

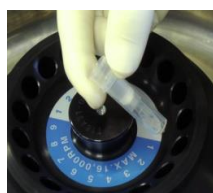
Examination of Loading Capacity of Proteins Using MonoSpin C18

It's very difficult to recover proteins with large molecular weight by reverse phase solid phase extraction, general silica base using small pore size. In this note, We introduce the loading capacity of proteins using MonoSpin C18 suitable for large molecular weight.

(Y.Yui, S.Ota)

What is MonoSpin?

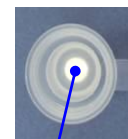
MonoSpin SPE centrifugal spin columns was developed to improve concentration of small-volume sample preparation. The low-pressure, high-flow, and low-liquid-retention properties of GL Sciences' monolith silica technology makes it uniquely suited for handling small amount samples.



Centrifuge operation

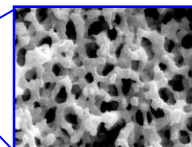


MonoSpin



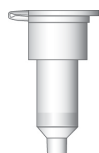
Silica Monolith Disk

Monolith structure



Line-up

MonoSpin series have cartridge with different size: Type S: Excellent for the pretreatment for samples of 50 - 800 μ L, Type L: Optimal for sample of 0.5 - 8 mL.



S Type (Small)

- Disc Size : ϕ 4.2 \times 1.5 mm
- Sample Volume : 0.5 - 800 μ L
- Elution Volume : 50 - 800 μ L
- Centrifugation Speed : 2,000 - 10,000 \times g



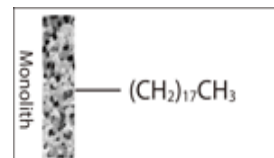
L Type (Large)

- Disc Size : ϕ 9 \times 3 mm
- Sample Volume : 0.5 - 8 mL
- Elution Volume : 0.5 - 8 mL
- Centrifugation Speed : 1,000 \times g

MonoSpin C18

Octadecyl functional group.

Optimal for drug, extraction in biological samples, and desalting & enrichment of peptide samples.



Examination of Loading Amount

Sample Preparation (Type S)

In this process, same sample volume with different concentration were applied. The concentration of each samples were gradually increased as shown below. The elution obtained in each samples were analyzed by HPLC, and calculated recovery by peak area. Finally, maximum loading amount is estimated by the transition of recovery rate.

- (1) 0.1, 0.5, 1.0, 2.0, 2.5, 4.0, 5.0 mg/mL of Insulin were applied.
 - (2) 0.05, 0.075, 0.10, 0.25, 0.50 mg/mL of BSA were applied.
- Adjusted by 0.1 % TFA solution

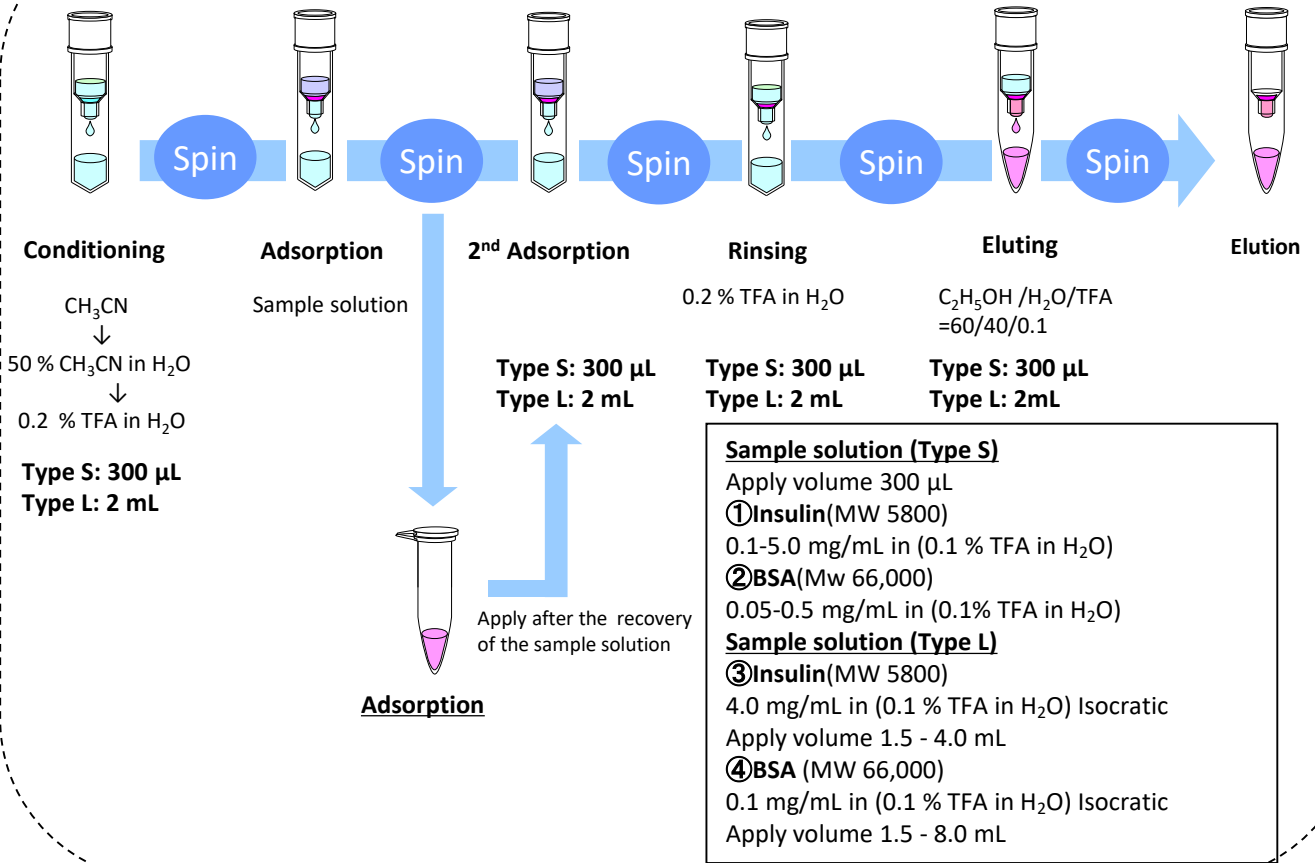
Sample Preparation (Type L)

In this process, the concentration were increased in stages, but sample volume were increased gradually. The sample volume are shown below. The elution obtained in each samples were analyzed by HPLC, and calculated recovery by peak area. Finally, maximum loading amount is estimated by the transition of recovery rate. The sample concentration is following the maximum loading amount estimated in Type S.

- (3) 1.5, 2.0, 3.0, 4.0 mL of Insulin 4.0 mg/mL were applied.
- (4) 1.5, 3.0, 5.0, 8.0 mL of BSA 0.1mg/mL were applied.

Operation of MonoSpin C18

Type S is centrifuged with 2,000xg 30sec.
Type L is centrifuged with 1,000xg 1 min.

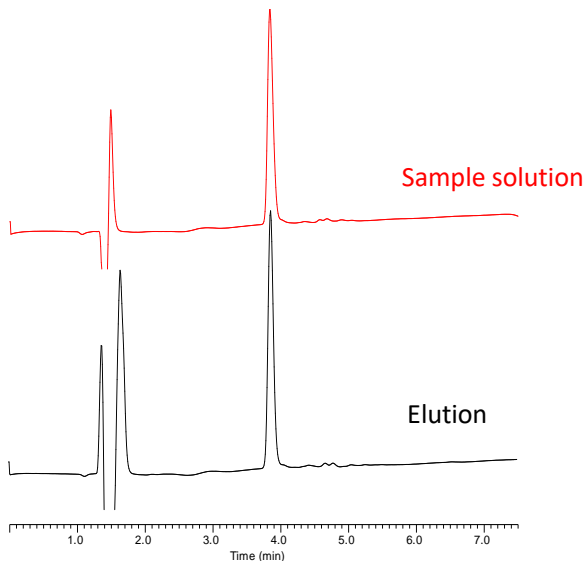


Examination of Recovery Rate

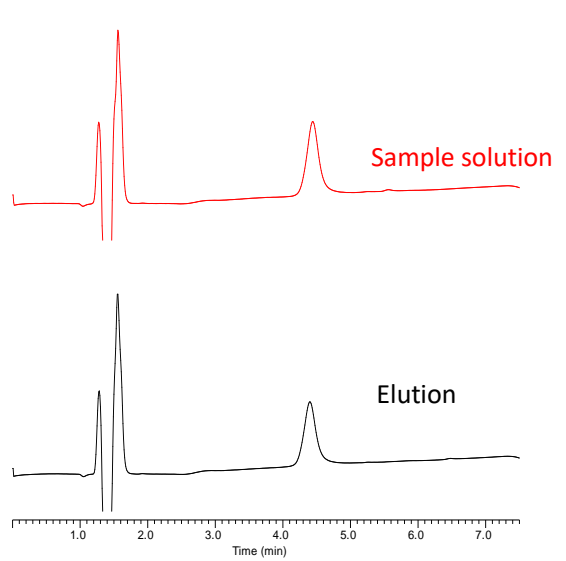
Conditions

Column: InertSustainBio C18 (1.9 µm, 100 × 2.1mmI.D.)
 Eluent: A) 0.1 % TFA in H₂O
 B) 0.1 % TFA in CH₃CN
 A/B=62/38 – 5 min - 50/50 - 0.1 min - 62/38 – 3 min hold, v/v
 Flow rate: 0.2 mL/min
 Col.Temp: 40 °C
 Detection: UV 220 nm
 Injection Vol: 2 µL

① Insulin 0.1 mg/mL



② BSA 0.1 mg/mL



Recovery and loading amount

MonoSpin C18 (Type S)

① Insulin (n=3)

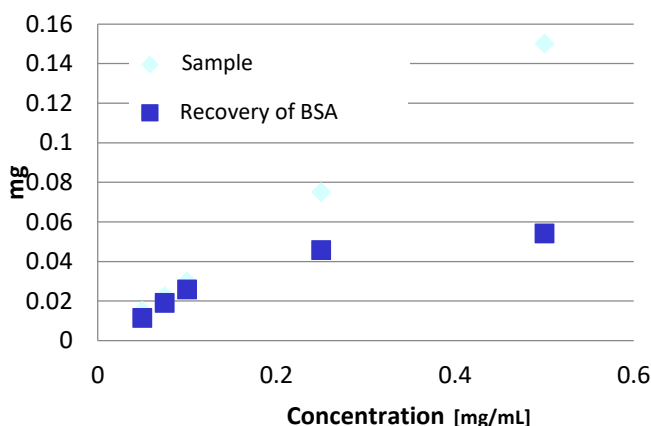
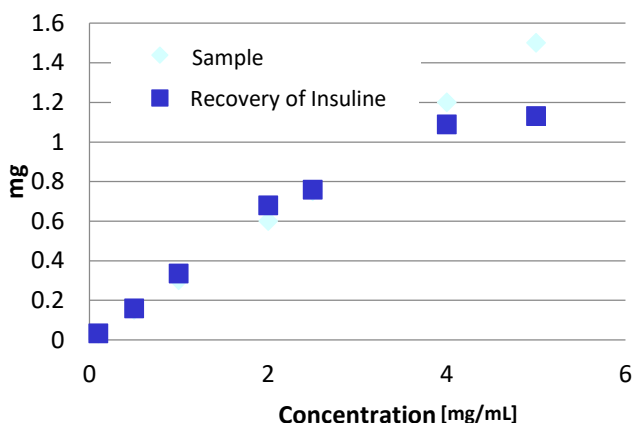
Concentration (mg/mL)	0.10	0.50	1.0	2.0	2.5	4.0	5.0
Recovery rate (%)	105.9	105.8	111.5	113.2	101.2	90.7	75.4
Sample (mg)	0.030	0.15	0.30	0.60	0.75	1.2	1.5
Recovery (mg)	0.032	0.16	0.33	0.68	0.76	1.1	1.1

Preferred reference quantities

② BSA (n=3)

Concentration (mg/mL)	0.05	0.075	0.10	0.25	0.50
Recovery rate (%)	76.7	84.8	86.1	61.1	36.2
Sample (mg)	0.015	0.023	0.030	0.075	0.15
Recovery (mg)	0.012	0.019	0.026	0.046	0.054

Preferred reference quantities



The amount of recovery becomes constant reaching at a certain point. The maximum loading amount shown below is calculated by the amount of sample recovered at the point.

Insuline= 1.2 mg (recovery rate=90.7 %)

BSA=0.03 mg (recovery rate=86.1 %)

MonoSpin C18 (L Type)

③ Insuline (n=3)

Applied Volume (mL)	1.5	2	3	4
Recovery rate (%)	103.6	89.1	66.9	53.4
Sample (mg)	6	8	12	16
Recovery (mg)	6.2	7.1	8.0	8.6

Preferred reference quantities

④ BSA (n=3)

Applied Volume (mL)	1.5	3	5	8
Recovery rate (%)	69.4	68.8	54.3	37.1
Sample (mg)	0.15	0.3	0.5	0.8
Recovery (mg)	0.10	0.21	0.27	0.30

Preferred reference quantities

In MonoSpin C18(L Type), the amount of maximum loading amount are,

Insuline= 8 mg (recovery rate=89.1 %)

BSA=0.3 mg (recovery rate=68.8 %)

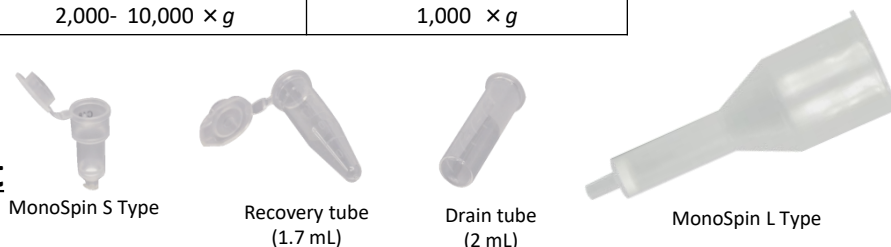
The difference of loading amount between Insuline and BSA arises from the difference in M/W. A compound with small molecular weight can easily get into through-pore and mesopore.

Specification and physical properties of MonoSpinC18

Product Description	Functional Group	S Type		L Type		Surface area (m ² /g)
		Through-pore (μm)	Mesopore (nm)	Through-pore (μm)	Mesopore (nm)	
MonoSpin C18	Octadecyl Groups	5	10	10	10	350

Type	MonoSpin S Type *1	MonoSpin L Type
Disk size	Φ4.2 × 1.5 mm	Φ9 × 3 mm
Sample Volume	up to 800 μL	up to 8 mL
Elution Volume	50 - 800 μL	0.5 - 8 mL
Centrifugation speed	2,000- 10,000 × g	1,000 × g

Product used in this experiment



MonoSpin S Type

Description	Qty.	Cat.No.
MonoSpin C18	50 pcs	5010-21700
	100 pcs	5010-21701

MonoSpin L Type

Description	Qty.	Cat.No.
MonoSpin C18	30 pcs	7510-11320

MonoSpin (S Type) Trial kit, Customization kit

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

Product Description	Available Phases/ Qty.	Cat. No.
MonoSpin Trial kit 1	C18, TiO, SCX, SAX 10 pcs each.	5010-21740
MonoSpin Trial kit 2	C18, Amide, CBA, NH ₂ 10 pcs each.	5010-21741
MonoSpin Trial kit 3	SCX, SAX, CBA, NH ₂ 10 pcs each.	5010-21742
MonoSpin Customization kit 20	2 different kinds, 10 pcs each	5010-01001

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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@glsc.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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