SHIMADZU APPLICATION NEWS

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY No. L229

Analysis of Biological Sample (2)

●Simultaneous Determination of Vanillylmandelic Acid (VMA), Homovanillic Acid (HVA) and Catecholamines in Urine
●Determination of Tryptophan Metabolites in Umbilical Serum

■ Determination of VMA, HVA and Catecholamines in Urine

Neuroblastoma (hereafter called NB) is a tumor that has developed from a cell differentiating to the sympathetic ganglian; it is known that the tumor cell, same as the sympathetic ganglian, has a function to produce and secrete catecholamines. As an NB grows, a large amount of catecholamines will be produced from it and will be discharged into urine. In fact, for the diagnosis of NB, the measurement of catecholamines and the derivatives including VMA or HVA in urine has become indispensable.

Conventionally, for the measurement of catecholamines in urine, extraction with activated alumina or extraction with boric acid gel was applied as pretreatment of the sample, and then for the detection of catecholamines, the electro-chemical detector was used. The advantage of this method is that chromatograms of high selectivity with minimized effect of co-existing substances can be obtained, but a low recovery rate and a rather complicated operational procedure are the drawback of it. In order to solve these problems, a method has been developed, whereby a sample of diluted urine is directly injected into the column and the sample is subjected to measurement for the analysis for VMA, HVA and catecholamines and the derivatives. Here, in this article, this mthod is introduced.

Fig.1 shows an example of analysis of a standard sample, Fig.2, analysis of a urine sample from a patient, and in Table 1, analytical conditions for these analyses. (Analytical data by courtesy of the Public Health Test Center, the Foundation of Hokkaido Pharmacists Association)

Table 1 Analytical Conditions

Instrument	: Shimadzu LC-10A System
Sample	: Urine
Column	: Shim-pack CLC-VMA (4.6mmID×150mmL) Shim-pack FLC-ODS (4.6mmID×50mmL)
Column Temp.	: 30°C
Flow Rate	: 1.0mL/min
Mobile Phase	: 0.1M Citrate buffer (pH 3.2) 6mM Octansulfonic Acid/Acetonitrile 100 : 2
Detection	: L-ECD-6A
Potential	: +0.8V

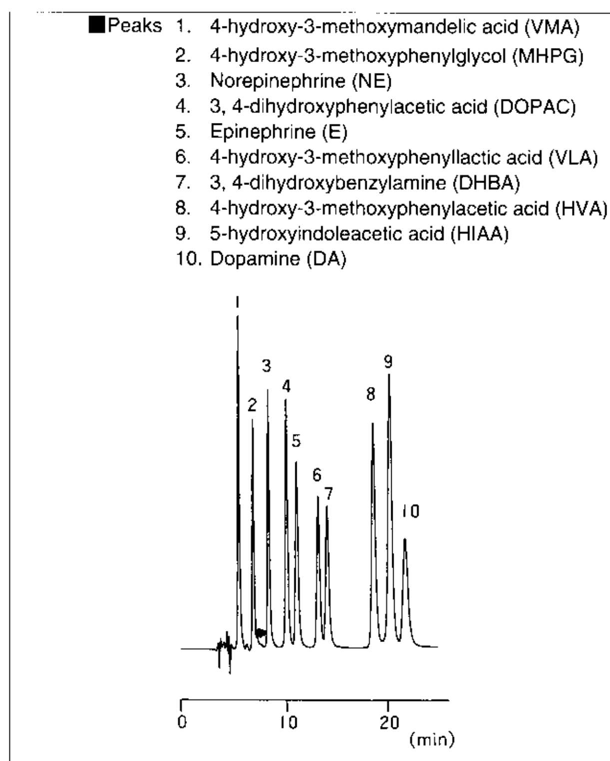

Fig.1 Chromatogram of Standard Mixture

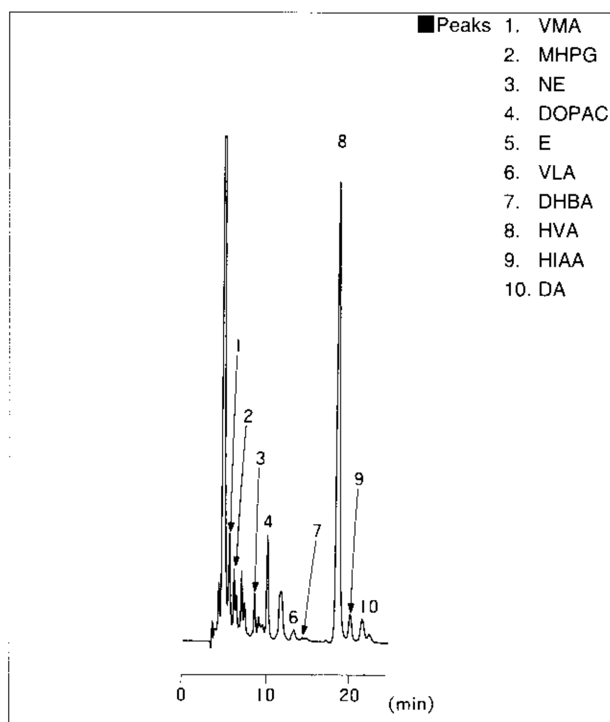

Fig.2 Chromatogram of Urine Sample

■ Determination of Tryptophan Metabolites in Umbilical Serum

Tryptophan and its metabolites are very important medically. Tryptophan is discharged through kynurenine and serotonine; as dysbolism is observed in case of such condition as phynylketonuria or bladder cancer, a convenient and high-accuracy anlaytical method is required for the diagnosis of such condition. By using the reversed phase chromatography, a mixture of tryptophan metabolites can be separated with high accuracy, and by fluorescence measurement of the tryptophan metabolites, high sensitivity and high selectivity analysis is possible.

Introduced in this article are analyses of a umbilical serum sample, whereby metabolites from kynurenine was measured by the UV detector, and metabolites from the indole pathways was by the fluorescent detector. As pretreatment of the serum sample, an ultrafiltration filter was used for removing proteins. Fig.3 shows a typical chromatogram of metabolites in kynurenine and Fig.4, a chromatogram of metabolites in the indole pathways. Fig.5 shows a chromatogram of metabolites in kynurenine pathways in ulbilical serum and in Fig.6, a chromatogram of metabolites in indole pathways in umbilical serum.

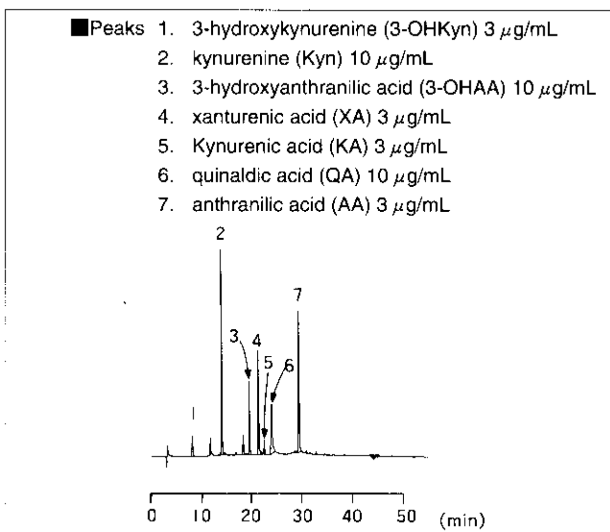

Fig.3 Typical Chromatogram of Metabolites in Kynurenine (Standard Mixture, 40 μL injected)

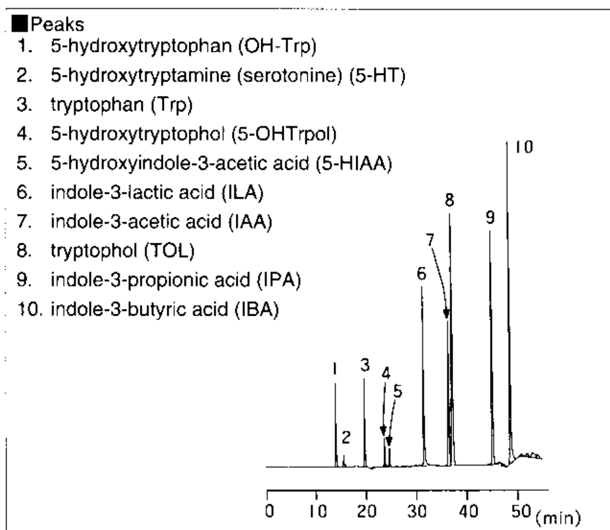

Fig.4 Typical Chromatogram of Metabolites in Indole Pathways (Standard Mixture, 500ng/mL ea., 40 μL injected)

Table 2 Analytical Conditions

Instrument	: Shimadzu LC-10A System
Sample	: Serum
Column	: Shim-pack CLC-ODS (6.0mmID × 150mmL)
Guard Column	: Shim-pack G-ODS (4) (4.0mmID × 10mmL)
Column Temp.	: 40°C
Flow Rate	: 1.0mL/min
Mobile Phase	: (A) 0.1M (Sodium) Phosphate (pH 3.3) (B) Acetonitrile Gradient Elution Time (min) 0-20-23-35-40-52-52-55-55-68 B. Conc (%) 0-12-22-22-37-37-70-70-0-0
Detection	: SPD-10A at 350nm (for Metabolites in Kynurenine Pathways) RF-10A λex=320nm, λem=400nm (for Metabolites in Indole Pathways)

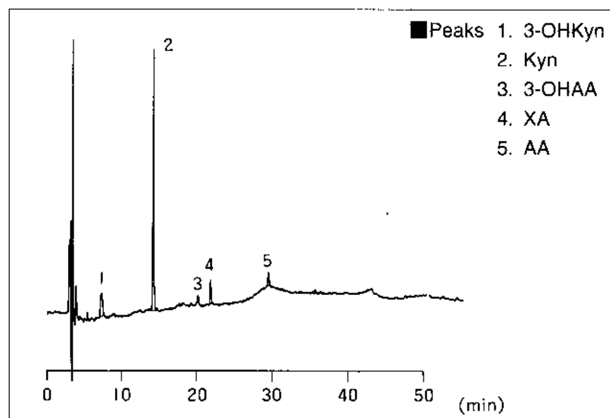

Fig.5 Chromatogram of Metabolites in Kynurenine Pathways in Umbilical Serum (80 μL injected)

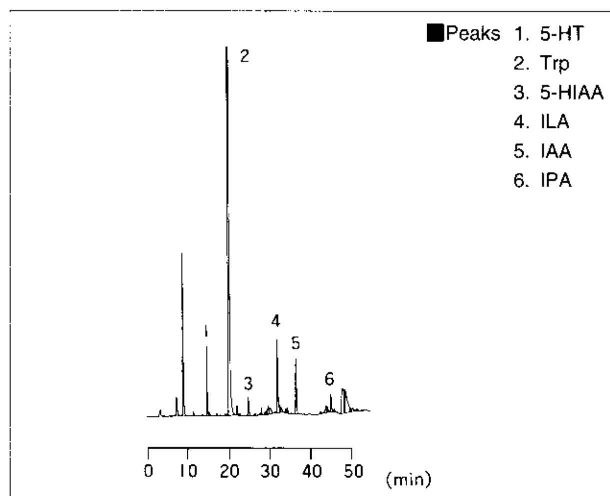

Fig.6 Chromatogram of Metabolites in Indole Pathways in Umbilical Serum

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