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Determination of Vanilmandelic Acid and Homovanillic Acid in Urine by Gas Chromatography

Vanilmandelic acid and homovanillic acid in a small quantity of human urine were purified by adsorption on Amberlite XAD-4, added with 3-methoxy-4-hydroxyphenylethanol as an internal standard, and converted to trifluoroacetyl-hexafluoroisopropyl derivatives. Their quantities were determined by the use of a gas chromatograph equipped with a 2% OV-17 column and an electron capture detector.

Urinary levels of vanilmandelic acid (VMA) and homovanillic acid (HVA) are closely related to diseases involved in catecholamine metabolism such as phaeochromocytoma and neuroblastoma. Many methods[1-4] to estimate VMA and HVA in urine have been studied using gas chromatography.

Here we present a new method for simultaneous determination of VMA and HVA in a small quantity of urine. An aliquot of 0.05—0.5 ml urine was acidified to pH 1.0 and saturated with sodium chloride. The solution was adsorbed on a column of Amberlite XAD-4 (5 mm × 3 cm), a macroreticular polystyrene resin. The column was washed with 10 ml of 0.1N acetic acid and subsequently 5 ml of water followed by elution with 30 ml of methanol–water (50: 50). The eluate was added with 21 μg of 3-methoxy-4-hydroxyphenylethanol (HMPE) as an internal standard, evaporated to dryness and kept over phosphorus pentoxide *in vacuo* for 10—20 min. One hundred μl of hexafluoroisopropanol and 100 μl of trifluoroacetic anhydride

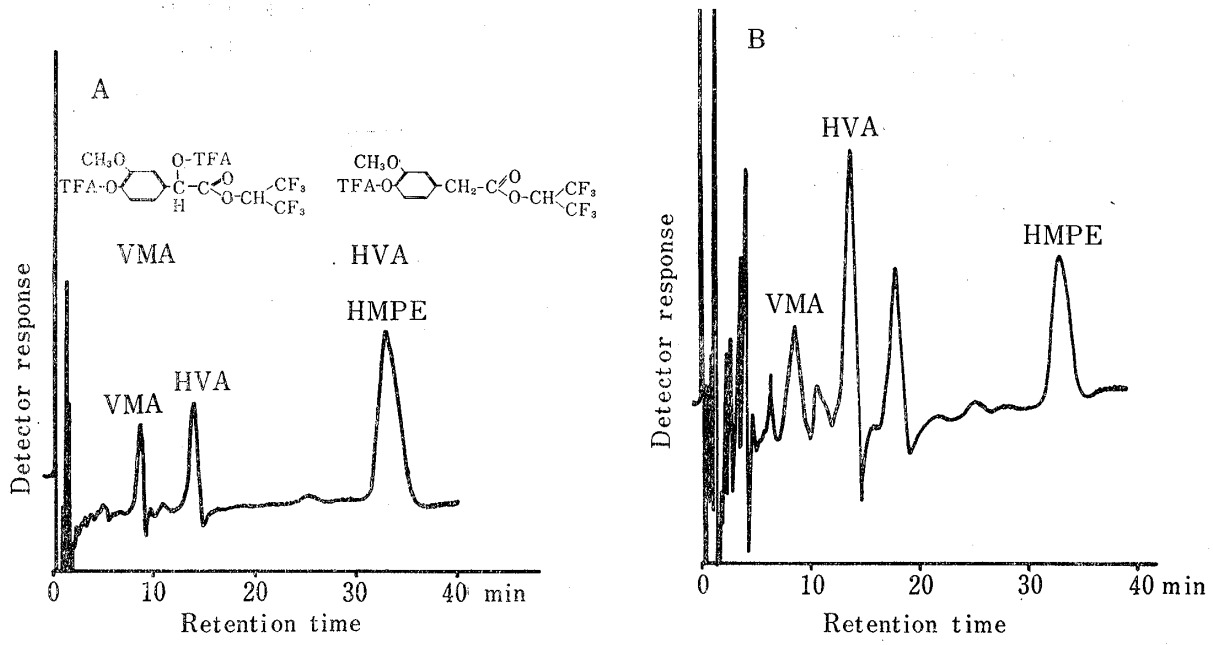

Fig. 1. Gas Chromatograms of Trifluoroacetyl-hexafluoroisopropyl Derivatives of VMA and HVA

A: authentic mixture B: urine from a patient with neuroblastoma
column: 2% OV-17 on Gas Chrom Q (80—100 mesh) in a glass tube (1.75m × 3mm), 100°; temperature at the injection port, 100°
detector: Electron capture detector (^{3}H, 300 mCi), 100°
flow rate: 40 ml/min.

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were added to the residue to react with VMA and HVA at 65° in oil-bath for 15 minutes, and the solution was condensed to dryness under a stream of nitrogen. The residue was dissolved in 0.5 ml of *n*-hexane, 1 μl of which was injected into a Shimadzu 3AE gas chromatograph equipped with an electron capture detector and a 2% OV-17 column. Gas chromatograms of authentic sample and urine specimen from a patient with neuroblastoma are shown in Fig. 1. The peak height ratio of VMA or HVA to HMPE was proportional to the quantity injected from 0.5 to 5 pmoles. These peaks were identified by the use of Shimadzu LKB 9000 mass spectrometer coupled to a gas chromatograph under the same condition as described above. The mass spectrum obtained from the peak due to the derivative of VMA or HVA from patient urines showed the same pattern as the one from the authentic sample, demonstrating the absence of impurity in each peak. The parent peaks supported the structures of the derivatives as shown in Fig. 1.

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Chemical Structure of QA_{II}, One of the Covalently Bound Adducts of Carcinogenic 4-Nitroquinoline 1-Oxide with Nucleic Acid Bases of Cellular Nucleic Acids[1)]

The chemical structure of the quinoline-adenine adduct, QA_{II}, which was isolated from deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) extracted from the cells treated with carcinogenic 4-nitroquinoline 1-oxide was proposed to be either 3-(N^6-adenyl)-4-aminoquinoline 1-oxide or 3-(N^1-adenyl)-4-aminoquinoline 1-oxide.

It has been reported that the cellular deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were chemically modified by treatment of the cells with carcinogenic 4-nitroquinoline 1-oxide (4NQO)[2-7)] or with its reduced metabolite, 4-hydroxyaminoquinoline 1-oxide (4HAQO). The quinoline moiety of 4NQO was thereby bound through a covalent bond to the nucleic acid bases.[4,5)] Four distinct adducts have been isolated by paper chromatography after acid-hydrolysis of DNA extracted from the cells thus treated and three of them were also found in RNA extracted simultaneously.[4,7)] Such a base modification can be produced *in vitro* by treatment of DNA, RNA, or synthetic polynucleotides with 4HAQO with the help

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