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Capillary Electrophoretic Determination of Jinggangmycin A in Formulations Using Direct UV Detection

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Abstract: A simple, fast and reliable method was developed for the analysis of jinggangmycin A (validamycin A) in commercial formulations. The running buffer used was acetate buffer (100 mmol/L, pH 4.7) with 15 kV as the applied voltage. The detection was achieved by using direct UV mode at 200 nm and the detection limit was 0.2 μg/mL. Linearity in the concentration range of 5-500 μg/mL was excellent ($R^2 > 0.999$). The run-to-run repeatability (n = 3), as expressed by the relative standard deviation (RSD) for migration times and peak areas were less than 0.5% and 3.0% respectively. The mean recovery ranged from 97.2% to 101.4%.

Keywords: Jinggangmycin A, validamycin A, capillary zone electrophoresis, direct UV detection.

Jinggangmycin A was separated from the culture broth of *Streptomyces hygroscopicus* var. *jinggangensis* and was identified as validamycin A[1]. It is the popular fungicide for the control of sheath blight disease, which is the main disease of rice. Because of its excellent control effect, low price and harmlessness, jinggangmycin A is widely used in Asia, and the controlled area is around 13 000 000 hm^2 in China.

The national standard method for the determination of jinggangmycin A in China is HPLC[2]. However, the tidious pretreatment of samples makes the analysis procedure time-consuming. Recently, we reported a novel method of capillary zone electrophoresis (CZE) with indirect UV detection (CZE-InUVD)[3]. Nevertheless, the detection sensitivity was not so satisfactory. Furthermore, there was potential interference from components in samples with complex matrixes. To overcome these disadvantages, we developed a new procedure for the determination of jinggangmycin A by CZE with direct UV detection (CZE-UVD).

Experimental

A standard sample for jinggangmycin A (purity ≥ 97%) was prepared as described previously[3]. Samples 1-3, manufactured by different companies, were soluble powders containing 20% jinggangmycin A. The chemicals used were of analytical grade and were obtained from Shanghai Chemical Reagents Co. (Shanghai, China).

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Electrophoresis was performed on P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) that was equipped with P/ACE UV detector or diode array detector, an autosampler and a temperature-controlled fluid cooled capillary cartridge. A computer with MDQ software (2.3 Version) was used for instrument control, data collection and processing.

The capillary was a 60 cm × 75 μm i. d. uncoated fused-silica capillary (Reafine Chromatography, Yongnian, Hebei, China) with a UV detection window placed on-column 50 cm from the injection end. The detection wavelength was set at 200 nm. The capillary temperature was controlled at 25℃. Samples were introduced from the anodic end of the capillary by pressure mode (0.5 p.s.i. for 5 s unless indicated otherwise, 1 p.s.i.＝6489.75 Pa).

Before each injection, a 3-min purge of the capillary with running buffer was programmed. All running buffer solutions were filtered through a 0.22 μm syringe membrane filter, and all samples were centrifuged (10 000 g for 10 min) before introduction into the system.

CZE-InUVD analysis was carried out on the same capillary and capillary electrophoresis system with the conditions as described previously[3].

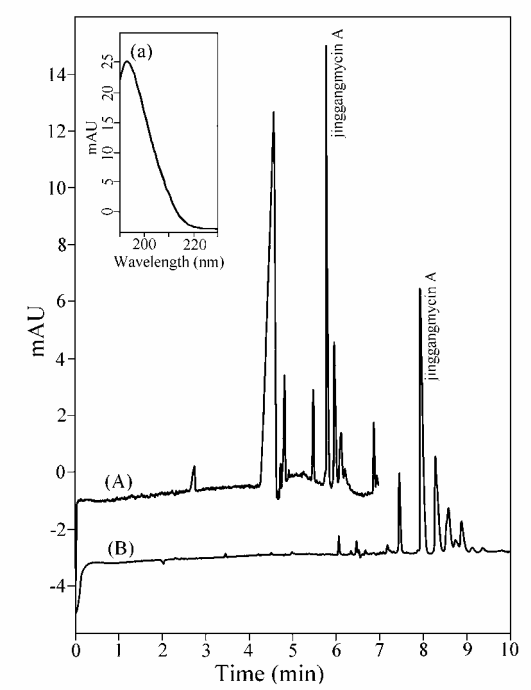
Results and Discussion

To achieve better separation, three factors are important. First, running buffer must have a low UV background signal. Second, optimum conditions must be selected to minimize variation in migration time and peak area from run to run in order to get reproducible results. Third, the conditions should guarantee good sensitivity and resolution. We used different buffers: acetate, phosphate and citrate. Acetate buffer was proved to be the most suitable one.

The detection for jinggangmycin A by CZE with UV detector is a challenging task because jinggangmycin A has no strong UV chromophore. Jinggangmycin A shows only end absorption in aqueous solution[4], therefore direct UV detection can only be done at a low wavelength. With the diode array detector, detection sensitivity was found optimal at the maximum absorbance wavelength of 193 nm in acetate buffer (**Figure 1** (a)). In this experiment, the detection wavelength was fixed at 200 nm with the UV detector. However, this led to a 24% reduction in sensitivity.

The pH value of the running buffer is an important parameter that can be manipulated to optimize selectivity in CZE, because it has a significant effect on the surface characteristic of the fused silica capillary and also influences the electric charge of the analyte[5]. As an aminoglycoside antibiotic, jinggangmycin A is positively charged in its protonated form at pH 3-8[6]. With the voltage at 15 kV and the concentration at 100 mmol/L, the pH levels of the running buffer were chosen at 4.0, 4.7, 5.0, 5.5 and 6.0 respectively. The corresponding numbers of theoretical plates were 69 000, 240 000, 289 000, 298 000 and 256 000 plates/meter. Although the separation efficiency was not the highest, the lowest relative standard deviations (RSDs) for both migration times and peak areas were achieved at pH 4.7. The reason is that the acetate buffer exhibits the highest buffer capacity at its pK_a value. The resolution for jinggangmycins(jinggangmy-

Figure 1 Electropherograms of sample 1



Experimental conditions: uncoated fused-silica capillary: 60 cm (effective length 50 cm) × 75 μm (i. d.); separation voltage: 15 kV; temperature: 25℃; pressure injection: 0.5 p.s.i. for 10 s*. (A) running buffer: 10 mmol/L aminopyrine-2 mmol/L EDTA (pH 5.2); indirect UV detection: 254 nm. (B) running buffer: 100 mmol/L acetate (pH 4.7); UV detection: 200 nm.

*In order to compare with CZE-InUVD, the injection time of CZE-UVD was doubled.

cin A and its analogs) at pH 4.7 was satisfactory. As a result, pH 4.7 was selected as the optimal pH. In addition, it is very easy to prepare this buffer.

The separation efficiency increased from 194 00 to 304 000 plates/m with the concentrations of acetate buffer increasing from 75 to 150 mmol/L. In general, a solution of higher concentration has higher buffer capacity, resulting in better reproducibility. However, due to the Joule heating effect, the RSDs for both migration times and peak areas were increased when the molarity became higher than 125 mmol/L. Since reproducibility is of critical importance for the analysis, a concentration of 100 mmol/L was chosen.

In addition, the most appropriate applied voltage was obtained at 15.0 kV (250 V/cm).

A good linear correlation ($R^2 = 0.9998$) between the peak area (X) and concentration (Y) was found in the concentration range of 5-500 μg/mL. The regression equation, $Y = 2.299 \times 10^{-3} \times X + 1.191$, was used to calculate the content of jinggangmycin A in the formulated products.

Table 1 Contents (%) of jinggangmycin A in commercial formulations (n = 3)

Sample	CZE-UVD	CZE-InUVD
Soluble Powder 1	24.95 ± 0.57	24.45 ± 0.45
Soluble Powder 2	21.36 ± 0.21	21.25 ± 0.30
Soluble Powder 3	24.27 ± 0.29	22.79 ± 0.54

The detection limit of jinggangmycin A was found to be 0.2 μg/mL. The sensitivity was about 10-fold higher than that of CZE-InUVD for the same injection amount, and was equal to that of HPLC[3].

The contents of jinggangmycin A in samples are listed in **Table 1**; they were all higher than the labeled content. The RSD values for products 1, 2 and 3 were 2.28%, 0.98%, and 1.19% respectively. These samples were also analyzed using CZE-InUVD. Both procedures yielded similar values for each sample (**Table 1**). Typical electropherograms of the two CZE methods are shown in **Figure 1**.

The average recoveries for samples 1, 2 and 3 were 100.8%, 97.2% and 101.4%, respectively with the corresponding RSD values of 0.82%, 1.71%, and 0.25%. This was verified in triplicate at a spiked level of 50 μg/mL.

Conclusion

A simple and reliable CZE method for the determination of jinggangmycin A was developed. The proposed protocol has several advantages compared to other procedures: (i) jinggangmycin A can be detected directly with minimal matrix interference; (ii) higher sensitivity and better resolution were obtained; (iii) The preparation of sample and running buffer was easy. It also provided excellent reproducibility, good linearity, lower run cost, and high separation speed.

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