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SHORT COMMUNICATION

Comparison of High Performance Capillary Electrophoresis and Liquid Chromatography for the Determination of Acyclovir and Guanine in Pharmaceuticals and Urine

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High-performance capillary electrophoresis (HPCE) and high-performance liquid chromatography (HPLC) were developed and applied to the determination of acyclovir (ACV) and guanine (G) in pharmaceuticals. The comparison study showed that two methods gave comparable results in linear range, recovery and reproducibility. HPCE was used for the determination of ACV and G in urine; the recovery was better than 81.3% and the RSD was less than 4.4%.

INTRODUCTION

Acyclovir (9-(2-hydroxyethoxylmethyl)guanine, ACV), an antiviral substance effective against herpes simplex and varicella zoster viruses, was introduced by Schaeffer *et al.* (1978) and Collins and Bauer (1979). Guanine (G) is an important biobase and a medical intermediate. There are many papers on the HPLC determination of ACV in plasma (Molokhia *et al.*, 1990), serum (Cronqvist and Nisson-Ehle, 1980) and ointments (Ashton and Ray, 1993), and of G in available products from DNA and RNA (Nakahara *et al.*, 1989 and Moltrasio and Lanzarotti, 1991). However, no report on the analysis of ACV and G by high-performance capillary electrophoresis (HPCE) has been seen.

In this paper, a simple, rapid and reproducible HPCE method for determining ACV and G using α-amino-5-mercapto-3,4-dithiazole (AMD) as internal standard was developed and compared to high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Reagents. ACV (99%) and ACV pharmaceuticals were obtained from Xinxiang Pharmaceutical Factory (Xinxiang, Henan Province, China), G was purchased from Sigma (St. Louis, MO, USA). AMD was from Dazhong Pharmaceutical Factory (Shanghai, China). All other reagents (A.R.) were purchased from Beijing Chemical Factory (Beijing, China). ACV (1 mg/mL), G (1 mg/mL) and AMD (20 mg/mL) stock solutions were prepared with 0.1 M NaOH solution.

HPLC and HPCE. An LC–6A HPLC system with a CLC–ODS column (150 × 6.0 mm i.d., 10 μm) (Shimadzu, Tokyo, Japan) was used for the separation of ACV and G. Samples were introduced by an injector with a 20 μL loop, and eluted with a mobile phase of 2% HAc:MeOH (90:10, v/v) at a flow-rate of 1.0 mL/min. Column temperature was maintained at 25°C, and the detection wavelength was set at 280 nm.

HPCE separations were carried out by using a 1229 HPCE analyser (Beijing Institute of New Technology and Application, Beijing, China) with a fixed wavelength UV detector at 280 nm. Bare fused silica capillaries were from Yongnian Optical Factor (Yongnian, Hebei Province, China). Capillary dimensions were 50 μm i.d., 375 μm o.d. and 50 cm length (38.5 cm to detector). The pH of buffers was measured using a No. 5994 pH meter (Cole-Parmer Instrument Co., Chicago, USA). The HPCE system was operated in the conventional mode with the anode injecting by applying 15 kV for 20 s and maintained at 24°C throughout. A voltage of 25 kV was applied. The capillary was cleaned with 0.1 M NaOH and double-distilled water after each run.

RESULTS AND DISCUSSION

The optimal HPCE buffer. The effects of pH and concentration of borax phosphate buffer and the proportion of organic modifier EtOH on the HPCE behaviours of ACV and G were investigated.

When the pH value was decreased from 3.0 to 1.7, the negative charge on the capillary inner wall was suppressed, so the migration time was decreased and the peak shape was improved. The capillary current was increased from 65 to 94 μA with the borax concentration increasing from 20 to 60 mM, and the peak shape became asymmetric owing to the temperature step caused by joule heat. When EtOH was added to the buffer up to 10%, the increased adhesiveness and analyte solubility and decreased electrophoretic mobility resulted in a migration time increase and peak shape improvement.

Taking into consideration the migration time, peak

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symmetry and capillary current, 40 mM Borax-H_3PO_4 (pH2):5% EtOH was selected as the running buffer for the HPCE separation of ACV and G with a capillary current of 72 μA, and an asymmetry factor of 1.00 was achieved.

Comparison of HPCE and HPLC for the separation of ACV and G. The HPCE reproducibility was affected by many factors such as applied voltage, injection volume, ζos (electrodynamic potential of innerwall). The effects of those factors could be minimized by adapting an internal standard method. Experimental results showed that the migration time (6.91 min) of internal standard AMD was between G and ACV in the optimal HPCE conditions, and its peak was sharp and symmetric. AMD was a good internal standard. Fig. 1 A shows the typical electropherogram of ACV, AMD and G completely separated within 10 min.

HPLC has better reproducibility of peak-area and t_R, it is not necessary to use an internal standard. Fig. 1B shows the typical chromatogram of standard ACV and G completely separated within 15 min.

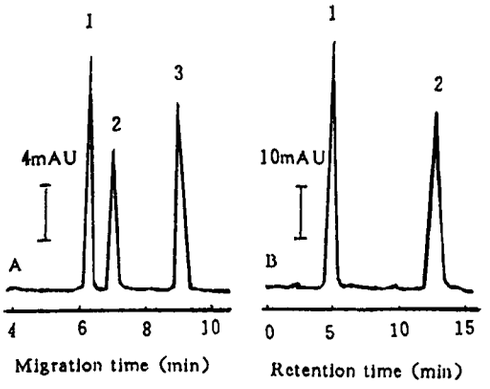

Figure 1. Typical separation of ACV and G by HPCE (A) and HPLC (B). (A) The detection wavelength was 280 nm. A 38.5 cm effective length×50 μm i.d. uncoated fused silica capillary was used. The running buffer was 40 mM borax H_3PO_4 (pH2):5% ethanol and the voltage was 25 kV. Peaks: 1, G (50 μg/mL); 2, AMD (4 mg/mL); 3, ACV (50 μg/mL). (B) The detection wavelength was 280 nm. ODS (10 μm) column (150×6.0 mm) was used and temperature maintained at 25°C. The mobile phase was HAc (2%):methanol (90:10, v/v) and flow-rate was 1.0 mL/min. Peaks: 1, G (20 μg/mL); 2, ACV (20 μg/mL).

Comparison of HPLC and HPCE in determination of ACV and G in ACV pharmaceuticals. In the HPCE, the concentrations of ACV from 10 to 300 μg/mL and G from 5 to 300 μg/mL were proportional to the relative heights (H) with detection limits (S/N=2) of 8.52 μg/mL for ACV and 2.80 μg/mL for G, respectively.

In HPLC, the concentrations of AVC from 2 to 200 μg/mL and G from 0.5 to 200 μg/mL were proportional to the peak-area (A) with detection limits (S/N=2) of 1.22 μg/mL for ACV and 0.30 μg/mL for G, respectively. The linear regression equations are $H=0.0126C+0.193$ ($r=0.9918$, $n=7$) and $A=547C-632$ ($r=0.9962$, $n=7$) for ACV; $H=0.0143C+0.268$ ($r=0.9945$, $n=7$) and $A=1140C-458$ ($r=0.9984$, $n=7$) for G.

The recoveries of 95.3–106.3% for ACV and 94.4–97.3% for G were obtained with RSD of 1.5–3.0% by HPCE. The recoveries were 96.4–101.1% (ACV) and 98.6–100.7% (G) with RSD of 0.5–2.4% by HPLC.

The results indicated that the detection limit of HPLC was lower than HPCE. There was no significant difference in recovery and RSD between HPLC and HPCE. The amounts of ACV and G in five ACV pharmaceuticals were determined with both HPCE and HPLC, the two methods gave similar results.

HPCE determination of ACV and G in urine. There was some interaction between the internal standard, AMD and protein in the urine. Urine samples were heated in boiling water for 10 m, and filtered with 1.2 μm filter to remove the protein. AMD, ACV and G were added to 25 mL of filtered urine and recovered by HPCE.

In the range of 10–200 μg/mL, the recoveries of ACV and of G were 81.3–100.9% and 86.7–97.4% with RSD of 2.8–4.4% and 1.7–3.0%, respectively.

CONCLUSION

HPCE and HPLC were developed to determine ACV and G in pharmaceuticals. The comparison study showed that the accuracy and reproducibility of both methods were similar. HPLC had a lower detection limit while the separation time of HPCE was slightly shorter. HPCE was also applied to the determination of ACV and G in urine.

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