Original Article

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Stability of extemporaneously compounded 5-fluorouracil utilizing high performance liquid chromatography

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SUMMARY The goal of the current study is to determine stability of compounded 5-fluorouracil (5-FU) in Intravia™ bags and CADD™ cassettes stored up to 15 days under refrigeration (2-8°C) and room temperature (25°C with 60% relative humidity), with four different concentrations (20 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL) and two diluents (0.9% sodium chloride and 5% dextrose). A stability-indicating high-performance liquid chromatography (HPLC) method was developed to analyze the 5-FU concentrations. The stability of compounded 5-FU infusions was investigated using this method. Two samples from each storage condition were assessed for stability on days 0, 4, 7, 10, and 15 as per United States Pharmacopeia (USP) guidelines. The assay of 5-FU was done utilizing a calibrated stability-indicating HPLC method. The stability-indicating HPLC assay showed 5-FU completely degraded within 1 hour in basic conditions. No cloudiness or color change was observed during the stability study. Precipitation was observed in the CADD™ cassettes at day 15 in both storage conditions and at day 10 in a single room-temperature CADD™ cassette for 40 mg/mL in 5% dextrose (D5W). HPLC assay revealed the infusions in CADD™ cassettes retained greater than 90% of the initial concentrations of 5-FU for 15 days stored at room temperature (25°C and 60% relative humidity) and for 10 days at refrigeration (2-8°C). Intravia™ bags retained stability through 15 days for all the compounded 5-FU concentrations and both the storage conditions. 5-FU infusions in both CADD™ cassettes and Intravia™ bags were stable for extendable periods in multiple concentrations compared to recommended guidelines for hospital use.

Keywords 5-Fluorouracil, compounding, stability, high performance liquid chromatography

1. Introduction

5-Fluorouracil (5-FU), a nucleotide analog and antimetabolite drug, has been approved in the treatment of head and neck cancer, breast cancer, and gastrointestinal cancer, and is one of the oldest known oncologic agent. It works by competing with uracil in RNA with one active metabolite, 5-fluoroxyuridine monophosphate (F-UMP), and inhibiting the synthesis of thymidine-phosphate products *via* inhibition of thymidylate synthase with another active metabolite, fluorodeoxyuridine monophosphate (F-dUMP). Together, these processes stop growth of cancer cells by inhibiting new DNA and RNA formation, thus preventing the cancer cells from replicating. Dosing varies by type of cancer, but ranges from 200 mg/m^2 to 3,000 mg/m^2 per the patient's body surface area (BSA) (*1*). 5-FU is commonly compounded in various concentrations at up to 50 mg/mL, either in CADD™ cassettes or Intravia™ bags, as well as other chemotherapy devices such as the Braun Easypump® (*2*). In order to ensure stability and quality of the compounded product and appropriate assignment of beyond-use dating (BUD), stability studies are essential. Health system pharmacies have often struggled to confidently extrapolate stability data from widely varied drug concentrations, differing diluents, and studies specific to unique elastomeric devices, which may or may not be composed of materials similar to the bags or cassettes utilized by the particular pharmacy. While manufacturers often do these studies for their commercial pre-made products in house, extemporaneously compounded 5-FU requires its own studies for BUD assignment as required by the joint commission and United States Pharmacopeia (USP) guidelines (*3*).

In this study, we present validated stability studies utilizing high performance liquid chromatography (HPLC) of extemporaneously compounded 5-FU in both 5% dextrose (D5W) and 0.9% sodium chloride (NS), respectively, over the course of 15 days at room

temperature and under refrigeration, as they would be used in the hospital setting. Validation of the method used was achieved using International Council for Harmonisation (ICH) guidelines for stability studies for publication and the studies by Sinha *et al*. (*4*) and Galanti *et al*. (*5*) demonstrated long term stability of 90-95% of 5-FU in polyvinyl chloride (PVC) and ethylene-vinyl acetate (EVA) bags and for use during freeze thaw cycles, by use of HPLC methodologies. Both PVC and EVA containers produced no precipitation when the drug was frozen for 79 days and then thawed in the microwave and stored for 28 days under refrigeration. This resulted in little to no loss of the efficacious form of the drug in 0.9% sodium chloride. Roberts *et al*. (*2*) also demonstrated the stability of 5-FU for up to 21 days in both sodium chloride and dextrose in the Braun Easypump®.

Additionally, the stability of 5-FU admixtures with folinic acid was studied by Milano *et al*. (*6*) and remained within 10% of the initial concentrations. While the previous studies were consistent regarding stability in EVA bags and PVC bags, it is worth noting that 5-FU precipitated in PVC reservoirs when stored under refrigerated conditions (as demonstrated by Martel *et al*. (*7*)), and in irradiated plastic bags (as demonstrated by Dine *et al*. (*8*)). Barberi-Heyob *et al.* (*9*) studied 5-FU in cassettes and found them to be stable for 7 days at refrigeration and 14 days at room temperature. Previous studies often utilized only one or two concentrations of 5-FU, typically in one device and diluent. However, in our study we utilized three different concentrations and undiluted 5-FU, in two different reservoirs, and in two different diluents. This allows for the analysis of the stability in each device, and at each concentration and diluent as it would be compounded and provides a more comprehensive understanding of 5-FU stability. As 5-FU is dosed by weight (*1*), institutions will often utilize multiple concentrations for compounding to optimize dosing regimens.

5-FU, as an analytical molecule, is particularly susceptible to alkaline conditions when initially put into solution, as demonstrated by Sinha *et al.* (*4*). Formulations with proven stability past manufacturer recommendations would reduce drug waste, potentially mitigate drug shortages, and improve the efficiencies of hospitals and health systems. Hence, in the present study we developed a simple and robust stability-indicating HPLC method to determine the stability of the intravenous 5-FU formulations compounded at the Jefferson Home Infusion Services (JHIS). The objective of this study was to determine whether injectable 5-FU prepared at 50 mg/mL, 40 mg/mL, 30 mg/mL, and 20 mg/mL concentrations in 5% dextrose (D5W) and normal saline (NS) and stored in CADD™ cassettes and Intravia™ bags were stable for up to 15 days when stored at refrigeration (2-8°C) and room temperature conditions (25°C and 60% relative humidity).

2. Materials and Methods

2.1. Materials

All chemicals were of analytical grade. 5-FU (Lot# A0305173, Acros Organics), potassium dihydrogen phosphate (Lot#Q18D028), sodium hydroxide (Lot# 995312), hydrochloric acid (Lot#167712), methanol (HPLC grade, Lot#197075), and all other chemicals were procured from Fisher Chemicals (Fair Lawn, NJ, USA). Fluorouracil for Injection USP, 5,000 mg/100 mL (Lot# P2002967 and expiration: 05/22) per pharmacy bulk package, was purchased from Fresenius Kabi (Lake Zurich, IL, USA). IntraviaTM bags, dextrose 5% (Lot# Y343428, and expiration: 12/21), and 0.9% sodium chloride (Lot# Y344029, and expiration: 12/21) were procured Baxter Healthcare Corporation (Deerfield, IL, USA). CADDTM cassettes were purchased through Smiths Medical (Minneapolis, MN, USA). IntraviaTM bags are composed of a polyolefin-based material. The solution contact layer is a blend of polypropylene, polyethylene, polyamide, and SEBS (Styrene Ethylene Butylene Styrene). The fluid path of CADDTM cassettes are composed of polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), and polypropylene.

2.2. Compounding of the 5-FU formulations

All compounding was performed in a certified, Class II, Type B2 biological safety cabinet by Jefferson Home Infusion Services (JHIS) pharmacy personnel. All study bags and cassettes were filled to 100 mL total volume with the appropriate volumes of drug (for 50 mg/mL) and drug/diluent (for 20, 30, and 40 mg/mL concentrations), packaged in chemotherapy transport bags, and immediately brought to the study site for proper storage and initiation of analysis.

2.3. Test groups and sampling timeline

Two temperature conditions (room temperature (25°C and 60% relative humidity) and refrigeration (2-8°C)) were used for all compounded preparations of 5-FU. Samples included 5-FU preparations as follows: 50 mg/mL (undiluted), 40 mg/mL, 30 mg/mL, and 20 mg/mL, one for each reservoir, diluent (either D5W or NS), and temperature, with a total number of 140 samples. To most closely reproduce the true compounding procedures and likely volumes, all reservoirs (cassettes and bags) were filled to 100 mL of all of the above concentrations. Due to the exorbitant cost that would have been incurred for additional drug and reservoirs, the study was limited to the above-mentioned 140 samples, deeming that number more than adequate to determine stability across multiple concentrations and storage conditions.

2.4. HPLC conditions

All chromatographic studies were performed on Waters® HPLC system (Milford, MA, USA), Alliance 2695 separations module, attached to the Waters® 2998 photodiode array detector. 5-FU concentration in all the samples was assessed by HPLC. The separations were performed on a Phenomenex Luna 5 µm 100Å (H16-221298) 100 × 4.6 mm column. The mobile phase was a 70:30 ratio of 5 mM potassium phosphate buffer (pH 6) and methanol, with an absorbance wavelength of 254 nm for a run time of 4.0 minutes at 25°C. The mobile phase was filtered and degassed before use. The flow rate was 0.5 mL/min, and the injection volume was 5 µL.

2.4. 5-Fluorouracil stability assay

Samples were incubated at respective times for the temperatures they were assigned. Samples included both preparations in CADD™ cassettes and Intravia™ containers. Specific quantity of samples was (from cassettes and bags) were aliquoted and dilution of 1:100 was achieved taking 1 mL of sample in volumetric flask (using analytical pipette) then q.s. to 100 mL using HPLC-grade water. The percentage of 5-FU remaining in each of the injectable formulations after Day 0 was calculated as a percentage based on the expected 5-FU concentration of the sample. The concentration was calculated from the area under the curve in the chromatogram utilizing the equation derived in the standard curve. The drug concentration was considered stable if the concentration of 5-FU was more than 90% of the initial concentration. Chromatogram peak areas were used to determine 5-FU concentrations in the CADD™ cassettes and Intravia™ bags. Samples were run as per the sampling timeline listed above and were run in duplicate per the HPLC protocol listed below.

2.5. Development of standard curve

Standard curve was created using a 5-FU analytical standard (Acros Organics, LOT A0305173, and CAS: 51-21-8) diluted to 100, 200, 400, 600, 800, and 1,000 µg/mL in HPLC-grade water. Stock solution for dilution was made *via* creating a 1,000 µg/mL solution by the addition of 10 mg (10,000 µg) of the standard into 10 mL of HPLC grade water to ensure enough stock could be utilized for dilutions. This solution was then mixed until the 5-FU had been dissolved. Later the dilutions were made in HPLC grade water, for creating calibration curve in the range of 100 to 1,000 µg/mL. Triplicate data was utilized to calculate both intraday variation and interday variations. HPLC was run as described above. The accuracy was calculated at each concentration as the ratio of the measured concentration to the nominal concentration multiplied by 100%. The limit of quantitation (LOQ) of the method was defined as the lowest concentration that could be quantitatively determined with acceptable precision and accuracy. Acceptance limits were defined as accuracy of 80-120% and % coefficient of variance (%CV) of ≤ 10%.

2.6. Physical evaluation

The pH is an important parameter that governs product stability, as changes in pH can affect the solubility of the product and cause precipitation. Thus, pH was measured at each sampling point, beginning at day 0 (initial). The SevenEasy™ pH meter (Columbus, OH, USA) attached to Routine Pro-ISM pH electrode (Mettler Toledo, Columbus, OH, USA) was used after three-point standardization with standard buffer solutions (pH 4.0, 7.0, and 10.0).

2.7. Stability under oxidative, alkaline, and acidic environments

The suitability of the present HPLC conditions to be used as a stability-indicating method was tested by accelerating the degradation of 100 µg/mL 5-FU in 1 N HCl, 1 N NaOH, and hydrogen peroxide solutions. Samples were withdrawn before and after heating each of the solutions at 80°C for 1 hour, 2 hours, and 5 hours. Each sample was analyzed by HPLC using the conditions explained above. Due to rapid hydrolysis in basic conditions, percent of initial values calculated for base was based on the amount in standard concentrations.

2.8. Data analysis

Stability of 5-FU was determined by calculating the percentage of the initial amounts. Wherever possible, the data are presented as mean ± standard deviation.

3. Results

3.1. Standard curve

The chromatogram of 5-FU standards shows a peak at the retention time (Rt) of 2.5 minutes (Figure 1a (blank) and 1b (sample)). A blank sample was also injected into the HPLC system and no peak was observed (Figure 1a). As shown in Figure 1c, a good linearity was exhibited in the concentration range (100-1,000 µg/mL) using the developed HPLC method. The average coefficient of determination of (r^2) observed was 0.99 for the standard curve. The slopes of the curves illustrated an excellent agreement with the coefficient of variability. The limits of quantitation (LOQ) and detection (LOD) were found to be 78 µg/mL and 39 µg/mL, respectively.

The intra- and inter-day relative standard deviations (RSD) were calculated to be 0.32% and 2.8%, respectively. The relative error (RE) for each standard concentration studied was found to be less than ± 10%.

Acceptable precision and accuracy were demonstrated by this method for all the standards and quality controls based on the recommended criteria (*10*). The percentage recovery of 5-FU using the HPLC method was also calculated from the peak areas obtained.

3.2. Stability-indicating HPLC method

The current HPLC method met all acceptance criteria and was reproducible for the study of 5-FU in unknown samples. Failure to recognize degradation profiles of drug or degradation products, or lack thereof, is a most common point leading to erroneous data reporting in stability studies (*11*). The established HPLC method was able to determine the degradation peaks of 5-FU under various stress conditions (Figure 2a). The blanks were found to be non-interfering in the retention time range of 1.4 to 2.3 min. The data suggest that basic conditions (Figure 2b) cause significant degradation of 5-FU almost immediately. Less oxidative degradation occurred when treated with hydrogen peroxide (Figure 2d), and even less degradation occurred under acidic conditions (Figure 2c). Degradation was confirmed by a reduction in concentration over time, and no degradation peaks could be identified likely due to lack of chromophores, which has been reported in previous studies (*4*). Hence, this HPLC assay was used to determine the stability of 5-FU in an injectable formulation prepared in JHIS.

3.3. Drug content

5-FU is a treatment for many types of cancer and must be compounded; it has no pre-packaged product available due to BSA dosing. Hospitals compound injectable 5-FU into many types of sterile containers. At JHIS, 5-FU injectable formulations are routinely compounded in Intravia™ bags and CADD™ cassettes.

The drug content analysis of 5-FU from the Intravia™ bags stored at 2-8°C (Figure 3a) found contents to be in the range of 90-110% of the labeled drug amount by the end of 15 days, with no significant difference in stability between formulations. This indicates that formulation was stable in nature. The Intravia™ bags kept at 25°C with 60% humidity contained 5-FU in the range of 90-110% at the end of 15 days (Figure 4a). This indicates that 5-FU is stable through 15 days in either refrigeration or room temperature in Intravia™ bags in concentrations from 20 mg/mL to 50 mg/mL.

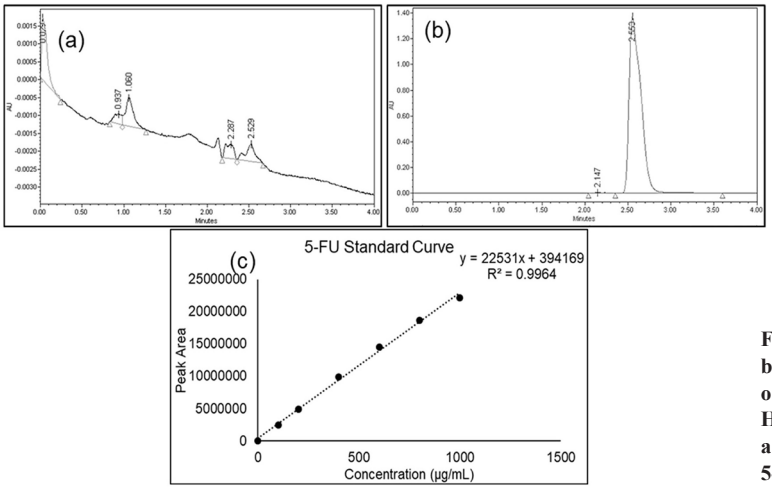

Figure 1. (a) Representative HPLC chromatogram of blank sample shows background peaks and absence of 5-fluorouracil at ~2.5 min; (b) Representative HPLC chromatogram of 5-fluorouracil showing peak at 2.5 minutes; (c) Standard calibration curve of 5-fluorouracil assay.

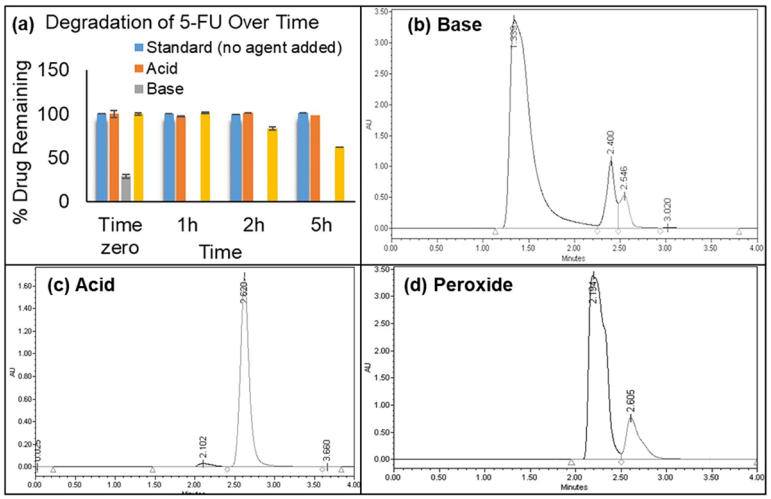

Figure 2. (a) Degradation of 5-FU over time under various stress conditions. It was observed that 5-FU gets completely degraded by the end of 1 h in base. (b-d) Degradation spectra or peaks for the 5-FU in presence of acid, base, and peroxide.

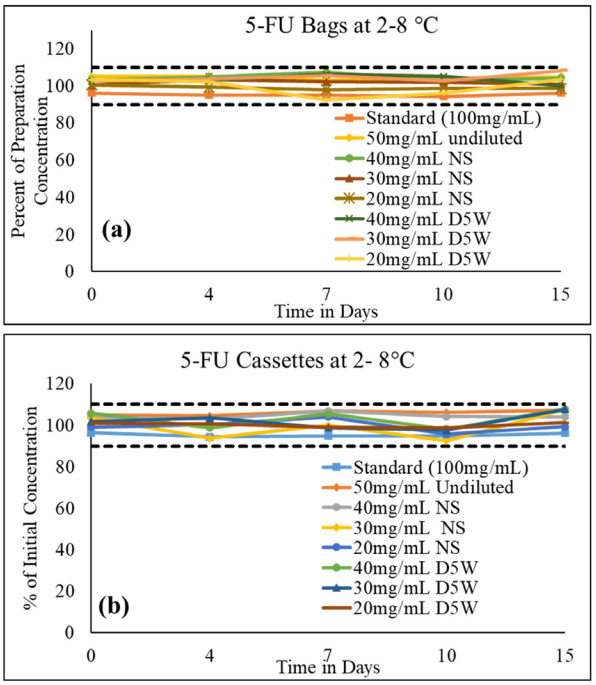

Figure 3. Percentage of initial concentration versus time profile for the 5-FU infusions in (a) Intravia™ bags and (b) PVC CADD™ cassettes at 2-8°C.

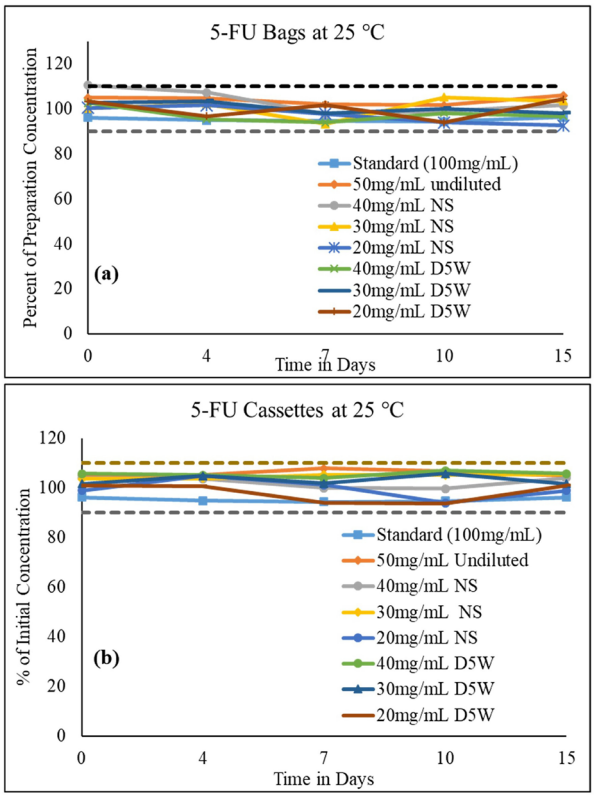

Figure 4. Percentage of initial concentration versus time profile for the 5-FU infusions in (a) Intravia™ bags and (b) PVC CADD™ Cassettes at controlled room temperature conditions (25°C and 60% Relative Humidity).

The drug content analysis of 5-FU from the CADD™ cassettes stored at 2-8°C (Figure 3b) found contents to be in the range of 90-110% by day 15. This indicates that formulation was stable in nature up until the 15-day mark; however, precipitation in the CADD™ cassettes at day 15 suggests, 10 days as the maximum stability for refrigerated samples. The CADD™ cassettes kept at 25°C with 60% humidity contained 5-FU in the range of 90-110% (Figure 4b) for 15 days. No further sampling was carried out at controlled room temperature conditions or refrigeration conditions after 15 days. The drug content analysis suggests that the Intravia™ bag and CADD™ cassette formulations of 5-FU with concentrations from 20 mg/mL to 50 mg/mL may be stable for 15 days at room temperature. However, previous reports have suggested presence of precipitates at high concentration around 50 mg/mL, hence is important to perform physical evaluations to verify this (*12,13*). In addition, refrigeration, CADD™ cassettes are stable for 10 days, and Intravia™ bags are stable for 15 days.

3.4. Physical evaluation

The Intravia™ bags remained clear throughout the study when stored under refrigeration or at room temperature and no precipitation was seen. In the CADD™ cassettes, at day 15 precipitation was observed for all the concentrations at room temperature and refrigeration. At day 10, precipitates were observed in the 40 mg/mL concentration stored at room temperature sample only.

The initial pH of the 5-FU infusion solutions ranged from 9.15-9.26. As shown in Figures 5 and 6, there was no significant change ($p < 0.05$) in the pH values under the two storage conditions.

4. Discussion

A robust and highly sensitive HPLC method was developed for the quantitation of 5-FU from the extemporaneously formulated injectable solutions in Intravia™ bags and CADD™ Cassettes. The method was able to efficiently quantitate 5-FU concentrations with % CV < 10% and 100 ± 10% accuracy for inter- and intra-day accuracies as per FDA guidelines.

In the present study, 5-FU was exposed to acid, base, and hydrogen peroxide at high temperature conditions. The assay clearly showed that 5-FU degrades in basic and to a lesser extent in peroxide conditions and the HPLC method was able to detect the degradation by a decrease in concentration. The drug completely degraded within 1 hour of exposure to basic conditions in the presence of heating, and even at the time zero point, registered at around 30% of the other initial concentrations on average, suggesting a nearly immediate degradative process when exposed to basic conditions. Previous reports suggested that treatment with base causes complete degradation of 5-FU and peroxide may cause

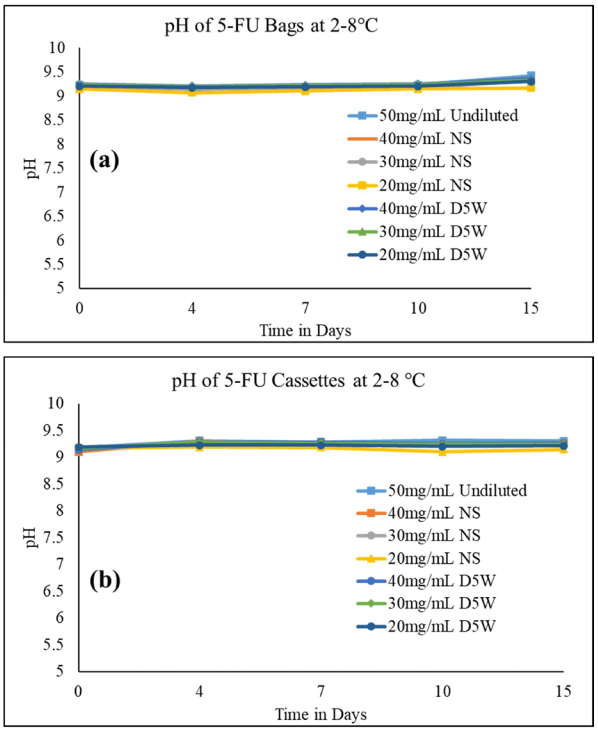

Figure 5. pH analysis of 5-FU solutions over time (in hours) in (a) Intravia™ bags, and (b) PVC CADD™ cassettes at 2-8°C.

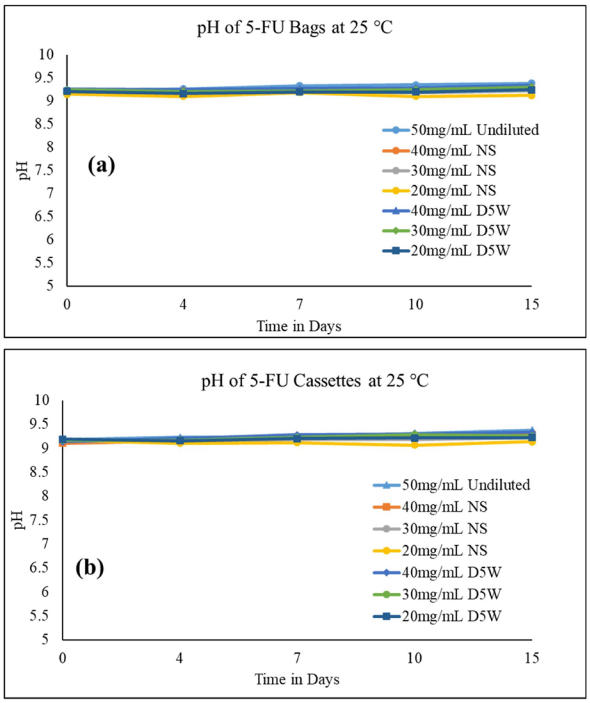

Figure 6. pH analysis of 5-FU solutions over time (in hours) in (a) Intravia™ bags, and (b) PVC CADD™ cassettes at controlled room temperature conditions (25°C and 60% relative humidity).

incomplete degradation of 5-FU (*4*). Moreover, exposure of 5-FU to thermal heating alone does not cause any degradation. As a result, the previous study may provide limited information about the stability of 5-FU. Hence, in our study we performed heat-induced treatment of 5-FU with acid, base, and peroxide. This extensive evaluation shows that 5-FU gets degraded completely, probably due to base hydrolysis, whereas hydrogen peroxide treatment with heating provides relatively lesser degradation profiles. The parent peak does not interfere with peaks of the peroxide, acid, or base, which indicates that HPLC assay is stability indicating.

Unlike previous evaluations, this stability-indicating method was later employed in studying the stability of compounded injectable formulations in Intravia™ bags and CADD™ Cassettes. Galanti *et al.* (*5*) evaluated 8 mg/mL formulations frozen and then thawed at 4°C for up to 28 days, and found that these preparations were stable for 28 days. Martel *et al.* (*7*) also evaluated 10 mg/mL at 4°C, 21°C, and 33°C in ambulatory pump reservoirs (both PVC and EVA) and EVA bags. They found these to be stable for 14 days at 21°C and 33°C for the reservoirs but only 3 days at 4°C for the PVC reservoirs and 5 days for the EVA reservoirs due to precipitation. In the EVA bags, they found all formulations were stable up to 14 days. Finally, Dine *et al*. (*8*) found 5-FU to be stable in PVC bags after irritation of the bags at 50 mg/mL and 25 mg/mL through 14 days.

In the present study, we evaluated drug content from seven formulations utilizing two different reservoirs, two different diluents, and four different concentrations for up to 15 days for 5-FU preparations. Intravia™ bags compounded with concentrations from 20 mg/mL to 50 mg/mL may be stable up to 15 days under both refrigeration and room temperature conditions. Overall, our findings were consistent with the previous literature, although different reservoirs were used in previous studies and our study. Differences from the findings of Galanti *et al*. (5) for beyond 15 days may be attributed to the freezing and microwaving protocols of the preparations in Galanti's study, which is not commonly done in practice with hazardous preparations.

Precipitation was observed in the CADD™ cassettes at day 15 in the tubing and in the body of the CADD™ cassette in 50 mg/mL concentration at both temperature conditions. The fluid is less mobile in the tubing and can sit for prolonged periods; this may account for the higher likelihood of precipitation at lower temperatures. Precipitation seen in the body of the CADD™ cassette can be attributed to the high concentration in the 50 mg/mL CADD™ cassette. This is consistent with the findings of Martel *et al*. (*7*) and Dine *et al*. (*8*) also observed precipitation in the 50 mg/mL concentration, although they attributed it to HCl formation due to radiation from the irradiated PVC bags.

Based on the present study we can conclude that injectable 5-FU in CADD™ cassettes are stable for up to 10 days at both, room temperature and under refrigeration. In Intravia™ bags, all the 5-FU

compounded preparations were stable at both the storage conditions for 15 days. These recommendations are longer than the present recommendation by the manufacturers and provide a more comprehensive picture than previous literature has given with the diversity of reservoirs, diluents, and concentrations studied. For instance, our study result of 10-day stability (at 2-8°C) for CADD™ cassettes and 15-day stability for Intravia™ bags at higher concentrations goes beyond the FDA-recommended manufacturer guidelines of 4 hours, the previous research reports of 120 hours for cassettes in admixtures, 7 days for cassettes, or 14 days in bags at room temperature (*9*). Using our study, efficiencies within the pharmacy can be enhanced. All stability findings and utilization of those findings are subject to BUD limits set forth in the USP Chapter 797.

5. Conclusion

A sensitive HPLC method was developed for the detection and quantitation of 5-FU. This method is stability indicating in nature as it can detect decreases in concentration with sensitivity up to 1,000 µg/mL. The 5-FU injectable Intravia™ bags are stable for 15 days when stored at room temperature (25°C and 60% relative humidity) and refrigeration (2-8°C) for concentrations ranging from 20 mg/mL to 50 mg/mL. The 5-FU injectable CADD™ cassettes are stable for 10 days at refrigeration (2-8°C) for concentrations ranging from 20 to 50 mg/mL. These cassettes are stable for 10 days for concentrations ranging from 20 to 30 mg/mL and 7 days for concentrations ranging 20 to 50 mg/mL at room temperature (25°C and 60% relative humidity). Overall, present study suggests the extended stability of the 5-FU preparations (beyond usual guidelines) in PVC bags and cassettes.

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