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Monitoring Homovanillic Acid and Vanillylmandelic Acid in Human Urine by Capillary Electrophoresis with Electrochemical Detection

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Abstract: A simple, rapid and low-cost method of separation and determination of homovanillic acid and vanillylmandelic acid in human urine was developed based on capillary zone electrophoresis / amperometric detection with high sensitivity and good resolution.

Keywords: Homovanillic acid, vanillylmandelic acid, capillary electrophoresis, electrochemical detection.

Homovanillic acid (HVA) and vanillylmandelic acid (VMA) are often tested in urine for the diagnosis of neuroblastoma, pheochromocytoma and psychosis, and for monitoring the response to therapy. High performance liquid chromatography (HPLC)[1], enzyme-linked immunosorbent assay (ELISA)[2] and capillary electrophoresis (CE)[3-4] have been used for their determination. However, its crossed reactivity of ELISA often makes the determinations semi-quantitative. CE has the advantages of high resolution, rapid separation, small injection volume and low cost over HPLC in bioanalysis. Since HVA and VMA have similar structures and similar ratios of charge to size, they are difficult to separate in CE. Coated capillaries were ever tried to separate them, but their runs of usage were limited[3]. UV detection in CE can not meet the real needs due to high limit of detection[4].

In the present work, we developed a simple, rapid and low-cost capillary electrophoresis / electrochemical detection for the separation and determination of HVA and VMA with high sensitivity and good resolution. The apparatus are the same as in Ref. 5. The optimum conditions found are as follows: buffer, 0.030 mol/L Na_2HPO_4–NaH_2PO_4 (pH 5.2); capillary, 51 cm×25 μm ID; injection, 5 kV for 10 s; separation voltage, 20 kV; detection potential, 1.1 V (*vs.* SCE). **Figure 1** shows the electropherogram of HVA and VMA in the presence of uric acid (UA, the main ingredient in urine) under optimum conditions. It can be found that HVA, VMA and UA can be separated very well. UA does not interfere with the determination of HVA and VMA. The responses for a series of six injections resulted in a relative standard deviation of 4.0% (t_m) and 6.6% (i_p) for HVA, and 4.4% (t_m) and 4.9% (i_p) for VMA. A linear relationship ranges from 5.00×10^{-6} mol/L to 5.00×10^{-4} mol/L for both HVA and

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VMA with a correlation coefficient of 0.994 for both HVA and VMA. The limits of detection are 1.3×10^{-6} mol/L and 7.9×10^{-7} mol/L for HVA and VMA, respectively, at a signal-to-noise ratio of 3.

Figure 2, curve 1 and 2 depicts the electropherogram of a urine sample without any standard HVA and VMA spiked. Before 7.5 min, one lower peak and two high peaks appear. Around 10 min, three low peaks and one higher peak elute in sequence. By comparison to the electropherogram of the standard HVA and VMA shown in **Figure 1**, the migration times of peak A and peak B in curve 2 are the same as those of standard HVA and VMA, respectively. In order to verify the conclusion, standard HVA or VMA was spiked into the urine sample, respectively. Curve 3 depicts the electropherogram of the urine sample with standard HVA spiked. Peak A increases without the increase of any other peak compared with curve 1. Curve 4 depicts the eletropherogram of the urine sample with standard VMA spiked. Compared with curve 1, peak B increases, but the other peaks do not. Therefore, peak A and peak B are the peaks of HVA and VMA, respectively. The contents of HVA and VMA in urine can be calculated according to the calibration curve. The results are listed in **Table 1**. The concentrations of HVA and VMA in human urine samples are determined to be 22.9 μmol/L and 16.9 μmol/L, respectively. They obviously fall into the range of healthy persons reported by literature from 8.2 to 41 μmol/L for HVA, from 11.6 to 28.7 μmol/L for VMA[6]. The recovery is 98% for HVA and 96% for VMA by the analysis of samples spiked with known quantities of HVA and VMA.

Table 1 Results of determination of HVA and VMA in a urine sample

Analyte	Concentration (10^{-5}mol/L)	Average concentration (10^{-5} mol/L)	Recovery (%)
HVA	2.32, 2.27, 2.29	2.29	98
VMA	1.72, 1.66, 1.70	1.69	96

Figure 1 The electropherogram of HVA and VMA (each 1.00×10^{-4} mol/L) in the presence of 6.00×10^{-5} mol/L UA under optimum conditions.

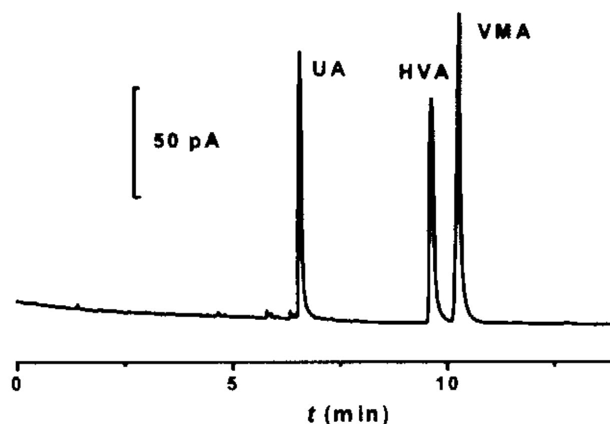
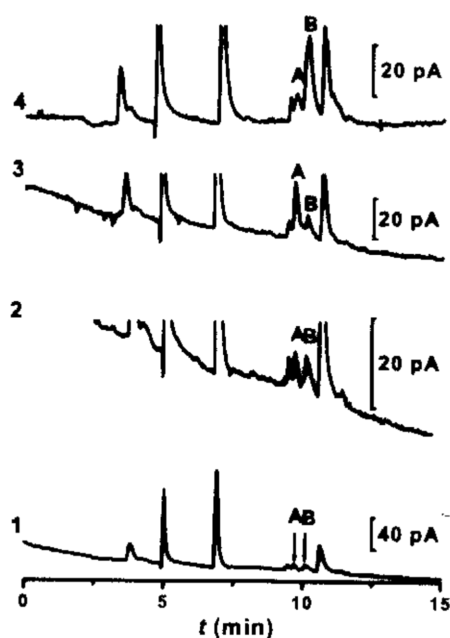


Figure 2 Electropherograms of (1) the urine sample, (2) amplification of curve 1, (3) the urine sample with 1.5×10^{-5} mol/L HVA and (4) the urine sample with 1.5×10^{-5} mol/L VMA.



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