# Analysis of Paraquat and Diquat in Urine

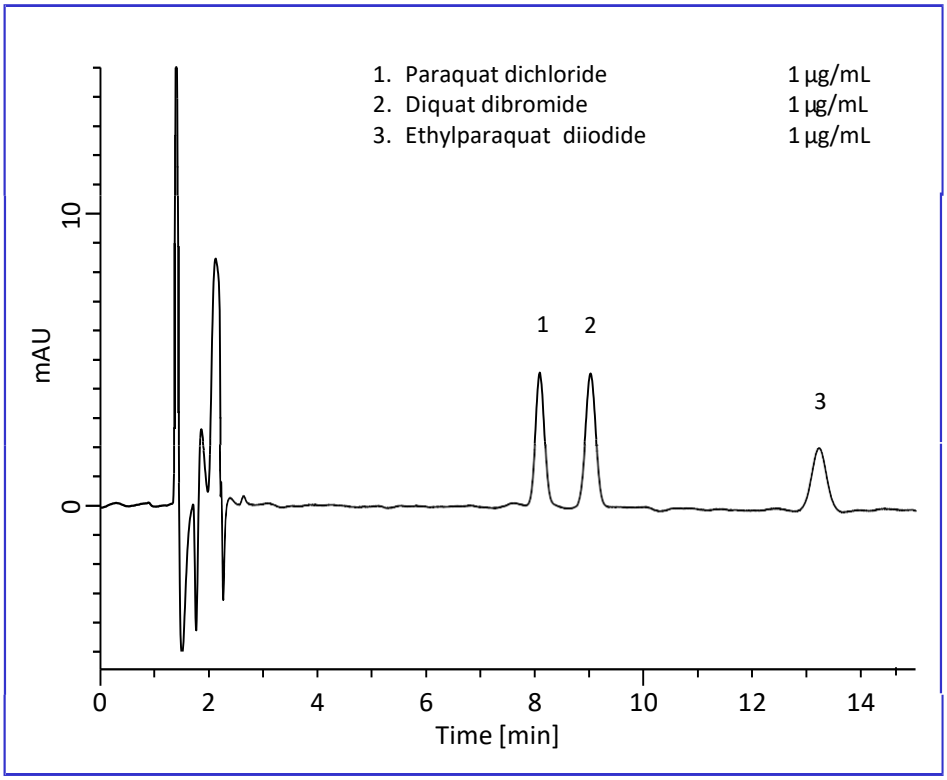
In this note, a determination method for paraquat and diquat using HPLC is described. Paraquat and diquat, which are quaternary ammonium herbicide widely used in the world, are also toxic to human being. Their concentration in urine or serum after ingestion is determined for accurate prognosis.

To determine the concentration in physiological sample, purification by solid-phase extraction is required before HPLC analysis. However, it is difficult to obtain good recovery with C18 cartridge because alkaline solution, in which paraquat and diquat is unstable, should be used to retain the compounds to the solid-phase.

Strong cation- exchange type is also not desirable because solution containing high concentration of salt has to be used for elution from the solid-phase. If the eluted sample is injected into an HPLC system without any dilution or desalting, peak shape often gets worse owing to the salt. In this note, MonoSpin PBA, which has carboxylate group for weak cation-exchange mode, was used. As a result, excellent recoveries were obtained from urinary sample with simple procedures.

(S. Ota)

## A Chromatogram Obtained from Standard Solution

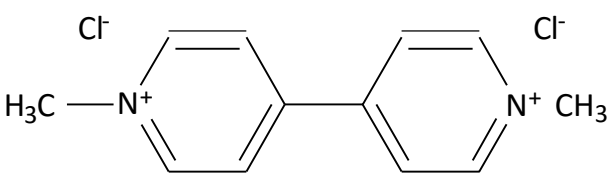



**Conditions**

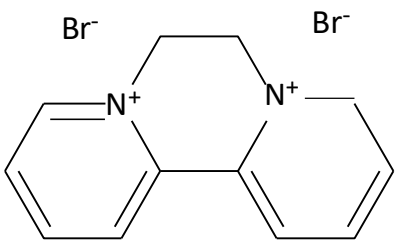
| | |
|---|---|
| **Column** | : Inertsil ODS-3 (5 µm, 150 x 4.6 mm I.D.) |
| **Eluent** | : A) Phosphate buffer * B) $CH_3CN$ A/B = 89/11, v/v |
| **Flow rate** | : 1.0 mL/min |
| **Temp.** | : 40 ℃ |
| **Detection** | : UV 290 nm |
| **Inj. Vol.** | : 50 µL |

* Phosphate buffer ; Phosphoric acid (200 mM), diethylamine (100 mM), and sodium 1-octanesulfonate (7.5 mM) was dissolved in water.

## Chemical Structures




Paraquat



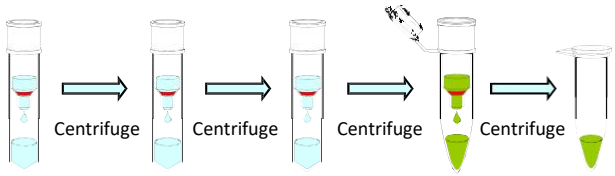

Diquat

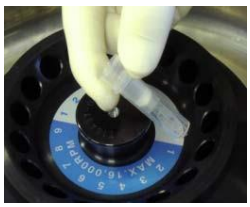
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

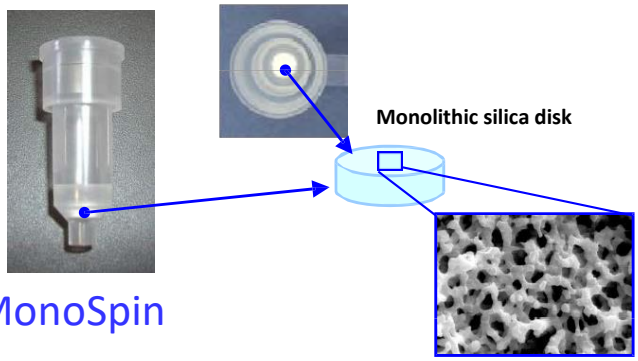
## What is MonoSpin ?

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.

An example of procedures



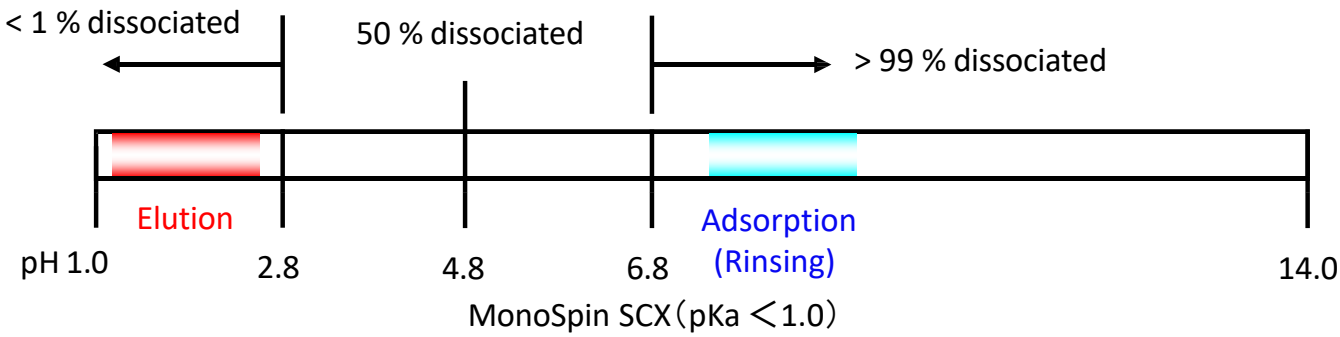



Enlarged view of monolithic silica

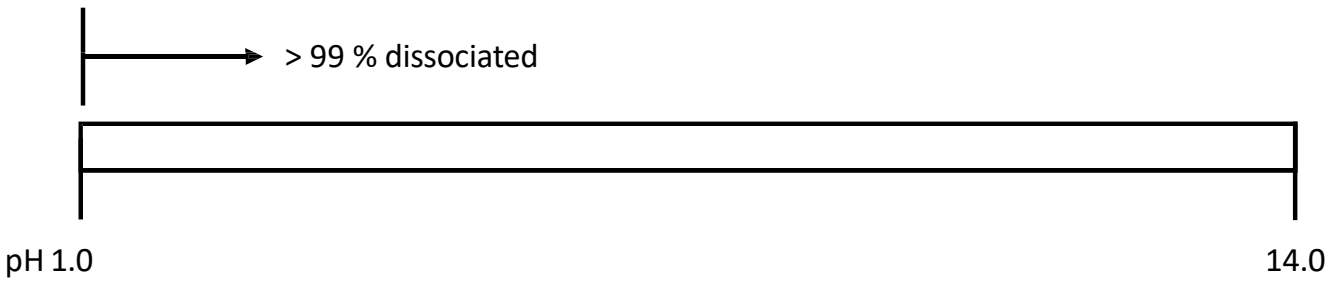
## Background of pretreatment using MonoSpin CBA

MonoSpin CBA has carboxylate group (COOH). This functional group is a typical weak acid, and its ratio of COO- anion changes depending on the pH of solvent passing through. When cationic paraquat and diquat are loaded onto MonoSpin PBA, basic solvent should be passed through to dissociate carboxylate group. In contrast, strong acid solvent is used for elution to inhibit dissociation of COOH group.
In case of MonoSpin SCX, its functional group is almost all dissociated even at pH 1. Therefore, elution of paraquat and diquat from MonoSpin CBA is much easier than that from MonoSpin SCX.

**MonoSpin CBA (pKa ≒ 4.8)**

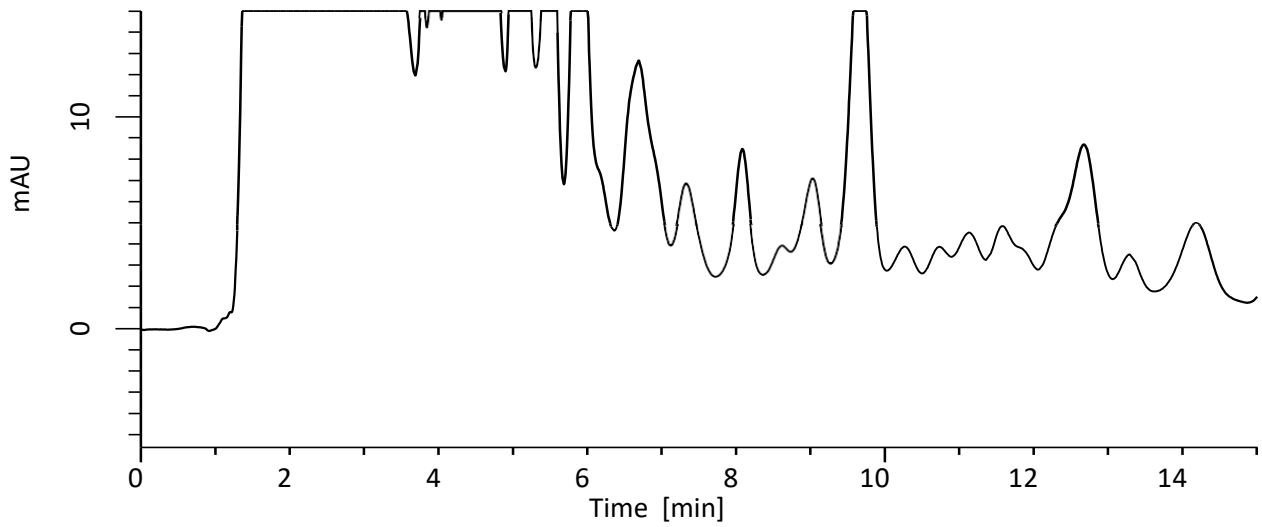



MonoSpin SCX（pKa ＜1.0）

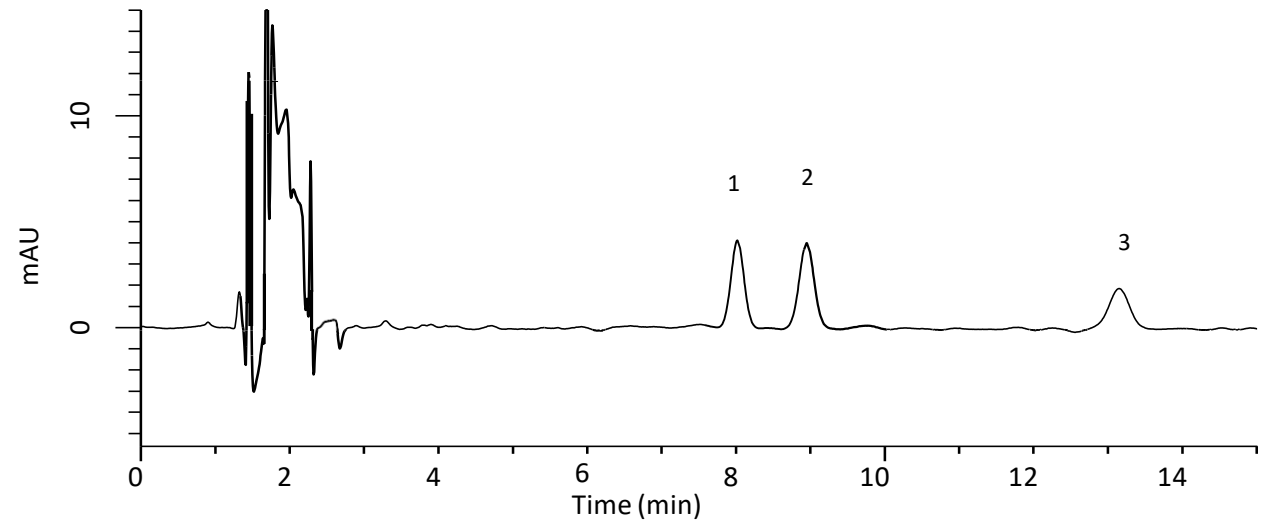


## Chromatograms obtained from urinary sample (1 □g/mL each standard addition)

＜Before purification with MonoSpin CBA＞




＜After purification with MonoSpin CBA＞




### Procedures for purification of paraquat and diquat

Preparation of buffer :
Buffer A : 10 mM Phosphate buffer (pH 7.0) Hydrochloric
Buffer B : acid : Methanol : Water = 1 : 30 : 69

Attach the spin column to tube for waste fluid
↓ + Buffer A 200 µL
Centrifuge*
↓ + Buffer A 400 µL
↓ + Sample 200 µL
Centrifuge
↓ + Buffer A 200 µL
Centrifuge
↓
(to top right)

(from bottom left)
↓
Put the spin column into collection tube
↓ + Buffer B 200 µL
Centrifuge
↓
Collected solution was injected into HPLC system

* Centrifugation was carried out at 10,000 g for 30 seconds.

## Validation of this method

Excellent recovery was obtained because strong basic solution, under which the compounds are unstable, was not used. MonoSpin series is also suitable for pretreatment of basic compound adsorptive to glass because each tube used is made of plastic.

| | Concentration (µg/mL) | Intra-day (*n*=5) Recovery (%) | RSD (%) | Inter-day (*n*=3) Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Paraquat | 1.0 | 97.5 | 6.5 | 95.4 | 2.6 |
| | 0.1 | 99.1 | 3.5 | 95.4 | 3.3 |
| Diquat | 1.0 | 94.3 | 8.9 | 97.4 | 3.0 |
| | 0.1 | 98.7 | 3.6 | 97.7 | 3.1 |
| Ethylparaquat | 1.0 | 88.0 | 5.9 | 89.8 | 6.0 |
| | 0.1 | 101.6 | 3.2 | 97.3 | 4.0 |

The spin column used in this note;

MonoSpin CBA 50/pk

Cat.No. 5010-21729

MonoSpin CBA 100/pk

Cat.No. 5010-21730

Detailed instructions for using MonoSpin spin columns can be viewed in the following link.
http://www.youtube.com/watch?v=uVh0Bw8QiGg

The HPLC column used in this note; Inertsil ODS-3 (5 µm, 4.6 x 150 mm)

Cat.No. 5020-01731



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