

Analysis of Catecholamines in Urine with HPLC-ECD

Catecholamines play an important role as neurotransmitter and adrenal gland hormones in the human body. Concentration of three major catecholamines (norepinephrine, epinephrine, and dopamine) in urine are significant in diagnosis and treatment of several diseases.

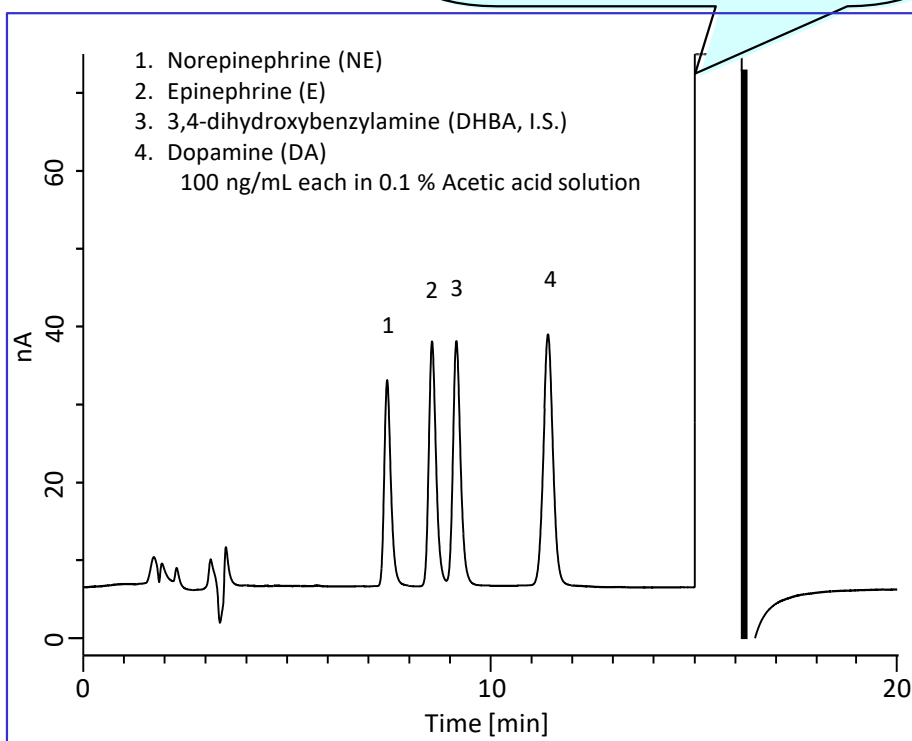
In this note, a determination method for urinary catecholamines using HPLC-ECD (Electrochemical detector) and MonoSpin PBA, which is a spin column

for sample pretreatment, is described. The advantage of this method is not only its simplicity but also high sensitivity and selectivity. Furthermore, this method is quite easy-to-use because cleaning of conductive diamond electrode used as the working electrode can be performed routinely without removing it from the flow cell. Manual polishing of the working electrode is not necessary at all.

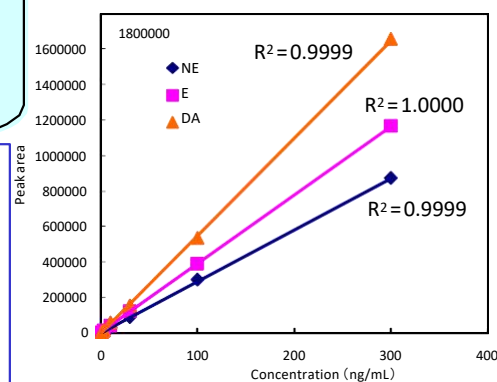
(C.Aoyama)

A Chromatogram Obtained from Standard Solution

To keep the working electrode clean, high oxidation potential (+ 4000 mV) was applied between 15 min and 16 min after every sample injection. This convenient automated cleaning can be carried out owing to the extreme stability of the boron-doped diamond electrode.



Calibration curves for three catecholamines

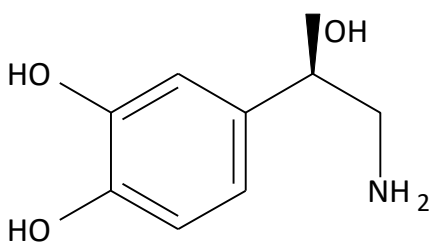


HPLC Conditions

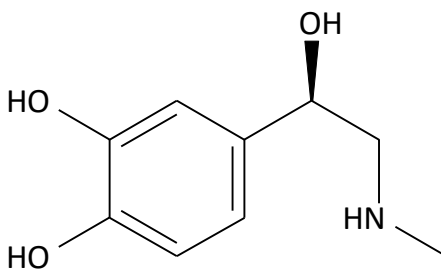
Column : Inertsil ODS-4
(5 μ m, 250 \times 3.0 mm I.D.)
Eluent : A) Acetate-citrate buffer*
B) CH₃CN
A/B = 100/16, v/v (Premix)
Flow rate : 0.5 mL/min
Col. Temp. : 35 $^{\circ}$ C
Detection : ECD 800 mV vs. Ag/AgCl
(ED703, Diamond)
Inj. Vol. : 20 μ L

* Acetate-citrate buffer :
To 500 mL of water, 0.82 g of sodium acetate (anhydrous),
2.10 g of citric acid (monohydrate), and 0.5 g of sodium 1-octanesulfonate was dissolved.

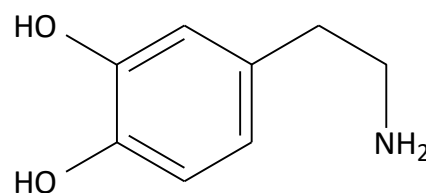
Chemical Structure



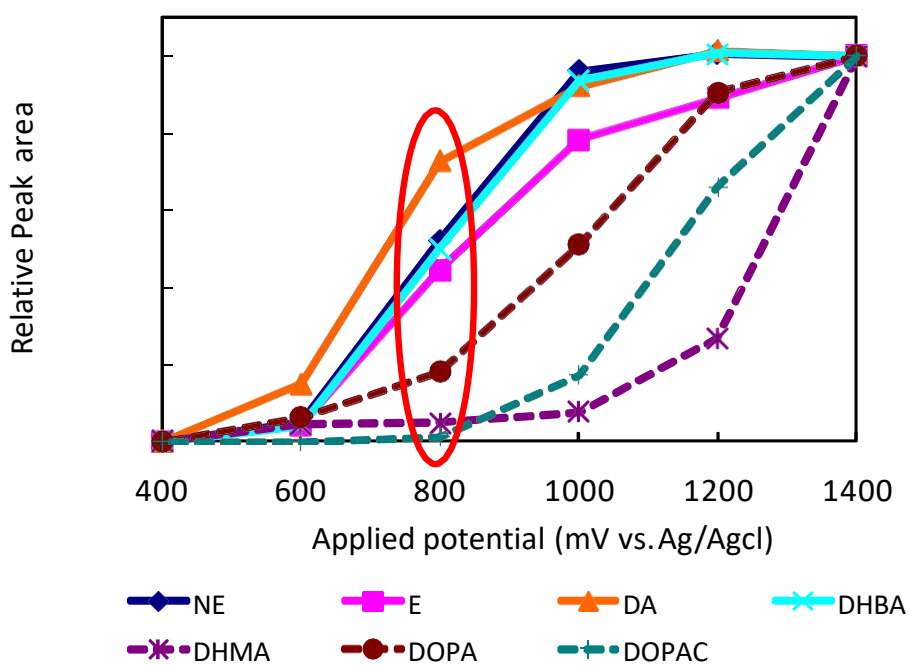
Norepinephrine (NE)
or noradrenaline



Epinephrine (E)
or adrenaline

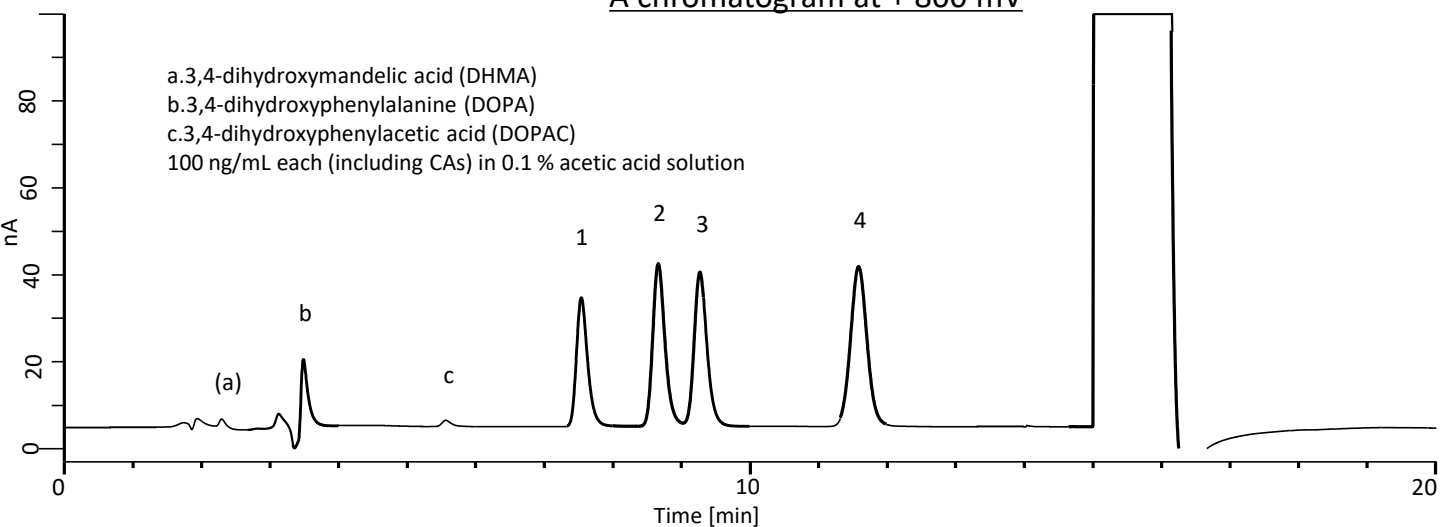


Dopamine (DA)

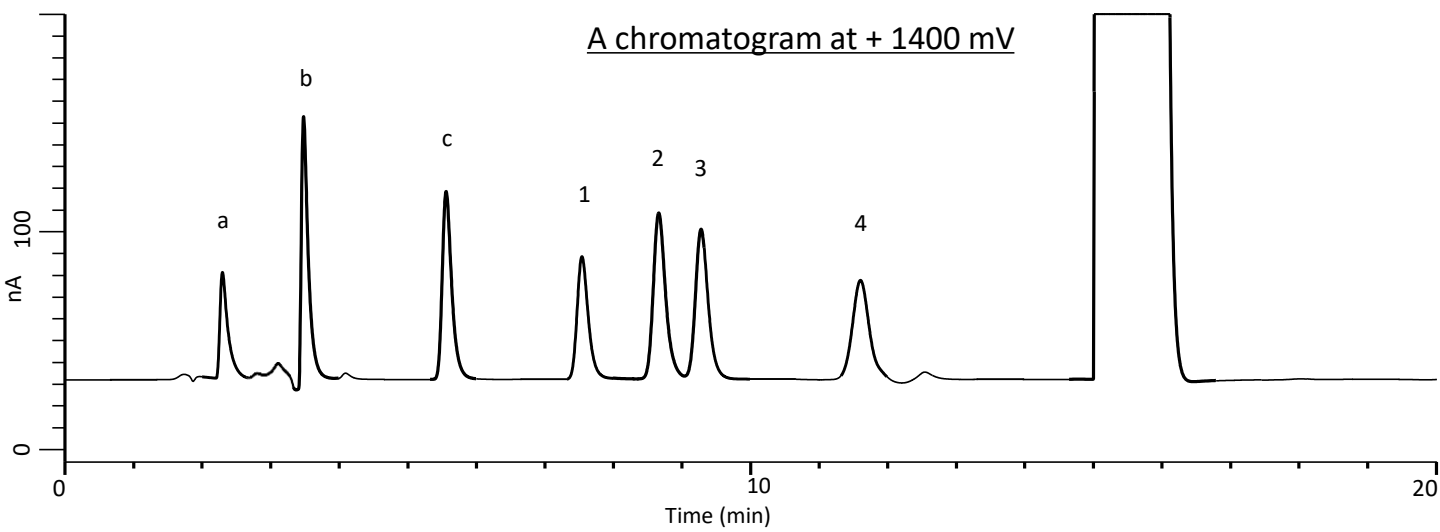


Setting the potential at + 800 mV enables us to diminish peaks of other compounds in sample solution and to detect catecholamines with high selectivity.

A chromatogram at + 800 mV



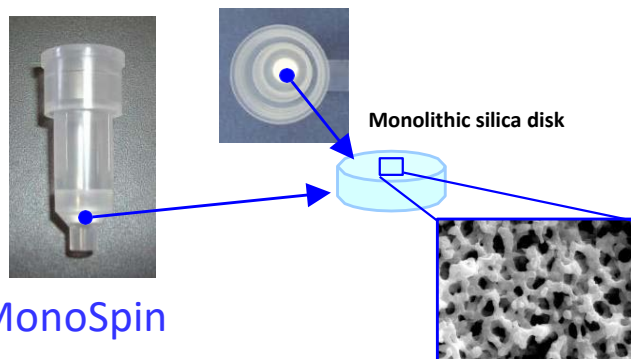
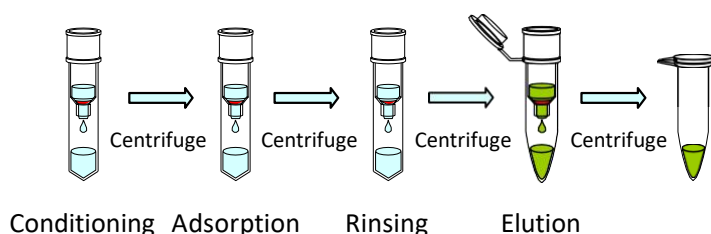
A chromatogram at + 1400 mV



Pretreatment of urinary sample using MonoSpin PBA

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.

An Example of Procedures



Preparation of Sample and Buffer

Urine sample: Add DHBA solution as internal standard in advance. Buffer A: 1 % acetic acid aqueous solution

Buffer B : 100 mM di-potassium hydrogen aqueous solution
(The pH was adjusted to 8.0 by adding phosphoric acid)

Buffer C : 1 M di-potassium hydrogen aqueous solution
(The pH was adjusted to 8.0 by adding phosphoric acid)

Procedures for purification of catecholamines

Attach the spin column to tube for waste fluid

↓ + Buffer A 200 μL Centrifuge at 10,000 g for 1 min

↓ + Buffer B 200 μL Centrifuge at 10,000 g for 1 min

↓ + Urine sample 200 μL

↓ + Buffer C 50 μL Centrifuge at 10,000 g for 1 min

↓ + Buffer B 200 μL Centrifuge at 10,000 g for 1 min

Put the spin column into collection tube

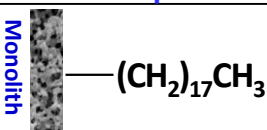
↓ + Buffer A 200 μL Centrifuge at 10,000 g for 1 min

↓

Collected solution was injected into HPLC system

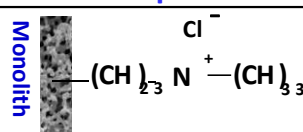
The series of MonoSpin

MonoSpin C18



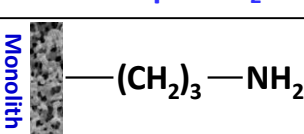
Octadecyl group is chemically bonded, and non-polar compounds can be retained because of its hydrophobic interaction. It can be used for extraction or desalting.

MonoSpin SAX



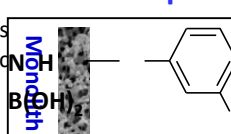
Trimethylaminopropyl group is bonded. It offers strong anion-exchange and weak hydrophobic interaction. It is suitable for extraction of acidic drugs.

MonoSpin NH₂



Aminopropyl group is bonded. It is suitable for extraction of hydrophilic compounds, such as sugar chain.

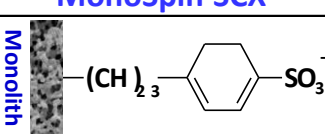
MonoSpin PBA



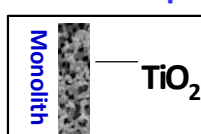
(used in this note)

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

MonoSpin TiO



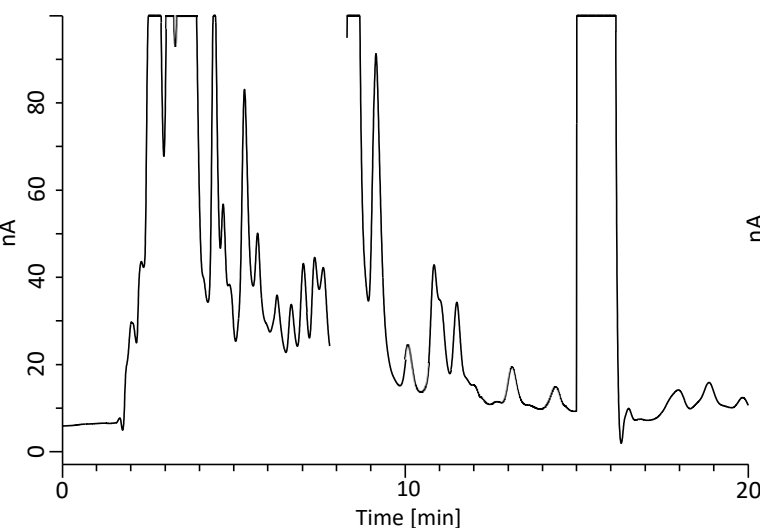
Propylbenzenesulfonic acid is bonded. It offers strong cation-exchange and hydrophobic interaction. It is suitable for extraction of basic drugs.



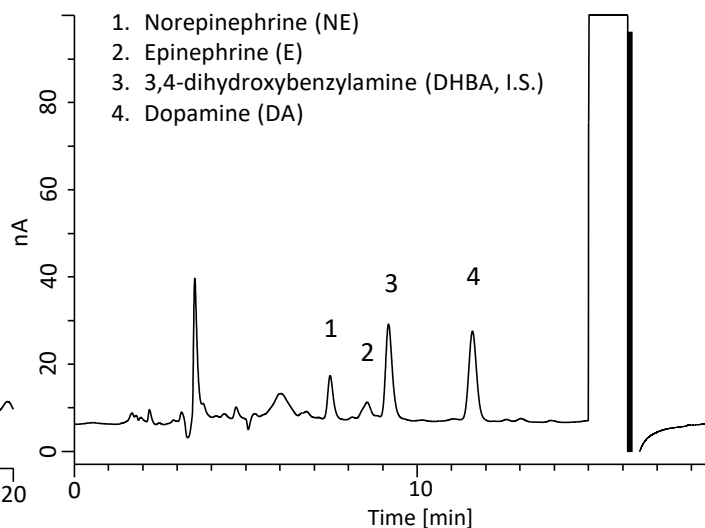
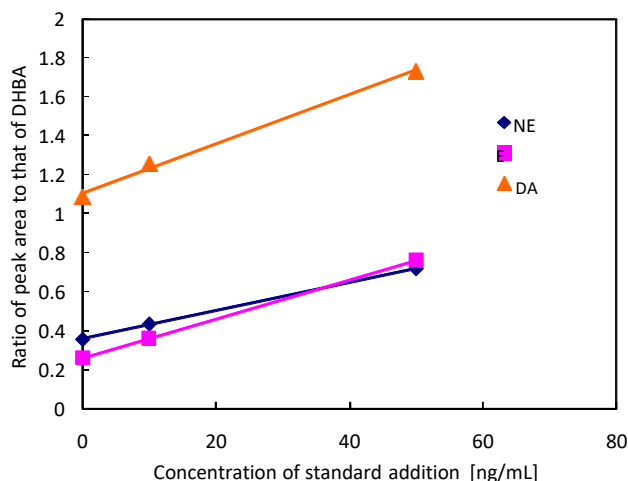
Monolithic silica is coated with titanium dioxide. It is suitable for extraction of phosphate-containing compounds.

Without any pretreatment

Many interfering peaks were detected.

After pretreatment using MonoSpin PBA

Almost all interfering peaks were eliminated by the purification.
The recovery ration of DHBA was 96.4 %.

A standard addition plot

Coefficient of variation Obtained concentration of
peak area ($n=4$) [ng/mL]

NE	2.4 %	49.0
E	6.9 %	25.9
DA	0.8 %	85.5

* We are grateful to Dr. Makoto Tsunoda (University of Tokyo) for his valuable suggestion and discussion.

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