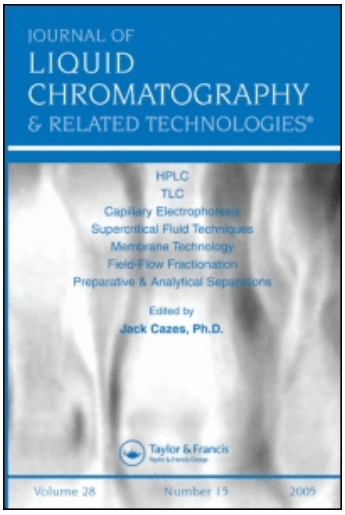

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Liquid Chromatographic Determination of Excretion Patterns of Urinary Phenolic Compounds

Yoshihisa Yamaguchi[a]

[a] College of Biomedical Technology and Nursing Biochemical Analysis Osaka University, Osaka, Japan

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LIQUID CHROMATOGRAPHIC DETERMINATION OF EXCRETION PATTERNS OF URINARY PHENOLIC COMPOUNDS

Yoshihisa Yamaguchi

College of Biomedical Technology and Nursing
Biochemical Analysis
Osaka University
1-1 Machikaneyamacho
Toyonaka, Osaka , Japan

ABSTRACT

Excretion patterns of urinary phenolic compounds were determined by means of chromatography with Sephadex G-10, with 4-aminoantipyrine for the detection of phenolic compounds.

Derivatization for phenolic compounds is based on a coupling reaction with 4-aminoantipyrine in the presence of sodium metaperiodate. The reaction is complete within a few minutes and, thus, provides a simple detection method.

Excretion patters of samples with normal subjects and patients of catecholamine-producing tumor were determined and the results were compared.

From the data obtained, this method is shown to be useful as a screening test of some catecholamine-producing tumors.

INTRODUCTION

Numerous techniques have been developed for detection of catecholamine metabolites in urine, such as colorimetric methods preceded by solvent extraction, a gas liquid chromatographic method, gas chromatograph-

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ic - mass spectrometry and high performance liquid chromatography (1-4).

For spot tests and some semiquantitative methods for urinary catecholamine metabolites, a coupling reaction with diazotized p-nitroaniline is widely used. On the other hand, determination of urinary total phenolic compounds with use of 4-aminoantipyrine has been described previously by Yamaguchi (5).

Recently, high performance liquid chromatography, with use of Sephadex G-10 for isolation of urinary catecholamine metabolites, has been reported (4).

In this paper, a new method for determination of urinary phenolic compounds excretion patterns is described and proposed as a diagnostic aid in patients with suspected cases of pheochromocytoma and neuroblastoma.

MATERIALS AND METHODS

All compounds used for this study were purchased from Sigma Chemical Co., St. Louis, Mo. 63178.

Preparation of reagent:

Carbonate-bicarbonate buffer, 0.05 mol/L, pH 10.1. Dissolve 3.18 g of anhydrous sodium carbonate plus 1.68 g of sodium bicarbonate in water and dilute to 1 liter.

Solution A. Dissolve 90 mg of 4-aminoantipyrine in 200 ml of carbonate -bicarbonate buffer.

Solution B. Dissolve 2.6 g of boric acid and 0.4 g of sodium metaperiodate in water and dilute to 200 ml.

Preparation of urine sample:

A 24-h urine specimen is collected and aliquot of the 24-h urine is centrifuged for 3 min at 2500 g. One ml of the supernatant of urine is directly applied to the Sephadex G-10 column (1 x 30 cm).

Sephadex gel chromatography:

Sephadex G-10 is swollen by heating a suspension of the particles in acetate buffer (0.05 M, pH 5.0) for 4 h at 90 °C under constant stirring. The fines are removed by several decantations and the resultant slurry is poured directly into the column. The column is then washed for 3 h with acetate buffer solution. After application of sample, chromatographic separation is performed with acetate buffer (0.05 M, pH 5.0). Each fraction of effluent contains 1.3 ml; 10 fractions are run within 30 min, and 40 fractions are collected.

Procedure for a detection of phenolic compounds:

To 0.5 ml of each chromatographic effluent is added 0.6 ml of solution A and shaken well; then 0.6 ml of solution B is added. Absorbance at 500 nm is read against the first fraction of effluent.

Total excretion value of urinary phenolic compounds is determined by the method previously reported (5).

Preparation of diazo reagent:

a) 100 mg of p-nitroaniline and 2 ml of conc. HCl are dissolved in 98 ml water.
b) 200 mg of Na_2NO_2 is dissolved in 100 ml of water.
c) 10 g of Na_2CO_3 is dissolved in 100 ml of water.
Mix the reagent of a, b and c at a ratio of 1:1:2 immediately before use.

Color development of phenolic compounds by diazo reagent :

To 0.5 ml of each effluent fraction, 1 ml of diazo reagent is added, and then allowed to stand for 30 min at room temperature. Measure absorbance at 540 nm.

RESULTS

Specificity of the reaction with 4-aminoantipyrine:

Table 1 shows the specificity of reaction with various biological phenolic compounds.
Precision and analytical recovery have already described in previous paper (5).

Excretion patters of urinary phenolic compounds

Chromatograms of standard compounds and of samples of normal subjects and patients of catecholamine-producing tumor are shown in Figure 1. Chromatograms of some other patients are also shown in Figure 2, in which samples from patients with diabetes mellitus and hypertension are analyzed.

TABLE 1.

Specificity of the reaction

Compounds tested	Absorbance (20 μg/tube)
Vanilmandelic acid	0.330
Normetanephrine	0.145
Epinephrine	0.138
3,4-Dihydroxyphenylalanine	0.100
Octopamine	0.095
Homovanillic acid	0.077
Noradrenaline	0.057
Dopamine	0.050
p-Hydroxyphenylacetic acid	0.000
Tyramine	0.000
Vanillin	0.000
Salicylic acid	0.000
Phenylalanine	0.000
Xanthurenic acid	0.580
Thyroxine	0.225
Guaiacol	0.290
Catechol	0.146
Serotonin	0.035

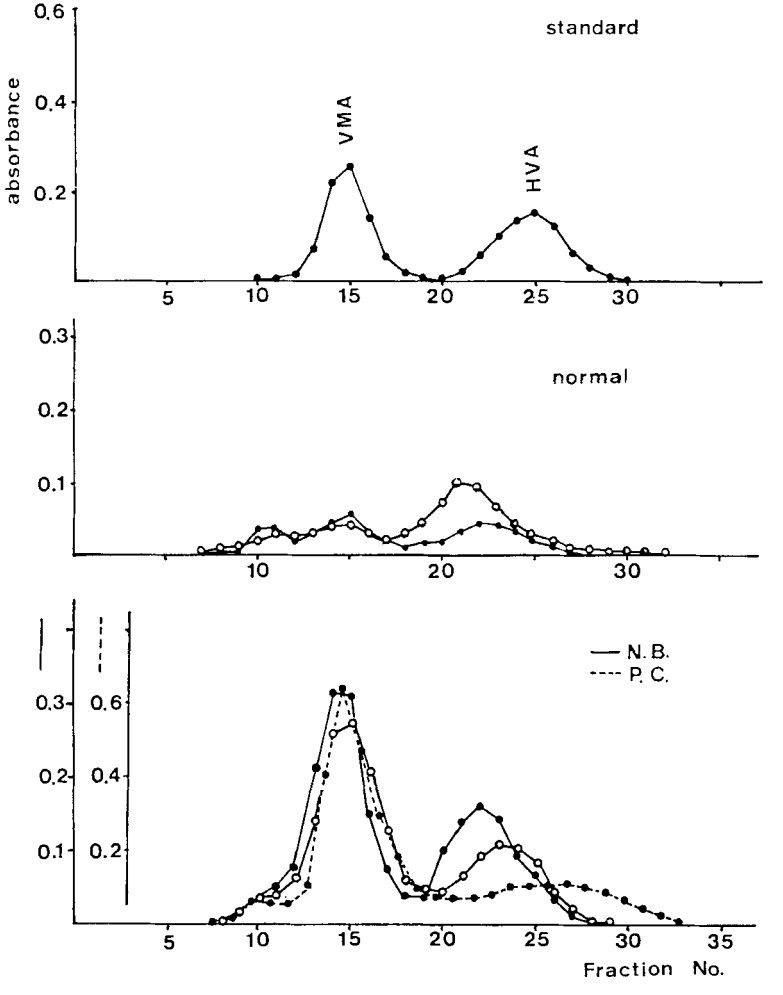

FIGURE 1. Excretion patterns of urinary phenolic compounds; chromatograms of standards, normal subjects and some patients of catecholamine producing tumor (N.B., Neuroblastoma, P.C., Pheochromocytoma)

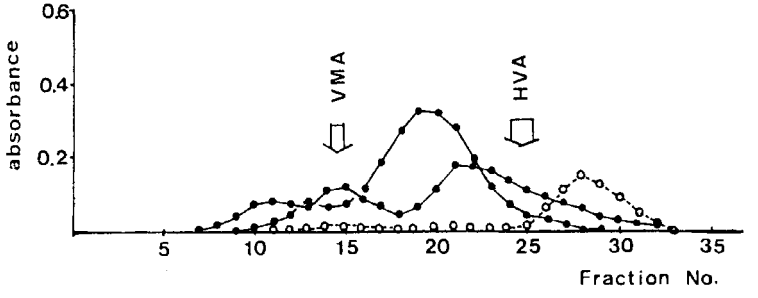

FIGURE 2. Excretion patterns of urinary phenolic compounds: ●——●, diabetes mellitus, and o---o, hypertension.

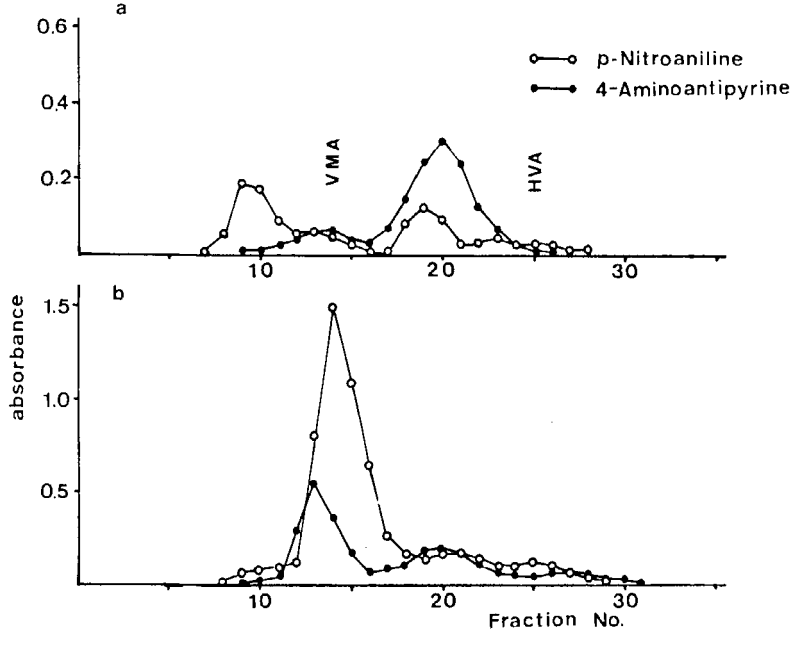

FIGURE 3. Comparison between the present method and the diazo reaction method. ●——●, present method and o——o, diazo reaction method. a) normal subject, and b) pheochromocytoma.

DISCUSSION

Comparison of excretion patterns of urinary phenolic compounds with diazo reaction method was also performed and the chromatograms were also shown in Figure 3, in which diazotization with p-nitroaniline showed high sensitivity and specificity for VMA (vanilmandelic acid) fraction but not for HVA (homovanillic acid) fraction.

Chromatograms between two methods are comparable; so the detection method using 4-aminoantipyrine can also applied to the diagnosis of catecholamine-producing tumors as shown in Figure 1.

The major advantage of this method is in its procedural simplicity and in the stability of reagents. Thus, this procedure provides new method that is useful for diagnostic purposes.

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