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Quantitative Determination of Anticancer Drug Heptaplatin by HPLC

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A method for the direct determination of anticancer drug heptaplatin by HPLC has been described. Phenomenex ODS column (150 mm × 4.6 mm i.d., 5 μm) was used as the stationary phase and a mixture of methanol and water (40:60, v/v) as the mobile phase. The flow rate was 1.0 mL min^{-1}, T = 40 ºC and the detection wavelength was 230 nm. Under these analytical conditions, the linear regression coefficient was found to be 0.9999, the average recovery exceeded 99.9 % with RSD 0.85 %. The method is accurate, reliable and rapid. It can be applied to the quality control of heptaplatin.

Key Words: HPLC, Anticancer drug, Heptaplatin, Analysis.

INTRODUCTION

Heptaplatin, *cis*-malonato[(4R,5R)-4,5-*bis*(amino-methyl)-2-isopropyl-1,3-dioxolane] platinum(II) (SKI-2053R, Sunpla) (Fig. 1) is a third generation anticancer platinum drug, which has shown equivalent antitumor activity and less toxicity compared to cisplatin and was approved for advanced gastric cancer in Korea[1-3]. To the best of our knowledge, there are no reports about its analytical method. As part of our drug development program, a HPLC method has been successfully established to analyze the purity of heptaplatin. The present paper describes the HPLC procedures for quantitative determination of heptaplatin in our laboratory.

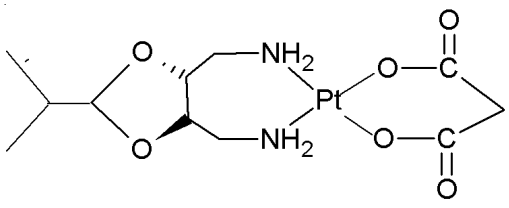

Fig. 1. Chemical structure of heptaplatin

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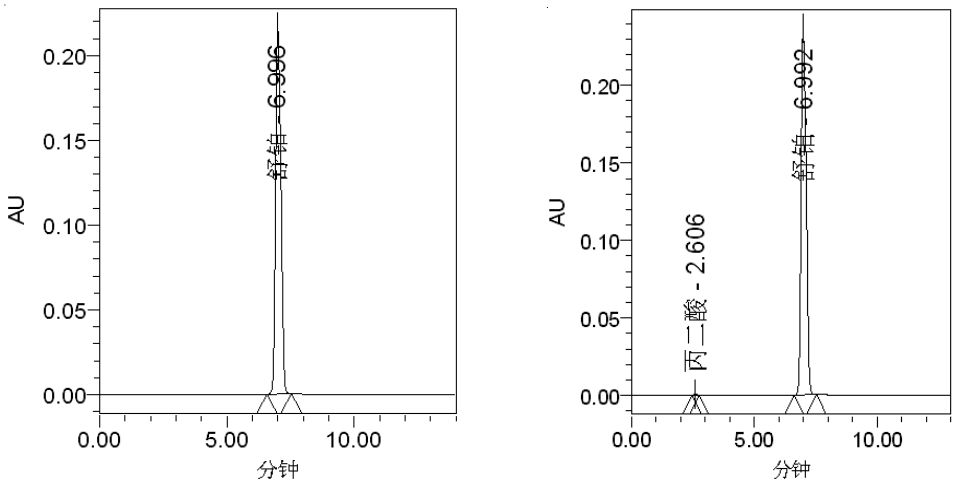
EXPERIMENTAL

The chromatographic system consisted of Waters 1525 binary HPLC pump, 717 plus auto-sampler, 2998 PDA detector. The chromatographic data were collected and processed with Empower 2 software. Heptaplatin standard and sample were synthesized in our laboratory. Malonic acid was purchased from Sigma-Aldrich (purity = 98 %).

Chromatographic systems and conditions: Chromatographic column was Phenomenex ODS column (250 mm × 4.6 mm, 5 μm). Mobile phase was a mixture of methanol and water (v/v = 40/60) with the flow rate of 1 mL min^{-1}. The detection wavelength, column temperature and injection volume was 230 nm, 40 ºC and 10 μL, respectively. Under these conditions, the retention time of heptaplatin was determined to be 6.99 min and the theoretical plate number was 4830 (Fig. 2).



(a) Heptaplatin standard (b) Heptaplatin sample

Fig. 2. Chromatograms of (a) heptaplatin standard and (b) sample

Preparation for the working solution[4]: A certain amount of the test sample was accurately weighed, dissolved in mobile phase and diluted with mobile phase to obtain a test solution having a mass concentration of 1.2 mg mL^{-1}, which was filtered through a 0.20 μm needle-type filter before injecting.

Peak purity assessment[5]: On the basis of the chromatographic conditions, PDA detector was used to check the purity of heptaplatin chromatographic peak. The detection result indicated that it was a single component. Purity of reference substance was calculated by normalization method. Heptaplatin is stable within 8 h, so analysis must be completed within the period and the solution has better to be prepared freshly. Under the conditions, heptaplatin and its related substance malonic acid are effectively separated, but the malonic acid peak does not reach the requirement of a quantitative analysis. Because of little toxicity of malonate to human, we only need to limit its content.

System suitability test: Take a precise amount of heptaplatin, dissolve it in the mobile phase and replicate injections (10 μL) of the solution. The chromatogram was recorded in Fig. 2 which shows that heptaplatin was well separated from malonic acid. The retention time of heptaplatin was 6.99 min, theoretical plates number was 4830, tailing factor was 1.23. Rs between heptaplatin and malonic acid was 14.86.

Linearity: Accurately weigh 29.72 mg of heptaplatin in 25 mL flask and dissolve in and dilute with mobile phase to a volume as a stock solution. Separately take 1, 2, 3, 4, 5 and 6 mL of the stock solution, place in six 10 mL flasks, add the mobile phase to the volume. Each was injected 10 μL, record peak areas, plot the peak area on the ordinate against heptaplatin concentration in the abscissa. As a result, within the range of 0.1-0.8 mg mL^{-1}, the chromatographic peak area A and the concentration of heptaplatin C showed good linear relationship, $A = 5.49 \times 10^6 C - 1.30 \times 10^4$, r = 0.9999.

Precision: Within the linear range, take three solutions of heptaplatin standard sample having concentrations of 0.72, 0.80 and 0.77 mg/mL, each concentration duplicate inject three times, each 10 μL. The relative standard deviation (RSD) of peak area of heptaplatin was determined to be 0.1, 0.5 and 0.1 %, respectively, which indicated good precision.

Stability of the solution: Prepare a 0.7 mg mL^{-1} heptaplatin standard solution, which was measured once every 0.5 h and continuously determined for 8 h to study its stability. RSD of the peak areas within 8 h was 1.6 %, which showed that storage of heptaplatin solution under room temperature for 8 h did not cause any loss of heptaplatin.

Accuracy: Accuracy was evaluated by the average recovery method. Take three sets of heptaplatin samples in three 10 mL flasks, add with different amount of standard heptaplatin separately to prepare three concentration gradients. Each solution was injected in triplicate into the chromatography under the above chromatographic conditions, measured their respective peak area and calculated the average recovery. The mean recovery was 100.27 %, RSD = 0.85 %, the results are shown in Table-1.

TABLE-1
RECOVERY RATE OF HEPTAPLATIN

Background (mg)	Added (mg)	Found (mg)	Recovery (%)	RSD (n = 3) (%)
1.29	1.69	2.96	98.8	0.4
2.00	2.48	4.50	100.8	1.0
2.89	3.47	6.40	101.2	0.7

Instrument detection limit: The instrument detection limit is defined as the concentration of analyte that provides signal-to-noise ratio of 3:1. In this HPLC method, the detection limit of heptaplatin was 0.015 mg mL^{-1}.

Specificity[6]: The specificity is the ability of the analysis method to determine the analyte specifically and accurately in the presence of other substances which might be included in the samples and in the system. The method specificity was assessed by comparing the chromatograms obtained from heptaplatin sample and its related substance malonic acid.

Quantitative determination of heptaplatin: Three samples were separately dissolved into mobile phase and prepared as 1 mg/mL. Separately inject equal volumes (10 μL) of the three solutions, each solution was injected in triplicate. Measure the peak areas, calculate the content of heptaplatin by the calibration curve and average the three injections. The test results are listed in Table-2.

TABLE-2
ANALYSIS RESULTS OF HEPTAPLATIN

Sample analysis results (%)	RSD (%)
99.78	0.3
98.66	0.6
98.31	0.1

RESULTS AND DISCUSSION

Heptaplatin is slightly soluble in water (the solubility is 4 mg/mL at room temperature) and not soluble in ordinate organic solvents such as methanol, isopropanol, ethanol, acetonitrile and acetone. The drug is a Pt(II) complex with a malonate group as the leaving group. Different volume ratios of methanol/water, acetonitrile/water, methanol/acetonitrile were tested as the mobile phase[7] and C_{18} column, C_8 column and CN column as the stationary phase were tried to separate heptaplatin and its related impurity-free malonic acid. Fig. 3 is the UV spectrum of heptaplatin in the mobile phase. Although the maximum absorption is at 194 and 230 nm was chosen as the detection wavelength, because the complex and malonic acid show considerable absorption at this wavelength and the absorption of the mobile phase

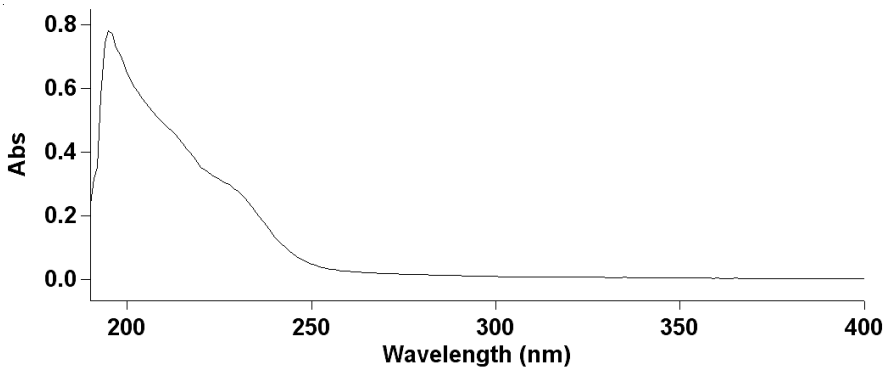


Fig. 3. UV spectrum of heptaplatin

can be eliminated. It was found that when methanol/water (40:60, v/v) was used as the mobile phase at 1 mL min^{-1} and 230 nm was chosen as the detection wavelength with ODS column (250 mm × 4.6 mm, 5 μm) at 40 ºC, Rs was greater than 1.5 and the linear regression coefficient for heptaplatin analysis was 0.9999, the average recovery exceeded 99.9 % with RSD 0.85 %. The method is accurate, reliable and rapid. It can be applied to the quality control of heptaplatin.

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