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Extended stability of 5-fluorouracil and methotrexate solutions in PVC containers

B.J. Vincké, A.E. Verstraeten, D.I.D. El Eini and T.M. McCarthy

Pharmaceutical Sciences Group, Baxter R & D Europe, Nivelles (Belgium)

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Summary

The extended physical and chemical stability of 5-fluorouracil and methotrexate in 0.9% sodium chloride injection in PVC containers was monitored for periods of up to 3 months and compared to solutions stored in glass containers. For 5-fluorouracil, 50 mg/ml solutions stored at room temperature are stable for up to 42 days and 5 mg/ml solutions for up to 13 weeks of refrigerated storage ($5 \pm 3°$ C) plus an additional week at room temperature. Methotrexate solutions at concentrations ranging from 1.25 to 12.5 mg/ml are stable for up to 15 weeks of refrigerated storage followed by an additional week at room temperature. No physical or chemical modifications of the solutions were observed, the only limiting factor for long-term stability being a progressive increase in drug concentration due to evaporative water loss from the PVC containers. Except for this slight increase in drug concentration, the same results were obtained for both plastic and glass containers.

Introduction

In an environment of economics, severe staffing problems and balancing the costs and benefits associated with technical and clinical programmes, the hospital pharmacy manager is faced with a continual challenge. One aspect of this is the problems in providing a 24-hour/7-day-week pharmacy-based i.v. additive service (Anderson et al., 1983). To overcome some of the dilemmas a demand for extended shelf-lives of reconstituted and repackaged parenteral drugs has developed.

To maximise the efficiency of i.v. additive services, the contents of two or more vials of antineoplastic drug solution may be aseptically combined into a 50 or 150 ml small volume container. The advantages of using premixed solutions of cytotoxic drugs in multiple-dose form allows individual patient-specific doses (often non-standard) to be withdrawn, thus reducing the need for drug reconstitution and the consequent aerosol exposure of hospital personnel, reducing drug waste and the potential for dose miscalculation (Dozier and Ballentine, 1983; Kleinberg and Duafala, 1988; Murphy et al., 1987).

5-Fluorouracil and methotrexate are widely used antineoplastic agents for the treatment of malignant diseases. Stability data for these drug substances are normally available for their original presentation with very limited extension to their admixed form in other drug delivery systems.

Correspondence: D.I.D. El Eini, Pharmaceutical Sciences Group, Baxter R&D Europe, Nivelles, Belgium.

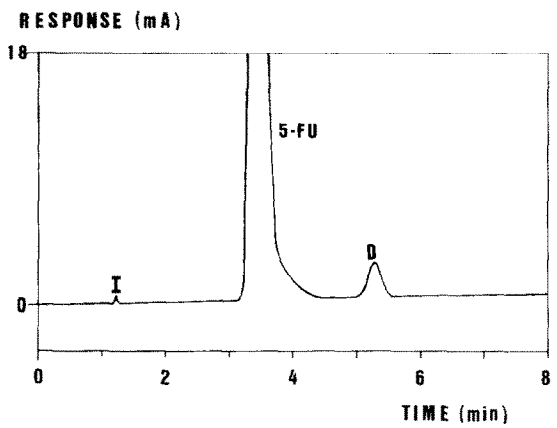

Fig. 1. Typical chromatogram of 5-fluorouracil USP reference standard and its impurities. Injection size: 5 μg 5-fluorouracil. Peaks I and D are unidentified impurities with a relative retention time of 0.35 and 1.54 min versus 5-fluorouracil at 1 min.

In this study, we report on the chemical and physical stability of 5-fluorouracil and methotrexate when diluted with 0.9% sodium chloride injection and stored in PVC Viaflex Minibag containers for extended periods of up to 3 months. A comparison of their stability in glass containers is also reported.

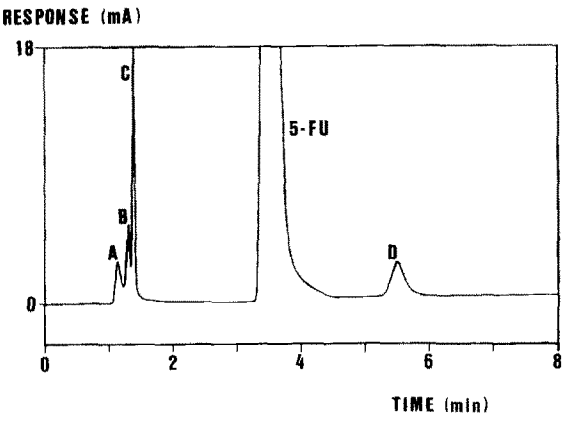

Fig. 2. Chromatogram of alkaline degradation of 5-fluorouracil: treatment – 2.5 h at 100 °C in 0.1 N NaOH. Injection size: 5 μg 5-fluorouracil. Peaks A, B, C and D are unidentified impurities with their relative times of 0.32, 0.36, 0.38 and 1.55 min versus 5-fluorouracil at 1 min.

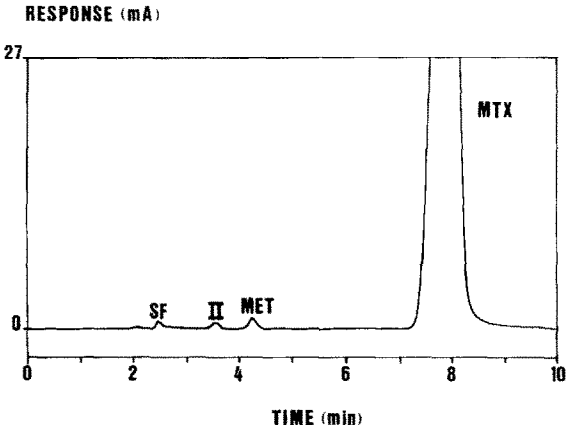

Fig. 3. Chromotagram of commercial Methotrexate USP reference standard and its impurities. Injection size: 1.25 μg. SF = solvent front (relative RT: 0.31); II = unidentified impurity (relative RT: 0.45); MET = methopterin impurity (relative RT: 0.54); MTX = Methotrexate (1.00).

Materials and Methods

Samples from two different product batches of 5-fluorouracil solution 50 mg/ml (Fluoroblastin) and methotrexate solution 25 mg/ml (Farmitrexate) were obtained from Farmitalia (F.R.G.). Also tested was 5-fluorouracil 25 mg/ml from Roche (Switzerland). 5-Fluorouracil and methotrexate reference standards were those of the United States Pharmacopoeia. All the solvents used in the mobile phase were of chromatographic grade and all other chemicals and buffer substances were of reagent grade. All solutions were prepared with

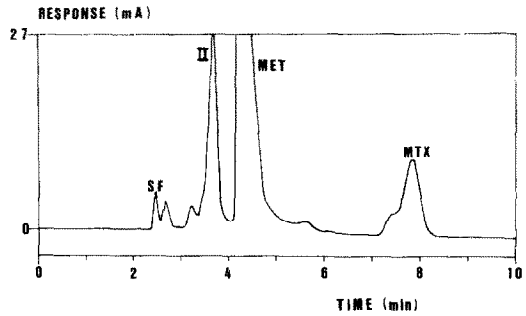

Fig. 4. Chromatogram of alkaline-degraded methotrexate (1 h at 85 °C in 1 M NaOH). Injection size = initial methotrexate content = 2.5 μg. SF = solvent front (relative RT: 0.31); II = unidentified impurity (relative RT: 0.47); MET = methopterin impurity (relative RT: 0.54); MTX = methotrexate (1.00).

twice-distilled water, purified on a Milli-Q system (Millipore).

Synthesis of N-10 methyl folic acid (methopterin)

Pure methopterin was prepared using a method similar to that described by Hansen et al. (1983); 800 mg of methotrexate dissolved in 57 ml 1 N NaOH, was heated to 85 °C for 60 min and protected from light. After cooling, the pH of the solution was adjusted to 3–4 using a 5 M HCl solution. The yellowish precipitate was filtered off (on fritted glass, disc no. 3), washed with water and dried under vacuum over P_2O_5 for a period of 4 h.

The yellowish precipitate was tested by HPLC and TLC techniques, showing a purity of more than 99%, and was identified as pure methopterin by Fast Atomic Bombardment (FABXe) ^{1}-H-NMR and ^{13}C-NMR.

Apparatus

HPLC analysis was carried out using a Hewlett Packard model HP1090A equipped with a diode array detector, model HP1040A, and integrator, model HP3392A (quantitative determinations were carried out using the peak area method). Readings of pH were done with an Orion Research 501 pH meter with a microelectrode (Orion 91–03).

Visual inspection of the samples was carried out against a black and white background cabinet equipped with four 100 W lamps.

Sample preparation

To determine the stability of 5-fluorouracil and methotrexate in admixtures stored in PVC containers, 1 litre stock solutions of 5 and 50 mg/ml of 5-fluorouracil and 1.25 and 12.5 mg/ml of methotrexate were prepared in 0.9% NaCl Injection (Baxter Viaflex). Each stock solution was analysed by HPLC for initial drug concentration, and its pH and visual appearance determined. Aliquots of 70 ml of each dilution of 5-fluorouracil and 100 ml of methotrexate were transferred to separate empty, sterile PVC minibags (Baxter Viaflex Minibags) and each container placed in a black polythene overpouch (Black

TABLE 1

Stability of 5-fluorouracil in 0.9% saline in 50 ml minibag containers stored at room temperature (theoretical = 50.0 mg/ml)

Storage intervals (days)	5-FU content (% of theoretical) [a]		Impurity content corresponding to peak 1 in chromatograms [a,b]		pH at 25 °C [c]	Water loss [c] (%)
	Mean	S.D.	Mean	S.D.		
Batch 6005						
0	98.9	0.4	0.15	0.03	8.93	0.00
7	100.2	0.3	0.16	0.03	8.96	0.40
14	102.1	0.2	0.19	0.02	8.90	1.01
21	103.7	0.7	0.17	0.05	8.97	1.46
28	104.4	1.4	0.20	0.05	9.01	1.83
36	103.8	0.5	0.19	0.02	8.99	2.32
42	105.0	1.3	0.17	0.05	9.01	2.73
Batch 6006						
0	99.8	0.4	0.11	0.02	8.93	0.00
7	100.1	0.5	0.19	0.03	8.96	0.40
14	101.0	0.2	0.19	0.02	8.88	0.95
21	102.0	0.1	0.18	0.03	8.98	1.43
28	103.8	0.4	0.20	0.03	9.01	1.81
36	101.6	0.8	0.19	0.02	8.99	2.31
42	105.2	0.8	0.17	0.02	9.01	2.76

[a] 2 bags injected in duplicate.

[b] Expressed as percent of 5-FU peak area.

[c] Mean of 2 bags.

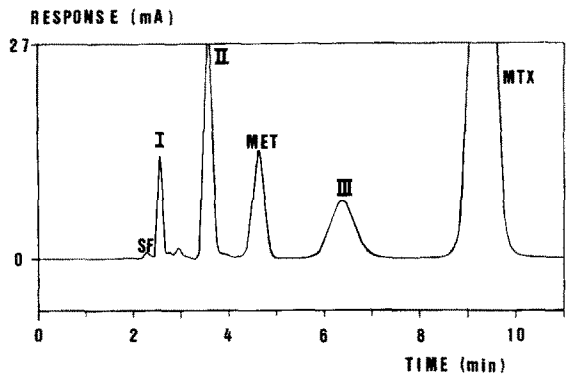

Fig. 5. Chromatogram of the photodegraded methotrexate (0.125 mg/ml in glass, exposed to neon light (178 ftCd) for 24 h). Injection size = initial methotrexate content: 1.25 μg. SF = solvent front (relative RT: 0.29); **I** and **III** = unidentified typical photodegraded products with relative retention times of 0.32 and 0.72 min; **II** = unidentified degradation product (relative RT: 0.43); MET = methopterin impurity (relative RT: 0.53); MTX = Methotrexate (1.00).

Minigrip, Minigrip SA, Belgium). Similarly, 20 ml aliquots of each 5-fluorouracil and methotrexate dilution were transferred to brown glass vials to determine the stability in glass containers.

Both PVC and glass containers were randomly assigned to storage under refrigeration (2–8 °C) or at ambient room temperature (25 ± 3 °C). Samples of both 5-fluorouracil and methotrexate were additionally tested for up to 7 days at ambient room temperature following 4 and 13 or 15 weeks' storage at refrigerated temperature.

Sample analysis

Admixture samples at each time point were analysed for drug concentration and by-product content by HPLC techniques, visually inspected against black and white backgrounds for solution clarity and their pH measured at 25 °C.

TABLE 2

Stability of 5-fluorouracil in 0.9% saline in 50 ml minibag containers stored in refrigerated conditions (theoretical = 5.0 mg/ml)

Storage intervals (days)		5-FU content (% of theoretical)		Impurity content corresponding to peak 1 in chromatograms [b]		pH at 25 °C [c]	Water loss [c] (%)
Refr.	RT	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]		
Batch 6005							
0	0	99.9	0.4	0.17	0.01	8.82	0.00
14	0	100.6	0.6	0.13	0.01	8.79	0.17
28	0	101.8	1.2	0.15	0.01	8.90	0.35
42	0	102.3	0.6	0.13	0.02	8.89	0.49
56	0	102.2	1.7	0.17	0.02	–	0.63
70	0	102.4	0.5	0.16	0.03	8.87	0.80
91	0	101.7	0.4	0.16	0.03	8.87	0.97
91	1	102.6	0.5	0.16	0.03	8.87	1.04
91	3	102.2	1.2	0.17	0.02	–	1.21
91	7	103.4	0.2	0.15	0.02	8.82	1.47
Batch 6006							
0	0	99.5	0.4	0.17	0.01	8.82	0.00
14	0	99.4	0.4	0.13	0.02	8.79	0.15
28	0	101.8	1.8	0.15	0.03	8.89	0.39
42	0	102.6	0.6	0.11	0.01	8.88	0.56
56	0	101.0	0.4	0.17	0.03	–	0.69
70	0	103.0	1.3	0.16	0.02	8.86	0.85
91	0	101.3	0.4	0.16	0.02	8.88	1.04
91	1	101.7	0.5	0.16	0.01	8.87	1.11
91	3	102.3	0.9	0.15	0.03	–	1.29
91	7	103.2	0.9	0.16	0.03	8.83	1.56

[a] Mean and S.D. of 2 bags injected in duplicate.
[b] Expressed as percent of 5-FU peak area.
[c] Mean of 2 bags.
– = not performed.

5-fluorouracil and its by-product were determined using a 10 μm C18 Alltech Associates column, 25 cm × 4.6 mm (600RPC), thermostated at 40 °C. Isocratic conditions were used to elute the column with a mobile phase consisting of 0.013 M phosphate buffer, pH 6.0, at a flow rate of 1.5 ml/min and the effluent was monitored at 254 nm. Under these conditions the capacity factor and the tailing factor for 5-fluorouracil is 2.5 and 1.4, respectively.

5-fluorouracil content is determined versus the linear regression analysis of 3-point 5-fluorouracil USP standard calibration (1.25, 2.5 and 5.0 μg injected). The method is linear between 1.25 and 5.0 μg with a correlation of 0.999, a relative standard deviation of 0.4% on 6 replicates and 0.8% on duplicates and recoveries of 100.3% (S.D. = 0.4%) and 100.2% (S.D. = 0.7%), respectively, on 5 replicates of 50.0 and 5.0 mg/ml solutions.

Methotrexate and its main by-product, methopterin, were determined using a 10 μm C18 Alltech Associates column, 25 cm × 4.6 mm (600RPC), thermostated at 40 °C. The column was eluted with a mobile phase consisting of 0.1 M citric acid and 0.2 M phosphate buffer, pH 6.0 (37 : 63 v/v), together with acetonitrile at a ratio of 91 : 9 v/v, at a flow rate of 0.8 ml/min and the effluent was monitored at 302 nm. Under these conditions the capacity factor and the tailing factor for methotrexate is 2.3 and 1.1, respectively.

The method is linear between 0.5 μg and 5 μg with a correlation of 0.9998, a relative standard deviation of 0.2% on 6 replicates and 0.4% on duplicates. Recoveries of 100.4% (S.D. = 0.2%) and

TABLE 3

Stability of 5-fluorouracil in 0.9% saline in glass containers stored in refrigerated conditions (theoretical = 5.0 mg/ml and 50.0 mg/ml)

Storage intervals (days)		5-FU content (% of theoretical)		Impurity content corresponding to peak 1 in chromatograms [b]		pH at 25 °C
Refr.	RT	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]	
Batch 6005 (5 mg/ml)						
0	0	101.4	0.3	0.14	0.01	8.87
28	0	102.4	0.2	0.14	0.03	8.90
28	8	99.7	0.3	0.13	0.01	8.88
91	0	101.6	0.2	0.17	0.03	8.92
91	7	101.5	0.1	0.17	0.03	8.87
Batch 6005 (50 mg/ml)						
0	0	100.8	0.3	0.21	0.04	8.99
0	28	101.9	0.8	0.21	0.02	9.03
0	36	99.9	0.2	0.17	0.04	9.01
0	42	101.3	0.6	0.17	0.04	9.02
Batch 6006 (5 mg/ml)						
0	0	101.3	0.1	0.16	0.01	8.88
28	0	102.2	0.4	0.16	0.03	8.91
28	8	100.7	0.3	0.13	0.02	8.89
91	0	101.9	0.1	0.16	0.03	8.91
91	7	101.9	0.1	0.15	0.01	8.87
Batch 6006 (50 mg/ml)						
0	0	100.3	0.3	0.23	0.03	8.98
0	28	100.2	0.1	0.22	0.03	9.02
0	36	98.5	0.5	0.17	0.01	9.00
0	42	98.9	0.7	0.17	0.03	9.01

[a] Mean and S.D. of 1 vial injected in duplicate.

[b] Expressed as percent of 5-FU peak area.

99.7% (S.D. = 0.3%) were obtained respectively on 5 replicates of 12.5 mg/ml and 1.25 mg/ml solutions.

During the study a sample size of 1.25 μg was injected for both concentrations and the methotrexate concentration determined against a 1.25 μg standard calibration point of Methotrexate USP reference standard expressed as dry weight (the water content in the standard was determined using the USP XXI).

Results and Discussion

Assay specificity

Assay specificity for 5-fluorouracil and methotrexate was demonstrated by chromatography separation of each compound from its major decomposition products obtained by the intentional degradation of solutions by heating, exposing to light and by degradation under alkaline conditions. The resulting chromatograms were examined to ensure adequate peak resolution between the active drug and other components of the mixture.

For 5-fluorouracil, solutions containing 5 mg/ml of the USP reference standard in 0.9% saline were refluxed at 100 °C alone or in the presence of 0.1 N NaOH for 2.5 h or exposed to daylight for up to 14 days. Fig. 1 shows a typical chromatogram for 5-fluorouracil USP reference standard which contains trace impurities (peaks I and D). The resolution factor between 5-fluorouracil and the main alkaline degradation product

TABLE 4

Stability of methotrexate in 0.9% saline in 50 ml minibag containers stored in refrigerated conditions (theoretical = 12.5 mg/ml)

Storage intervals (days)		MTX content (% of theoretical)		Methopterin content in % w/w of MTX		Other impurity content [b]		pH at 25 °C [c]	Water loss [c]
Refr.	RT	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]		
Batch 6001									
0	0	98.4	0.3	2.01	0.03	0.31	0.07	8.29	0.00
14	0	100.3	0.8	1.98	0.03	0.25	0.04	8.21	0.17
28	0	97.6	0.6	1.94	0.02	0.21	0.06	8.14	0.33
42	0	97.9	0.2	1.97	0.11	≤ 0.15	–	8.10	0.47
56	0	97.5	0.7	1.96	0.01	≤ 0.15	–	–	0.59
70	0	98.7	0.3	1.91	0.03	≤ 0.15	–	8.08	0.74
91	0	100.1	0.4	1.94	0.07	0.16	0.02	–	0.90
105	0	99.4	0.4	1.95	0.07	0.17	0.02	7.97	1.03
105	1	100.1	0.9	1.96	0.05	0.17	0.02	8.00	1.08
105	4	100.0	0.4	1.97	0.08	0.16	0.01	7.96	1.21
105	7	100.8	1.2	1.94	0.08	0.17	0.04	7.99	1.34
Bch LE901BG									
0	0	101.7	1.0	2.02	0.03	0.35	0.02	8.33	0.00
14	0	101.3	0.6	1.98	0.05	0.20	0.01	8.29	0.14
28	0	99.9	0.5	1.97	0.05	0.17	0.01	8.18	0.31
42	0	101.1	0.6	1.93	0.02	≤ 0.15	–	8.17	0.45
56	0	100.1	1.3	1.93	0.01	≤ 0.15	–	–	0.59
70	0	100.4	0.3	1.95	0.06	0.15	0.02	8.13	0.75
91	0	100.3	0.2	1.95	0.07	0.19	0.02	–	0.93
105	0	101.1	0.7	2.09	0.08	0.22	0.03	8.03	1.08
105	1	101.9	0.2	1.91	0.03	0.15	0.03	8.07	1.13
105	4	101.9	0.7	1.90	0.03	0.16	0.02	8.02	1.25
105	7	103.0	1.1	1.96	0.06	0.19	0.06	8.05	1.39

[a] Mean and S.D. of 2 bags injected in duplicate.
[b] Expressed as percent of methotrexate peak area.
[c] Mean of 2 bags.
– = not performed.

peak is 4.7 and between 5-fluorouracil and the main impurity in the USP reference standard is 3.6 (peaks C and D, respectively, in Fig. 2). Fourteen days exposure to daylight did not significantly affect the stability of 5-fluorouracil in the solutions examined.

Solutions containing 12.5 mg/ml of the methotrexate were degraded by heating to 85 °C alone or in the presence of 1 N NaOH for 1 h or exposed to neon light (178 ft.Cd) for 24 h. The main impurity of methotrexate in the USP reference material and the alkaline degradation of methotrexate solution is methopterin (Figs. 3, 4). The resolution factor between methotrexate and methopterin is 5.5.

Significant degradation was noted when methotrexate solutions were exposed to neon light (178 ftCd) for 24 h (Fig. 5). The photodecomposition kinetics show a preliminary increase in methopterin levels and product **II** content, followed by two other degradation products corresponding to peaks **I** and **III** which become the principal degradation products.

These are possibly 2,4-diamino-6-pteridinecarbaldehyde and *p*-aminobenzoylglutamic acid as described by Chatterji et al. (1978) and Jozwiakowski (1986). It seems that the photodecomposition of methotrexate is concentration-dependent. After 84 h exposure to daylight, the percentage of methotrexate remaining in 12.5, 1.25 and 0.125 mg/ml solutions in Minibags is 97%, 58% and less than 2%, respectively.

TABLE 5

Stability of methotrexate in 0.9% saline in 50 ml minibag containers stored in refrigerated conditions (theoretical = 1.25 mg/ml)

Storage intervals (days)		MTX content (% of theoretical)		Methopterin content in % w/w of MTX		Other impurity content [b]		pH at 25 °C [c]	Water loss [c]
Refr.	RT	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]		
Batch 6001									
0	0	98.2	0.4	2.04	0.03	0.36	0.03	7.29	0.00
14	0	99.2	0.1	2.08	0.03	0.32	0.03	7.13	0.16
28	0	98.2	0.4	1.99	0.08	0.27	0.07	7.08	0.33
42	0	98.3	0.3	1.94	0.06	0.18	0.06	7.05	0.46
56	0	97.3	0.3	1.96	0.07	≤ 0.15	–	–	0.61
70	0	98.7	0.2	1.98	0.04	0.17	0.01	7.07	0.76
91	0	98.6	0.8	1.99	0.06	0.20	0.02	–	0.93
105	0	98.0	0.5	2.03	0.09	0.28	0.11	6.99	1.06
105	1	99.8	0.2	1.97	0.01	0.15	0.01	7.02	1.11
105	4	100.4	0.3	2.00	0.09	0.18	0.01	7.10	1.24
105	7	101.6	0.2	1.92	0.03	0.18	0.02	7.08	1.37
Bch LE901BG									
0	0	101.4	0.4	2.04	0.01	0.41	0.03	7.33	0.00
14	0	101.4	0.2	1.99	0.02	0.30	0.03	7.16	0.14
28	0	100.6	0.3	1.97	0.03	0.19	0.02	7.08	0.31
42	0	101.0	0.3	1.94	0.04	≤ 0.15	–	7.07	0.43
56	0	100.9	1.1	1.93	0.03	0.25	0.02	–	0.60
70	0	101.7	0.5	2.01	0.03	0.17	0.01	7.12	0.79
91	0	100.6	0.7	2.04	0.03	0.17	0.02	–	0.98
105	0	101.7	0.3	2.05	0.15	0.22	0.07	6.95	1.14
105	1	102.4	0.1	1.95	0.03	0.15	0.01	7.05	1.19
105	4	101.8	0.2	1.99	0.05	0.19	0.03	7.10	1.31
105	7	103.3	0.8	1.93	0.02	0.19	0.03	7.12	1.46

[a] Mean and S.D. of 2 bags injected in duplicate.

[b] Expressed as percent of methotrexate peak area.

[c] Mean of 2 bags.

– = not performed.

Additionally, the peak purities of 5-fluorouracil, methotrexate and methopterin were confirmed using photodiode array spectroscopy. A spectral overlay comparison at the leading edges, apex and trailing edges was performed between 400 and 190 nm for 5-fluorouracil and between 400 and 210 nm for methotrexate and methopterin.

Extended stability of admixed 5-fluorouracil and methotrexate in 0.9% NaCl solution

The stability of 5-fluorouracil and methotrexate in the admixtures was assessed by examining the percentage changes from the theoretical concentrations for each concentration/solution/temperature/container study condition.

5-Fluorouracil, which is sparingly soluble in water, may precipitate from high concentration preparations at low temperatures. It was therefore decided in this work to study the stability of the high concentration system at room temperature only. The stability data for 5-fluorouracil are shown in Tables 1–3. In both concentrations studied, minimal changes in 5-fluorouracil content, pH and related product levels were observed throughout the storage periods in both glass and PVC containers. For the 5 mg/ml solution there is an increase in 5-fluorouracil concentration of approximately $3.6 \pm 0.6\%$ (S.D.) w/v after 91 days storage at 4°C plus 7 days at room temperature, and for the 50 mg/ml solution the increase in 5-fluorouracil concentration is approximately 5.7

TABLE 6

Stability of methotrexate in 0.9% saline in glass containers stored in refrigerated conditions (theoretical = 1.25 mg/ml and 12.5 mg/ml)

Storage intervals (days)		MTX content (% of theoretical)		Methopterin content in % w/w of MTX		Other impurity content [b]		pH at 25°C
Refr.	RT	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]	Mean [a]	S.D. [a]	
Batch 6001 (12.5 mg/ml)								
0	0	100.6	0.2	1.96	0.02	0.21	0.02	8.32
28	0	98.1	0.1	1.90	0.01	0.16	0.01	8.25
28	7	99.2	0.2	2.01	0.02	0.21	0.01	8.27
105	0	99.1	0.8	1.97	0.05	0.17	0.01	7.82
105	7	98.5	0.6	1.94	0.04	0.15	0.02	7.88
Batch 6001 (1.25 mg/ml)								
0	0	99.5	0.3	2.02	0.04	0.29	0.01	7.35
28	0	97.1	1.0	2.10	0.01	0.22	0.04	7.15
28	7	97.8	0.1	1.90	0.05	0.24	0.01	7.20
105	0	97.3	0.3	2.00	0.04	0.25	0.07	7.24
105	7	98.3	0.2	1.91	0.06	0.18	0.02	7.14
Batch LE901BG (12.5 mg/ml)								
0	0	101.8	0.2	1.95	0.02	0.27	0.02	8.40
28	0	100.0	0.4	1.99	0.01	0.18	0.01	8.29
28	7	100.2	0.2	1.92	0.02	0.22	0.01	8.30
105	0	101.0	1.8	2.00	0.06	0.19	0.03	8.19
105	7	100.5	0.1	1.91	0.02	0.16	0.01	8.21
Batch LE901BG (1.25 mg/ml)								
0	0	102.2	0.1	2.04	0.01	0.35	0.03	7.34
28	0	102.3	0.1	1.97	0.09	0.24	0.01	7.17
28	7	99.6	0.2	1.94	0.02	0.31	0.01	7.19
105	0	99.5	0.1	2.04	0.04	0.22	0.04	7.29
105	7	100.6	1.0	1.93	0.03	0.21	0.01	7.15

[a] Mean and S.D. of 1 vial injected in duplicate

[b] Expressed as percent of methotrexate peak area

$\pm$ 0.6% w/v after 42 days storage at room temperature (RT).

This increase in concentration is directly related to the evaporative water loss from the PVC containers which, under the conditions of this study, is estimated at 1.51 $\pm$ 0.06% w/w for the 5 mg/ml solution after 91 days storage at 4°C and 7 days at RT and 2.8 $\pm$ 0.1% w/w after 42 days storage at RT for the 50 mg/ml solution.

In the glass containers, where no water loss occurs, the concentration of 5-fluorouracil remained essentially unchanged throughout the study for both concentrations.

The level of impurity expressed as a percentage of the 5-fluorouracil peak area remained unchanged at below 0.2% and no other peaks appeared during the study, regardless of 5-fluorouracil concentration, container and storage temperature (Tables 1 to 3). Similarly, the pH of the solutions remained essentially unchanged for all the 5-fluorouracil systems studied.

For methotrexate, stability data similar to those of 5-fluorouracil were obtained (Tables 4–6). No apparent change in methotrexate content was observed, regardless of concentration, container and storage temperature condition. A slight increase in methotrexate concentration with time was observed in the PVC bags and not in the glass containers. A total concentration increase of 2.3 $\pm$ 1.2% w/v methotrexate after 105 days of refrigerated storage plus 7 days at RT was observed for both concentrations. The water loss for the same period of storage from these systems is 1.4 $\pm$ 0.1% w/w.

No apparent changes occurred in the levels of the main impurity, methopterin, and the other main impurity (peak II in the chromatograms, see Fig. 3). Methopterin levels remained at around 1.96 $\pm$ 0.05% w/w of the methotrexate content and the impurity peak II remained at 0.20 $\pm$ 0.08% when expressed as a percentage of the methotrexate peak area. No other peaks appeared throughout the study, regardless of concentration, container or storage temperature. The pH of the methotrexate solutions depended on the drug concentration; for the 1.25 mg/ml solutions the initial pH is around 7.3 and for the 12.5 mg/ml solution the initial pH is 8.9. Decreases of 0.26 $\pm$ 0.05 pH units were observed after 105 days' refrigerated storage plus 7 days at room temperature for both types of container and for both concentrations.

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