ACCOUNT OF EXPERIENCE

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Stability of cyclophosphamide containing infusions

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The stability of cyclophosphamide containing Normal Saline admixture solution for infusion at various temperatures has been studied. The difference in stability during storage at room temperature and at 2–8 °C was observed based on an HPLC assay. Stored in a refrigerator, the decomposition of the active agent was less than 1% on the seventh day, and remained under 1.11% on the 14th day. Under room temperature the decomposition exceeded 10% on the seventh day and was over 25% on the 14th day.

1. Introduction

Commercially available cyclophosphamide tablets and powder for injection shold be stored at a temperature not exceeding 25 °C; the preparations will withstand brief exposure to temperatures up to 30 °C, but should be protected from temperatures exceeding 30 °C. Following reconstitution as directed with sterile water for injection or bacteriostatic water for injection containing parabens, solutions of cyclophosphamide are stable for 24 h at room temperature or 6 d when refrigerated at 2–8 °C; however, because steril water for injection does not contain preservatives, some manufacturers recommend that solutions should be reconstituted with this solvent to be used promptly, preferably within 6 h. Reconstituted solutions of the drug are compatible with 5% dextrose alone and combined with 0.9% sodium chloride, 5% dextrose and lactated Ringer's, lactated Ringer's, 0.45% sodium chloride, or 1/6 M sodium lactate injection [1].

Extemporaneous oral liquid preparations containing 1 or 5 mg of cyclophosphamide per ml, prepared by dissolving the powder for injection or lyophilized powder for injection in aromatic elixir, are stable for 14 d refrigerated at 2–8 °C [1].

The stability examination of infusion mixtures of these compounds is important to assure appropriate storage and safe therapy. Since aseptic technology and sterile conditions needed for the preparation of these infusion mixtures are not always available at hospitals and clinical institutions the establishment of a central cytostatic service is a practical option.

2. Investigations, results and discussion

The HPLC retention time of cyclophosphamide under experimental conditions described in 3.4 is 5.6–5.8 min and the decomposition products appeared at 2.8, 4.02 and 4.39 min on the chromatogram. On the seventh day, 99.28 ± 1.57%, and 89.14 ± 1.23% of the original compound could be measured in the infusion mixture when stored in the refrigerator and at room temperature, respectively (Table 1).

The data in Table 2 are characteristic for all the stability data of cyclophosphamide in aqueous parenteral vehicles. In all cases the solutions remained clear and colorless. These facts are also described in the literature [2].

The data available indicate that the loss of cyclophosphamide follows first-order kinetics. The values for the calculated first-order rate constants with 99% confidence limits are shown in Table 3 (room temperature) and in Table 4 (refrigerator temperature) [2].

From the semilogarithmic plot of the decomposition process, the linear regression coefficients of the data are $r^2 = 0.96$, and 1.00 measured at room temperature and at

Table 1: Stability of cyclophosphamide in sodium chloride 0.9% intravenous solution

Time* (d)	Content in % ± SD of the initial concentration**	
	22–25 °C	2–8 °C
0	100	100
1	98.98 ± 0.78	99.98 ± 0.89
2	98.24 ± 1.87	99.80 ± 0.92
3	98.02 ± 0.08	99.82 ± 1.02
4	97.37 ± 1.56	99.78 ± 1.26
5	95.45 ± 0.89	99.65 ± 1.45
6	92.53 ± 0.77	99.40 ± 0.68
7	89.14 ± 1.23	99.28 ± 1.57
14	72.66 ± 1.08	98.89 ± 0.96

* after dilution
** each value represents the mean of 6 determinations

Table 2: Stability of cyclophosphamide in sterile water for injection

Room Temperature (24–27 °C)		Refrigerator Temperature (approximately 5 °C)	
Time (d)	Assay (mg/ml)	Time (d)	Assay (mg/ml)
0	20.22	0	20.37
0	20.17	0	20.37
0	20.42	0	20.22
0	20.30	0	20.20
4	18.25	4	19.58
4	18.23	4	20.05
4	18.42	4	20.62
4	18.37	4	20.38
14	14.44	52	19.40
14	13.95	52	19.05
14	14.25	52	19.13
14	14.34	52	19.10
55	4.26	119	18.27
55	4.40	119	18.25
55	4.50	119	17.92
55	4.38	119	18.68

Table 3: First-order rate constants with 99% confidence intervals for disappearance of cyclophosphamide from solutions at room temperature

Vehicle	Initial concentration (mg/ml)	Rate constant (d^{-1})	99% Confidence interval (d^{-1})
5% Dextrose	3.1	0.0270	0.0229–0.0311
5% Dextrose	0.1	0.0167	0.0053–0.0280
Dextrose-saline	3.1	0.0216	0.0153–0.0279
Dextrose-saline	0.1	0.0229	0.0152–0.0306
Paraben preserve water	21	0.0263	0.0257–0.0268
Benzyl alcohol preserved water	21	0.0405	0.0382–0.0428
Sterile Water for Injection USP	21	0.0280	0.0273–0.0287

Table 4: First-order rate constants with 99% confidence intervals for disappearance of cyclophosphamide from solutions at 5 °C

Vehicle	Initial concentration (mg/ml)*	Rate constant (d^{-1})	99% Confidence interval (d^{-1})
5% Dextrose	3.1	0.000564	0.000211–0.000917
5% Dextrose	0.1	0.000594	0.000202–0.000986
Dextrose-saline	3.1	0.000755	0.000065–0.0014445
Dextrose-saline	0.1	0.00067	−0.00063–0.00197
Paraben preserve water	21	−0.00018	−0.00078–0.00042
Benzyl alcohol preserved water	21	−0.00039	−0.00096–0.00018
Sterile Water for Injection USP	21	0.00087	0.00066–0.00108

* Theoretical

2–8 °C, respectively. The half-life of the drug in solution is 82.5 and 2882.5 d stored at 22–25 °C and at 2–8 °C, respectively.

A slight decrease in pH in aqueous solutions of the drug during the study apparently did not affect the kinetics of decomposition. Such a conclusion is supported by the results of Hirata et al., which showed that the rate constant at 75 °C for loss of the drug was independent of pH between pH values of 2 and 10 [3].

According to these results we may conclude that since the degree of decomposition of cyclophosphamide at 2–8 °C did not increase above 1% on the seventh day, the infusion mixture can be stored for seven days.

3. Experimental

3.1. Materials

Cytoxan® 200 mg liophilized cyclophosphamide (monohydrate) powder for injection (Bristol-Myers Squibb), 0.9% NaCl infusion solution used for diluting cyclophosphamide is the product of Human RT (Human Serum production and Research PLC), Salsol® A 500 ml).

3.2. Preparation of solution

The compound (Cytoxan® 200 mg) has been dissolved in 0.9% NaCl infusion solution. The examined concentration was 0.4 mg/ml. The infusion mixture, prepared under aseptic conditions in a laminar box using protective clothing, was stored in the refrigerator (2–8 °C) and at room temperature (21 to 25 °C) for 14 d. The values measured after the preparation of the right away infusion mixture are regarded as zero values.

Six samples of cyclophosphamide solution were measured for 14 hd every 24 h. The chemical analysis of cyclophosphamide was carried out by HPLC according to a known procedure [4].

3.3. Equipment

Merck Hitachi HPLC system, supplement by pump (L-6200 A model), injector (Rheodyne 7161 model), detector (L-4250 UV-VIS model) and D 6000 A interface.

3.4. Conditions of chromatography

Column Ultrasphere ODS BST 250 × 4 mm, 5 μm, mobile phase: CH_3CN/H_2O (3:7), flow rate: 1.5 ml/h, UV detector = 200 nm. Injection volume: 20 μl. Degradation products did not interfere with the determination of cyclophosphamide [5].

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