LC Technical Note
LT140
GL Sciences Inc.

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.

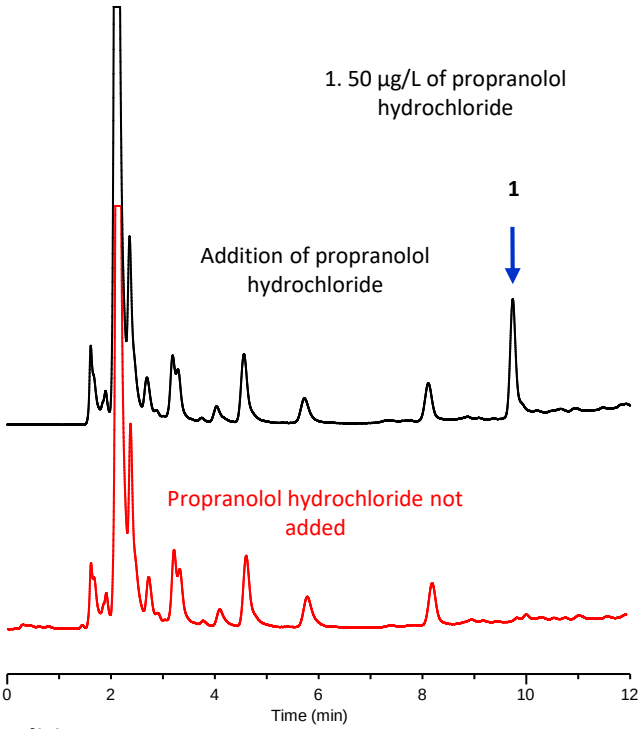
Propranolol hydrochloride is a β-blocker that is used to treat angina, arrhythmias, and high blood pressure. This is one of the drugs that require blood monitoring because of the close therapeutic and poisoning range of blood concentrations, in addition to the large individual differences in drug metabolism. The therapeutic range is determined to be 10 µg/L to 100 µg/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.



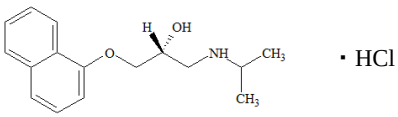
Example of Pretreatment

- Sample *
  - * Addition of propranolol hydrochloride
    - Control serum plus propranolol hydrochloride (100 µL)
    - Propranolol hydrochloride not added
    - Control serum (100 µL)
- Deproteinization
  - Acetonitrile 900 µL
  - Stirring
- Centrifugal separation
  - 3000 rpm, 10 min
  - 900 µL of the supernatant solution
- Centrifugation to dryness
  - Room temperature
- Constant volume
  - 90 µL H_2O
- HPLC

Conditions

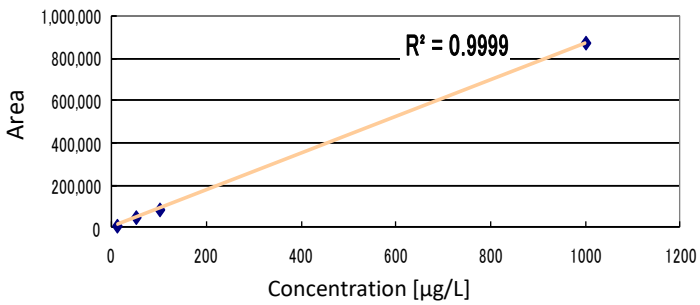
Column : Monocap C18 fast-flow (150 × 0.1 mm I.D.)
Eluent : A) CH_3CN/H_2O/ TFA = 10/90/0.1, v/v
B) CH_3CN/H_2O / TFA = 95/5/0.1, v/v
A/B = 90/10 – 15 min - 30/70, v/v (gradient mixer)
Flow rate : 1 µL/min
Col. Temp. : 20 ℃
Detection : LIF Ex. 266 nm Em. 300 - 350 nm (LIF726)
Injection Vol. : 20 nL

Structural Formula



Propranolol hydrochloride

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



Serum propranolol hydrochloride calibration curve

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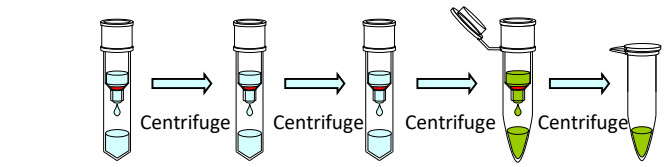
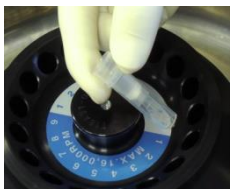
Since LIF726 is a highly selective detector, only simple deproteinization can be performed to analyze drugs in sera. However, there are many contaminants associated with plasma drugs compared with sera, so we present examples of using MonoSpin as a contrivant pretreatment method.

What is MonoSpin?

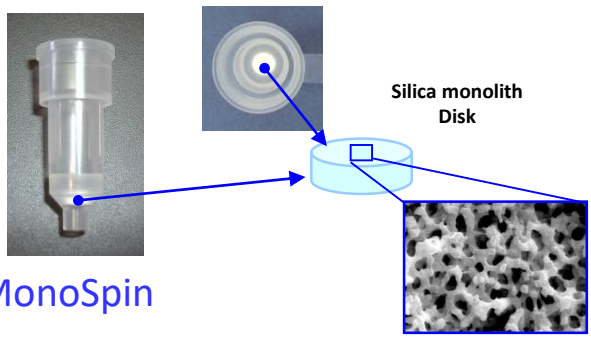
The MonoSpin series is a spin-column using silica monoliths with uniform continuum pores. Silica monoliths with high porosity are used as carriers, so they can be passed through by centrifugal manipulation alone.
Therefore, it is possible to purify and concentrate samples by a simple operation in a short 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



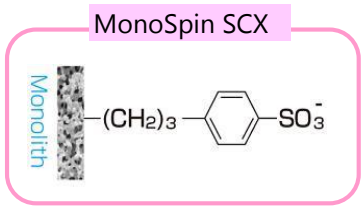


The procedure is centrifugation



Close-up view of the silica monolith.

About MonoSpin SCX



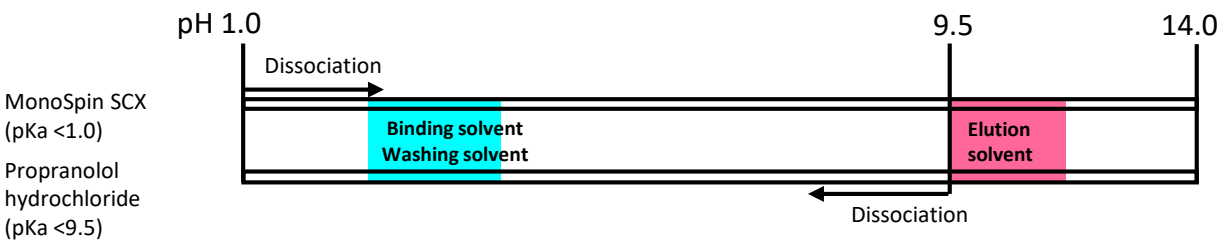
Strongly acidic functional groups have been modified in the MonoSpin SCX. It combines strong cation exchange and weak hydrophobic interactions and is therefore optimal for the extraction of basic drugs.

Principles of Pretreatment using MonoSpin SCX

In the MonoSpin SCX, the strongly acidic functional group propylbenzenesulfonic acid (SO_3H) is modified, and sulfonic acid is in the dissociated state (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.

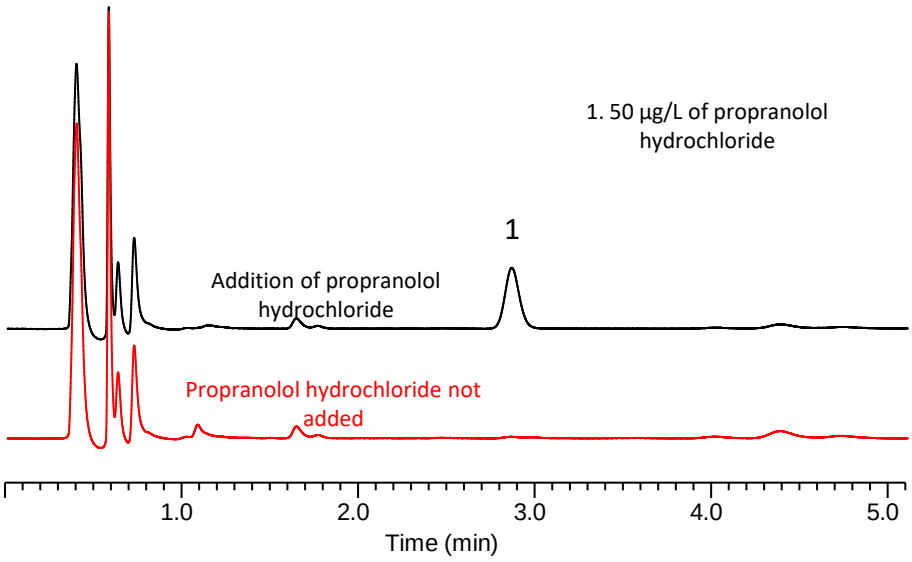
Protocol for the pretreatment process

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 µL
+500µg/L propranolol hydrochloride solution 10µL
+ H_2O 400 µL
500 µL

Attach waste liquid tubes to the MonoSpin SCX
Washing solvent 450 µL
Centrifuge
Washing solvent 450 µL
Centrifuge
Sample solution 450 µL
Centrifuge
§ +(b) 450 µL of washing solvent.
Centrifuge
↓
Replace with a retrieval tube
Elution solvent 90 µL
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 µm, 100 × 2.1 mm I.D.)
Eluent : A) CH_3CN
B) 20 mM KH_2PO_4 (pH 2.5, H_3PO_4)
A/B = 25/75, v/v (gradient mixer)
Flow rate: 0.6 mL/min
Col. Temp. : 40 ℃
Detection :LIF Ex. 266 nm Em. 300 - 350 nm (LIF726)
Injection Vol. :5 µL

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

GL Sciences, Inc. Japan
22-1 Nishishinjuku 6-Chome
Shinjuku-ku, Tokyo,
163-1130, Japan
Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@gls.co.jp
Web: www.glsciences.com

GL Sciences B.V.
De Sleutel 9
5652 AS Eindhoven
The Netherlands
Phone: +31 (0)40 254 95 31
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (ShangHai) Ltd.
Tower B, Room 2003,
Far East International Plaza,
NO,317 Xianxia Road,
Changning District.
Shanghai, China P.C. 200032
Phone: +86 (0)21-6278-2272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn

GL Sciences, Inc. USA
4733 Torrance Blvd. Suite 255
Torrance, CA 90503
Phone: 310-265-4424
Fax: 310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

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