

# Analyzing Propranolol Hydrochloride in Biological Samples by HPLC

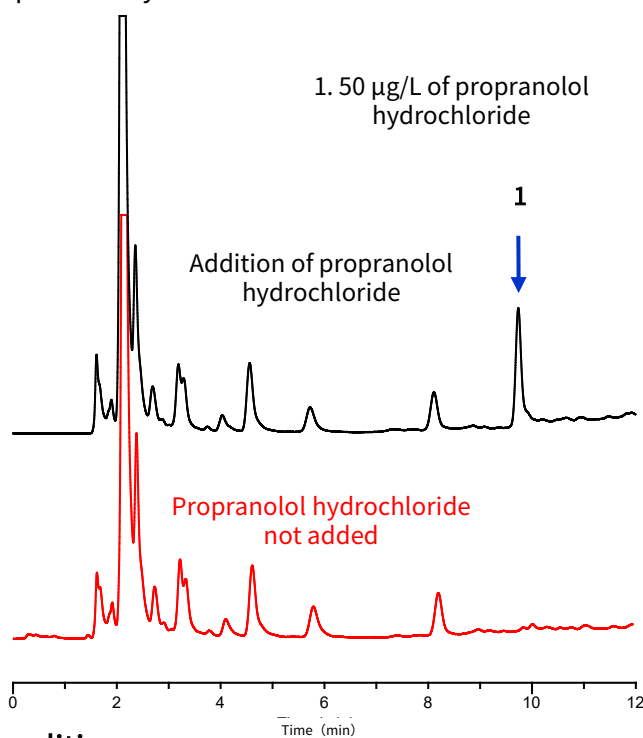
We present a preprocessing method using MonoSpin SCX and propranolol hydrochloride analyses in biological samples using a laser-excited fluorescent detector LIF726. Propranolol hydrochloride is a  $\beta$ -blocker that is used to treat angina, arrhythmias, and high blood pressure. This is one of the drugs that require blood monitoring because of the close therapeutic and poisoning range of blood concentrations, in addition to the large individual differences in drug metabolism. The therapeutic range is determined to be 10  $\mu\text{g/L}$  to 100  $\mu\text{g/L}$  in plasma,

which requires a highly sensitive detection method. The laser-excited fluorescence detector used here enables sensitive and selective detection by using a laser as the source of the fluorescence detector. 10 n pages, we added propranolol hydrochloride to sera, then deproteinized them with organic solvents, and measured them HPLC. On p. 3, plasma was pretreated with propranolol hydrochloride followed by MonoSpin SCX and analyzed.

(A. Tamura)

## 1. Analysis of Serum Propranolol Hydrochloride

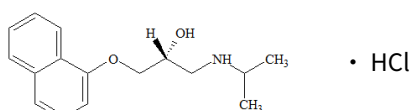
Propranolol hydrochloride solution was added to the control serum, followed by deproteinization and analysis.



### Conditions

**Column** : Monocap C18 fast-flow (150 × 0.1 mm I.D.)  
**Eluent** : A) CH<sub>3</sub>CN/H<sub>2</sub>O/ TFA = 10/90/0.1, v/v  
 B) CH<sub>3</sub>CN/H<sub>2</sub>O / TFA = 95/5/0.1, v/v  
 A/B = 90/10 - 15 min - 30/70, v/v (gradient mixer)  
**Flow rate** : 1  $\mu\text{L}/\text{min}$   
**Col. Temp.** : 20 °C  
**Detection** : LIF Ex. 266 nm Em. 300 - 350 nm (LIF72)  
**Injection Vol.** : 20 nL

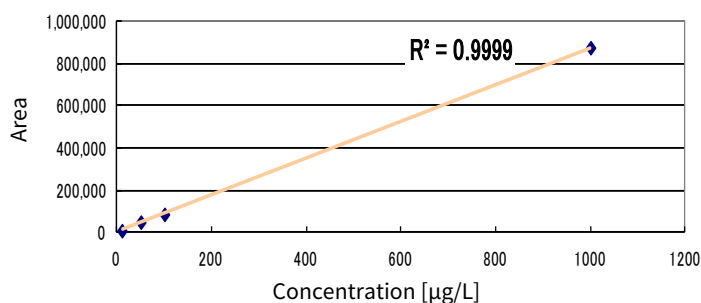
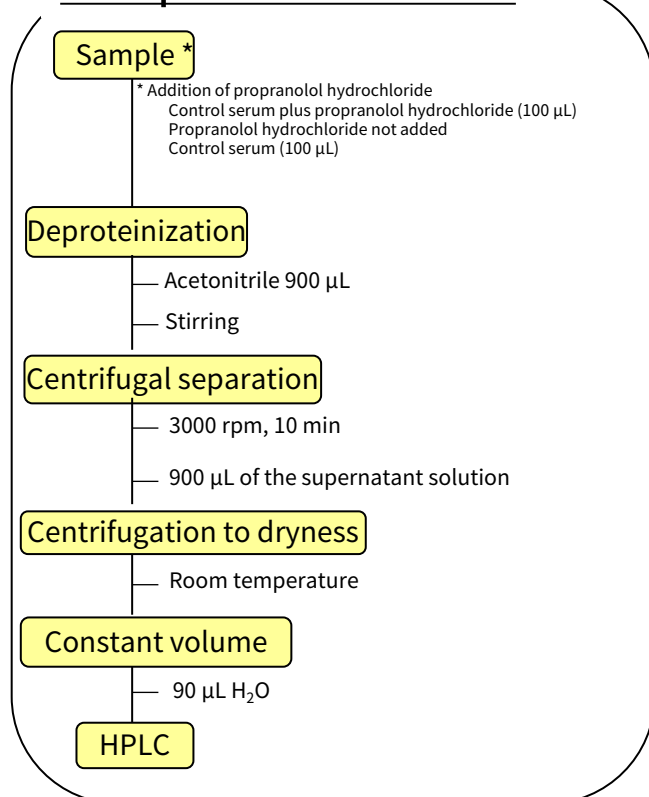
### Structural Formula



Propranolol hydrochloride

Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

### Example of Pretreatment



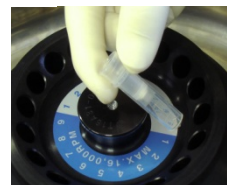
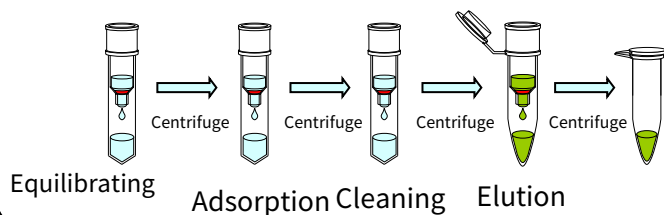
Serum propranolol hydrochloride calibration curve

Since LIF726 is a highly selective detector, only simple deproteinization can be performed to analyze drugs in sera. However, there are many contaminants associated with plasma drugs compared with sera, so we present examples of using MonoSpin as a contrivant pretreatment method.

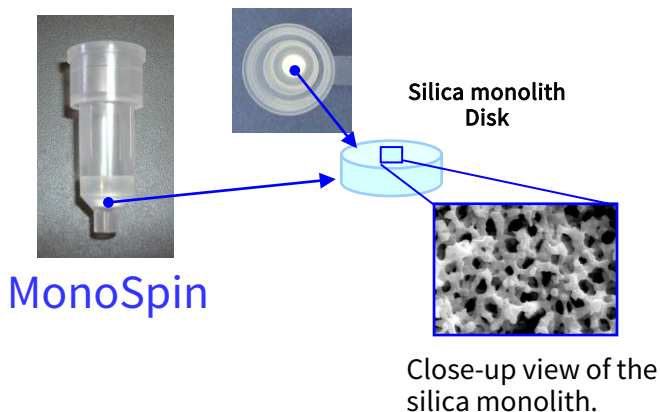
### What is MonoSpin?

The MonoSpin series is a spin-column using silica monoliths with uniform continuum pores. Silica monoliths with high porosity are used as carriers, so they can be passed through by centrifugal manipulation alone. Therefore, it is possible to purify and concentrate samples by a simple operation in a short time. It is also suitable when the sample volume is small because the bed volume is small and the liquid can be cut off.

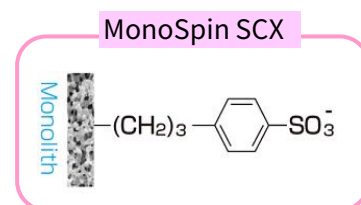
#### Example of the Operation Method



The procedure is centrifugation



### About MonoSpin SCX



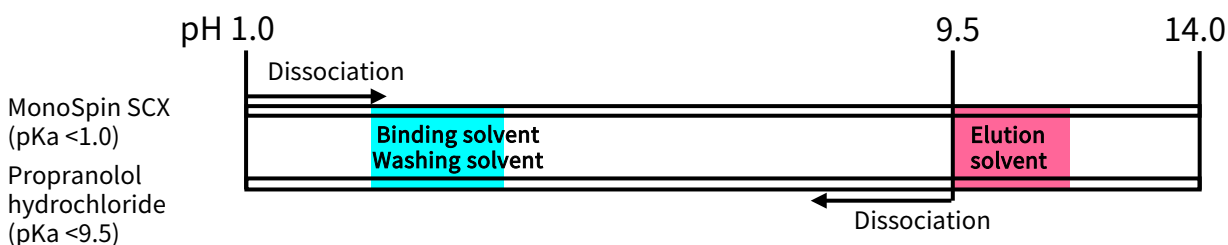
Strongly acidic functional groups have been modified in the MonoSpin SCX. It combines strong cation exchange and weak hydrophobic interactions and is therefore optimal for the extraction of basic drugs.

### Principles of Pretreatment using MonoSpin SCX

In the MonoSpin SCX, the strongly acidic functional group propylbenzenesulfonic acid ( $\text{SO}_3\text{H}$ ) is modified, and sulfonic acid is in the dissociated state ( $\text{SO}_3^-$ ) regardless of the pH of the solution through which it passes. On the other hand, basic compounds are in a dissociated state in acidic and neutral solutions.

First, when basic compounds are added to the MonoSpin SCX under acidic conditions, they adsorb by ion-exchange action. After washing with acidic aqueous solution, acidic to neutral and hydrophilic compounds are not retained and passed. Finally, an organic solvent with added ammonia water can suppress and elute the dissociation and hydrophobic adsorption of adsorbed basic compounds.

In basic compounds, the target components can be eluted selectively compared with the MonoSpin C18 of hydrophobic interactions alone.



## 2. Analysis of Propranolol Hydrochloride in Plasma

Plasma was pretreated and analyzed with MonoSpin SCX after adding propranolol hydrochloride solutions.

### Protocol for the pretreatment process

#### Preparations

- ① Washing solvent Methanol
- ② Washing solvent 2 % aqueous formic acid
- ③ Elution solvent 28 % ammonia water/methanol = 5/95, v/v

#### Sample solution

Plasma 90  $\mu\text{L}$   
 +500 $\mu\text{g/L}$  propranolol hydrochloride solution 10 $\mu\text{L}$   
 + H<sub>2</sub>O 400  $\mu\text{L}$   
 500  $\mu\text{L}$

Attach waste liquid tubes to the MonoSpin SCX

Washing solvent 450  $\mu\text{L}$

Centrifuge

Washing solvent 450  $\mu\text{L}$

Centrifuge

Sample solution 450  $\mu\text{L}$

Centrifuge

S + (b) 450  $\mu\text{L}$  of washing solvent.

Centrifuge

↓

Replace with a retrieval tube

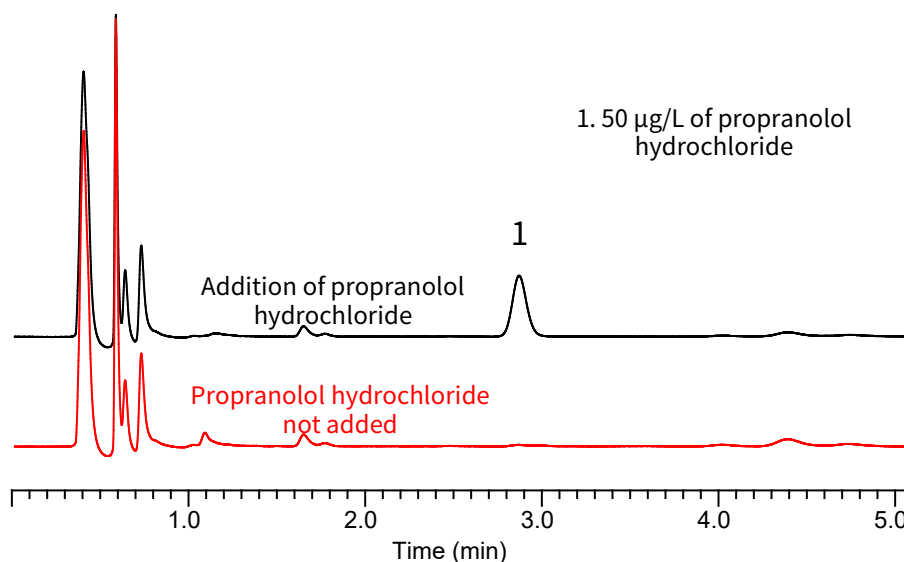
Elution solvent 90  $\mu\text{L}$

Centrifuge

↓

Inject the recovered solutions into HPLC systems

\* All centrifugations were performed at 10,000 g for 1 min.



### Conditions

**System** : LC800 System  
**Column** : Inertsil ODS-4 HP (3  $\mu\text{m}$ , 100  $\times$  2.1 mm I.D.)  
**Eluent** : A) CH<sub>3</sub>CN  
 B) 20 mM KH<sub>2</sub>PO<sub>4</sub> (pH 2.5, H<sub>3</sub>PO<sub>4</sub>)  
 A/B = 25/75, v/v (gradient mixer)  
**Flow rate** : 0.6 mL/min  
**Col. Temp.** : 40 °C  
**Detection** : LIF Ex. 266 nm Em. 300 - 350 nm (LIF726)  
**Injection Vol.** : 5  $\mu\text{L}$



## MonoSpin SCX

Cat. No. 5010-21725 (50)

Cat. No. 5010-21726 (100)

\* There are also trial kits for initial review!

MonoSpin Trial Kit 1: Optimal for Drug and Pesticide Analyses.  
(C18, SCX, SAX, and 10 TiO)  
Cat. No. 5010-21740

MonoSpin Trial Kit 2: Optimal for analyzing sugar chains and hydrophilic compounds.  
(10 each of C18, Amide, CBA, and NH2)  
Cat. No. 5010-21741

MonoSpin trial kit 3, optimal for analyzing ionic compounds.  
(10 SCX, SAX, CBA, and NH2)  
Cat. No. 5010-21742

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