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Determination of 5-Fluorouracil in 5-Fluorouracil Injection and Human Serum by HPLC

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ABSTRACT

A high-performance liquid chromatography (HPLC) method was developed for the determination of 5-fluorouracil (5-FU) in 5-FU injection and human serum. A Nova-park C_{18} column (3.9 × 150 mm, 4.6 μm) and mobile phase consisting of 5 mmol/L KH_2PO_4 (pH 6.0) – methanol (96:4, v/v) at a flow rate of 1.0 mL/min were used for separation. The detection wavelength was set at 254 nm. The human serum was treated with $AgNO_3$ (20%) and NaCl (20%). The method showed a good linear relationship in the range of 0.1 - 100 μg/mL (R^2 = 0.9999) with limits of quantification (LOQ) at 0.10 μg/mL and 0.85 μg/mL (S/N = 10) in 5-FU injection and human serum, respectively. The average recoveries of different concentrations in 5-FU injection and human serum were 95.79 - 105.1% and 90.2 - 108.8%, respectively. The relative standard deviation (RSD) of precision was 0.4%, and those of stability and repeatability were less than 1.3% and 2.1% (n = 5) in both samples, respectively. Impurities in the human serum did not interfere with the determination of 5-FU. The method is simple, rapid, sensitive and can accurately determine 5-FU in 5-FU injection and human serum.

Key words: high performance liquid chromatography, 5- FU, human serum, drug concentration

INTRODUCTION

5-Fluorouracil (5-FU) is one of the anti-metabolite and anti-tumor drugs that has been around and in use for decades. It is most commonly used in the treatment of the cancers of colon, breast, stomach and pancreas[1]. 5-FU injection is widely used and many products with the specification of 0.25 g/10 mL. Although 5-FU is an active medicine against many cancers, it has some side effects[2-3]. Some of the most common and important side effects include soreness of the mouth, difficulty swallowing, diarrhea, stomach pain, low white blood counts, low platelet counts and anemia. 5-FU has anti-tumor function only after it metabolizes into the corresponding nucleotide in the body and there are metabolic differences between different people. It had been proven that if there were large amounts of 5-FU in the human serum after injecting for certain hours, 5-FU would not metabolize completely and would endanger health [2-3]. Therefore, developing a simple, rapid, economical and accurate method for the determination of 5-FU in human serum is necessary. So far, gas chromatography-mass spectrometry (GC-MS)[4-5], high performance liquid chromatography (HPLC)[6-12] and high performance capillary electrophoresis (HPCE)[13] had been used for the determination of 5-FU in human serum. Methods which had been used for the determination of 5-FU injection were HPLC[14], RP-HPLC[15] and HPCE[16]. The method developed in this study is simple, rapid, economical and accurate, and it may be applied for the rapid determination of 5-FU in injection and human serum.

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MATERIALS AND METHODS

I. *Materials and Chemicals*

5-FU was purchased from Sinopharm Chemical Reagents Limited Company, China. Methanol was of chromatographic grade. All other reagents were from standard sources. Ultrapure water was used. 5-FU injection was purchased from Shanghai Xudong Haipu Pharmaceutical Co., Ltd. Human serum was offered by the Central Hospital of LiaoYuan in Jilin Province, China.

II. *Preparation of Standard and Sample Solutions*

5-FU standard (100 mg, accurately weighed) was placed in a 50-mL volumetric flask. To this, 40 mL of water was added and the mixture was sonicated for 30 s. The mixture was made up to volume with water to obtain a final concentration of 2 mg/mL. This was used as the standard stock

solution. The standard stock solution was diluted with water to obtain a series of standard solutions (0.1 - 100 μg/mL). The 5-FU injection was diluted with water and filtered through a 0.22-μm membrane before analysis. $AgNO_3$ (20%, 600 μL) was added into a mixture containing human serum (1.0 mL) and the standard stock solution of 5-FU (4 μL). The resulting mixture was subjected to vortex for 3 min and placed to stand for 5 min. NaCl (20%, 700 μL) was added and the mixture was subjected to vortex for another 3 min. After centrifugation at 13,000 rpm for 12 min, the supernatant (0.5 mL) was diluted with water to 1 mL in centrifuge tube and filtered through a 0.22-μm membrane.

III. *HPLC Conditions*

A 600-Controller HPLC system (Waters, MA, USA) with UV detector was used for the analysis. A Nova-park C_{18} column (3.9 × 150 mm, 4.6 μm) was used for separation. The column temperature was maintained at 25°C. The standards and samples were determined using a mobile phase consisting of 5 mM KH_2PO_4 solution (pH = 6.0) and methanol (96 : 4) at the flow rate of 1 mL/min. The injection volume was 20 μL. The peak of 5-FU was detected at the wavelength of 254 nm.

RESULTS

I. *Calibration curve*

5-FU was shown to exhibit a good linearity in the test range. The standard stock solution was diluted to a series of appropriate concentrations with water for the construction of the calibration curve. At least ten concentrations of the solution were analyzed in triplicate, and the calibration curve was constructed by plotting the peak areas versus the concentrations of 5-FU. The linearity results are shown in Table 1 and the HPLC chromatogram of 5-FU is shown in Figure 2.

II. *Precision*

The standard solution of 5-FU was analyzed continuously (n = 5), under the present chromatographic conditions. The RSD calculated from the peak areas was 0.4%.

III. *Repeatability*

5-FU injection and human serum spiked with 5-FU were analyzed (n = 5). The average content in the 5-FU injection was 24.45 mg/mL and the RSD was 0.2%. The average content in the human serum spiked with 5-FU was 5.97 μg/mL and the RSD was 2.1%.

IV. *Stability Test*

Sample solutions were injected and analyzed every 2 h. The results showed that 5-FU was stable in both samples during the test period (14 h). The RSDs were 1.3 and 1.8% in 5-FU injection and human serum, respectively.

V. *Determination of Real Samples*

5-FU injection and human serum were analyzed in triplicate. The concentration of 5-FU was calculated using the regression equation of the calibration curve. The HPLC chromatograms of the real samples are shown in Figures 2 and 3.

Table 1. Linear range, regression equation and limit of quantification of 5-FU calibration curve

Compound	Linear range (μg/mL)	Regression equation	Correlation coefficient (r^2)
5-FU	- 100	y = 74250 x + 7592.8	0.9999

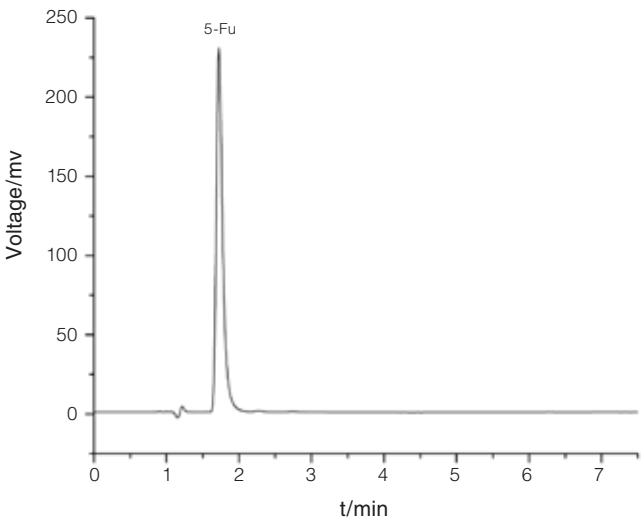

Figure 1. HPLC chromatogram of 5-FU standard. (The concentration of 5-FU is 20 μg/mL)

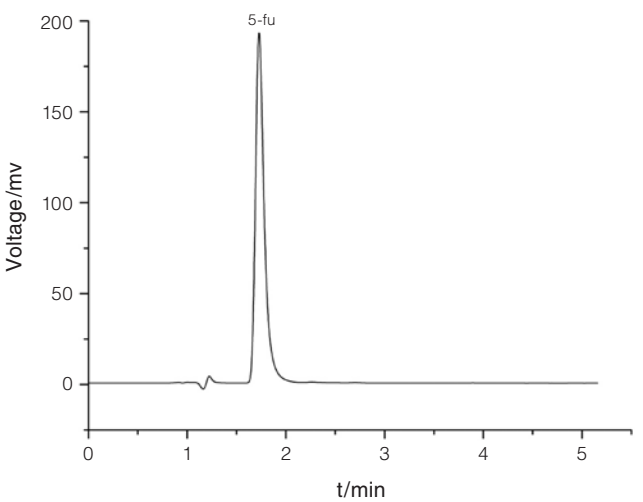

Figure 2. HPLC chromatogram of the 5-FU injection sample. (The concentration of 5-FU is 20 μg/mL)

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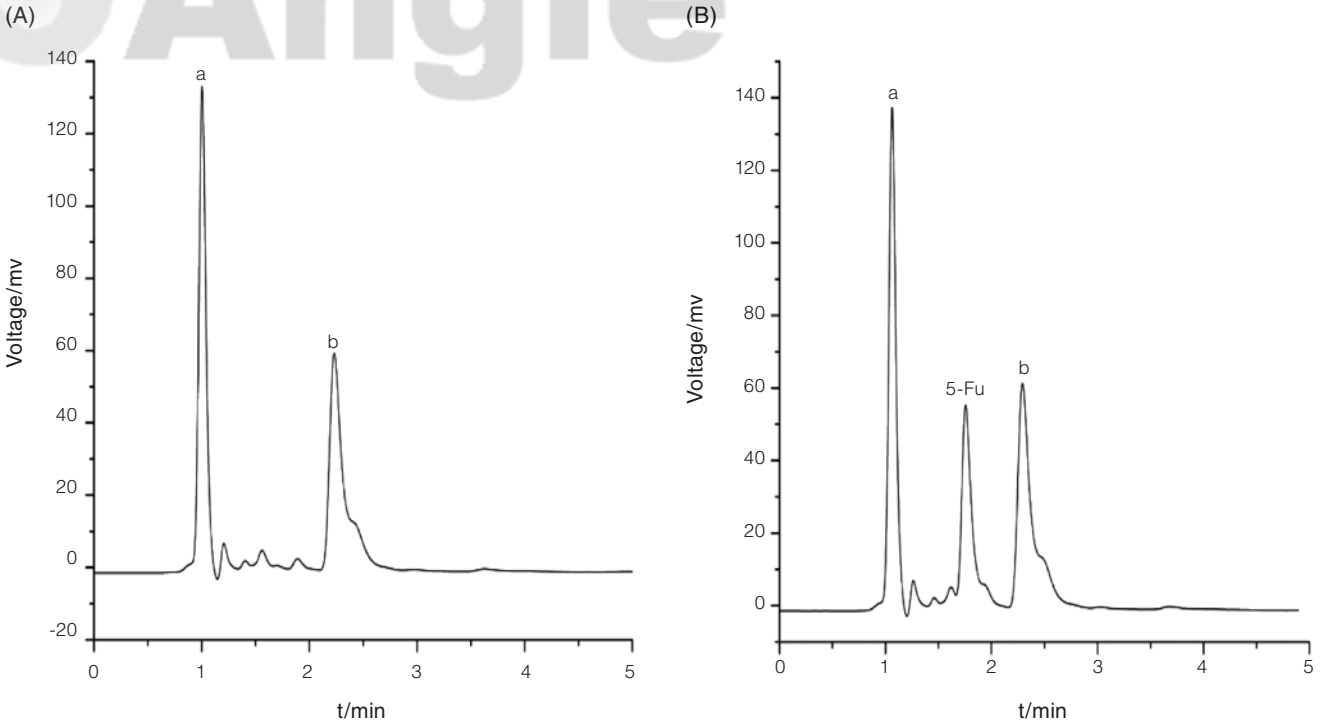

Figure 3. HPLC chromatograms of the (A) human serum sample, and (B) human serum sample spiked with 5-FU standard at the level of 4 μg/mL. * a and b are impurities.

Table 2. Relevant quantitative parameters of 5-FU in 5-FU injection

Compound	Content of sample (μg/mL)	Addition of standard (μg/mL)	Determination (μg/mL)	Average recovery* (%)	RSD (%)	LOQ (μg/mL)
5-FU	16.24	8	23.22	95.79	1.7	0.10
		16	32.30	100.2	0.9	
		24	42.30	105.1	2.3	

*Average of triplicate.

VI. *Recovery by Standard Addition*

The recovery of the method was evaluated by adding a known amount of the standard stock solution of 5-FU separately into a certain amount of 5-FU injection (2 mL) and human serum (1 mL). Three replicates were performed for the test. The mixtures were prepared and analyzed using the method mentioned above (MATERIALS AND METHODS II and III). The contents, average recoveries and limits of quantification (LOQ) of 5-FU in the 5-FU injection and human serum samples are shown in Tables 2 and 3, respectively.

Table 3. Results of average recoveries and LOQ of 5-FU in human serum

Addition (μg/mL)	Determination (μg/mL)	Average recovery* (%)	RSD (%)	LOQ (μg/mL)
6	5.97	99.50	0.7	0.85
12	10.82	90.17	0.3	
24	26.12	108.8	1.2	

*Average of triplicate.

DISCUSSION

The results of this study indicated that the HPLC run time of 5-FU in human serum was within 2 min. The method was shown to be simple, accurate and sensitive. The treatment of human serum with $AgNO_3$ and NaCl was convenient, and the impurities in human serum did not interfere with the determination of 5-FU. The method could be applied for the determination of 5-FU in injection and human serum. It is applicable for the examination of 5-FU in the serum of patients who have been injected 5-FU and for monitoring concentration of the drug.

The methods of previous studies[4-6,8,10,12-14] have disadvantages, including a longer analysis time (more than 2 min), the use of environmentally unfriendly solvents[6,8] or the treatment of human serum using organic solvent and

nitrogen[4,10]. In comparison, the method developed in this study required a shorter analysis time and relatively less solvent consumption. It also provided a simple and convenient way of sample preparation.

The optimization of the analytical conditions used in this study involved the evaluation of different mobile phases, concentrations and pH values of the KH_2PO_4 solution. It was found that these mobile phases resulted in longer analysis times and poor peaks. Methanol and ethyl acetate were tested as reagents for the treatment of human serum, but they were found to be unsuitable due to the complexity of the process, longer preparation times and interferences from some impurities. Therefore, the present conditions were chosen for this method.

CONCLUSIONS

A simple, accurate, rapid and sensitive high-performance liquid chromatographic (HPLC) method was developed for the determination of 5-fluorouracil (5-FU) in 5-FU injection and human serum.

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