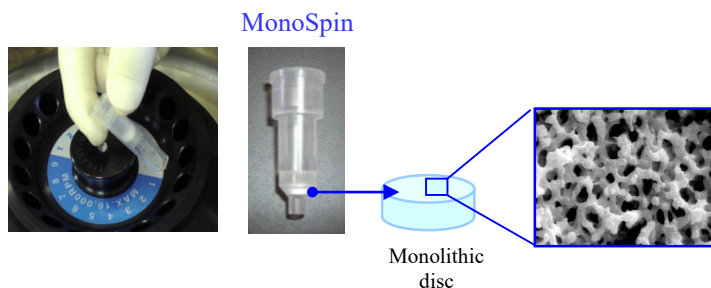


Cyclosporin A (Cyclosporin A) is a type of cyclic polypeptide antibiotic and is used as a medical drug. It is used to suppress the immune system of organ transplant patients, thereby reducing the risk of organ transplant rejection. However, due to the narrow effective therapeutic window and the potential for toxicity, it is necessary to monitor blood drug concentrations. In this study, cyclosporin A was added to human serum, extracted and purified using spin columns MonoSpin C18 and Ph, and analyzed by LC-MS/MS. (T.Kunieda)

1. Pretreatment with MonoSpin

◆ What is MonoSpin?

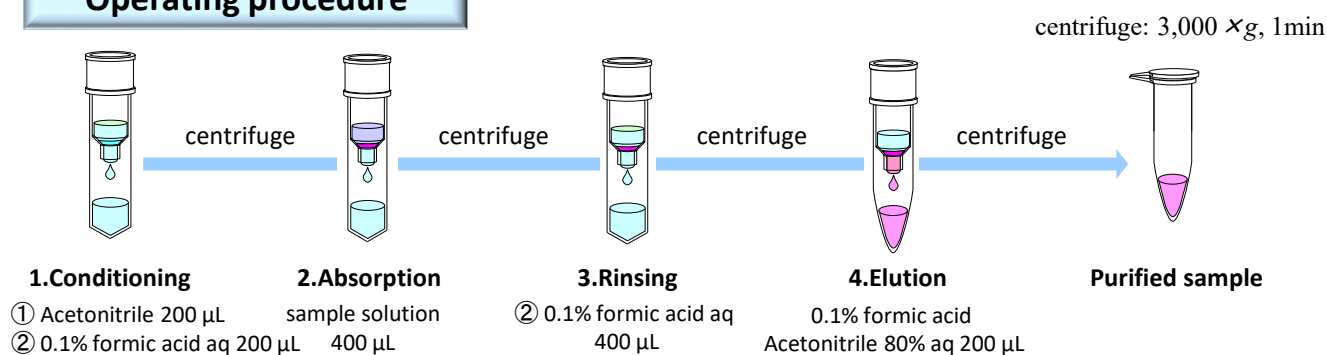
The MonoSpin series is a spin column using a silica monolith with uniform continuous pores. Silica monolith with high porosity is used as a carrier, so liquid can be passed through only by centrifugation. Therefore, it is possible to purify and concentrate the sample with a short and simple operation. In addition, the bed volume is small and the liquid is easily drained, so it is also suitable for small sample volumes.



how to use → <https://youtu.be/YDhZ1Dtwq3k>

application → <https://www.gls.co.jp/technique/lifescience/monospin/application/index.html>

Operating procedure



sample solution

uman serum: 0.1% formic acid: cyclosporin (50% acetonitrile containing 0.1% formic acid) = 200:190:10μL, centrifuged at 10,000 x g for 1 min, and the supernatant was used as the sample solution.

* By removing fine particles by centrifugation in advance, the flow of the spin column will be improved.

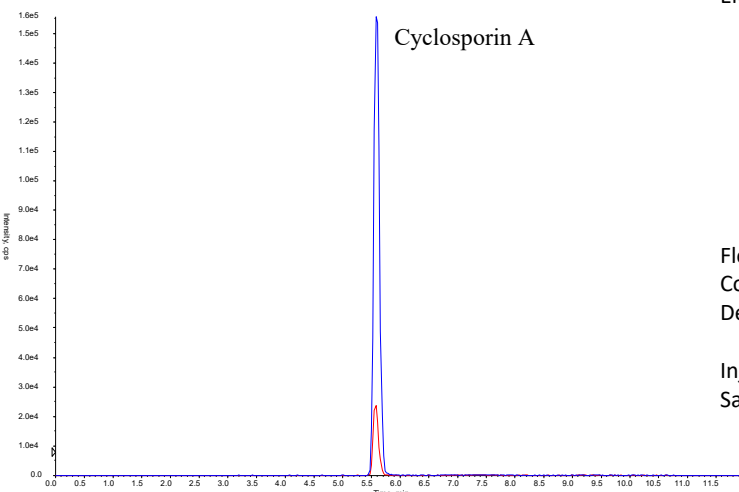
Spike recovery test results

| | | Rec (%) | RSD(%) |
|-----------|-----|---------|--------|
| 0.5 ng/mL | C18 | 94.8 | 2.4 |
| | Ph | 103.8 | 10.0 |
| 5 ng/mL | C18 | 98.6 | 12.1 |
| | Ph | 115.6 | 14.8 |

(0.5,5 ng/mL in sample, n=3 x 1 days)

2.Measurement condition

Chromatogram example of standard solution



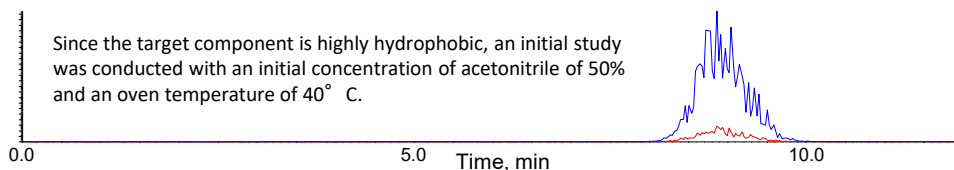
Column : InertSustain Phenyl (GL Sciences Inc.)
 (3 μm, 50 x 2.1 mm I.D.)
 Column Cat.No. : 5020-16433
 Eluent : A) CH₃CN
 B) 0.1% HCOOH in H₂O

| Time (min) | A (vol%) | B (vol%) |
|------------|----------|----------|
| 0.0 | 10 | 90 |
| 7.0 | 90 | 10 |
| 10.0 | 90 | 10 |
| 10.1 | 10 | 90 |
| 13.0 | 10 | 90 |

Flow Rate : 0.4 mL/min
 Col. Temp. : 50 °C
 Detection : MS/MS(SCIEX)
 (QTRAP 6500+: ESI, Positive, SRM)
 Injection Vol. : 5 μL
 Sample : Cyclosporin A (20 ng/mL)
 Q1/Q3 = 1220/1203
 Q1/Q3 = 1220/1185

Examination of LC column and analysis conditions

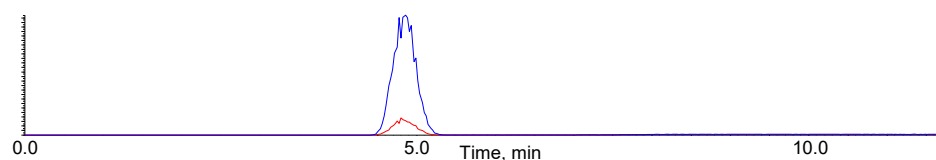
■ InertSustain AQ-C18 (1.9 μm 100 x 2.1 mm I.D.)



Since the retention was strong with the ODS column and the peak was broadened, we investigated with the C8 column.



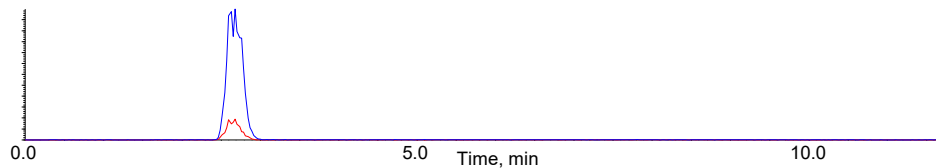
■ InertSustainSwift C8 (3 μm 100 x 3.0 mm I.D.)



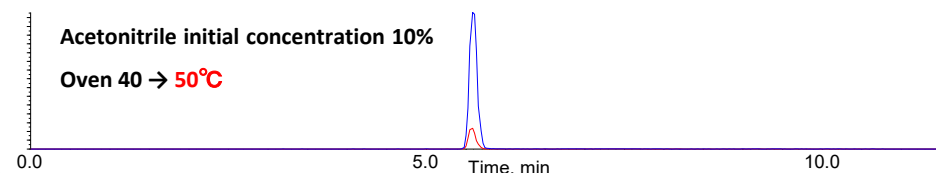
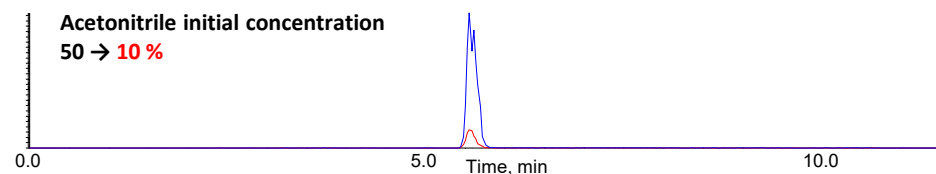
Since the peak width was wide even with the C8 column, we next investigated with the phenyl column.



■ InertSustain Phenyl (3 μm 50 x 2.1 mm I.D.)



The phenyl column gave narrower peak widths, and changing the eluent gradient resulted in sharper peaks. The peak shape was further improved by increasing the column oven temperature to 50°C.



3.Related product

InertSustain Phenyl

Phenyl-bonded columns give a different separation pattern than C18 columns due to π -electron interactions. It is effective as a column of choice when separation is insufficient with a C18 column. In particular, InertSustain Phenyl has a phenyl group directly bonded to silica gel, so it has the characteristic of recognizing the difference in the electronic state of aromatic compounds to a greater extent than a general phenyl column (alkylphenyl group-bonded columns). In addition, since the phenyl group is chemically modified at high density, it has excellent stereoselectivity and exhibits unprecedented separation performance.

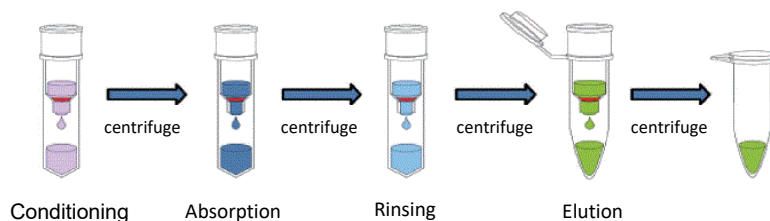


| Column | Size | Cat.No. |
|---------------------|-----------------------------|------------|
| InertSustain Phenyl | 3 μ m, 50 x 2.1 mm I.D. | 5020-16433 |

MonoSpin(Solid-phase extraction column for minute samples)

MonoSpin is a pretreatment spin column that uses a silica monolith with uniform continuous pores. All operations can be performed with a short centrifugation process.

The S type is ideal for samples up to 800 μ L, the L type is ideal for larger 1-8 mL samples, and the 96-well plate type is available for multi-sample processing.



MonoSpin C18

A column with octadecyl groups and reversed-phase partitioning interaction.

Ideal for extracting drugs from biological samples and desalting/concentrating peptide samples.

| Product name | Quantity | Cat.No. |
|--------------|----------|------------|
| MonoSpin C18 | 50 | 5010-21700 |



MonoSpin S type

MonoSpin Ph

Reversed-phase mode type with chemically modified phenyl group. It is possible to perform pretreatment with higher selectivity by utilizing the weaker hydrophobic interaction than C18. Suitable for recovering hydrophobic drugs from biological samples.

| Product name | Quantity | Cat.No. |
|--------------|----------|------------|
| MonoSpin Ph | 50 | 5010-21733 |

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