# GLC Determination for Ftorafur in Biological Fluids

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**Abstract** □ A GLC analysis for free ftorafur was developed to follow the drug disposition in body fluids of patients. The free drug was extracted from aqueous biological samples with chloroform, derivatized by methylation, and chromatographed on 1% HI-EFF 8BP using flame-ionization detection. The analysis is sensitive (0.25 μg/ml of plasma) and specific for the intact molecule, and it does not interfere with subsequent fluorouracil analysis of the same sample.

**Keyphrases** □ Ftorafur—GLC analysis, biological fluids □ GLC—analysis, ftorafur in biological fluids □ Antineoplastic agents—ftorafur, GLC analysis in biological fluids

The antitumor agent ftorafur [1-(tetrahydro-2-furanyl)-5-fluorouracil] (I) (1) is a prodrug form of fluorouracil (2, 3). It slowly decomposes *via* aqueous and enzymatic hydrolysis with the release of free fluorouracil. Thus, I has much lower toxicity than fluorouracil (five to seven times less) (4, 5) but possesses similar activity. Additionally, it has a much longer half-life. These properties make I an ideal chemotherapeutic alternative to fluorouracil, which has the attendant difficulties of toxicity and a short half-life (30 min) (6).

Despite substantial clinical work utilizing I (7–9), pharmacokinetic studies have been limited by the lack of a generally available analytical method specific for free intact I. Microbiological assays have been used in disposition studies in animals and humans (10, 11). These methods are indirect in that they measure the amount of fluorouracil resulting from the I decomposition, I itself being essentially inactive, and the results may be high when compared to those obtained by instrumental methods (3). Additionally, the microbiological assay has low sensitivity (0.5 μg/ml) and is time consuming, requiring 24 hr at 5° and 18 hr at 37°. Recently, pharmacokinetic studies of $^{14}$C-labeled I were conducted using chromatographic and radiochemical techniques to separate and quantitate the drug and its metabolites (12). The reported half-life for $^{14}$C-labeled I was 16.8 hr.

The present work describes an analytical method based on chloroform extraction of I with subsequent derivatization by methylation with a 30% solution of dimethylformamide in dimethylformamide dimethyl acetal (II)[1]. The method is applicable to blood and urine samples and can be performed routinely.

## EXPERIMENTAL

**Reagents**—All chemicals were analytical reagent grade, and all solvents except nanograde chloroform[2] were redistilled. Pyridine was distilled first from *p*-toluenesulfonyl chloride and then from potassium hydroxide. Both pyridine and dimethylformamide were stored over molecular sieves[3]. Compound I[4] and 6-$^{3}$H-fluorouracil[5] (specific activity of 7.3 Ci/mmole) were used as received. 6-$^{3}$H-Labeled I was synthesized by sodium tritoxide–tritium oxide exchange[5] (13). Subsequent purification by instant TLC[6], using methanol–chloroform (1:9), yielded 6-$^{3}$H-I with a specific activity of 0.46 Ci/mmole.

Synthesis of 6-$^{2}$H-fluorouracil was accomplished by sodium deuteroxide–deuterium oxide exchange (13). Subsequent purification by recrystallization from methanol and ether gave 6-$^{2}$H-fluorouracil deuterated to the extent of 93% as determined by mass spectral analysis of the trimethylsilyl derivative.

All labeled and nonlabeled standards were prepared in absolute ethanol at concentrations permitting use of 10–20-μl aliquots.

**Extraction of Biological Samples**—Blood samples were collected in heparinized containers[7] and centrifuged to separate the plasma. The plasma was then frozen until analyzed. Urine samples were collected and frozen directly.

To 1.0 ml of plasma was added 4000 dpm of 6-$^{3}$H-I and 0.758 μg of 6-$^{2}$H-fluorouracil to serve as internal standards. After dilution with 1 ml of water, the pH was adjusted to 7.4 with 10 μl of pH 4.7, 0.5 *M* acetate buffer/ml of plasma. The sample was then extracted three times at 0° with 15, 8, and 8 ml of chloroform to remove I. The first two extracts were filtered through cotton, evaporated to dryness under nitrogen, and transferred with chloroform to a 6 × 50-mm test tube[8] for derivatization. The third extraction was included only when levels of I were anticipated to be low. Traces of chloroform were removed from the aqueous phase by bubbling nitrogen through the plasma.

The procedure of Cohen and Brennan (14) was then used to extract fluorouracil. This extract was further purified by instant TLC with methanol–chloroform (6:94) followed by instant TLC with methanol–chloroform (40:60). All elutions and transfers of fluorouracil were with methanol. When sufficient plasma was available, analyses for I and fluorouracil were carried out on separate portions of the same plasma sample.

With only minor variations, urine samples were extracted in the same manner as described, and analyses for I and fluorouracil were performed on separate portions of the same sample. Prior to the chloroform extraction of I, 1.0-ml aliquots were adjusted to pH 7.4 with 1 *N* NaOH or 0.5 *M*, pH 4.7 acetate buffer. Fluorouracil extraction was performed on 0.5-ml urine aliquots. The addition of sodium sulfate was eliminated from the Cohen and Brennan (14) procedure, and the first instant TLC was carried out in methanol–chloroform (3:97).

**Derivatization and Measurement of Radioactivity**—The evaporated I sample was dissolved in 30 μl of dimethylformamide. Following dissolution of the sample, 70 μl of II was added; the reaction vessel was then sealed in a flame without prior cooling of the solution. The reaction vessel was then cooled to room temperature and totally immersed in an oil bath at 95° for 15 min. After cooling, 10 μl was injected into the gas chromatograph for analysis. An aliquot also was added to toluene phosphor before determining radioactivity in a liquid scintillation spectrometer[9] equipped with automatic external standardization to correct for quenching. The percent extracted was then calculated.

Immediately prior to analysis, the dried fluorouracil sample was reacted with 10 μl of pyridine and 20 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (III)[10] for 10 min at room temperature to form the trimethylsilyl derivative.

**GLC[11] and Quantitation**—The 3-*N*-methyl derivative of I was chromatographed on a glass column, 1.83 m (6 ft) × 0.63 cm (0.25 in.), packed with 1% HI-EFF 8BP on 100–120-mesh Gas Chrom Q[12] and conditioned at 220° for 48 hr. A flame-ionization detector also was used. Nitrogen carrier gas flow was maintained at 40 ml/min. The injection port and detector temperatures were 225 and 250°, respectively.

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Mallinckrodt Chemical Works, St. Louis, Mo.
[3] Linde Type 3A, MC & B Manufacturing Chemists, Cincinnati, Ohio.
[4] Microbiological Associates, Washington, D.C.
[5] New England Nuclear, Boston, Mass.
[6] ITLC SA, Gelman Instrument Co., Ann Arbor, Mich.
[7] B-D Vacutainers, Scientific Products, McGaw Park, Ill.
[8] T 1320-1, Scientific Products, McGaw Park, Ill.
[9] Packard Tri-Carb model 3380 and absolute activity analyzer model 554, Packard Instrument Co., Downers Grove, Ill.
[10] Regisil, Regis Chemical Co., Morton Grove, Ill.
[11] Packard Instrument Co., Downers Grove, Ill.
[12] Applied Science Laboratories, State College, Pa.

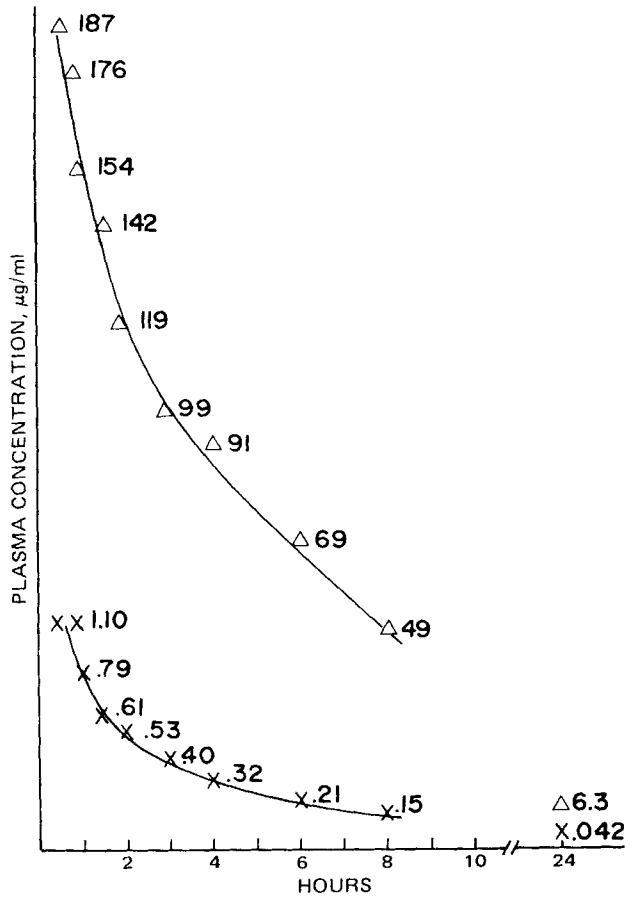


**Figure 1**—*Plasma concentration of I (△) and fluorouracil (×) in a patient receiving I at 2.25 g/m² iv over 30 min.*

The sample was injected at a column oven temperature of 207°, and the derivative eluted at approximately 7 min. Quantitation was accomplished by prior and subsequent injection of a standard sample of 3-*N*-methyl I and peak height comparison. Blanks were periodically injected to test for any possible sample carryover.

The trimethylsilyl derivative of fluorouracil was chromatographed on a glass column, 1.83 m (6 ft) × 0.63 cm (0.25 in.), packed with 1% SE-30 on 100–120-mesh Gas Chrom Q and conditioned at 300° for 24 hr. The column and injection port temperatures were 85 and 300°, respectively. Helium carrier gas flow was approximately 30 ml/min. The retention time under these conditions was approximately 6.5 min.

Isotope dilution was used to quantitate fluorouracil. Mass spectra[13] from *m/e* 250 to 300 were recorded every 2 sec while the derivative was eluting. The fraction of deuterium in all samples was determined from the average of the abundances of the M − 15 ions and their associated isotope peaks (*m/e* 259, 260, 261, and 262) in the five most intense spectra recorded. Spectral data for both deuterated and nondeuterated standards were recorded at the beginning and end of each day, and these data were used in calculating the fraction of deuterium and the amount of fluorouracil in the plasma samples determined on that day. The variation in the spectral data obtained from the standard samples was small; for example, the average value of 16 measurements of the fraction of deuterium in the deuterated standard made over 45 days was 0.932 ± 0.006 (± 1 *SD*).

## RESULTS AND DISCUSSION

The extraction procedure (14) yielded an interference which, upon repeated injection into the gas chromatograph, led to a severe loss of sensitivity. This interference was eliminated by instant TLC using methanol–chloroform (6:94) followed by instant TLC using methanol–chloroform (40:60). The first instant TLC removed most of the interference and all of I; removal of I is crucial since any amount will interfere with subsequent fluorouracil analysis. The second instant TLC further reduced the interference.

[13] Model 01SG-2, Jeol, Cranford, N.J.

**Table I—Quantitation of I and Fluorouracil Added to Human Plasma**

| I | | Fluorouracil | |
|---|---|---|---|
| Amount Added, μg/ml | Amount Found, μg/ml[a] | Amount Added, μg/ml | Amount Found, μg/ml |
| 5.0 | 4.9 ± 0.1 | 0.100 | 0.105 ± 0.006[b] |
| 15.0 | 15.4 ± 0.3 | 0.153 | 0.161[c] |
| 50.0 | 52 ± 2 | 0.229 | 0.237[c] |
| 100 | 105 ± 3 | 0.382 | 0.384 ± 0.011[b] |
| 200 | 201 ± 2 | 0.764 | 0.759 ± 0.029[b] |
| | | 1.15 | 1.15[d] |
| | | 1.53 | 1.49[d] |

[a] Average of five determinations ± *SD*. [b] Average of four determinations ± *SD*. [c] Average of two determinations. [d] One determination.

Separate control experiments with both $^3$H-I and 6-$^3$H-fluorouracil showed that the fractionation of the two drugs by chloroform extraction was essentially complete. No detectable amount of fluorouracil was extracted into the chloroform layer, in agreement with previous results (11), while less than 5% of I was picked up in the subsequent fluorouracil extraction.

The thermal instability of I presents special problems in derivatization and instrumental analysis. Attempts to chromatograph free I on various columns (1% SE-30, 3% OV-1, and 3% OV-17) resulted in very low sensitivity. Attempts to form a trimethylsilyl derivative with a 50:50 mixture of III and pyridine were unsuccessful. TLC of the evaporated reaction mixture showed only I, while injection of this reaction mixture onto the gas chromatograph (injection port temperatures of 150, 200, 250, and 300°) resulted in I decomposition with the formation of the trimethylsilyl derivative of fluorouracil to the extent of 10–20%, necessitating the complete removal of I prior to subsequent fluorouracil analysis. The trimethylsilyl derivative of I was not detected. Treatment of the methyl derivative of I in the same manner did not result in the formation of a detectable quantity of 1-trimethylsilyl-3-*N*-methyl-5-fluorouracil, even though such a derivative is formed from 3-*N*-methyl-5-fluorouracil. Thus, the methyl derivative is stable under the GLC conditions.

The methylation reaction is quantitative [>97% complete by TLC on silica gel F-254[14] using methanol–chloroform (1:4 v/v)] with no apparent decomposition of I or its derivative. GLC analysis showed that varying the amount of dimethylformamide from 20 to 40% had no effect on the reaction; however, use of pure II alone gave erratic results. At 90°, the reaction was complete after 15 min; at 95 and 100°, only 5 min was required. Running the reaction at 95 or 100° for 20 min caused no detectable decomposition of the methyl derivative.

The derivative structure was established as 1-(tetrahydro-2-furanyl)-3-*N*-methyl-5-fluorouracil, in agreement with previous results with the same reagent and similar compounds (15). NMR[15] analysis in deuterochloroform showed a doublet at 442 Hz with a relative area of 1 ($H_6$), a multiplet at 357 Hz with a relative area of 1 ($H_1'$), a multiplet at 245 Hz with a relative area of 2 ($H_4'$), a singlet at 200 Hz with a relative area of 3 ($NCH_3$), and a multiplet at 123 Hz with a relative area of 4 ($H_2'$ and $H_3'$). The UV spectrum had a maximum at 270 nm ($\epsilon$ 7991) and a minimum at 237 nm ($\epsilon$ 1854). Characteristic ions in the mass spectrum of the derivative were the molecular ion at *m/e* 214 (13% relative abundance), the base + H and base + 2H at *m/e* 144 (2) and 145 (4), and the tetrahydrofuranyl moiety at *m/e* 71 (100). The corresponding ions occurred in the mass spectrum of I at about the same relative intensities.

Figure 1 shows data obtained from a patient receiving 2.25 g/m² iv of I over 30 min. Its plasma disappearance was biphasic with a $\beta$-phase half-life of 5 hr, which differed significantly from the value of 18.6 hr obtained for the median half-life of $^{14}$C-labeled I in humans receiving 50 mg/m² (9). Data obtained from seven patients in this laboratory indicate an average half-life of 6.7 hr for I. The plasma fluorouracil level from 2 to 24 hr was similar to levels previously obtained in patients receiving a continuous intravenous infusion of fluorouracil at 1100 mg/m²/24 hr for 72–96 hr[16].

Compound I (90 mg) isolated from 1.5 liters of combined urine from nine patients showed no optical activity, indicating no enzymatic pref-

[14] Brinkmann Instruments, Westbury, N.Y.
[15] Varian A-60, Palo Alto, Calif.
[16] Unpublished data obtained in this laboratory in cooperation with the Milton A. Darling Memorial Center, The Grace Hospital, Detroit, Mich.

erence for either enantiomer. This result is in agreement with results obtained previously with human cell cultures and each pure enantiomer as well as the racemic mixture. No significant difference in the cytotoxic effects or the relative abilities to prevent an increase in cell numbers was observed with the three forms (16).

Control runs with plasma blanks showed no interferences with either the I or fluorouracil procedure. The accuracy and the concentration range over which both methods were tested are indicated in Table I. The correlation coefficients for the amount added *versus* the amount calculated in Table I are 0.999 for both sets of data, indicating that both analyses are linear in the concentration ranges tested. The methods as outlined permit the detection of 0.25 μg of I and 0.025 μg of fluorouracil/ml of plasma and are specific for the intact molecules. The sensitivity and applicability of these analyses indicate that they are suitable for these types of studies, and clinical investigations utilizing these procedures are currently being performed.

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[17] *Note added in proof:* Similar methods for the concurrent determination of I and fluorouracil were reported recently by A. T. Wu, H. J. Schwandt, and W. Sadee, *Res. Commun. Chem. Pathol. Pharmacol.* **14**, 89 (1976).

# Estimation of Pharmacokinetic Parameters from Postinfusion Blood Level Data Obtained after Simultaneous Administration of Intravenous Priming and Infusion Doses


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**Abstract** □ Occasionally, it is desirable to attain steady-state blood drug levels rapidly in pharmacokinetic studies as well as in the treatment of certain diseases. In these cases, it is useful to administer an intravenous priming dose in combination with continuous drug infusion. Mathematical relationships are presented for the determination of pharmacokinetic parameters in these situations using postinfusion blood drug level data. The parameters obtained by this method are identical to the parameters obtained after a rapid intravenous injection of a drug.



**Keyphrases** □ Pharmacokinetic parameters—determined from postinfusion blood level data after simultaneous intravenous priming and infusion doses □ Dosage regimens—simultaneous intravenous priming and infusion doses, pharmacokinetic parameters determined from postinfusion blood level data


Methods for the assessment of pharmacokinetic parameters from postinfusion blood level data obtained after continuous intravenous infusion were presented previously (1). However, in practice, a rapid intravenous priming dose is often given simultaneously with the beginning of a continuous infusion to achieve steady-state blood drug levels rapidly in the body. This paper presents the treatment of postinfusion blood concentration data for the estimation of pharmacokinetic parameters in those instances.

## THEORY AND DISCUSSION

**Two-Compartment Model**—For a drug that exhibits the characteristics of a two-compartment open model (Scheme I), the decay of blood concentration, $C_{p(\text{bolus})}$, with time $t$, after a rapid intravenous injection (assuming first-order elimination and distribution kinetics) can be expressed as (2):

$$C_{p(\text{bolus})} = \frac{X_0(\alpha - k_{21})}{V_c(\alpha - \beta)} e^{-\alpha t} + \frac{X_0(k_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta t} \qquad \text{(Eq. 1)}$$

If:

$$A = \frac{X_0(\alpha - k_{21})}{V_c(\alpha - \beta)} \qquad \text{(Eq. 2)}$$