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Short Communication

Rapid analysis of 5-fluorouracil in plasma or formulations by high-performance liquid chromatography

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Introduction

5-Fluorouracil (5-FU) is an antimetabolite frequently employed in the treatment of carcinomas of the gastrointestinal tract, breast and ovary [1]. Treatment with this drug can be optimized and placed on more rational basis by defining the time course of 5-FU in plasma for individual patients [1, 2]. Additionally, analysis of 5-FU in its pharmaceutical formulations is an important tool for testing the content uniformity of these products and ensures quality.

Several techniques have been employed for the analysis of 5-FU in plasma. These techniques include gas chromatography [2–8], high-performance liquid chromatography (HPLC) [9–16], and microbial assays [17, 18]. None of these methods have been applied for the analysis of this drug in formulations. The use of radial-compression liquid chromatography for the quantitation of 5-FU has not been previously reported. As demonstrated in this report, with this approach a rapid and accurate analysis with high chromatographic efficiency is achieved for 5-FU.

Experimental

Reagents

A pharmaceutical grade 5-fluorouracil (Roche Lab., Nutley, NJ), was used; analytical grade cytidine (Boehringer, Mannheim GmbH, West Germany) was used as an internal standard. 1-hexane sulfonic acid (PIC-B6™) (Millipore Co., Milford, MA) was HPLC grade. Deionised water was passed through a Norganic™ trace organic removal

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cartridge and 45-μm membrane filter (Millipore Co., Milford, MA) before use. Adriamycin (Farmitalia Carlo Erba, Milan, Italy), bleomycin (Bristol Lab., Syracuse, NY), carmustine (BCNU) and lomustine (CCNU) (Laboratoire Roger Bellon, Neuilly-Paris, France), cyclophosphamide (Mead Johnson Co., Evansville, IN), etoposide (Sandoz Ltd., Basle, Switzerland), methotrexate (American Cyanamid Co., Pearl River, NY), prednisone (Philips Roxane Labs., Columbus, OH), and vincristine-sulphate (Eli Lilly and Co., Indianapolis, IN) were either of reagent or pharmaceutical grade.

Pharmaceutical preparations

The content uniformity of three commercially available formulations of 5-FU viz. 5% injectable solution (10-ml), 5% topical solution, 5% topical cream (all from Roche Lab., Nutley, NJ) was examined according to the described assay.

Apparatus

A radial-compression liquid chromatograph (Waters Associates, Milford, MA) consisting of a controller (Model 720), data processor (Model 730), HPLC pump (Model 45), automatic injector (WISP), radial-compression separation module (Z-Module) equipped with a 10-μm 8 mm × 10 cm MB^{TM} C_{18} Radial Pak cartridge and a variable wavelength UV/visible detector (model 480). The wavelength was set at 270 nm, the absorption maximum for 5-FU.

Chromatography

The mobile phase was a 0.025 M solution of 1-hexane sulfonic acid in water, filtered and degassed before use. The flow rate was 4 ml min^{-1} and typical back-pressure was 1100–1200 psi.

Standard curves for 5-FU in plasma

Because of the wide range of concentrations of 5-FU in plasma found following therapeutic doses of this drug, standard curves in the ranges 0.025–1.0 (low range) and 1–25 μg ml^{-1} (high range) were constructed. Blank plasma samples (1 ml) were supplemented with appropriate volumes of 0.1 or 0.02 μg ml^{-1} stock solutions of 5-FU in water to yield different concentrations in the above ranges. The samples were then made up to 1.3 ml with HPLC grade water, and 50 μl of 0.5 μg ml^{-1} (for low range) or 50 mg ml^{-1} (for high range) of internal standard solutions in water was added. The sample was subjected to the extraction procedure as detailed below.

Plasma extraction

The plasma was extracted by adding 9 ml of ethyl acetate to the sample and vortex-mixing for 1 min. After the tube was centrifuged for 10 min at 1746 **g**, the ethyl acetate layer was carefully transferred to a clean tube and brought to dryness under a gentle steam of nitrogen gas at ambient temperature. The residue was reconstituted with 200 μl of the mobile phase by placing the tube in an ultrasonic water bath for 1 min. The reconstituted residue was then transferred to an autosampler microvial, and the autosampler was programmed to inject 50–100 μl of each microvial in duplicate.

Analysis of patient's samples

Plasma samples collected at preselected intervals from a 42-year-old male patient (59 kg) with malignant lymphoma were analyzed for 5-FU by adding the internal

standard and subjecting the samples to the above detailed procedure. In addition to the 5-fluorouracil dose (600 mg m^{-2}), the patient received 750 mg m^{-2} of cyclophosphamide, and 10 unit m^{-2} of bleomycin all by intravenous bolus administration.

Analysis of 5-FU in formulations

One millilitre of each ampoule of the 5% injectable solution or an accurately weighed quantity (about 1 g) of each bottle of 5% topical solution was transferred to a 50-ml volumetric flask and the volume was brought to the mark with water. The flask was then shaken vigorously for 2 min and placed in an ultrasonic bath for 15 min. From each flask, 1 ml of the solution was transferred to a 25-ml volumetric flask containing 1.3 mg of internal standard and the volume was brought to the mark with water. The flask was then shaken for 2 min and a portion was transferred to an autosampler vial. The autosampler was programmed to inject 100 µl of solution into the cartridge in duplicate.

The 5% water-base topical cream was analyzed by accurately weighing an amount (20–40 mg) of the cream and placing it in a 50-ml volumetric flask containing 1.3 mg of the internal standard. The volume was brought to the mark with distilled water, and the flask was shaken and ultrasonicated as described above. The actual amount in each sample was calculated using standard curves (10–80 µg ml^{-1} of 5-FU) prepared with the same amount of internal standard as used for each sample. Three units (ampoule or bottle) from each of 5 lots for each dosage form were tested.

Results and Discussion

Chromatography

Figure 1 shows typical chromatograms of intact plasma blank, plasma blank supplemented with 5-FU and internal standard, and patient's plasma samples supple-

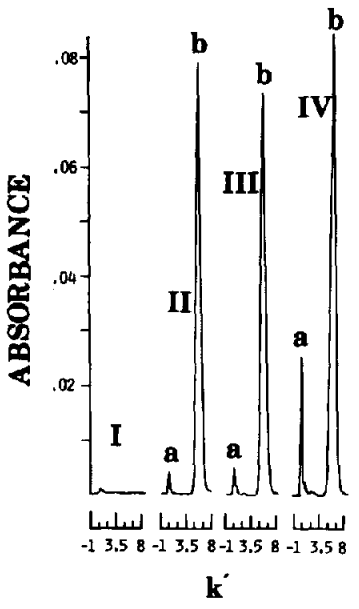

Figure 1
Typical chromatograms [absorbance versus capacity factor (k')] of an intact plasma blank (I), a plasma blank (0.5 ml) supplemented with 0.25 µg of 5-FU and 625 µg of internal standard (II), and patient's plasma samples (0.5 ml) collected 2 h (III) and 0.5 h (IV) after intravenous bolus administration of 600 mg m^{-2} of 5-FU and each supplemented with 625 µg of internal standard. Key: 5-Fu (a); internal standard (b).

mented with internal standard. Similarly, Fig. 2 demonstrates representative chromatograms for 5-FU in the pharmaceutical formulations examined. As demonstrated in these figures, the retention times of 5-FU and internal standard were 1.11 [capacity factor (k') = 0.66] and 4.85 min (k' = 6.2), respectively. The efficiency of chromatography under the conditions used as expressed in number of theoretical plates (N) for the above compounds were 242 and 208, respectively; and the resolution between these two peaks was 4.65. These parameters were computed according to standard equations [19].

Specificity

The specificity of the described method was investigated by determining the retention times of anticancer drugs commonly employed with 5-FU in combined chemotherapeutic modalities (Table 1). As shown in Table 1, some of these compounds were not detected during the run, and others had retention times distant from that of 5-FU. Even with carmustine which has a retention time of 1.52 min, a complete resolution from 5-FU was achieved.

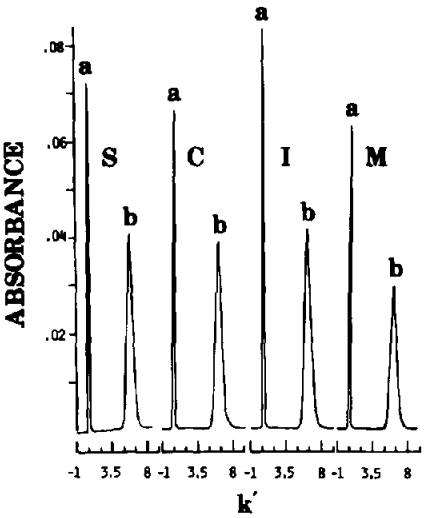

Figure 2
Representative chromatograms for the 5-fluorouracil formulations examined. Key: 5% topical solution (S); 5% topical cream (C); 5% injectable solution (I); standard sample (M) containing 40 μg/ml of 5-FU (a) and 120 μg/ml internal standard (b).

Table 1
Retention times of drugs commonly used with 5-fluorouracil in combined chemotherapeutic modalities

Drug	Retention time (min)
Methotrexate	2.81
Bleomycin	1.87
Etoposide	ND
Lomustine	1.72
Carmustine	1.52
Cyclophosphamide	ND
Cis-platinum	2.54
5-Fluorouracil	1.11
Cytidine	4.85
Vincristine	ND
Prednisone	ND
Adriamycin	ND

Linearity

The linearity of the assay for 5-FU in plasma was examined by constructing standard curves for 15 different days, based on peak height ratio (drug/internal standard) versus concentration of the drug. The assay was highly linear with correlation coefficients (r) > 0.99 [viz. mean (SD) = 0.9934 (0.0057), n = 6 for high range; mean (SD) = 0.9975 (0.00236), n = 9 for low range]. An equally good linearity (r > 0.999) was obtained for standard curves employed for the analysis of 5-FU in formulations. When the peak area was used in lieu of the peak height, the linearity of the standard curves was not as good (i.e. smaller r values).

Analytical recovery and precision

The analytical recovery of 5-FU from plasma was investigated by supplementing 1-ml portions of blank plasma with 0.2, 2 and 20 μg of this drug and analyzing the samples in quadruplicate as described above.

The percentage recovery obtained at the above low, medium, and high concentrations were 96.1, 99.7 and 110%, respectively. We investigated the precision of the assay for 5-FU in plasma by performing four similar experiments at each of the above concentrations, and computing the relative standard deviation (RSD). The RSD values at 0.2, 2, and 20 μg ml^{-1} were 1.7, 5.3, 4.9%, respectively. The precision of the assay when used for formulations was equally good, i.e. RSD <3.5% (Table 2).

Patient's samples

The described method was used for the analysis of plasma samples collected from a cancer patient treated with 5-FU by rapid intravenous injection. A semilogarithmic plot of the concentration versus time data obtained (Fig. 3) shows that the disappearance of 5-FU in this patient exhibited the two-compartment open model characteristics with an initial rapid distribution phase followed by slow post-distribution phase. This is in agreement with previous reports [20, 21] dealing with the pharmacokinetics of 5-FU in humans.

Table 2
Content uniformity data acquired for 5-fluorouracil formulations according to the described assay

Lot no.	Percent of the labelled claim Injectable solution*	Topical solution†	Topical cream‡
1	97.6 (2.3)§	80.9 (3.5)	87.5 (0.9)
2	91.0 (0)	86.2 (2.0)	87.9 (2.0)
3	95.0 (1.1)	85.0 (2.5)	87.8 (2.7)
4	93.7 (0.6)	85.1 (1.6)	98.8 (0.6)
5	93.0 (0)	96.9 (0.7)	98.8 (0.5)
Mean	94.1	86.8	92.2
SD	2.5	6.0	6.0
Range	91–97.6	80.9–96.9	87.5–98.8

*Efudex 5% injectable solution (10-ml) (Roche Lab., Nutley, NJ).
†Efudex 5% topical solution (Roche Lab., Nutley, NJ).
‡Efudex 5% topical cream (Roche Lab., Nutley, NJ).
§Mean (RSD) of three units.

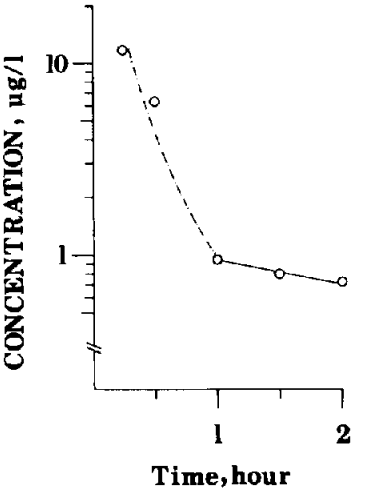

Figure 3
A semilogarithmic plot of the concentration of 5-FU in plasma versus time obtained for a cancer patient treated with 600 mg m^{-2} of 5-fluorouracil by intravenous bolus administration.

Content uniformity

The applicability of the described assay for the analysis of 5-FU in formulations was examined by testing the content uniformity of three commercially available products of this drug. Three units from each of 5 lots of each formulation were analyzed and the mean (SD) percent of the labelled claim was calculated and presented in Table 2. With the exception of one lot of topical solution, the labelled claims of all products were within the range 91–99%, which coincide with the U.S.P. content uniformity requirement (100 ± 15%).

In conclusion, a rapid and accurate method for the analysis of 5-fluorouracil has been described that can be employed for therapeutic monitoring or pharmacokinetic studies as well as for testing the content uniformity of its products.

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References

[1] B. A. Chabner, C. E. Meyers and V. T. Oliverio, *Sem. Oncol.* **4**, 165–191 (1977).
[2] R. E. Finch, M. R. Bending and A. F. Lant, *J. Pharm. Sci.* **67**, 1489–1490 (1978).
[3] R. M. Kok, A. P. de Jong, C. T. van Groeningen, G. J. Peters and J. Lankelma, *J. Chromatogr.* **343**, 59–66 (1985).
[4] W. M. Williams, B. S. Warren and F.-H. Lin, *Anal. Biochem.* **147**, 478–486 (1985).
[5] E. A. De Bruijn, O. Driessen, N. van den Bosch, E. van Strijen, P. H. Slee, A. T. van Oosterom and U. R. Tjaden, *J. Chromatogr.* **278**, 283–289 (1983).
[6] N. Kawabata, S. Sugiyama, T. Kuwamura, Y. Odaka and T. Satoh, *J. Pharm. Sci.* **72**, 1162–1165 (1983).
[7] C. Aubert, J. P. Sommadossi, P. Coassolo, J. P. Cano and J. P. Rigault, *Biom. Mass. Spectrom.* **9**, 336–339 (1982).
[8] M. C. Cosyns-Duyck, A. A. Cruyl, A. P. Leenheer, A. De Schryver, J. V. Huys and F. M. Belpaire, *Biom. Mass. Spectrom.* **7**, 61–64 (1980).
[9] L. J. Schaaf, D. G. Ferry, C. T. Hung, D. G. Perrier, I. R. Edwards, *J. Chromatogr.* **342**, 303–313 (1985).
[10] P. L. Stetson, U. A. Shukla and W. D. Ensminger, *J. Chromatogr.* **344**, 385–390 (1985).
[11] M. Iwamoto, S. Yoshida and S. Hirose, *J. Chromatogr.* **310**, 151–157 (1984).
[12] J. L. Au, M. G. Wientjes, C. M. Luccioni and Y. M. Rustum, *J. Chromatogr.* **228**, 245–256 (1982).
[13] D. C. Sampson, R. M. Fox, M. H. Tattersall and W. J. Hensley, *Ann. Clin. Biochem.* **19** (part 2), 125–128 (1982).

[14] C. L. Hornbeck, R. A. Floyd, J. C. Griffiths and J. E. Byfield, *J. Pharm. Sci.* **70**, 1163–1166 (1981).
[15] A. R. Buckpitt and M. R. Boyd, *Ann. Biochem.* **106**, 432–437 (1980).
[16] T. Marunaka, Y. Umeno, K. Yoshida, M. Nagamachi, Y. Minami and S. Fujii, *J. Pharm. Sci.* **69**, 1296–1300 (1980).
[17] B. H. Min, W. A. Garland, T. M. Lewinston and B. M. Mehta, *Biom. Mass. Spectrom.* **12**, 238–240 (1980).
[18] E. R. Garrett, G. H. Hurst and J. R. Green, Jr, *J. Pharm. Sci.* **66**, 1422–1429 (1977).
[19] C. Horvath and W. R. Melander, in *Chromatography Fundemental and Applications of Chromatographic and Electrophoretic Methods* (E. Heftmann, Ed.), pp. A27–A135. Elsevier Scientific Publishing Co., Amsterdam, 1983.
[20] J. M. Kirkwood, W. Ensminger, A. Rosowsky, N. Papathanasopoulos and E. Frei, III. *Cancer Res.* **40**, 107–113 (1980).
[21] W. E. Macmillan, W. H. Wolberg and Peter G. Welling, *Cancer Res.* **38**, 3479–3482 (1980).

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