Monoamine Analysis in Tissue Homogenate

AM04-0418

Analytical Condition

Summary

Column	Eicompak SC-30DS (ID 3.0 x 100 mm)
Precolumn	AC-ODS packing material in ID 3.0 x 4.0 mm
Flow Rate	340-400 µl/min
Working Electrode	Graphite, WE-3G (Gasket GS-25)
Applied Potential	+750 mV vs. Ag/AGCl
Temperature	25ºC

Mobile Phase

80% 0.1 M citrate-acetate buffer (pH 3.5)

20% methanol

Include 220 mg/l sodium octane sulfonate (SOS) and 5 mg/l EDTA-2Na

Preparation of the Mobile Phase

1. Dissolve 8.84 g citric acid monohydrate (FW 210.14) and 3.10 g sodium acetate unhydrate (FW 82.03) in 800 ml of ultrapure water (>18.2 MΩ/cm). This gives Sol. 1.
2. Measure 200 ml of HPLC grade methanol separately. (Do not add the methanol directly to the solution as methanol dissolves into the water.) Add the 200 ml of methanol to Sol. 1.
3. Add 220 mg Sodium Octane Sulfonate (SOS) and 5 mg EDTA-2Na.
4. Keep this solution in a glass bottle. Shake well. DO NOT refill into used glassware.

Quality and Function of Reagents in the Mobile Phase

MilliQ water

Use fresh MilliQ water (>18.2 MΩ/cm resistance). Do not store this in a plastic bottle. Do not use distilled water or double distilled water.

Methanol

Please use HPLC grade methanol. A higher methanol level reduces the retention time of each compound. To short a retention time results in low resolution from peak to peak. We recommend Wako methanol for LC/MS (code 134-14523).

SOS, Sodium Octane Sulfonate

Please use Nacalai Tesque (code 31729-62). This reagent is used for increasing the retention time of amines specifically. SOS forms an ion pair with $-NH_2$ at $-SO_3$. The octyl group in SOS partitions to ODS on the column. SOS enhances elution of cations but most of the -COOH is not ionized at pH 3.5. In other words, SOS does not affect non-amine compounds at pH3.5.

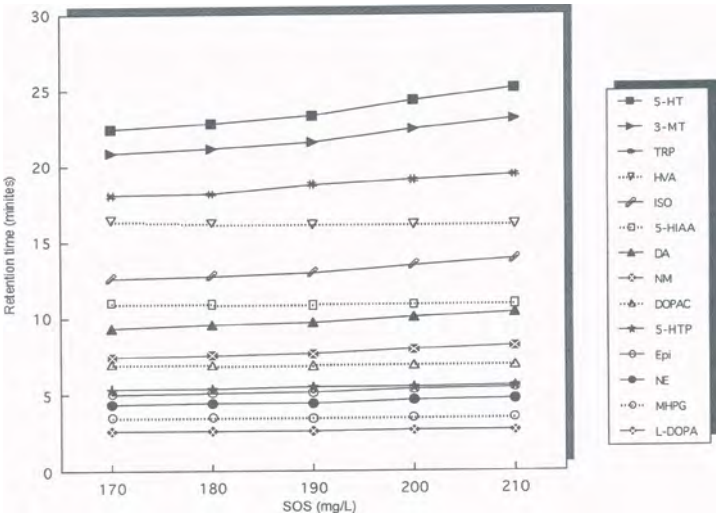

Fig. 1. **SOS Concentration in Mobile Phase and Retention Time** The amine groups retention time is prolonged by a higher SOS level. This data was obtained from conditions using SC-5ODS (5 µm particle ODS) and not by SC-3ODS

EDTA-2Na

This reagent is used to form metal chelates with metal ions in columns. If EDTA-2Na is not present in the mobile phase, the background current will increase. Please do not add too much EDTA-2Na because it is ECD active and can also raise the background current.

Standard Samples

Catecholamines are stable in acids such as hydrochloric acid. Indoleamines are stable at pH 3 but hydrolyzed by strong acids and are light sensitive.

Prepare the Following Solutions

Solution A	0.1 M acetic acid including 1 mg/ml EDTA-2Na
Solution B	0.1 M hydrochloric acid including 1 mg/ml EDTA-2Na
Solution C	0.02 M acetic acid including 10 µM EDTA-2Na

Stock 1s, 100 ng/µl

Prepare 100 ng/µl for each compound (100 ng/µl = 10 mg/100 ml). For chloride or other salts, the real concentration of your target substance can be calculated by the following:

Desired Weight × Molecular Weight of Salt / Molecular Weight of Free = Dissolving Weight

Examples

10 mg MHPG:	10 mg × 454.52/368.38 = 12.34 mg MHPG-piperazine
10 mg DA:	10 mg × 189.64/153.18 = 12.38 mg DA-HCl
10 mg 5-HT:	10 mg × 387.4/176.22 = 23.01 mg 5-HT-creatine sulfate

Dilute 5-HT and 5-HIAA salts in separate containers of 100 ml of Solution A to make 10 mg/100 ml. Dilute the other salts in Solution B. Different compounds should not be mixed together. Store at 4°C. You may find EDTA precipitation and if so use the supernatant.

Stock 2s, 1 ng/µl

Use Solution A to dilute each compounds Stock 1s at a ratio of 1:100 to make 1 ng/µl of a standard mix solution.

If you have separation problems, it may be helpful to make 2 types of mix solutions; an amine solution and a non-amine solution.

Final Solution

Before injection, dilute Stock 2s to the desired concentration using Solution C. It is normal to see separation at a concentration of 100 pg/µl.

Sample Preparation

1. Dissect the sample area from the animal and weight.
2. Add 0.5 ml of 0.2 M perchloric acid to the sample per 100 mg wet tissue.
3. Add 100 ng isoproterenol /100 mg wet tissue as an internal standard (Using 1 ng/µl ISO in Solution A is preferable).
4. Homogenize at a constant speed and duration.
5. Denature the protein by keeping the homogenate in an ice bath for 30 min.
6. Spin at 20,000 G for 15 min at 4°C.
7. Remove the supernatant.
8. Modify the pH of the supernatant to become pH3.0 by using 1 M sodium acetate.
9. Filter using a 0.45 µm syringe filter (after filtering, please store at -80°C if it is required).
10. Analyze by HPLC.

Sample Chromatograms

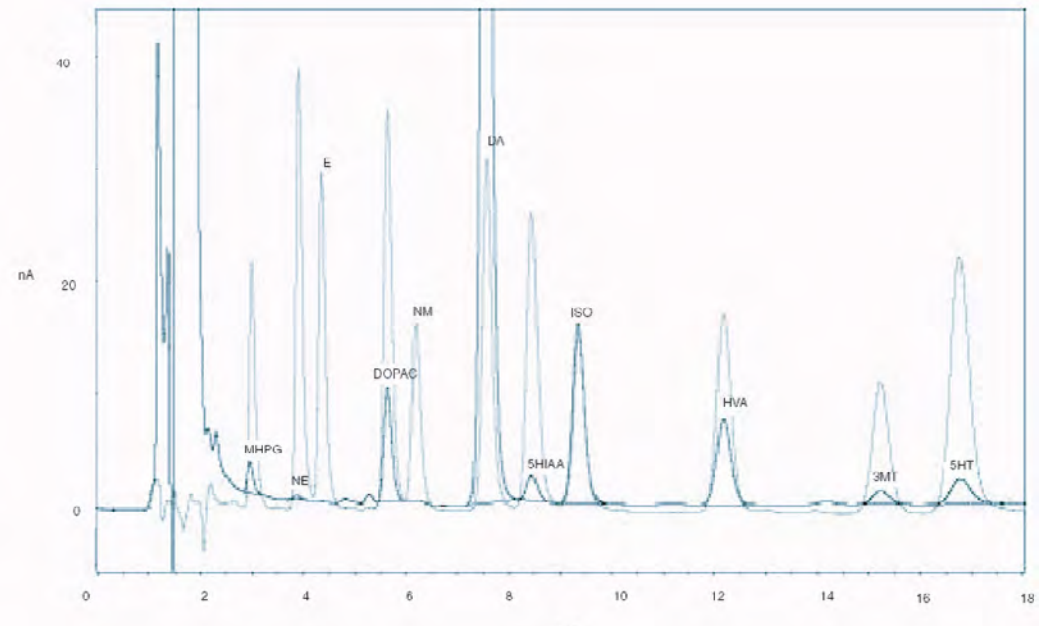

Fig. 2. The chromatogram drawn in black is obtained from the homogenate of a rat striatum.
The grey chromatogram is from a standard solution at a concentration of 1 ng on the column (40 nA = 400 mV).

Sample chromatograms obtained from four different brain regions.

(All of these data were obtained at different conditions to those described in Analytical Condition.)

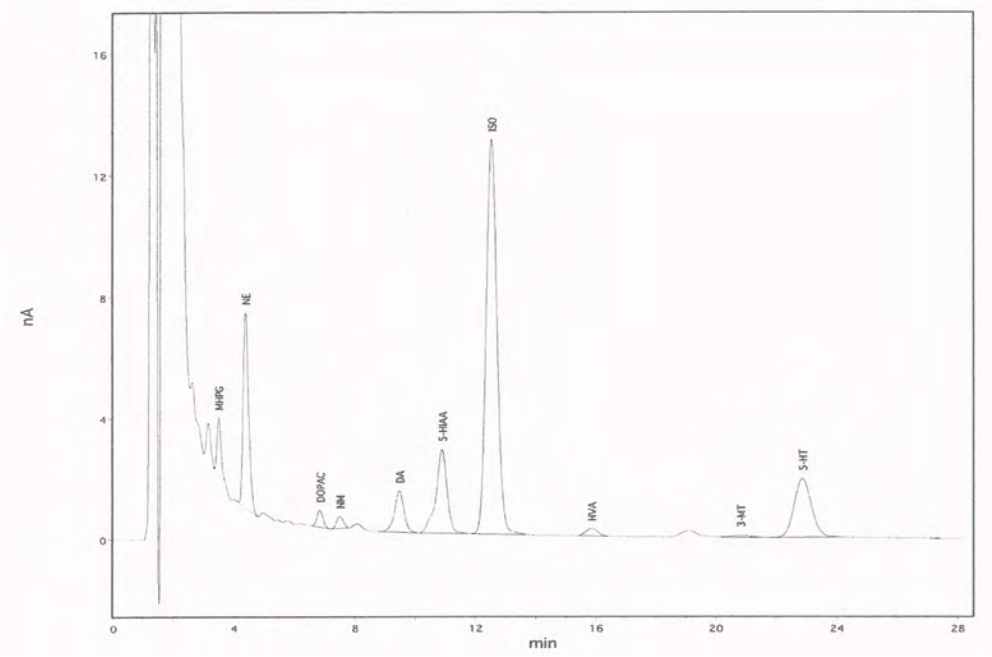

Fig. 3. Rat Cerebrum (10 µl injection)

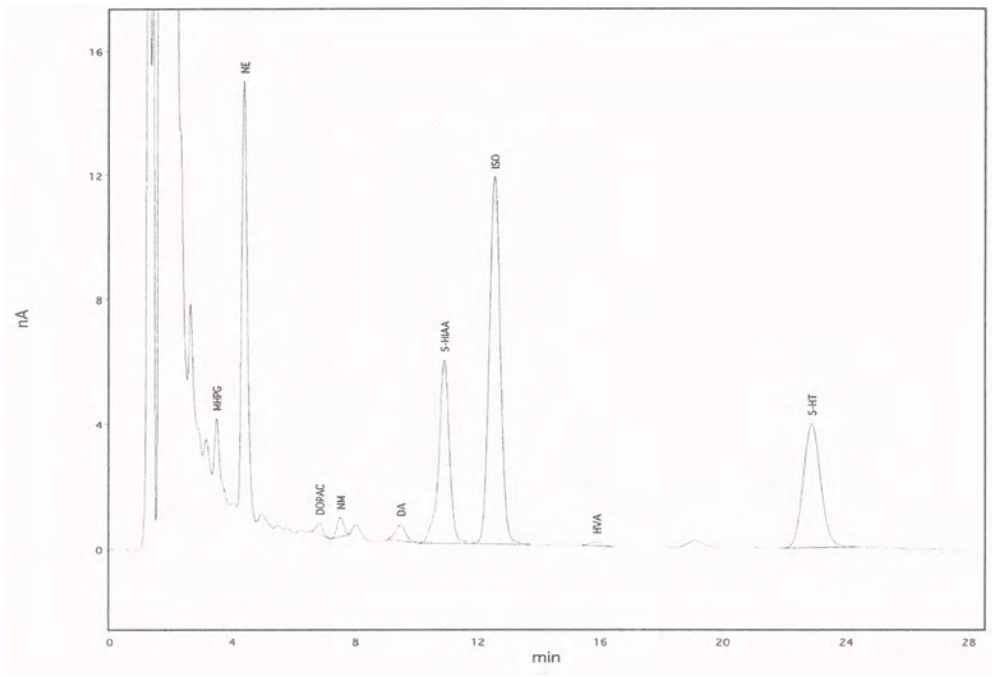

Fig. 4. Rat Hippocampus (10 µl injection)

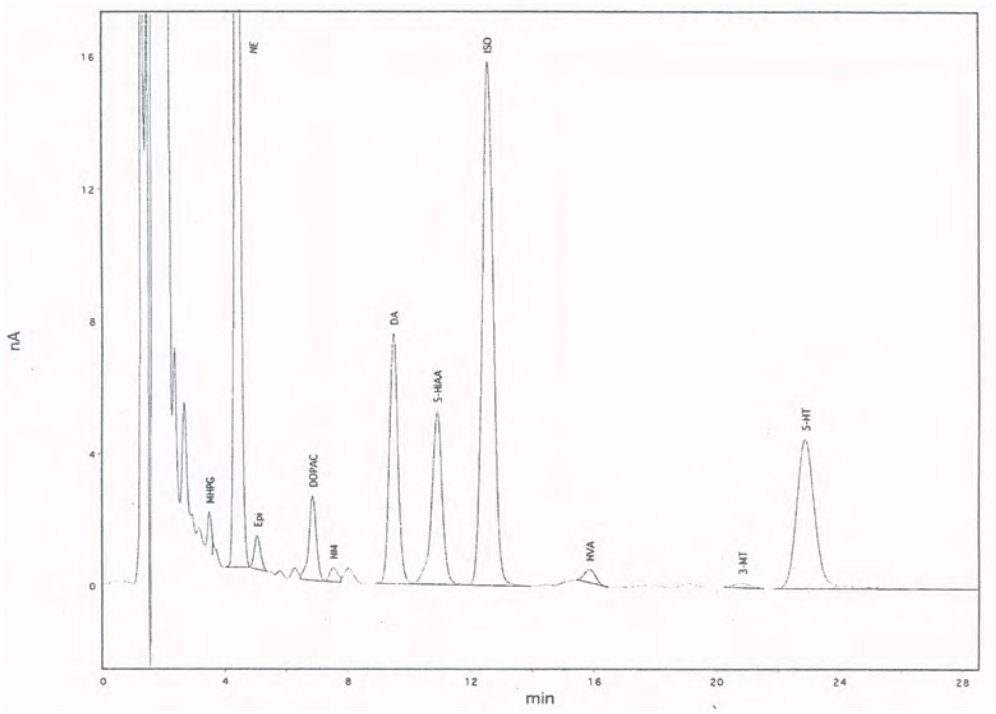

Fig. 5. Rat Hypothalamus (10 µl injection)

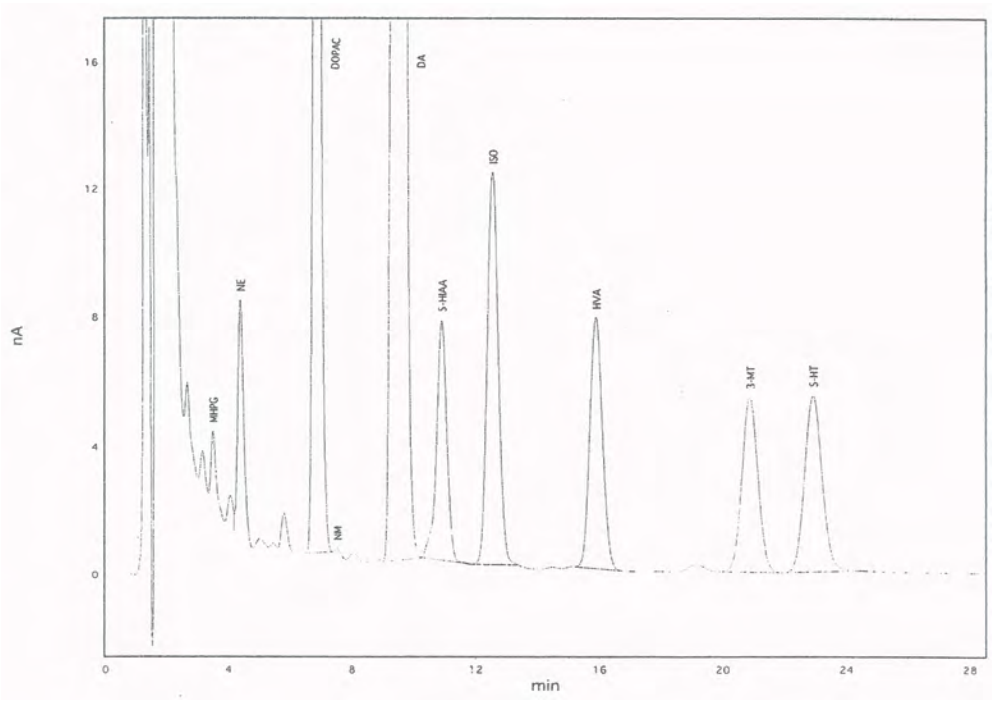

Fig. 6 Rat Striatum (10 µl injection)