

Long-Term Stability of 5-Fluorouracil in 0.9% Sodium Chloride after Freezing, Microwave Thawing, and Refrigeration

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ABSTRACT

Objective: To investigate the stability of 5-fluorouracil diluted in 0.9% sodium chloride (normal saline [NS]) after freezing, microwave thawing, and storage for 28 days at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Methods: Polyvinylchloride (PVC) infusion bags ($n = 5$) containing 5-fluorouracil 800 mg/100 mL were frozen for 79 days at -20°C . The bags were then thawed in a microwave oven and stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 28 days. The concentration of 5-fluorouracil was measured by high-performance liquid chromatography. Visual and microscopic inspections were performed and pH was measured periodically during storage. Solutions were considered stable if the lower limit of the 95% confidence interval of the concentration versus time profile remained greater than 90% of the initial concentration.

Results: No colour change or precipitation was observed in any of the solutions. Slight changes in pH were observed during refrigeration. 5-Fluorouracil solutions were stable during storage at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 28 days, as indicated by the results of high-performance liquid chromatography.

Conclusion: 5-Fluorouracil 8 mg/mL in NS may be prepared in advance, frozen and stored in PVC bags, and thawed before use. The solutions remained stable after freezing at -20°C for 79 days followed by storage at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for up to 28 days.

Key words: 5-fluorouracil, freezing, thawing, stability, microwave

RÉSUMÉ

Objectif : Analyser la stabilité de solutions de 5-fluorouracile dilué dans du chlorure de sodium à 0,9 % (solution physiologique salée) une fois congelées, puis décongelées au micro-ondes, et entreposées pendant 28 jours à $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Méthodes : Des solutions de 5-fluorouracile à raison de 800 mg dans 100 mL ont été préparées dans des sacs de polychlorure de vinyle (PVC) pour perfusion ($n = 5$) qui ont été congelés pendant 79 jours à -20°C . Les sacs ont ensuite été décongelés au micro-ondes, puis entreposés à $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ pendant 28 jours. La concentration du 5-fluorouracile a été mesurée par chromatographie liquide à haute performance. Des inspections visuelles et microscopiques ont été pratiquées et le pH mesuré périodiquement pendant la période d'entreposage. Les solutions étaient considérées stables si la limite inférieure de l'intervalle de confiance à 95 % de la courbe de la concentration en fonction du temps demeurait supérieure à 90 % de la concentration initiale.

Résultats : Aucun changement de couleur ni précipité n'ont été observés dans aucune des solutions. De légers changements de pH ont été notés pendant la période de réfrigération. Les solutions de 5-fluorouracile étaient stables lorsqu'elles étaient entreposées à $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ pendant 28 jours, comme l'ont montré les résultats de la chromatographie liquide à haute performance.

Conclusion : Les solutions de 5-fluorouracile à 8 mg/mL dans une solution physiologique salée peuvent être préparées à l'avance, congelées et conservées dans des sacs en PVC, puis décongelées avant l'utilisation. Les solutions demeurent stables après avoir été congelées à une température de -20°C pendant 79 jours, puis entreposées à $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ jusqu'à 28 jours.

Mots clés : 5-fluorouracile, congélation, décongélation, stabilité, micro-ondes

[Traduction par l'éditeur]

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INTRODUCTION

5-Fluorouracil, an analogue of the pyrimidine uracil and one of the oldest anticancer agents, acts as an antimetabolite drug.¹ It remains the standard therapy for advanced colorectal cancer and is also one of the primary agents used to treat head, neck, and breast cancer.² The drug is usually diluted for parenteral injection with 0.9% sodium chloride (NS) or 5% dextrose.³

Preparation of IV solutions by a centralized IV additive service has several potential benefits, including efficient and timely preparation and administration of drugs, reduction in medication errors, better health and safety control, high assurance of stability and sterility, and standardization of drug concentration.⁴ Freezing prepared solutions in ready-to-use formats can extend the long-term stability of injectable drugs.⁵ The use of a microwave oven reduces the time required to thaw frozen infusion solutions.⁶⁻¹⁵

The stability of 5-fluorouracil has been tested in several solutions and containers and at various temperatures ranging from 4°C to 35°C. 5-Fluorouracil was stable when prepared in NS and stored in polyolefin bags at 5°C ± 3°C for 28 days.¹⁶ The purpose of the current study was to evaluate the stability of 5-fluorouracil diluted in NS (800 mg in 100 mL) in polyvinylchloride (PVC) bags, frozen for 79 days at -20°C, thawed in a microwave oven, and stored for a further 28 days at 5°C ± 3°C.

MATERIALS AND METHODS

Preparation of Solutions

5-Fluorouracil (Fluracedyl, 1000 mg in 20 mL; Teva Pharma Belgium SA Wilrijk, Belgium, lot 06 H 28 PA) was diluted in 100 mL NS in a PVC bag (Macopharma Benelux, Mouscron, Belgium, lot 07 F 08 D, expiry June 2009) to obtain an approximate final concentration of 800 mg/100 mL (8 mg/mL).¹⁷ Five such bags were prepared under aseptic conditions in a vertical laminar air flow hood.

Stability Study

The 5 infusion bags containing 5-fluorouracil 800 mg/100 mL were frozen (freezer model GS5203, 520 l, Liebherr Comfort, Lienz, Austria) at -20°C in 2 steps. During the freezing step, which took place over the first 24 h, the bags were placed in the freezer until the contents reached the desired temperature of -20°C. For the storage step, the bags were moved to a second, identical freezer, which contained only the frozen infusion bags, and were stored for 79 days. After this storage period the bags were thawed at 270 W power in a carousel-equipped microwave oven (model NND998C/W with specifications 230 V, 800 W, 2450 MHz; Panasonic, Saint-Denis, France), according to a validated "light cycle" as

previously described.⁷⁻¹⁵ Briefly, this method consists of thawing for 13 min, mixing, and thawing for an additional 7 min. The bags were then stored at 5°C ± 3°C for 28 days.

Immediately after preparation of the bags and again immediately after thawing (designated as day 0), a 2-mL sample of the solution was withdrawn from each of the 5 bags for determination of 5-fluorouracil content. Samples were also withdrawn after 1, 2, 5, 7, 9, 12, 15, 21, and 28 days of storage at 5°C ± 3°C. Each sample was withdrawn by means of a 2-mL single-use sterile syringe (Terumo, Leuven, Belgium, batch numbers 0610004 and 0707007), equipped with a needle, and was placed in a glass container. The concentration of 5-fluorouracil was determined in triplicate for each sample.

Before sampling, each bag was inspected visually in room light without special equipment, and the colour and appearance of the solution and presence of any precipitate were recorded. A drop of the sample was placed under a cover slip and checked microscopically for crystallization at 100× magnification (model 4653549 microscope, Carl Zeiss, Oberkochen, Germany) immediately after preparation and after thawing. NS was used as the control solution.

The pH of each sample was measured with an Inolab Level 1 pH meter (WTW GmbH, Weilheim, Germany, batch number 01270007) equipped with a glass electrode (Hamilton Biotrode, Bonaduz, Switzerland, product code 1280809/165) and calibrated with 2 standard solutions (BDH Limited, Poole, England; lot 4345650J for pH 4.0 standard and lot 5082000J for pH 7.0 standard).

5-Fluorouracil Assay

Samples were assayed by high-performance liquid chromatography (HPLC; Alliance model 2695 chromatography system, Waters Corporation, Milford, Massachusetts) with a photodiode array detector (model 996, Waters Corporation) and data acquisition and processing module software (Empower 2 Software, Waters Corporation). We used a reverse-phase C18 column with a guard column (Hypersil ODS [C18] 3-µm, catalogue number 9868; guard column [C18] 5-µm, catalogue number 96013; Alltech Associates, Inc, Deerfield, Illinois, batch number 3/120/4307). The mobile phase was 5% methanol (Labscan Ltd, Dublin, Ireland, catalogue number C26C11X, batch number 0190/7) and 95% aqueous 0.01 mol/L potassium dihydrogen phosphate (Merck, Darmstadt, Germany, reference 1.04873.1000), adjusted to pH 7.50 ± 0.05 with aqueous 5.0 mol/L sodium hydroxide (Merck, reference 1.06498). The flow rate was 1 mL/min. The column temperature was set to 35°C and the wavelength to 267 nm. The retention time was about 1.7 min.

Control solutions containing 5-fluorouracil at 4 different concentrations (5, 10, 25, and 35 mg/mL) were assayed to calculate within-day variation. Between-day variation was

estimated with the calibration solution containing 5-fluorouracil at 7.14 mg/mL.

5-Fluorouracil solutions of 1, 5, 10, 15, 25, 30, and 35 mg/mL in sterile water for injection were prepared for use in evaluating the linearity of the assay.

Assay Validation

The stability-indicating capability of the chromatographic method was assessed using partially decomposed solutions of the drug. Solutions of 5-fluorouracil (25 mg/mL) were exposed to 3 different pH conditions: neutral pH (8.80); alkaline pH (11.03), obtained by adding 5 mol/L sodium hydroxide; and acidic pH (1.20), obtained by adding 12 mol/L hydrochloric acid. Each of these solutions was heated at 100°C for 60 min.

Test samples were diluted (1 in 100) with water for injection (B BraunMedical SA, Diegem, Belgium, lot 7425 A 192). Standard solutions were prepared by diluting 100 µL of the commercially available 5-fluorouracil solution (Fluracedyl, 1000 mg in 20 mL [50 mg/mL]; Teva Pharma Belgium SA, lot 06 H 28 PA) in 600 µL water for injection. This solution was diluted again (1 in 100) before the assay. A 10-µL aliquot of each test solution, along with 10-µL aliquots of the standard solutions, was injected onto the chromatograph. All samples were assayed in triplicate. All standard and assay solutions were taken from the same batch and package of 5-fluorouracil. The linearity of the analytical response allowed assay calibration with a single level (7.14 mg/mL).

Statistical Analysis

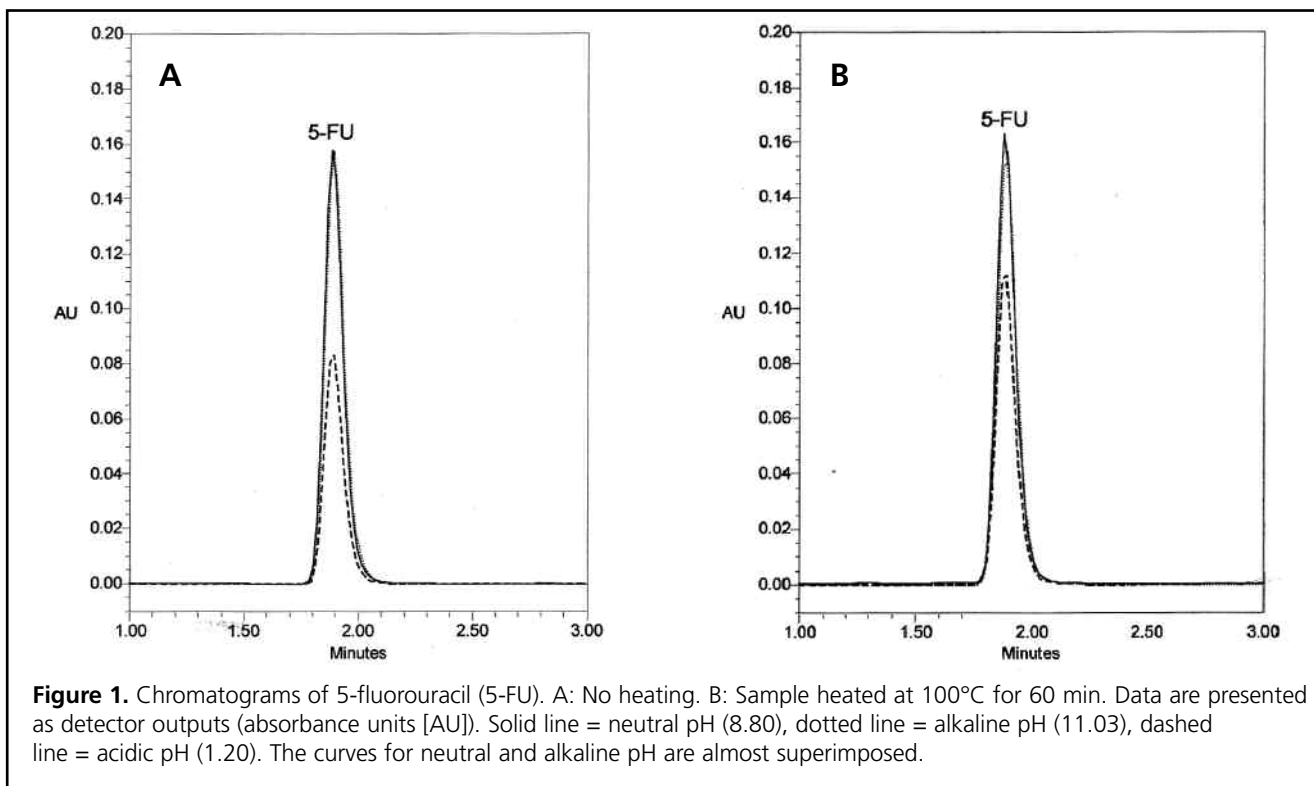
Data are expressed as mean and standard deviation (SD). Linear regression was used to analyze the drug concentration on each day over the study period. After verification of the homogeneity of the slopes of the concentration versus time curve obtained from each of the 5 test solutions, a common regression line was estimated. As recommended by the US Food and Drug Administration, the drug solutions were considered stable if the lower limit of the 95% confidence interval of this common estimated regression line remained above 90% of the initial concentration.¹⁸

RESULTS

Assay Validation

Linear regression analysis of the peak area yielded a correlation coefficient (r^2) of 0.9976 in the range of 1 mg/mL to 35 mg/mL, which indicates good linearity of the assay. The within-day relative SD values ($n = 10$) were 2.80% for the 35 mg/mL control solution, 2.90% for the 25 mg/mL control solution, 1.72% for the 10 mg/mL control solution, and 0.27% for the 5 mg/mL control solution. The between-day precision ($n = 11$) estimated for the standard solution (7.14 mg/mL) was 3.04%.

Degraded samples of 5-fluorouracil were assayed to confirm separation of the parent drug from its degradation products. Before and after heating at 100°C for 60 min, no



additional peaks from decomposition products were observed, but with acidic pH there was a decrease in the peak corresponding to the intact drug (Figure 1). Computerized spectral analysis of photodiode array detector signals confirmed the 5-fluorouracil peak.

Stability of 5-Fluorouracil Solutions

No colour changes or precipitation were observed visually, and no crystallization was observed microscopically after thawing or after 28 days of storage at 5°C ± 3°C. The pH of the solution (mean ± SD) increased slightly over time: the pH was 8.75 ± 0.04 before freezing, 8.86 ± 0.01 on day 0 after thawing, and 8.82 ± 0.01 on day 28 after thawing, but these changes were not clinically significant, since the solutions remained within the acceptable range for infusion (4 ≤ pH ≤ 10).¹⁹

The initial concentration of 5-fluorouracil after thawing (day 0) and the concentration after various periods of storage, expressed as percentage of the initial concentration, are shown in Table 1. The slopes of the 5 regression lines of the concentration of each bag over time did not differ significantly (*p* = 0.99). The common estimated regression line showed a statistically significant decrease in 5-fluorouracil concentration over time (*p* < 0.001). However, the lower limit of the 95% confidence interval at day 28 remained above 90% of the initial concentration.

DISCUSSION

In this study, solutions of 5-fluorouracil 8 mg/mL in NS in PVC bags were stable after freezing and storage for 79 days at -20°C, thawing in a microwave oven, and refrigeration for a further 28 days. We believe that the assay was stability-

indicating. Although the absence of degradation peaks after heating in acidic and alkaline conditions may indicate that the parent drug remained intact, computer analysis of photodiode array detector signals confirmed the 5-fluorouracil peak by spectral comparison. Other authors have observed the absence of degradation peaks despite a decrease in the peak of the parent drug.²⁰

The test concentration of 5-fluorouracil (8 mg/mL) was chosen because it represents 40% of the doses (± 5%) administered in the authors' institution, where a dose-banding technique is used for prescriptions of antineoplastic agents.²¹ With dose-banding, the required dose of an antineoplastic agent is calculated and then rounded up or down to a pre-determined dose within a defined range.²¹ Dose-banding with ready-to-use infusions has the potential to increase timely dispensing and administration of antineoplastic agents to patients²² without compromising quality and safety.²³

In this study, only chemical stability was evaluated; each centre must prospectively evaluate the sterility of its own products. However, 5-fluorouracil solutions prepared and stored as described in this study may be assumed to be at low risk of microbiological contamination and safe for administration within 14 days if stored under refrigeration according to USP/NF (United States Pharmacopeia/National Formulary) chapter 797 recommendations.²⁴

Standard and test solutions were taken from the same batch and package of commercially available 5-fluorouracil; therefore, the initial drug concentration was assumed to be the same for all test solutions. Overfilling of PVC bags, by up to 28% for 100-mL bags,⁸ makes it difficult to match the nominal to the expected final concentration, but expressing results as a percentage of the initial concentration permitted us

Table 1. Concentration and pH of 5-Fluorouracil (800 mg in 100 mL 0.9% Sodium Chloride) Stored in Polyvinylchloride Bags after Being Frozen and Thawed in a Microwave Oven

Storage Time* (days)	Mean Concentration ± SD (mg/mL)†	Mean % of Initial Concentration‡ (95% CI)§	Mean pH¶ ± SD
0	6.78 ± 0.12	100.00	8.86 ± 0.01
1	6.64 ± 0.10	99.79	8.82 ± 0.01
2	6.52 ± 0.12	99.58	8.84 ± 0.00
5	6.37 ± 0.16	98.94	8.83 ± 0.00
7	6.61 ± 0.13	98.52	8.82 ± 0.01
9	6.46 ± 0.10	98.10	8.81 ± 0.00
12	6.52 ± 0.12	97.46	8.80 ± 0.00
15	6.59 ± 0.11	96.83	8.85 ± 0.01
21	6.46 ± 0.13	95.56	8.84 ± 0.01
28	6.60 ± 0.10	94.08	8.82 ± 0.01

CI = confidence interval, SD = standard deviation.

*Samples were frozen, thawed in a microwave oven, then stored under refrigeration (temperature 5°C ± 3° C) for the specified periods.

†Mean of 5 samples.

‡Initial concentration, before freezing (mean ± SD): 6.44 ± 0.15 mg/mL.

§Based on the profile of concentration versus time obtained by linear regression.

¶Initial pH, before freezing (mean ± SD): 8.75 ± 0.04.



to bypass this pitfall. When the entire drug volume within a bag is administered, the overflow volume is considered clinically negligible.²⁵

In conclusion, 5-fluorouracil in NS (800 mg in 100 mL) in PVC bags may be frozen for up to 79 days, thawed in a microwave oven, and then stored for up to 28 days at 5°C ± 3°C without major changes in concentration. Advance preparation of batches of 5-fluorouracil IV solutions may therefore be considered.^{7-15,26-30}

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