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Short communication

Simple high-performance liquid chromatographic method for the quantitation of 5-fluorouracil in human plasma

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Abstract

A simple, rapid, specific and sensitive high-performance liquid chromatography method has been developed for quantitation of 5-fluorouracil (5-FU) in human plasma. The method involves deproteinization of a small sample volume of plasma (150 μl) followed by HPLC on a cation-exchange resin column, Aminex HPX-87H (300×7.8 mm I.D.), preceded by a similar guard cartridge with UV detection at 265 nm. This method allows a good separation of 5-FU with a retention time of 24 min and a detection limit at 25 ng/ml. The calibration curve was linear from 25 to 2000 ng/ml. The coefficient of variation was ≤4.4% for within-day reproducibility and ≤9.5% for day-to-day reproducibility.

Keywords: 5-Fluorouracil

1. Introduction

5-Fluorouracil (5-FU) is an antimetabolite antineoplastic agent, generally used adjunctly in the treatment of a wide variety of solid tumors, such as cancers of the gastrointestinal tract [1,2], head and neck [3,4], and lung [5].

The current analytical techniques for 5-FU use GC [6,7], GC–MS [8,9] and HPLC [10–19]. This paper describes a rapid, sensitive and selective method for the quantitation of 5-FU in plasma, using a small sample size without solvent extraction, with HPLC and spectrophotometric detection.

2. Experimental

2.1. Reagents

5-FU was kindly supplied by Hoffmann-La Roche (Basel, Switzerland). Uracil, hypoxanthine, xanthine, uric acid, barbituric acid, uridine, thymidine, cytosine, 5-fluorouridine (FUrd), 5-fluoro-2′-deoxyuridine (FdUrd), 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) were obtained from Sigma (La

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Verpillère, France). Analytical-grade sulfuric acid and trichloroacetic acid were purchased from Merck (Nogent-sur-Marne, France).

For stock solutions, 5-FU was dissolved in water and stored at 4°C in darkness.

2.2. Mobile phase

The mobile phase was composed of 0.005 *M* sulfuric acid filtered through a 0.2-μm membrane filter.

2.3. Sample preparation

Blood samples were collected on EDTA and immediately centrifuged at 4°C at 3000 *g* for 10 min. The plasma was stored at −20°C until analysis. Each sample was allowed to thaw at room temperature, and 150 μl of plasma were added to 150 μl of trichloracetic acid (diluted at 10% in distilled water). The tube was vortex-mixed for 30 s and centrifuged at 5000 *g* for 5 min; 50 μl of the clear supernatant were injected into the HPLC system. Standards were prepared from normal human plasma spiked with different amounts of 5-FU.

2.4. High-performance liquid chromatography

The HPLC equipment consisted of a Beckman Model 116 pump with a 210 A sample injection valve (Beckman, Gagny, France) fitted with a 50-μl sample loop, a Shimadzu SPD-6A spectrophotometric detector and a CR-5A integrator (Touzard-Matignon, Vitry-sur-Seine, France).

The column was a cation-exchange resin in form H^+ of sulfonated styrene–divinylbenzene copolymer (Aminex HPX-87H, 9 μm mean particle diameter, 300×7.8 mm I.D.; Bio-Rad, Ivry-sur-Seine, France) preceded by a similar guard cartridge thermostatted at 60°C. The flow-rate of the mobile phase was 0.5 ml/min. The absorbance of the eluent was monitored at 265 nm.

3. Results and discussion

Representative chromatograms from human plasma are shown in Fig. 1.

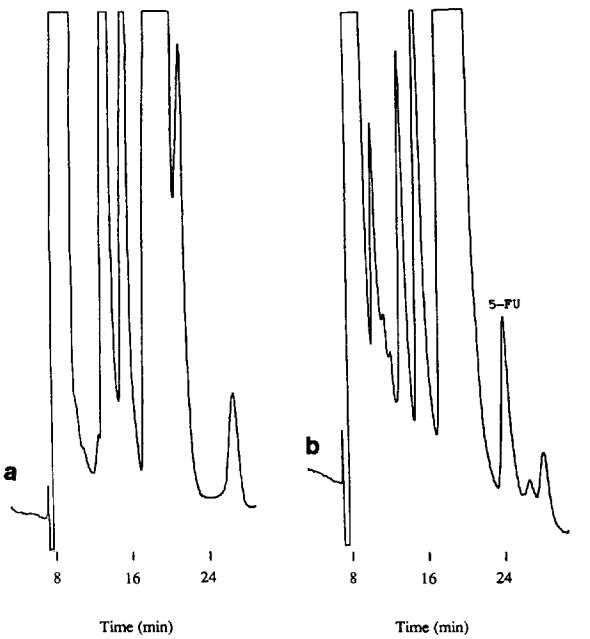

Fig. 1. (a) Chromatogram of blank plasma. (b) Chromatogram of a patient plasma sample containing 5-FU at 250 ng/ml.

At a signal-to-noise ratio of 3, the minimal detectable and quantifiable concentration after the precipitation procedure was 25 ng/ml.

The recovery was determined from the comparison of peak areas obtained after injection of aqueous standard of 5-FU and deproteinized plasma of equivalent concentrations. The studied concentrations were 25, 50, 100, 200, 500, 1000, 1500, 2000 ng/ml. Each determination was made in duplicate. The results range from 94.6 to 106.7%.

A linear relationship between 5-FU concentrations and peak areas was observed between 25 and 2000 ng/ml with a correlation coefficient of r=0.9994. Each point was determined from three assays.

The within-day highest coefficient of variation (C.V.) for 5-FU was 4.4% for a concentration of 50 ng/ml (n=6) (Table 1). The day-to-day C.V. values were determined in triplicate from spiked plasmas (200, 500 and 1000 ng/ml) for twelve days (Table 1).

In HPLC, reversed-phase (RP) [11,13,16,17,19] and reversed-phase ion-pair (RP-IP) [10,12,14,15,18] are the most widely used techniques for the analysis of 5-FU.

Table 1
Within-day and day-to-day coefficients of variation for human plasma spiked with different amounts of 5-FU

Concentrations (ng/ml)	Coefficient of variation (%)	
	Within-day (n=6)	Day-to-day (n=12)
50	4.4	8.7
200	3.7	8.6
500	3.5	9.2
1000	3.1	9.5

The disadvantage of the majority of these techniques is the poor retention of 5-FU, which appears close to the solvent front, even when the mobile phase is almost totally [10–12,15] or totally devoid [13,14,16–19] of methanol or acetonitrile.

Some authors have tried to circumvent this inconvenience by using two columns in line [10,16], by valve switching [12] or by gradient elution [17]. We chose to use an ion-exchange technique, which allowed us a longer retention time for 5-FU (24.2 min), and no interference with endogenous compounds or frequently associated drugs (Table 2) such as uridine, uric acid, xanthine, hypoxanthine, cytosine, thymidine, FUrd, FdUrd, FdUMP, cisplatin, methylprednisolone, alizapride, metoclopramide, methotrexate. The only product with a retention time close to that of 5-FU was uracil, without however impeding the quantitation of 5-FU.

In addition to its specificity, the method we describe has the advantage of simplicity, since it only requires a simple deproteinization, thereby avoiding time- and solvent-consuming extraction procedures. The deproteinization technique obviates the need for an internal standard and its risk of coelution with 5-FU or with endogenous compounds. Moreover, this method requires only a small volume of plasma (150 μl), but is sensitive enough for pharmacokinetic analysis of 5-FU. Fig. 2 shows the mean (eight patients) plasma concentration—time curve of 5-FU during 96 h continuous intravenous infusion (1000 mg m^{-2} 24 h^{-1}), in addition to cisplatin (20 mg m^{-2} 24 h^{-1}) started 24 h before.

Table 2
Retention times of other similar or frequently associated products

Compound	Retention time (min)
Uracil	24.9
Uridine	15.5
Uric acid	18.8
Xanthine	NR
Hypoxanthine	NR
Cytosine	NR
Thymidine	20.2
FUrd	15.1
FdUrd	17.5
FdUMP	8 and 17.6
Cisplatin	17.9
Methylprednisolone	NR
Alizapride	NR
Metoclopramide	NR
Methotrexate	NR

NR=no retention.

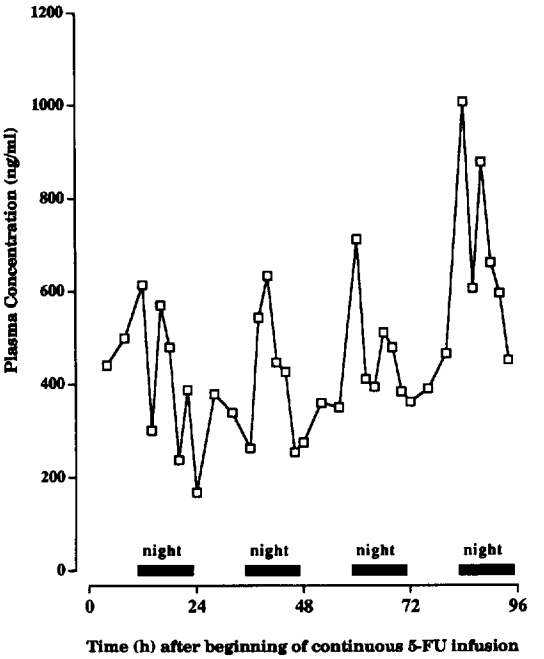

Fig. 2. Mean plasma 5-FU concentrations from eight patients during 96 h of continuous intravenous infusion of 5-FU (1000 mg m^{-2} 24 h^{-1}), in addition to cisplatin (20 mg m^{-2} 24 h^{-1}) started 24 h before. Dark bars represent night periods.

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