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NOTE

HPLC Determination of 5-Fluorouracil in Human Plasma

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The present study describes the HPLC method for the estimation of 5-fluorouracil in human plasma. Varying amounts of 5-fluorouracil (10 to 400 ng) were added to drug-free human plasma (0.5 mL) and mixed with 0.3 mL of acetonitrile. The mixture was vortexed for 5 min and centrifuged at 3,000 rpm for 10 min and the supernatant liquid was filtered. Twenty microlitres of the resultant filtrate was injected into a reverse phase C-18 column using a mobile phase consisting of HPLC grade water at a flow rate of 0.8 mL/min. The eluents were monitored at 260 nm. On conducting validation study, it was found that the method is simple, precise, specific, less time consuming and accurate for the estimation of 5-fluorouracil in human plasma samples.

Key words: 5-Fluorouracil, Human plasma, HPLC, Determination.

5-Fluorouracil is the drug of choice in the treatment of carcinoma of stomach, colon, rectum, breast and ovaries[1]. Several HPLC methods have been reported for the estimation of 5-fluorouracil in plasma[2–5]. Some of the reported HPLC methods[2, 3] required photo diode array detector and the process is considered tedious. However, the HPLC methods using the most commonly available columns are preferred. In the present study a sensitive, accurate and precise HPLC method has been developed for the estimation of 5-fluorouracil in human plasma using RP C-18 column and simple UV detection.

5-Fluorouracil was purchased from M/s Lancaster synthesis, UK. Acetonitrile and water used were of HPLC grade (Qualigens).

Instrumentation: A gradient High Pressure Liquid Chromatography (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP C-18 column (250 mm × 4.6 mm I.D.; particle size 5 μm; YMC, Inc., Wilmington, NC 28403, U.S.A) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

HPLC conditions: The mobile phase used was HPLC grade water and filtered through 0.2 m membrane filter and degassed with a helium spurge for 15 min before use and pumped from the solvent reservoir to the column at a flow rate of 0.8 mL/min which yielded column back pressure of 200–225 kg/cm^2. The

column temperature was maintained at 40°C. The detector sensitivity was set at 0.0001 a.u.f.s. The volume of each injection loop was 20 μL.

Method: Seven sets of plasma samples with varying drug concentrations were prepared by spiking drug-free plasma with an appropriate volume (100 μL) of a known amount of 5-fluorouracil at a concentration range of 10 to 400 ng/ 0.5 mL of plasma.

An aliquot of plasma (0.5 mL) was accurately measured into a 10 mL glass tube with a teflon-lined cap, followed by addition of 0.5 mL of acetonitrile. The mixture was vortexed to ensure complete mixing of contents for 5 min and centrifuged for 10 min at 3,000 rpm. The resultant supernatant liquid was filtered through 0.2 μm membrane filter and twenty microlitres of the filtrate was injected into a reverse phase C-18 column and the eluents were monitored at 260 nm. The peak areas of 5-fluorouracil were recorded and the regression of plasma concentration of the drug over its peak area was calculated using the least squares method of analysis.

Precision: Aliquots of blank plasma (0.5 mL) were spiked with 5-fluorouracil solutions (100 μL) so as to yield concentrations of 50, 100 and 200 ng/0.5 mL of plasma. Each plasma sample was treated, as described above, and the filtrate was injected in to the HPLC column (n = 5). Each sample was prepared in triplicate on three consecutive days and injected in to the HPLC column (n = 5) to observe the precision of the method.

Accuracy: The preanalyzed plasma samples containing 100 ng/ 0.5 mL were added with known quantity of 5-fluorouracil (50, 100 or 200 ng/0.5 mL) and subjected to the proposed HPLC method, in triplicate. The differences in the measured concentration and that of the added quantity (50, 100 or 200 ng/0.5mL) were expressed as per cent recovery.

5-Fluorouracil appeared on the chromatogram at 5.83 min (Fig. 1) wherein the run time was set at 10 min. When the same drug solution was injected 5 times, the retention time of the drug was same. Table-1 shows the mean peak area of 5-fluorouracil solutions for 7 such determinations. When the concentration of 5-fluorouracil and its respective peak area was subjected to regression analysis by least squares method, a high correlation coefficient was observed (r = 0.99983) in the range of 10 to 400 ng/mL. The regression of 5-fluorouracil concentration over its peak area ratio was found to be Y = 478.1112 + 60.07659X where 'Y' is the peak area ratio and 'X' is the concentration of 5-fluorouracil. This regression equation was used to estimate the amount of 5-fluorouracil in plasma or in validation study (precision and accuracy).

The present HPLC method was also validated for intra- and inter-day variation. To assess the precision, drug-spiked plasma samples (0.5 mL) containing known quantity of the drug (50, 100 and 200 ng/mL) were subjected to the HPLC method. The plasma samples were treated as per the procedure described above, and the resultant filtrate was repeatedly injected on the same day and on three different days. The coefficient of variation (CV) in the peak area ratio for five replicate injections was found to be less than 2.3%. Also, the inter-day variation (3 days and five injections) was found to be less than 2.2%. Thus, the results show that this HPLC method is highly reproducible. When a known amount of drug solution

(50, 100 or 200 ng/mL) was added to preanalyzed plasma samples (100 ng/mL), there was a high recovery (99.37%) of 5-fluorouracil indicating that this HPLC method is highly accurate.

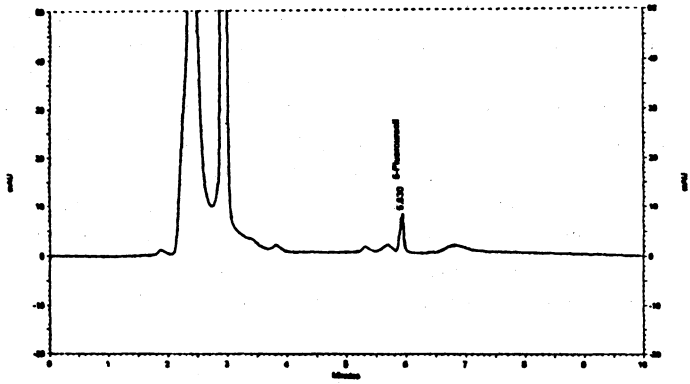

Fig. 1. Typical HPLC chromatogram of 5-fluorouracil in human plasma

TABLE -1
CALIBRATION CURVE FOR THE ESTIMATION OF
5-FLUOROURACIL IN HUMAN PLASMA BY HPLC METHOD

Amount of 5-fluorouracil added to 0.5 mL human plasma (ng)	Peak area *	C.V. (%)
0	0	0.00
10	855	2.52
20	1619	2.76
50	3482	0.98
100	6772	1.49
200	12621	0.68
300	18486	0.92
400	24392	0.82

*Mean of seven determinations
Regression equation: Y= 478.1112 + 60.07659X (r = 0.99983)

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