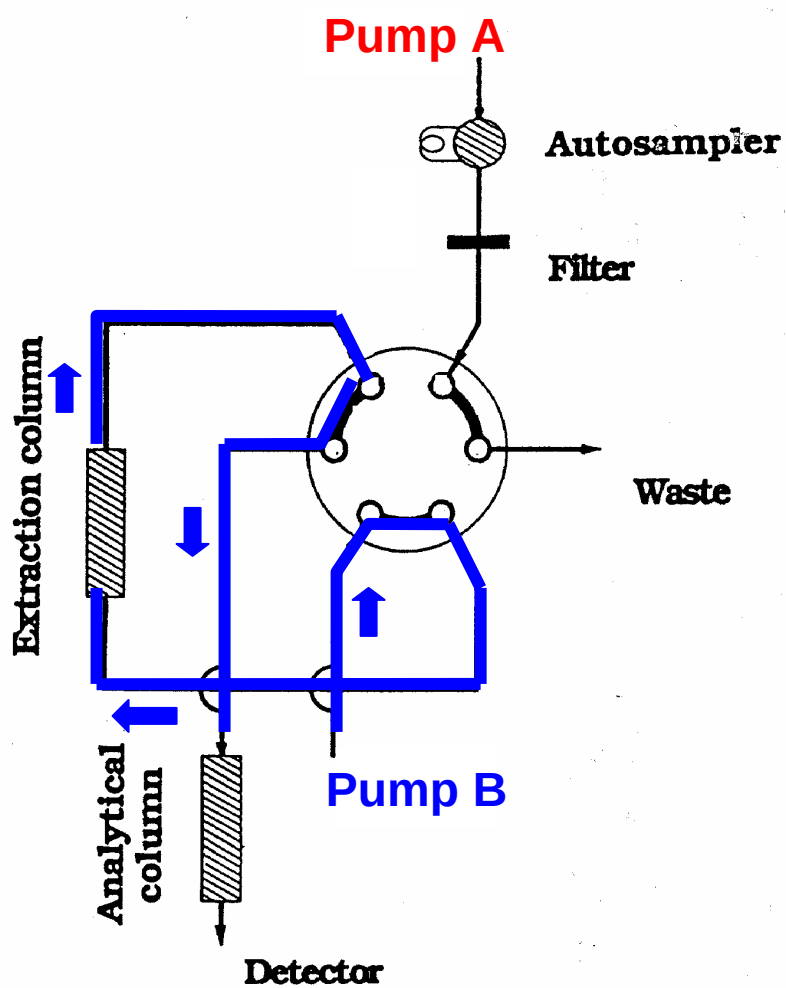
Protein (after extraction of the drug into the pores of the particle)

Online Bioanalysis System

Extraction position



Elution position



Different BioTrap 500 columns

Different types of phases: **BioTrap 500 MS**

BioTrap 500 C8

BioTrap 500 C18

Column dimensions: **13 x 4.0 mm**

20 x 4.0 mm

Micro 20 x 2.0 mm

General extraction methods for acidic and basic drugs with the BioTrap 500 MS column

Column characteristics

Internal surface: Hydrophobic polymer

Outer surface: α_1-acid glycoprotein

Extraction of acidic compounds:

4% 2-prop. in 100 mM formic acid

Extraction of basic compounds:

4% 2-propanol in 10 mM ammonium acetate pH 10

- The general mobile phases are compatible with the plasma proteins.
- The methods normally give high recovery of acidic and basic compounds.
- The mobile phase composition is compatible with MS detection as well as other detection systems.

Method development with the MS compatible extraction column

1. Choose a detection method, MS or MS-MS.

2. Develop a preliminary analytical method. Choose a column and a mobile phase composition giving a good chromatographic performance.

3. Connect the extraction column and the analytical column to the switching valve. Extract the analyte using one of the general extraction mobile phases.

Basic compounds:	4% 2-propanol in 10 mM ammonium ac. pH 10
Acidic compounds:	4% 2-propanol in 100 mM formic acid pH 2.4

4. Optimize the system.

   (changing anal. column, adjust the mobile phase composition)

5. Validate the method.

Direct injection of serum on the BioTrap column. MS/ES detection

Extraction column: **BioTrap 500 MS 20 x 4.0 mm**

Mobile phase: 10 mM ammonium acetate, pH 10.0 (pH adjusted with ammonium hydroxide)

Inj. vol.: 50 µl

Flow: 3.2 ml/min

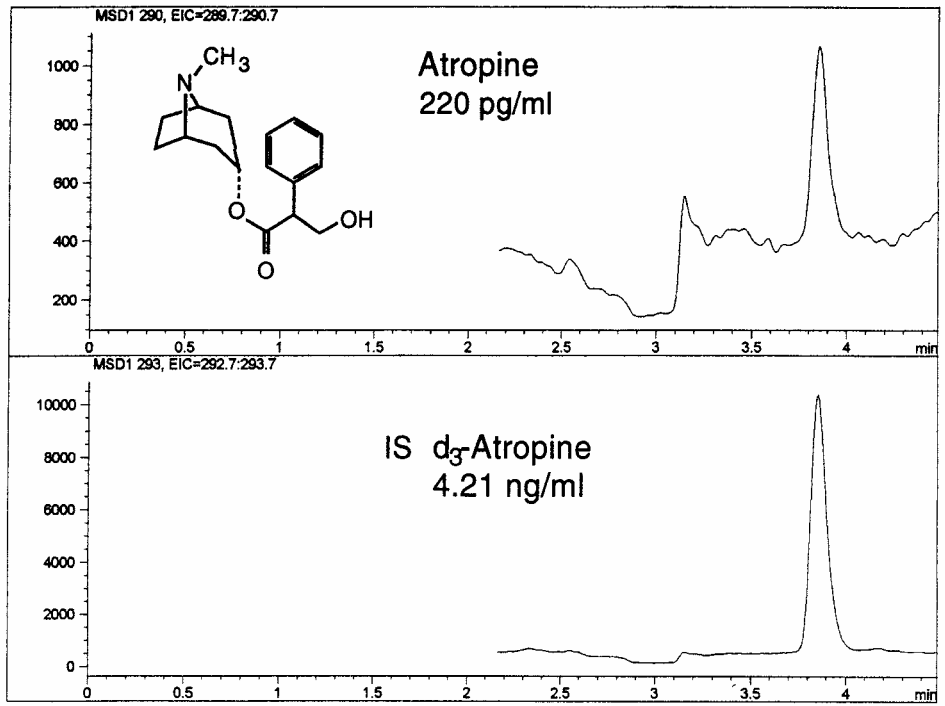
Extraction time:1 min

Analytical column: Zorbax SB-C18, 5 µm, 150 x 4.6 mm + guard column, 12.5 x 4.6 mm

Mobile phase: 25 % acetonitrile in 50 mM formic acid

Flow: 1 ml/min

Detection: HP 1100 series LC/MSD, API-ES positive



Precision in the determination of atropine

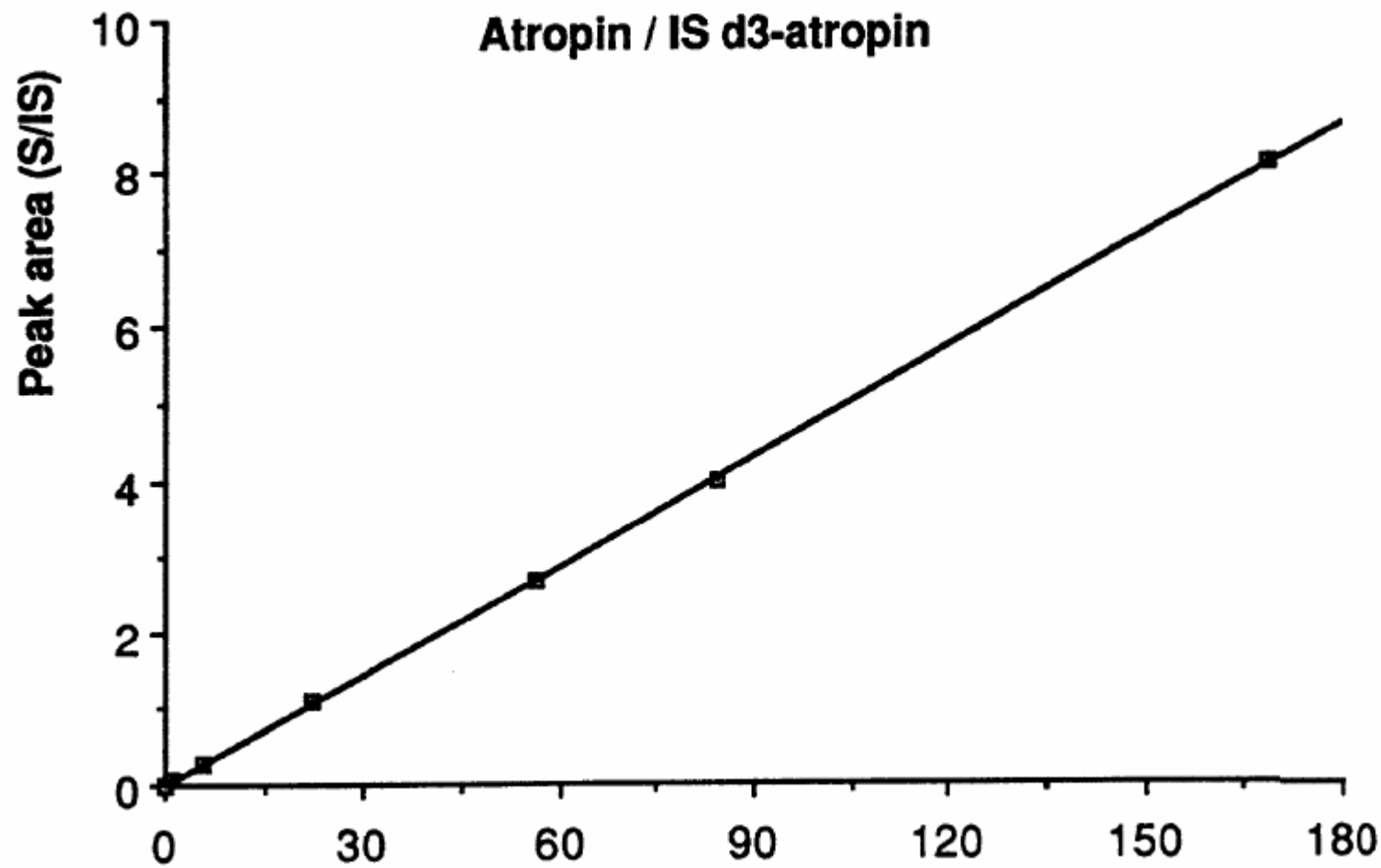
Intra-day variation

Conc. (ng/ml)	RSD %	n
0.225	**6.08**	7
1.12	**1.43**	7
22.5	**1.26**	7

Inter-day variation

Conc. (ng/ml)	RSD %	n
1.12	**5.23**	10
22.5	**2.68**	9

Calibration graph for atropine



Linear regression equation:

Y = 0.00038 + 0.04790X

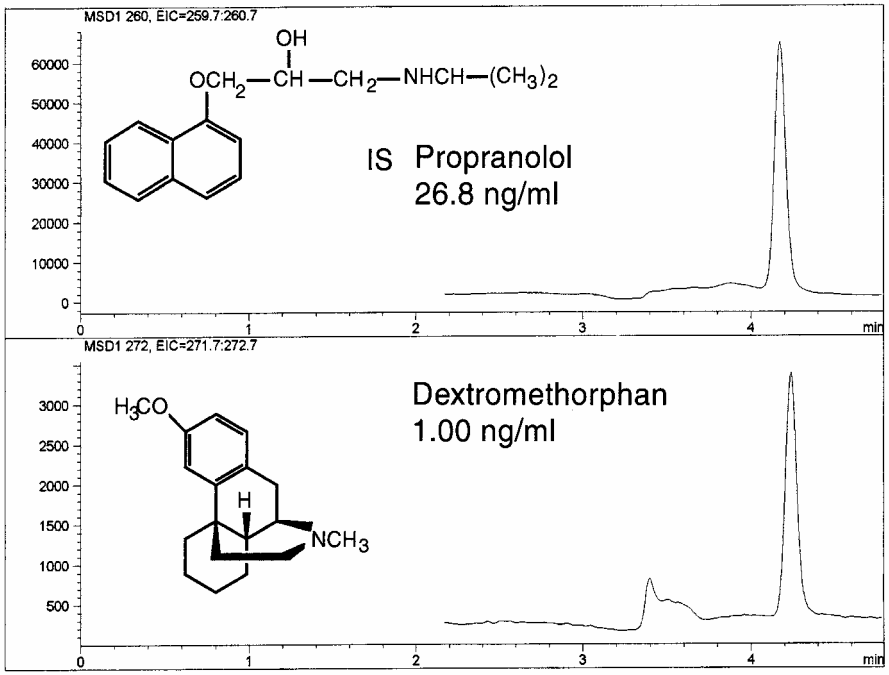
r = 0.99996

Recovery of atropine vs. modifier concentration in the extraction mobile phase

Mobile phase: 2-propanol in 10 mM ammonium acetate buffer pH 10.0

2-propanol	peak area	RSD %	n
0	67.1 x 10^3	**1.42**	5
2	62.5 x 10^3	**0.74**	3
4	60.2 x 10^3	**2.38**	3

Direct injection of serum on the BioTrap column.



MS/ES detection

Extraction column: **BioTrap 500 MS 20 x 4.0 mm**

Mobile phase: **4 % 2-propanol in 10 mM ammonium acetate, pH 10.0 (pH adjusted with ammonium hydroxide)**

Inj. vol.: **50 µl** **Flow:** **3.2 ml/min**

Extraction time: **1 min**

Analytical column: **Zorbax SB-CN, 5 µm, 150 x 4.6 mm + guard column, 12.5 x 4.6 mm**

Mobile phase: **30 % acetonitrile in 50 mM formic acid**

Flow: **1 ml/min**

Detection: **HP 1100 series LC/MSD, API-ES positive**

Linearity and intra-day variation of dextromethorphan

Linear regression equation

Concentration range: 0.20 - 56.0 ng/ml

$Y = -0.00157 + 0.0494\ X$

r = 0.99998

Intra-day variation

Conc. (ng/ml)	**RSD %**	n
0.996	**4.89**	7
24.9	**2.57**	7

Direct injection of serum on the BioTrap extraction column. MS/ESI detection of fluoxetine

Extraction column: **BioTrap 500 MS 20 x 4.0 mm**

Mobile phase: **4% 2-popanol in 10mM ammonium acet., pH 10.0**

Flow: **3.2 ml/min**

Extraction time : **1 min**

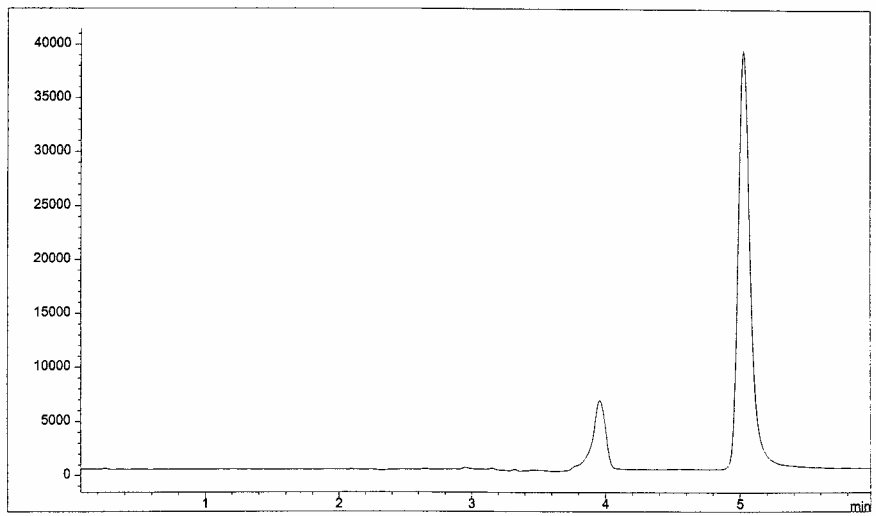
Anal. column: **Zorbax SB-CN, 150 x 4.6 mm, 5 µm + guard 12.5 x 4.6 mm**

Mobile phase: **35% acetonitrile in 25 mM formic acid**

Flow: **1.0 ml/min**

Detection: **HP 1100 series LC/MSD, API-ES positive, Adjusted to 310.1.**

Sample: **50 µl serum containing fluoxetine, 50 ng/ml.**



Analysis of propranolol using BioTrap 500 C18

Inj. vol.: 500 µl

Extraction col.: BioTrap 500 C_{18} (20 x 4.0 mm)

Mobile phase: 4 % 2-prop. in ph. b. pH 7.0

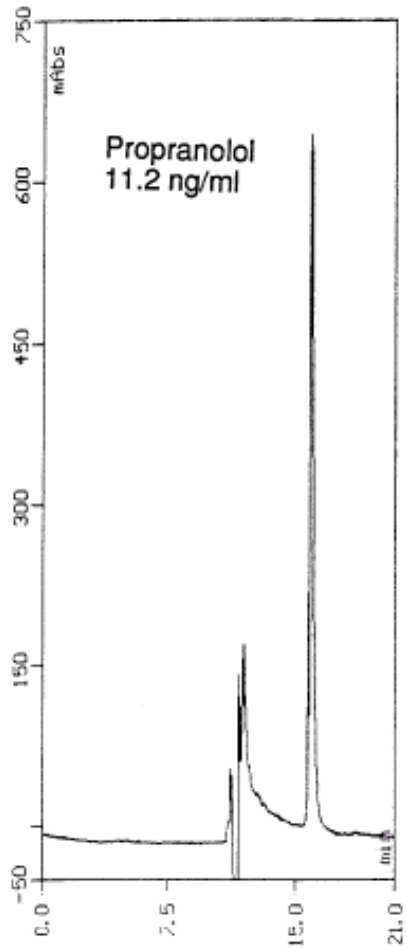
Analytical col.: CT-sil C_8 (100 x 4.6 mm) + 10 x 3.0 mm CT-sil C_8 guard

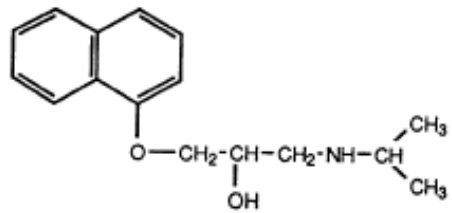
Mobile phase: 28 % acetonitrile in ph. b.

pH 2.8 (m=0.1)

Fluorimetric detection: Ex = 220 nm,

Em = 340 nm





Separation efficiency vs. number of injections

Sample: Propranolol (12 ng/ml) in serum **Inj. vol: 500 µl**

Extraction column: BioTrap 500 C18, 20 x 4.0 mm

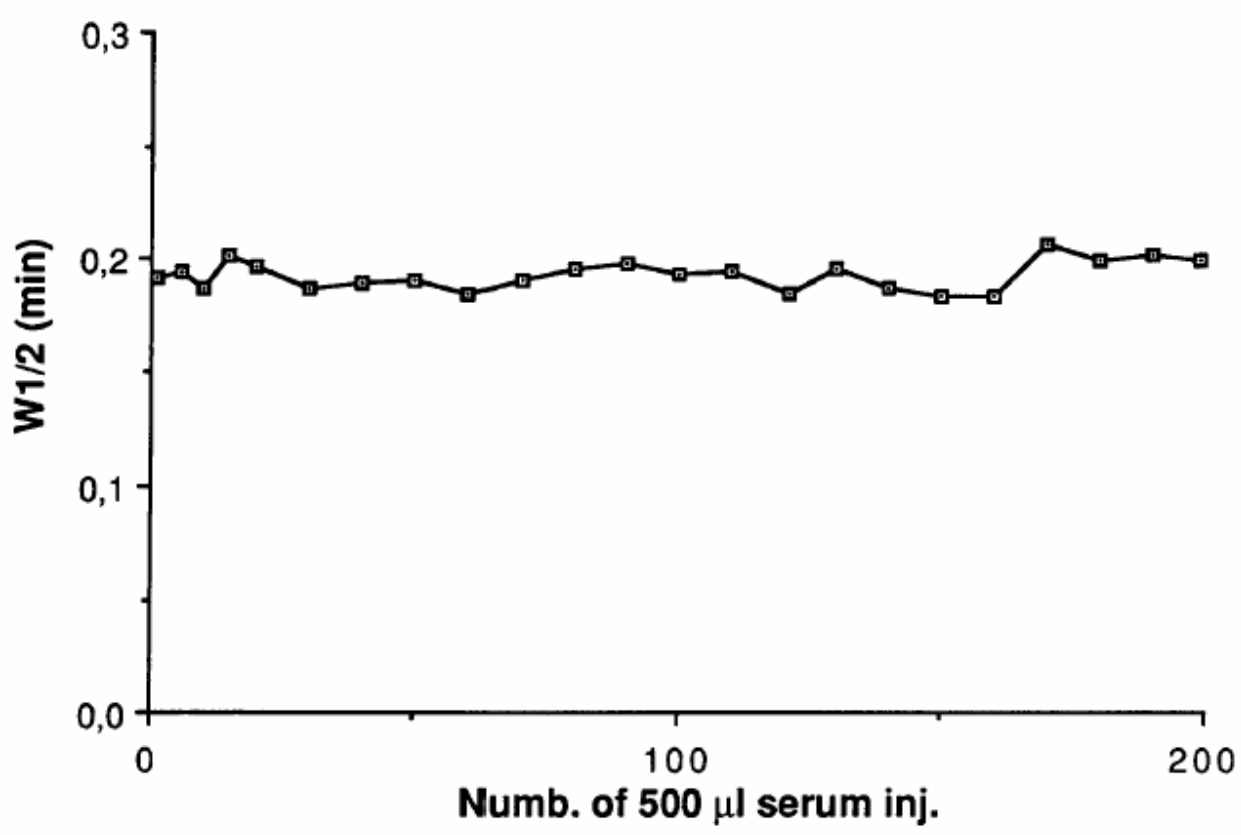
Extraction mobile phase: 4% 2-propanol and 5 mM sod. octanesulfonic acid in 30 mM sod. ph. b. pH 7.0

Analytical column: Hypersil Elite, 5 mm, 150 x 4.6 mm + guard

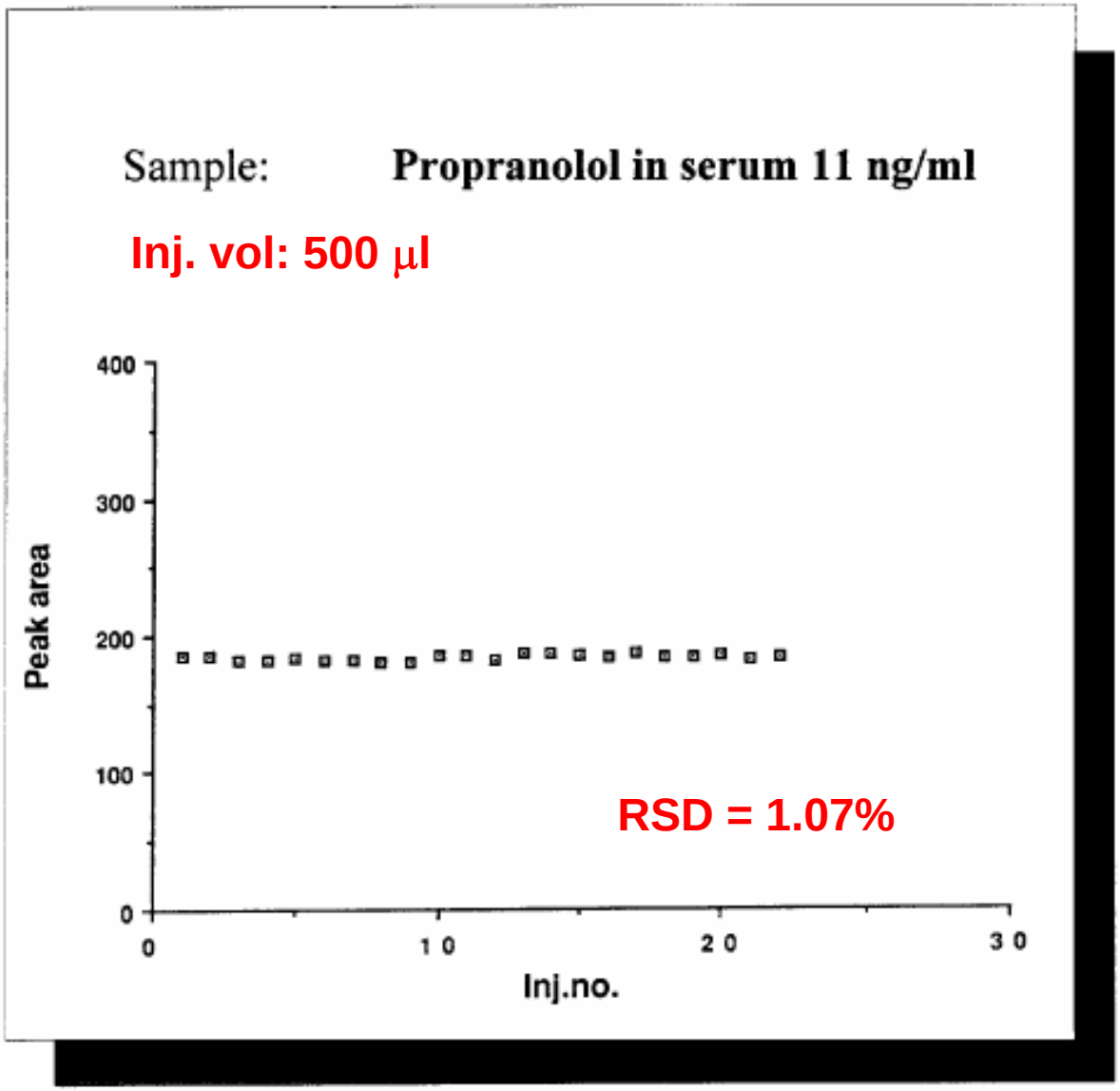
Analytical mobile phase: 33% acetonitrile and 2 mM sod. octanesulfonic acid in 116 mM sod. ph. b. pH 2.8

Detection: Fluorescence,

ex 220 nm, em 340 nm



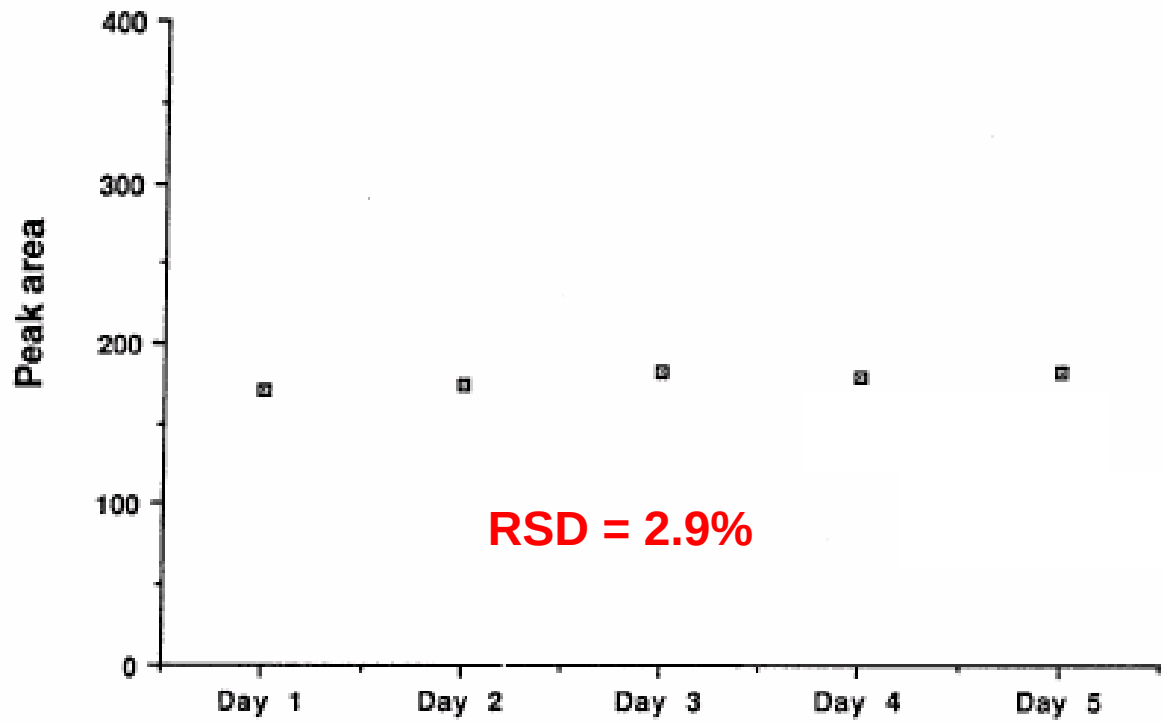
Intraday variation



Interday variation

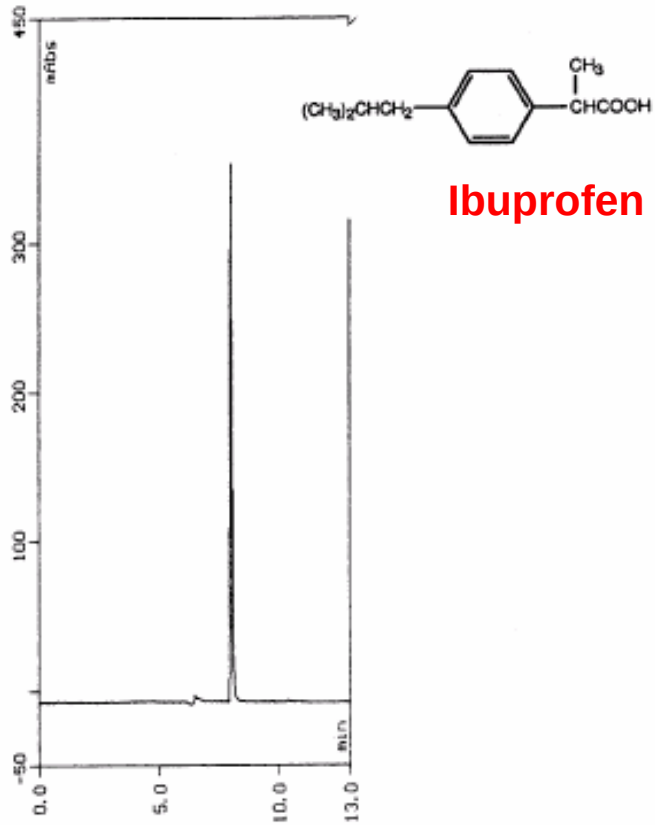
Sample: **Propranolol in serum 11 ng/ml**

Inj. vol: 500 µl



RSD = 2.9%

BioTrap 500 C18 application using the displacement technique



Ibuprofen

Extraction column: BioTrap 500 C18, 20 x 4.0 mm

Extraction mobile phase: 2% 2-propanol in 30 mM sod.ph.b. pH 7.0 with 10 mM octanoic acid

Flow rate: 0.8 ml/min **Inj. vol:** 10 µl

Analytical column: CT-sil C18, 5 mm, 150 x 4.6 mm with guard

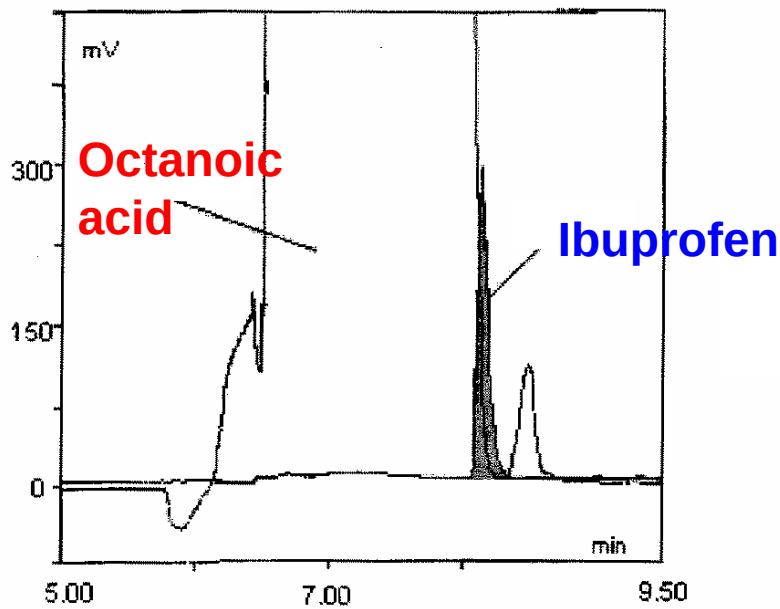
Analytical mobile phase: 35% acetonitrile in 30 mM sod.ph.b. pH 7.0

Flow rate:1.0 ml/min

Detection: Fluorescence ex. 225 nm, em. 535 nm

J. Hermansson et al.,
J. Chromatogr., 797 (1998)251

Overlay plot demonstrating peak compression



Extraction column: BioTrap C18, 20 x 4.0 mm

Extraction mobile phase: 2% 2-propanol in 30 mM sodium phosph. buffer with 10 mM octanoic acid pH 7.0

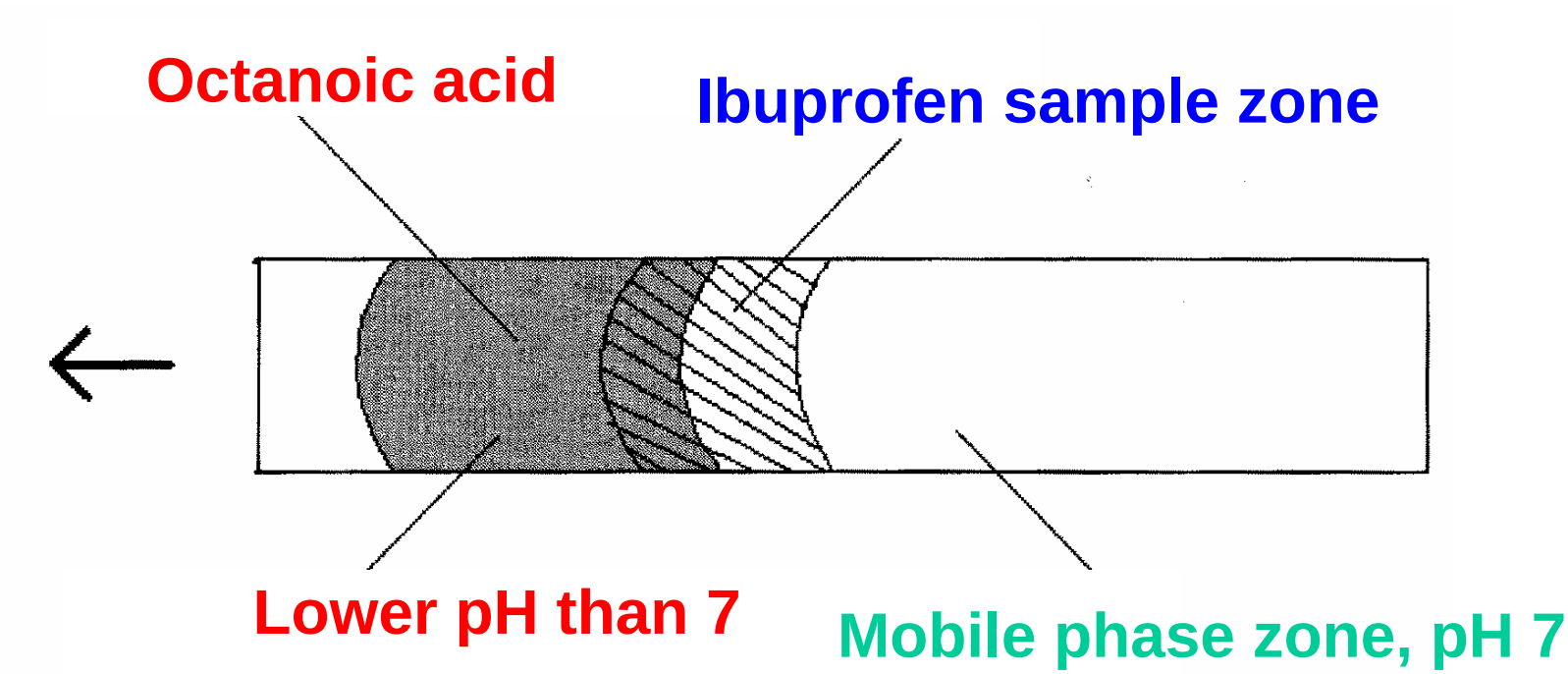
Analytical column: CT-sil C18 100 x 4.6 mm

Analytical mobile phase: 35% acetonitrile in 30 mM sodium phosph. b. pH 7.0

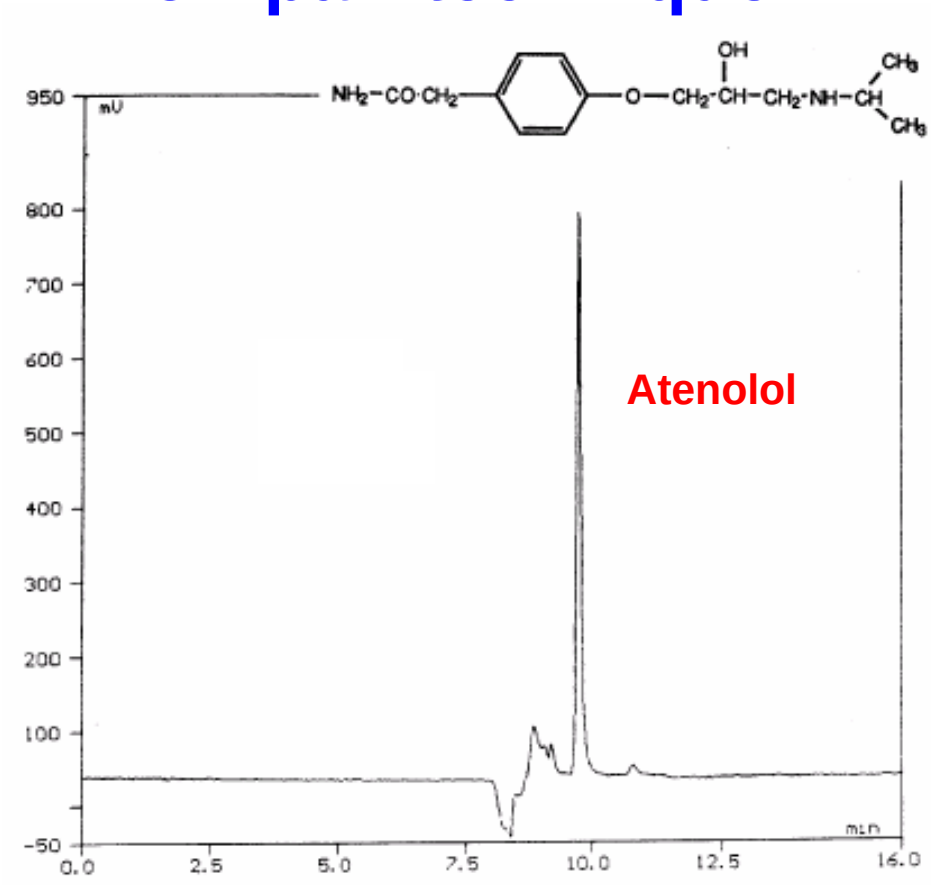
Fluorimetric det.: Ex = 225 nm, Em = 535 nm

J. Hermansson et al., J. Chromatogr., 797 (1998)251

Compression of Ibuprofen with octanoic acid



Online extraction of antenolol from serum using the ion-pair technique



Sample: Atenolol (126 ng/ml) in serum **Inj. vol.: 200 µl**

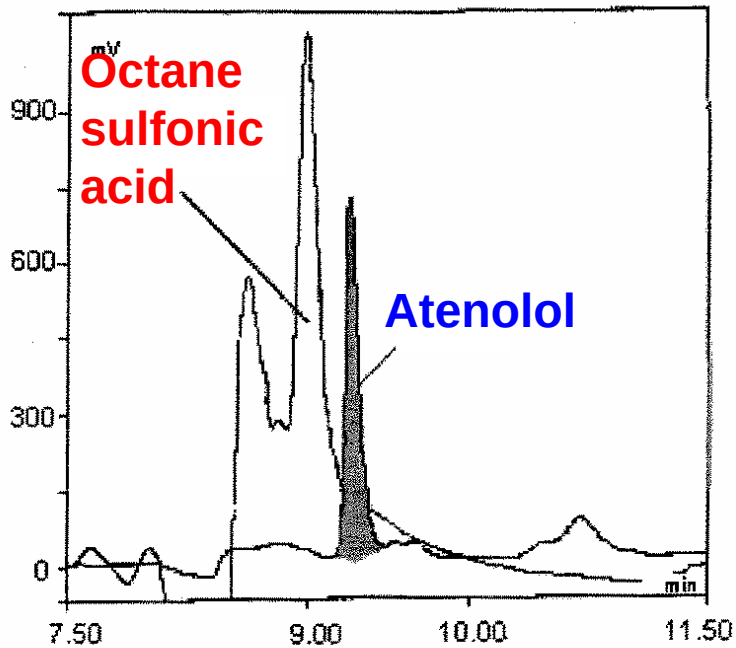
Extraction column: BioTrap 500 C18, 20 x 4.0 mm

Extraction mobile phase: 2% 2-propanol and 5 mM sod. octanesulfonic acid in 30 mM sod.ph.b. pH 7.0

Analytical column: Zorbax SB-CN, 5 mm, 150 x 4.6 mm, + guard

Analytical mobile phase: 25% acetonitrile and 2 mM sod. octanesulfonic acid in 116 mM sod.ph.b. pH 3.0

J. Hermansson et al., J. Chromatogr., 797 (1998)251



Peak compression and enrichment of atenolol

Overlay plot RI and fluorescence

Inj. vol: 200 µl

Extraction column: BioTrap C18, 20 x 4.0 mm

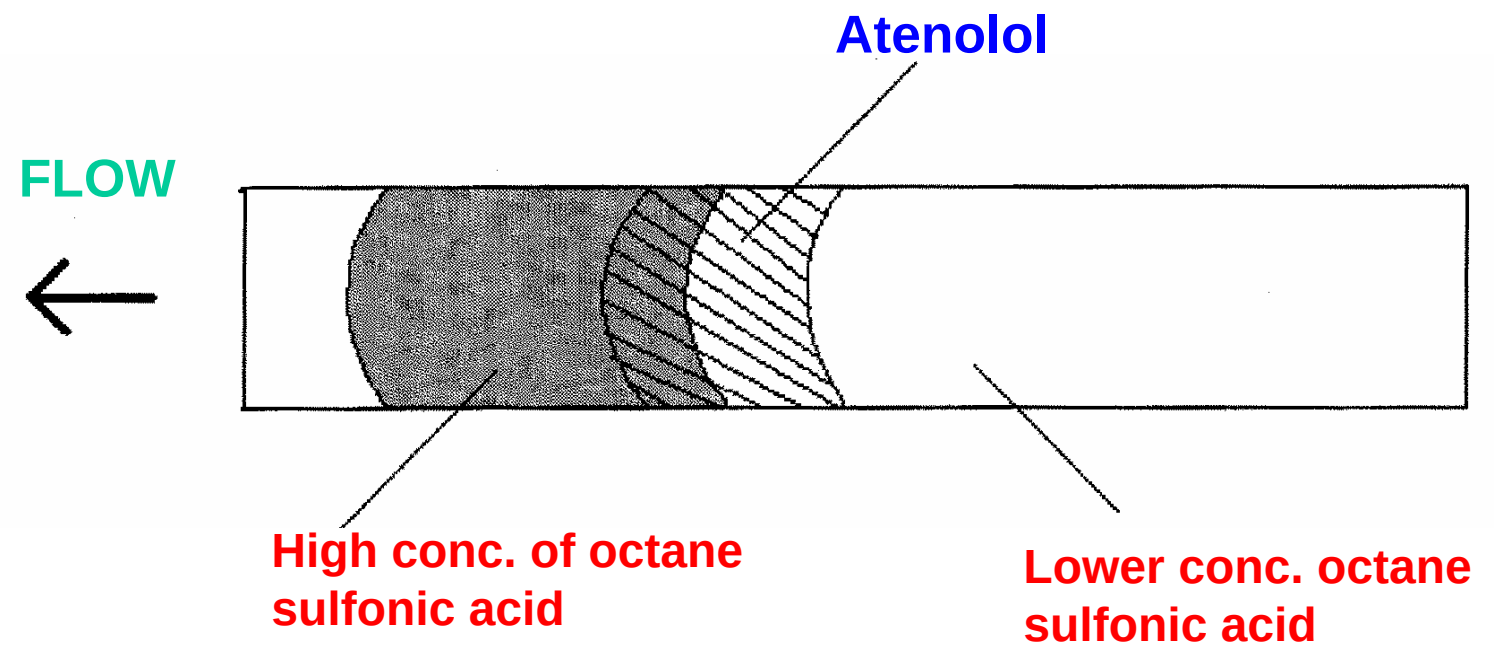
Extraction mobile phase: 2% 2-propanol in 30 mM sodium phosph. b. with 5 mM octanesulfonic acid pH 7.0

Analytical column: Zorbax SB CN 150 x 4.6 mm with guard

Analytical mobile phase: 25% acetonitrile in 30 mM sodium phosph. b with 2 mM sodium octanesulfonic acid, pH 3.0

Inj. volume: 200 µl

Compression of atenolol by octane sulfonic acid



Effect of an ion-pairing agent on recovery

Injection of 200 µl serum

	Recovery from plasma
Without ion-pairing agent	~ 50%
With sodium octylsulfate	~ 100%

Comparison of column efficiency for atenolol, with and without extraction column

	$W_{1/2}$
Coupled column system	0.094[1)]
Analytical column	0.091[2)]

1. 200 μl of serum injected
2. 10 μl sample dissolved in mob. ph. injected directly on the analytical column

The separation efficiency of atenolol is affected by

- More effective trapping on the extraction column
- Enrichment on top of the analytical column
- Compression of the sample zone during the migration on the analytical column

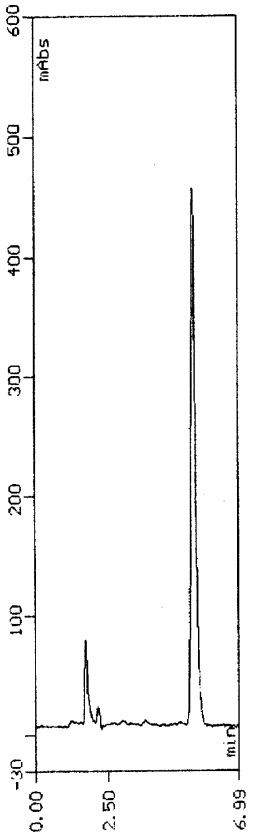
RePeat

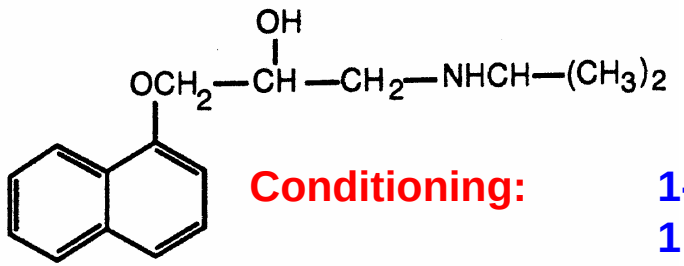
An off-line solid phase extraction cartridge for repeated use

Advantages:

- **A very large number of samples on the same cartridge**
- **Highly reduced cost per sample**
- **Polymer based particles (pH 2-13)**

Extraction procedure for propranolol in serum





Conditioning: 1-2 ml 1% triethylamine in ethylacetate
1 ml dist. water

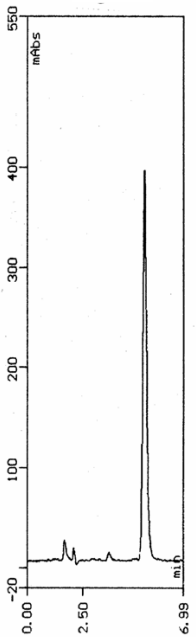
Sample application: 1 ml sample (serum mixed 1:1 with 4% 2-propanol in 30 mM sodium hydroxide)

Washing: 2x1 ml 4% 2-propanol in 30 mM sodium hydroxide
0.5 ml dist. Water

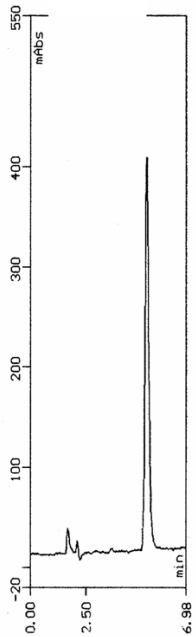
Elution: 1 ml 1% triethylamine in ethyl acetate. Evaporate and reconstitute

Sample: Propranolol 47.9 ng/ml in serum
Injection vol.: 100 ul
Column: Zorbax SB-CN 150x4.6 mm+guard
Mobile phase: 22% acetonitrile in 50 mM formic acid
Flow: 1 ml/min
Detection: Fluorescence ex=220 nm, em= 340 nm

Inj. 1



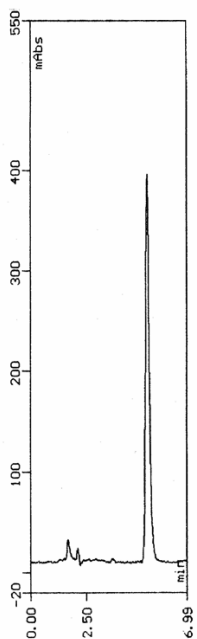
Inj. 31



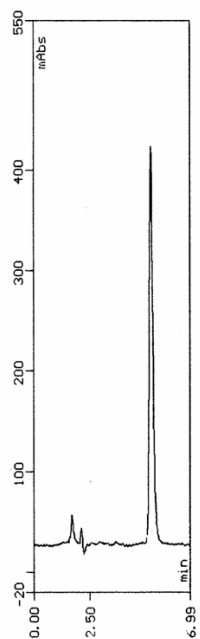
RePeat

Sample: Propranolol (48 ng/ml), 500 ul serum extracted on a RePeat 25 mg cartridge

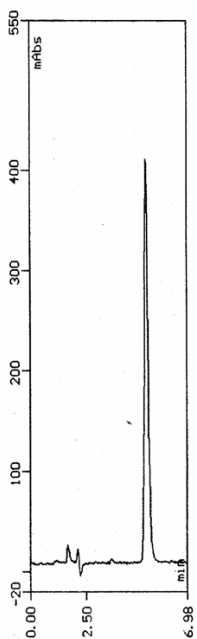
Inj. 62



Inj. 95



Inj. 133



Note

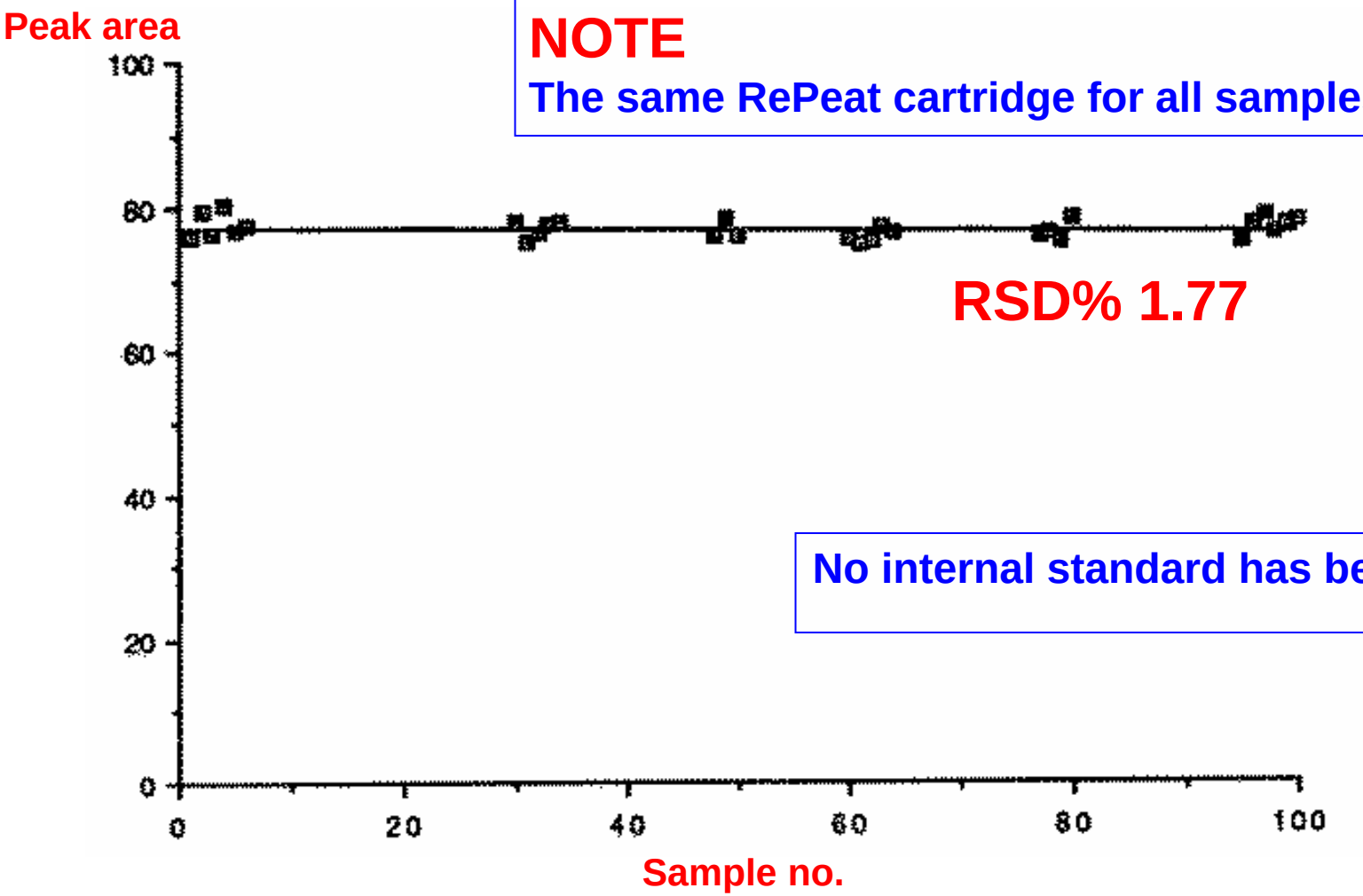
The same RePeat cartridge has been used for all 133 samples

Stability study RePeat

Sample: Propranolol 48 ng/ml

500 ul serum extracted on a RePeat 25 mg cartridge

NOTE
The same RePeat cartridge for all samples



RSD% 1.77

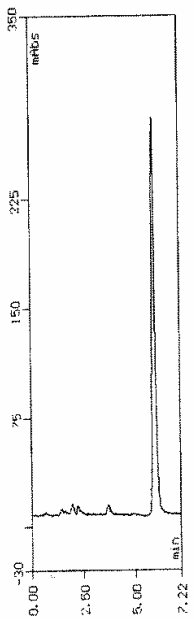
No internal standard has been used

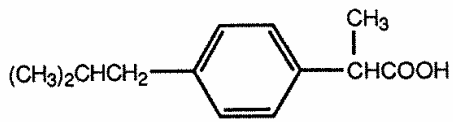
Extraction procedure for **ibuprofen** in serum

RePeat 25 mg cartridge

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

RePeat 25 mg cartridge





Sample:	Ibuprofen, 6.9 µg/ml, in serum
Injection volume:	100 µl
Column:	Zorbax SB-CN, 150x4.6 mm, 5 µm + guard
Mobile phase:	30% acetonitrile in 50 mM ammonium acetate pH 6.0
Flow:	1 ml/min
Detection:	Fluorescence: ex=225 nm, em= 555 nm

Conclusions

- Particles with a biocompatible external surface have been obtained by reaction with the human plasma protein α_1-acid glycoprotein.
- The surface within the pores is very hydrophobic giving a high recovery.
- The properties of the particles make possible the direct injection of large serum/plasma volumes (500 μl or more/sample).
- By the new generation extraction column(BioTrap 500 MS) general methods can be used for the extraction of basic and acidic drugs.
- Combination of the new extraction column with MS or MS-MS detection gives a general solution to an extremely broad range of bioanalytical separation problems.