

# Sample Preparation of Peptides and Proteins Using MonoSpin C18 - Obtain High Recovery with Ethanol Elution -

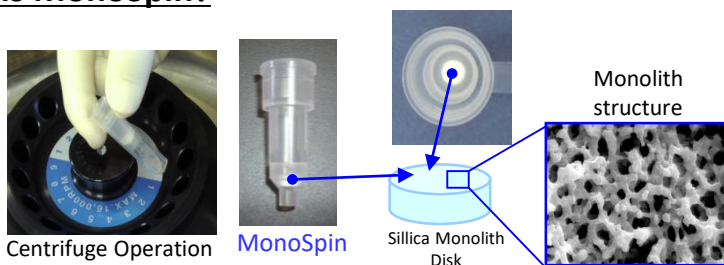
Reversed-phase HPLC is used for the quantitative analysis of proteins. Proteins are separated based on the difference of hydrophobicity. Sample preparation is necessary when analyzing biological samples to reduce the affect from matrix.

Purification of biological samples using revers-phase solid phase extraction requires acetonitrile or methanol contained solvent for analyte elution. However, there is a case high recovery can't be obtained due to a few factors: slow partition, precipitation by high concentration of organic solvent and strong adsorption to packing material.

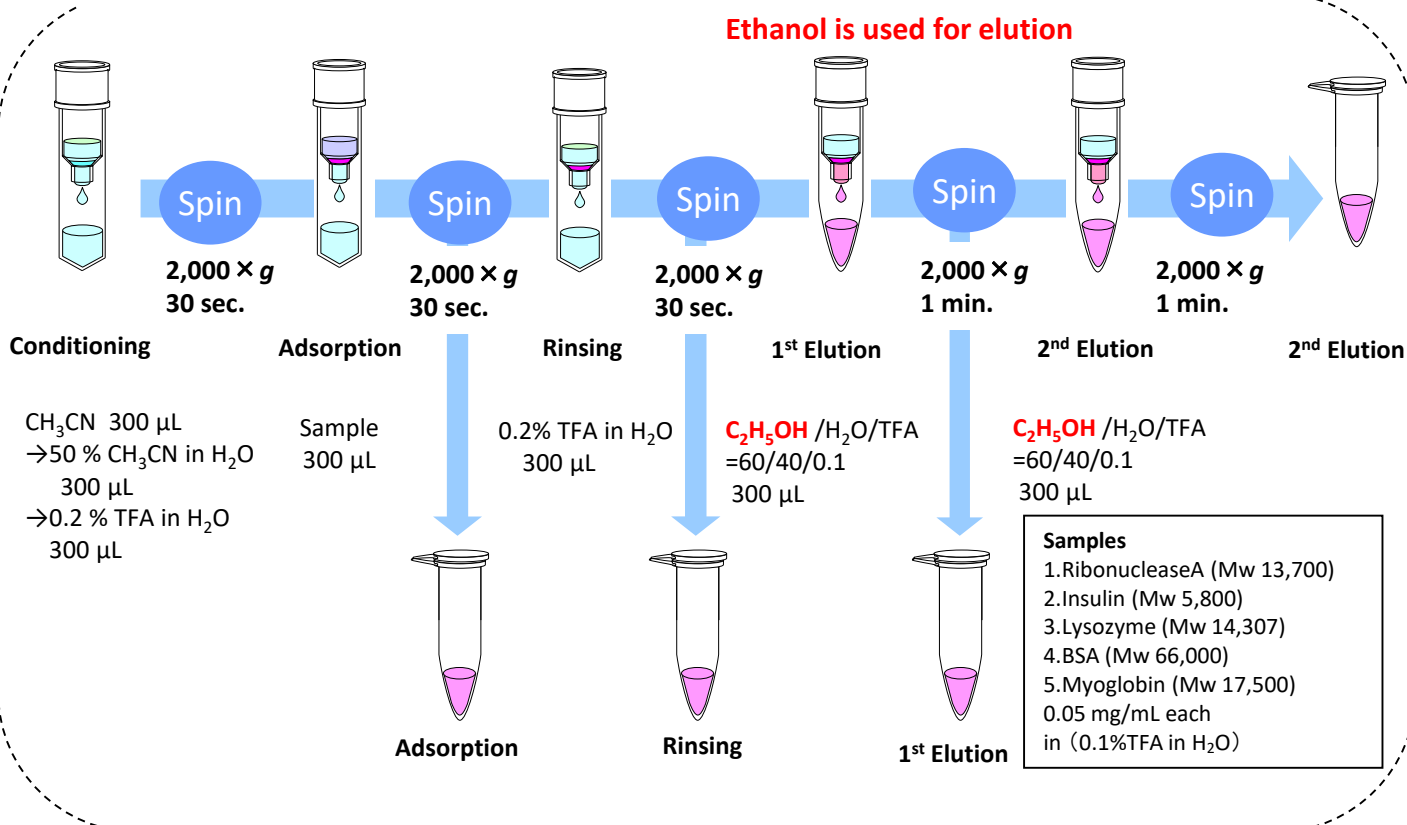
In this note, MonoSpin, uniquely suited for handling small amount samples, is used to purify the proteins. During the process, changes in selectivity and recovery are seen due to the usage of different kind of organic solvent. (Y. Yui, S.Ota)

## What is MonoSpin?

MonoSpin SPE centrifugal spin columns were 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.



## Operation of MonoSpin C18

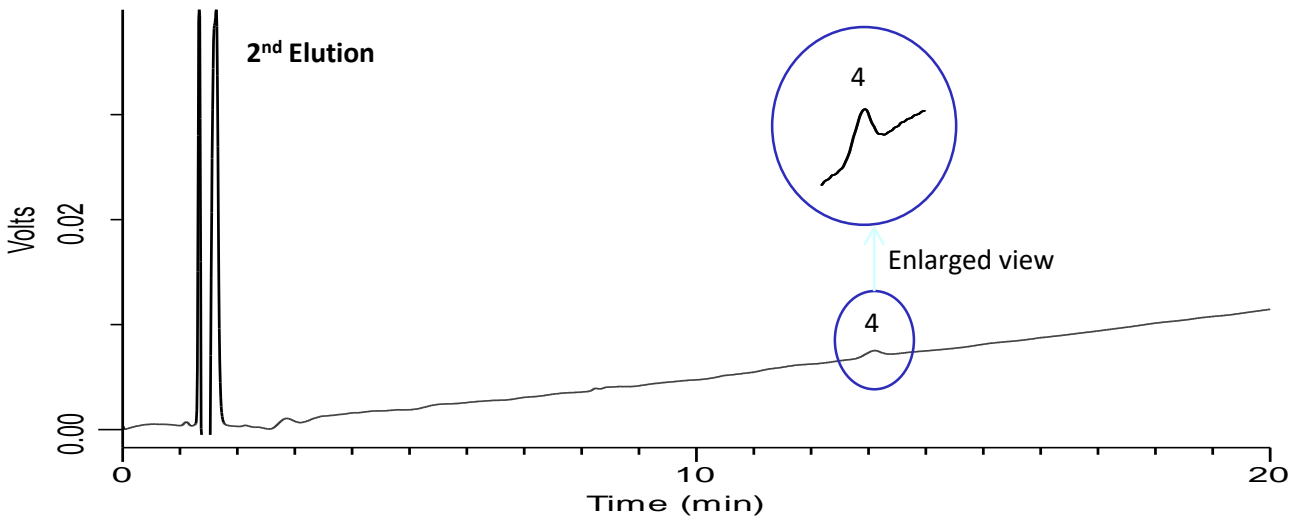
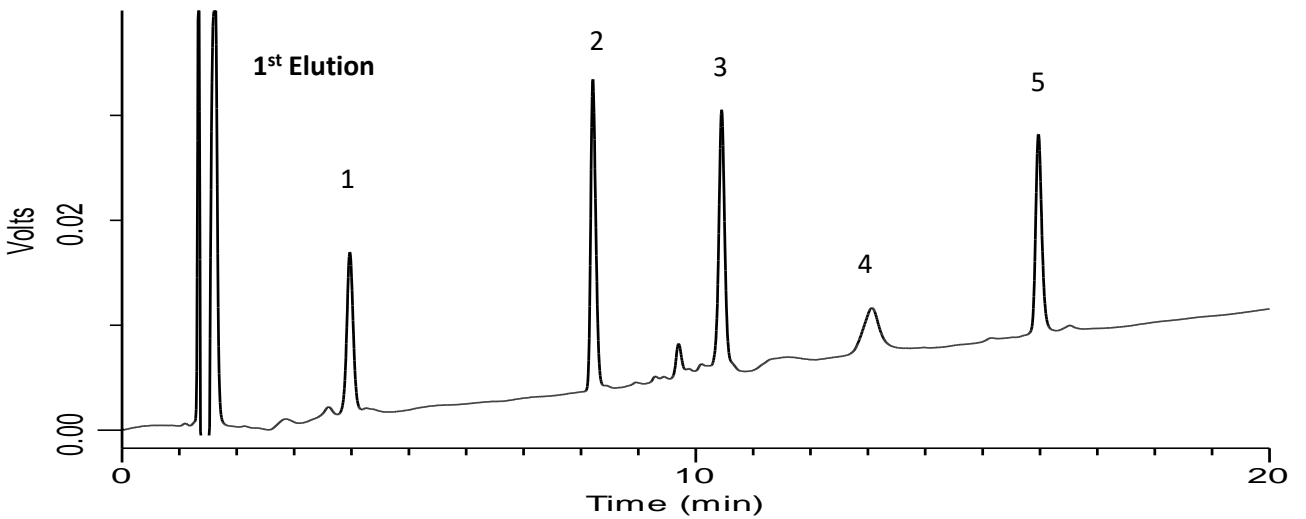
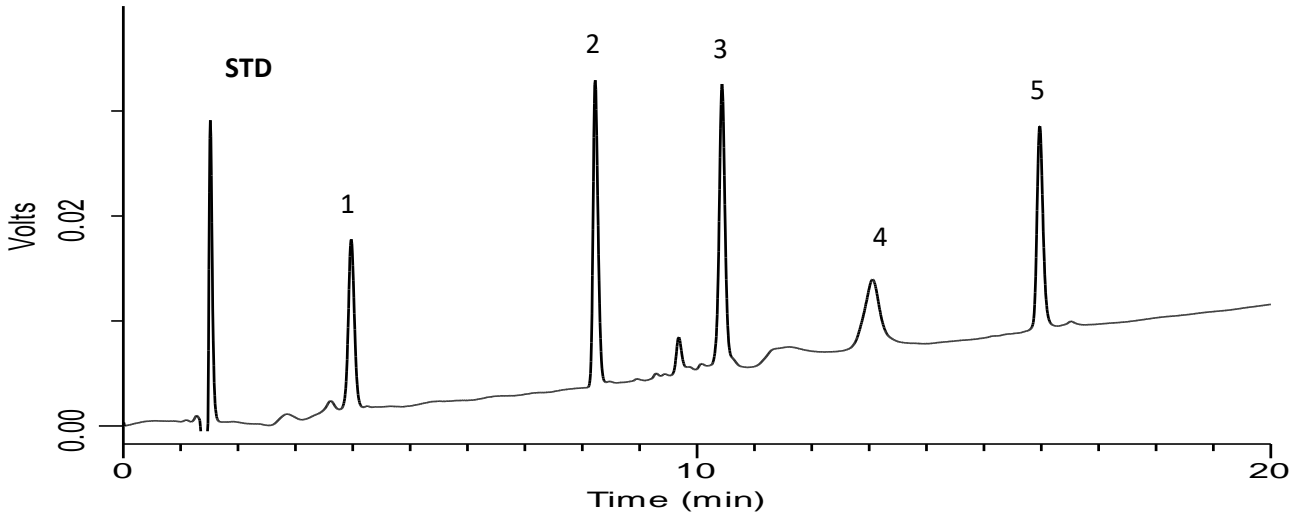


**Chromatogram**

- 1. RibonucleaseA (Mw 13,700)
- 2. Insulin (MW 5,800)
- 3. Lysozyme (MW 14,307)
- 4. BSA (MW 66,000)
- 5. Myoglobin (MW 17,500)

**Conditions**

Column: InertSustainBio C18 (1.9  $\mu$ m, 100  $\times$  2.1 mm I.D.)  
 Eluent: A) 0.1 % TFA in H<sub>2</sub>O  
 B) 0.1 % TFA in CH<sub>3</sub>CN  
 A/B=72/28 - 20min - 45/55 - 0.1 min - 72/28 - 5 min  
 Flow rate: 0.2 mL/min  
 Col.Temp: 40  $^{\circ}$ C  
 Detection: UV 220 nm  
 Injection Vol: 2  $\mu$ L



## Recovery (%)

Recovery ( $n=3$ ) is calculated by the peak area. Only BSA isn't recovered enough compared to other proteins. However, it makes possible to increase recovery by eluting twice.

Purification Process Compounds	Recovery (%)			
	Adsorption	Rinsing	1 <sup>st</sup> Elution	2 <sup>nd</sup> Elution
RibonucleaseA	0	0	<b>101.0</b>	0
Insulin	0	0	<b>104.9</b>	0
Lysozyme	3.6	0	<b>91.8</b>	0
BSA	<b>11.5</b>	0	<b>75.7</b>	<b>7.8</b>
Myoglobin	0	0	<b>99.9</b>	0

Also, poor recovery is seen when eluting with acetonitrile or methanol. Recovery rate changes depending on elution solvent.

## Acetonitrile elution

Acetonitrile is used at 1<sup>st</sup> and 2<sup>nd</sup> elution instead of ethanol.

Purification Process Compounds	Recovery (%)			
	Adsorption	Rinsing	1 <sup>st</sup> Elution	2 <sup>nd</sup> Elution
RibonucleaseA	0	0	<b>12.8</b>	<b>8.4</b>
Insulin	0	0	107.7	2.0
Lysozyme	2.5	0	93.8	8.5
BSA	9.7	0	<b>60.9</b>	<b>6.3</b>
Myoglobin	0	0	97.3	7.8

## Methanol elution

Methanol is used at 1<sup>st</sup> and 2<sup>nd</sup> elution instead of ethanol

Purification Process Compounds	Recovery (%)			
	Adsorption	Rinsing	1 <sup>st</sup> Elution	2 <sup>nd</sup> Elution
RibonucleaseA	0	0	83.2	8.7
Insulin	0	0	94.8	0.9
Lysozyme	4.6	0	<b>0</b>	<b>0</b>
BSA	12.0	0	<b>0</b>	<b>0</b>
Myoglobin	0	0	<b>0</b>	<b>1.3</b>

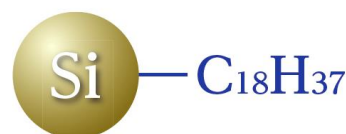
**Products****MonoSpin C18**

Cat. No. 5010-21700 (50 pcs)  
Cat. No. 5010-21701 (100 pcs)

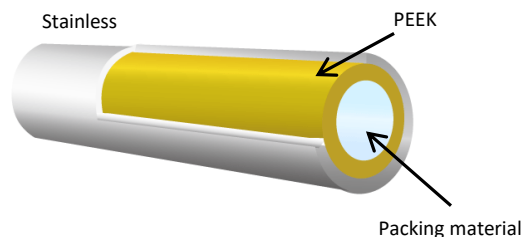
\*We have trial kit for initiating your analysis.

MonoSpin Customize 20:	You may choose 2 kinds of MonoSpin (10 pcs each) Trypsin is an exception Cat. No. 5010-01001
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HPLC • LC/MS Column  
**InertSustainBio C18**

**Rapid Separations of Proteins and Peptides**

Column sectional view

**Features**

- Separation of compounds from small to large molecules
- A radically new Steel-Coated-PEEK hardware preventing adsorption of peaks
- Suitable for analyzing Phosphate compounds

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