LC Technical Note
LT161
GL Sciences Inc.

Purification of Ribavirin in Human Serum using MonoSpin PBA

Ribavirin is an anti-viral medication used in combination with Interferon to treat Hepatitis C. Since it easily accumulates in human body, it is possible to forecast and study the therapeutic effects by pharmacokinetic analysis.

As Ribavirin is a hydrophilic drug, sample preparation by hydrophobic interaction using phases such as C18 spin will most likely not work. For this reason in the note below a MonoSpin PBA column, which shows selective adsorption for cis-diols contained in Ribavirin, was used for purification.

Ribavirin was added to 0.25 – 25 µg/mL of human serum which was then purified by using MonoSpin PBA and analyzed by HPLC.

The analytical column used is InertSustain AQ-C18 which provides stability even under highly aqueous mobile phase conditions.

The results show that the measurement of Ribavirin in Human Serum was conducted in an easy and selective way.

In this note other remarks are described which may help you developing your method.

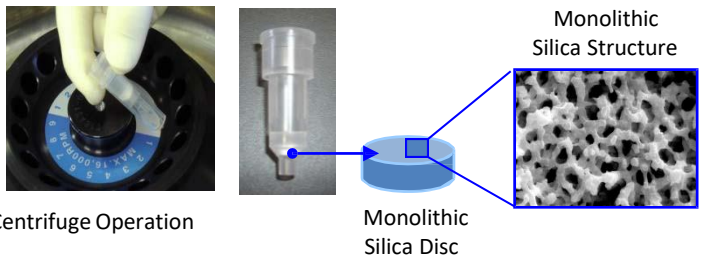
(Y. Yui, N. Iwanami)

◆MonoSpin

MonoSpin is a series of spin columns for solid phase extraction (SPE). Owing to the high permeability of monolithic silica disk packed into the spin column, the procedures, such as conditioning, sample loading, washing, and elution can be carried out only by centrifuging the column. It is also the advantage that the elution volume is only 200 µL.

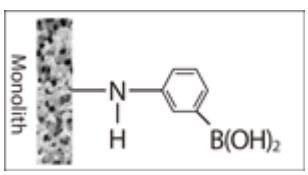
Among the series of MonoSpin, MonoSpin PBA, which has phenylboronic acid as a functional group, can adsorb *cis*-hydroxyl group containing compounds selectively.

Refer to the following link to see the video tutorial:
http://www.gls.co.jp/flv/monospin/monospin.html



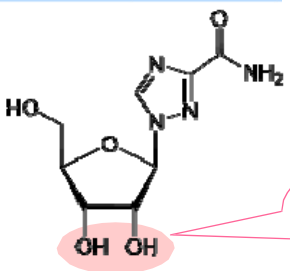
◆Purification by MonoSpin PBA

MonoSpin PBA



Phenylboronic acid is chemically bonded. Compounds containing *cis*-diol group can be retained with high selectivity.

Chemical Structure

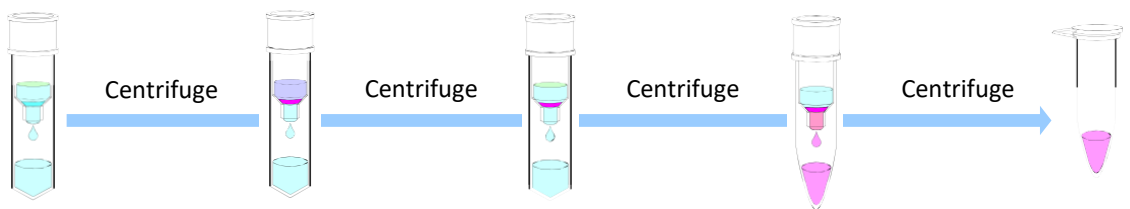


Ribavirin MW : 244
pKa : 12.25

The structure of MonoSpin PBA where it retains and adsorbs Ribavirin.

Purification Process Flowchart

Centrifuge: 2,300 × g, 2 min



1.Conditioning
400 µL
0.6 % ammonia aqueous solution

2.Adsorption
400 µL
sample solution

3.Rinsing
400 µL
0.6 % ammonia aqueous solution

4.Elution
200 µL
1 % formic acid aqueous solution

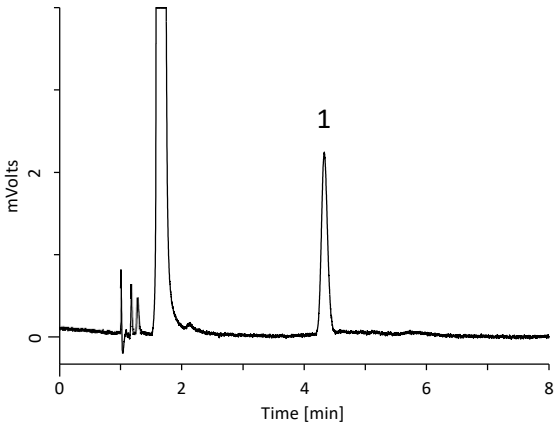
Purified sample

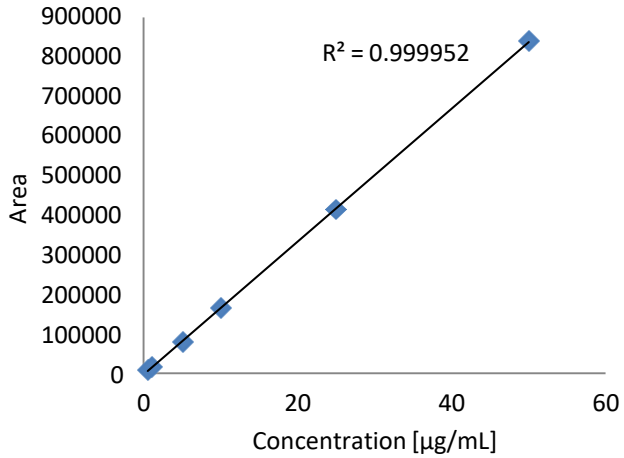
Sample preparation

0.6 % ammonia aqueous solution, human serum and Ribavirin were diluted with water and mixed together at 50 : 49 : 1, and centrifuged at 5,000 × g for 3 minutes. The supernatant was collected as a sample solution.

*It is important to collect and use the supernatant as a sample solution to prevent the MonoSpin PBA from clogging.

◆ Standard Solution





HPLC Conditions

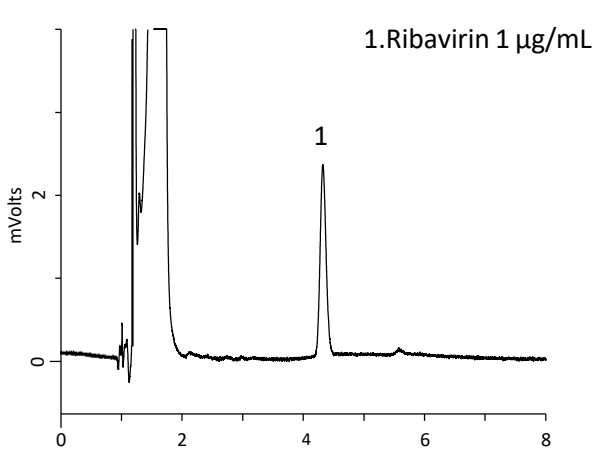
Column: InertSustain AQ-C18 HP (3 µm, 150 × 2.1 mm I.D.)
Eluent: A) Solution*
B) Solution/CH_3CN=19/1, (v/v)
gradient (v/v)
Flow rate: 0.3 mL/min
Column temp.: 25 ℃
Detection: UV 220 nm
Injection Vol.: 5 µL

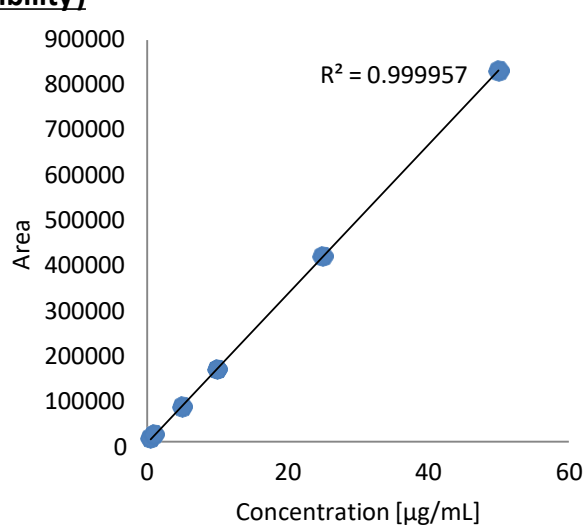
Time	A (%)	B (%)
0	100	0
6	100	0
6.1	0	100
11	0	100
11.1	100	0
20	100	0

*Preparation method of solution:
2 g of anhydrous sodium sulfate were dissolved in 300 mL of water and then 8 mL of phosphoric acid were added to 2 L.

◆ Spike and Recovery Test ① (Intra-day Reproducibility)

Chromatogram of the recovery solution (1 µg/mL)



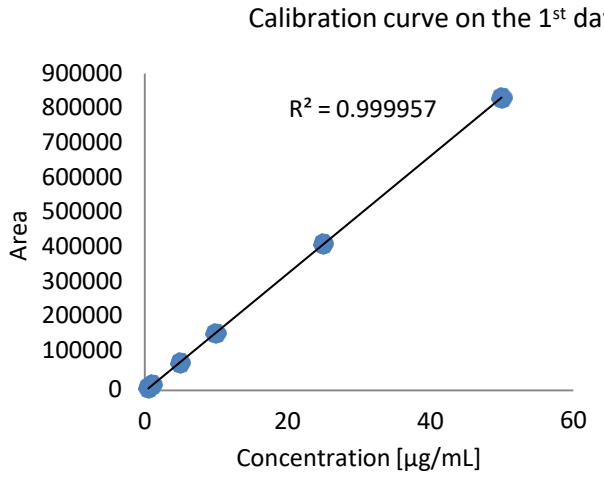


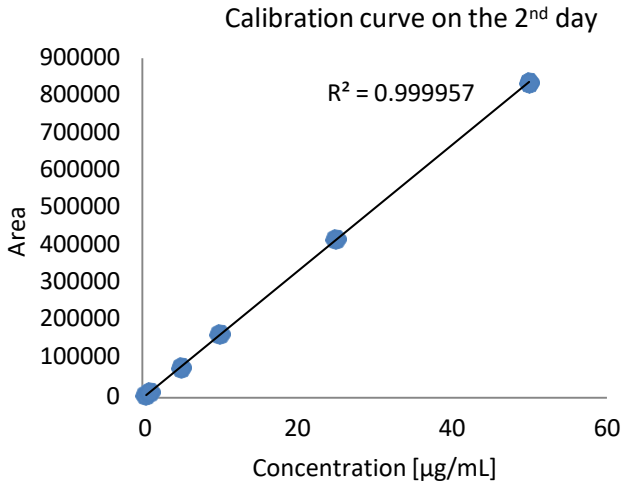
Recovery rate and intra-day reproducibility of each concentration level (*n*=6).

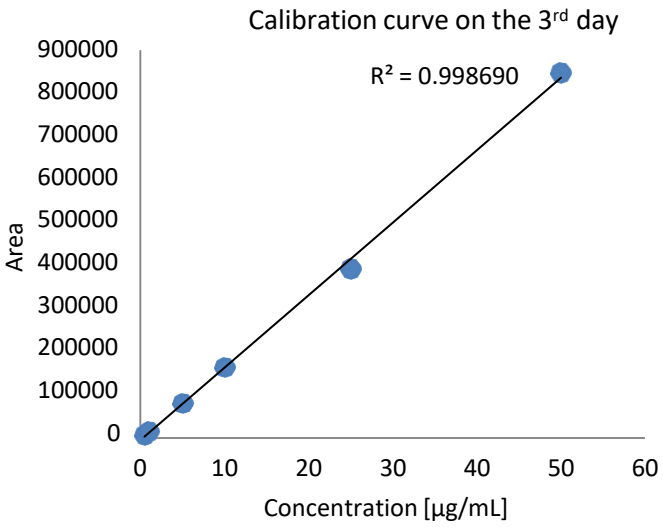
Spiked concentration,µg/mL	Recovery,%	RSD,%
0.25	100.7	2.4
0.5	102	3.5
2.5	97.5	1.7
5	97.7	2.5
12.5	100.7	0.9
25	98.9	1.2

The recovery rate and the intra-day coefficient of variation of each concentration level (n=6) elaborated after the analysis have shown excellent results with **97.5-100.7% recovery rate and 0.9-3.5% RSD (Relative Standard Deviation)**.

◆ Spike and Recovery Test ②（Inter-day Reproducibility during 3 days）







The graph show the calibration curve and the correlation coefficient of each concentration level (n=6) elaborated after the three days of analysis. The results obtained are excellent as the **correlation coefficient has been 0.998690- 0.999957**.

Recovery Rate and -day Reproducibility of each concentration level during the threedays.

Spiked concentration, µg/mL	1st day	2nd day	3rd day	RSD (%)
0.25	100.7	99.4	95.7	3.1
0.5	102	99.3	98.9	3.0
2.5	97.5	98.5	98.9	1.6
5	97.7	101.9	99.3	2.5
12.5	100.7	101.4	94.7	3.4
25	98.9	99.5	100.8	1.9

The recovery rate and the intra-day coefficient of variation of each concentration level elaborated after the analysis shown excellent results with **94.7-101.9% recovery rate** and **RSD 1.6-3.4%**.

Physical Properties and Specifications of MonoSpin PBA

Description	Bonded Phase	S Type (Small)		Surface Area (m^2/g)
		Through-pore (μm)	Meso-pore (nm)	
MonoSpin PBA	Phenyl boric acid	5	10	350

Products used for the Analysis

MonoSpin

MonoSpin S Type (Small)

Recovery Tube (1.7 mL)

Waste Tube (2 mL)

Each MonoSpin S Type (Small) columns are attached with 1.7 mL recovery tubes and 2.0 mL wastetubes.

Description	Qty.	Cat.No.
MonoSpin PBA	50 pcs	5010-21715
	100 pcs	5010-21716

MonoSpin S Type（Small）Trial Kits and Customized Kit

The following trial and customized kits are available for purchase to test a whole range of MonoSpin spin columns to make the best decision on which MonoSpin to use.

Description	Available phases	Cat.No.
MonoSpin Trial Kit 1	C18,TiO,SCX,SAX 10 pcs each.	5010-21740
MonoSpin Trial Kit 2	C18,Amide,CBA,NH2 10 pcs each.	5010-21741
MonoSpin Trial Kit 3	SCX,SAX,CBA,NH2 10 pcs each.	5010-21742
MonoSpin Customized Kit 20	Customize your kit by choosing 2 types* of phases 10 pcs each.	5010-01001

*MonoSpin Trypsin, MonoSpin ProA, MonoSpin ProG are not available for the MonoSpin Customized Kit 20.

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