

Background: To determine the stability of fludarabine phosphate in concentrate in glass containers and diluted with sodium chloride 0.9% in polyethylene (PE) bags.

Material and methods: Solutions of fludarabine phosphate 25 mg/ml (concentrate) in glass containers ($n = 6$) were opened and stored at refrigerator temperature (2–8°C) and at room temperature (15–25°C). Another six solutions of fludarabine phosphate were diluted in NaCl 0.9% and stored in PE bags in the same conditions (2–8°C and 15–25°C). Samples of each solution from the 12 containers were analysed for fludarabine phosphate concentration initially and after 0.5, 1, 1.5, 2, 2.5, 3, 13 and 15 days of storage. Samples were assayed by a high performance liquid chromatographic (HPLC) method with ultraviolet detection ($\lambda = 265$ nm) to determine the fludarabine phosphate concentration at each time of sampling.

Results: The concentration of fludarabine phosphate at each sampling time in the analysed solutions remained within 93.3–102.0% of the initial concentration, regardless of the storage temperature and container.

Conclusions: Fludarabine phosphate, both undiluted in glass containers and diluted with NaCl 0.9% in PE bags, remains stable (< 10% degradation) for at least 15 days at room and refrigerator temperature.

Key words: fludarabine, stability, concentrate, polyethylene, HPLC.

The stability of fludarabine phosphate in concentrate and diluted with sodium chloride 0.9%

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Introduction

Fludarabine phosphate (9- β -D-arabinofuranosyl-2-fluoroadenine monophosphate) is a fluorinated derivative of the nucleotide adenosine monophosphate. The molecular formula of fludarabine phosphate is $C_{10}H_{13}FN_5O_7P$ (MW 365.2) and the structure is provided in Fig. 1. Fludarabine is relatively resistant to deamination by adenosine deaminase. After intravenous administration, fludarabine is rapidly dephosphorylated to the primary metabolite, 9- β -D-arabinofuranosyl-2-fluoroadenine (F-ara-A), which is re-phosphorylated intracellularly by deoxycytidine kinase to fludarabine and subsequently to the active metabolite, 9- β -D-arabinofuranosyl-2-fluoroadenine triphosphate (F-ara-ATP). This active metabolite acts by inhibiting DNA polymerase α , ribonucleotide reductase and DNA primase, thus inhibiting DNA and RNA synthesis and apoptosis [2, 5].

Fludarabine is indicated for the treatment of patients with a variety of lymphoproliferative malignancies including chronic lymphocytic leukaemia (CLL) – the most common leukaemia in the Western world – and non-Hodgkin's lymphoma (NHL) [3, 6]. Fludarabine is often used in the treatment of CLL in combination therapy, e.g. with cyclophosphamide [7, 8], rituximab [9], or mitoxantrone [10, 11].

Fludarabine phosphate should be prepared for parenteral use by aseptically diluting in 5% glucose or 0.9% sodium chloride. The solution of fluda-

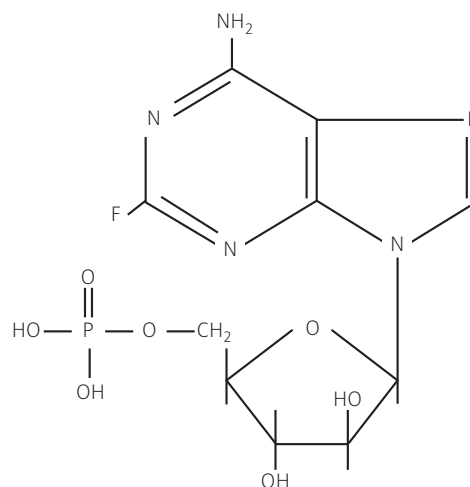


Fig. 1. The chemical structure of fludarabine phosphate

rabine phosphate contains no antimicrobial preservatives and thus should be used within 8 hours of preparation [4]. This does not mean that drug degradation occurs immediately beyond that period, but is rather connected with the sterility of the final solution. However, if the preparation of admixtures from the vials is made with aseptic techniques, sterility is maintained. Drug stability for a minimum of a few days is most beneficial for practical management. Therefore, stability studies are much needed to establish the period when the drug concentration remains within acceptable limits, i.e., when the drug concentration(s) on any day of analysis is not less than 90% of its initial concentration. Stability is defined as the time during which a reconstituted antineoplastic drug retains its integrity in terms of quantity and chemical identity [12].

The analytical procedures used in the stability study should be well validated. The concentration of fludarabine phosphate was carried out by means of a high pressure liquid chromatographic (HPLC) method with UV detection. HPLC-UV is the most popular method in analytical laboratories due to the relative simplicity of its operation and the lower costs of its instrumentation in comparison with more sophisticated techniques.

Material and methods

The research was done at the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań, Poland.

Reagents

Fludarabine phosphate standard was supplied by LGC Standards (Lomianki, Poland). Acetonitrile and methanol were liquid chromatography grade. Methanol was purchased from Merck and acetonitrile from Sigma-Aldrich. Water used in the mobile phase was deionized, distilled and filtered through a Millipore system before use. The dosage of fludarabine phosphate in commercial formulation was carried out with Fludarabine Teva® (TEVA Pharmaceuticals Polska, Warsaw, Poland). The composition of one 2-ml vial was: 50 mg fludarabine phosphate, mannitol and sodium hydroxide.

Chromatographic assay method

The concentration of fludarabine phosphate was carried out by means of a high pressure liquid chromatographic (HPLC) method with UV detection, which was a modified version of the method developed by Ficarra *et al.* [1]. An HPLC system consisting of a Waters Alliance liquid chromatograph (Milford, MA, USA; model 2695), a Waters 2487

PDA detector, an autosampler, a 100 µl syringe, degasser, column oven, and a data collection system running Waters' Empower Pro software was used for this analysis. The parameters of chromatographic separation were: Symmetry C18 column 250 × 4.6 mm, 5 µm particle size (Waters), mobile phase: acetonitrile – water (5 : 95), flow rate of the mobile phase 1 ml/min. The UV detector wavelength was set at 265 nm. The method used isocratic elution with a total run time of 6 min. The injection volume was 1 µl and the column was thermostated at 25°C. Under these conditions, the mean retention time for fludarabine phosphate was 1.8 min.

Standard preparation

Stock solution was prepared by weighing 10 mg of fludarabine phosphate into a 20-ml volumetric flask. The substance was dissolved and diluted to volume with water. The solution was kept at 4°C. Working standard solutions were prepared by appropriate dilutions of the stock solution in water to obtain concentrations across the range of 0.01–0.50 mg/ml. Quality control (QC) samples were also freshly prepared in a similar manner by separate weighing.

Calibration curve

Aliquots of the standard stock solution of fludarabine phosphate were pipetted into six different 10-ml volumetric flasks and solutions were diluted with water. The final concentrations of fludarabine phosphate were respectively 0.01, 0.05, 0.1, 0.2, 0.25, 0.5 mg/ml. Three determinations were carried out for each concentration. Peak areas were recorded for all the solutions. The linear regression analysis was carried out by plotting the peak areas (*y*) of the compound against the respective concentrations (*x*) of fludarabine phosphate. The linearity for the relationship between peak area and concentration was demonstrated by a correlation coefficient (*r*).

Precision and accuracy

The precision and accuracy parameters were determined from freshly made quality control standards in three different concentrations (0.025, 0.15, 0.3 mg/ml). Table 1 shows intra- and inter-day precision (% RSD) and accuracy of this assay method. The precision of the method at each concentration was calculated as the relative standard deviation of the mean (RSD) using the following equation: $RSD = (SD/mean) \times 100$. The accuracy of the procedure was determined as the relative mean error (RME) using the following equation: $RME = [(mean - spiked\ concentration\ of\ the\ analyte)/spiked\ concentration\ of\ the\ analyte] \times 100$.

Table 1. Intra- and inter-day precisions and accuracies of fludarabine phosphate (*n* = 3 in all cases)

Concentration [mg/ml]	Intra-day			Inter-day		
	Mean ± SD	% RSD	% RME	Mean ± SD	% RSD	% RME
0.025	0.026 ± 0.001	4.32	4.0	0.026 ± 0.001	2.2	4.0
0.150	0.152 ± 0.008	5.08	1.3	0.139 ± 0.018	13.2	7.33
0.300	0.269 ± 0.009	3.60	10.3	0.264 ± 0.018	6.9	12.0

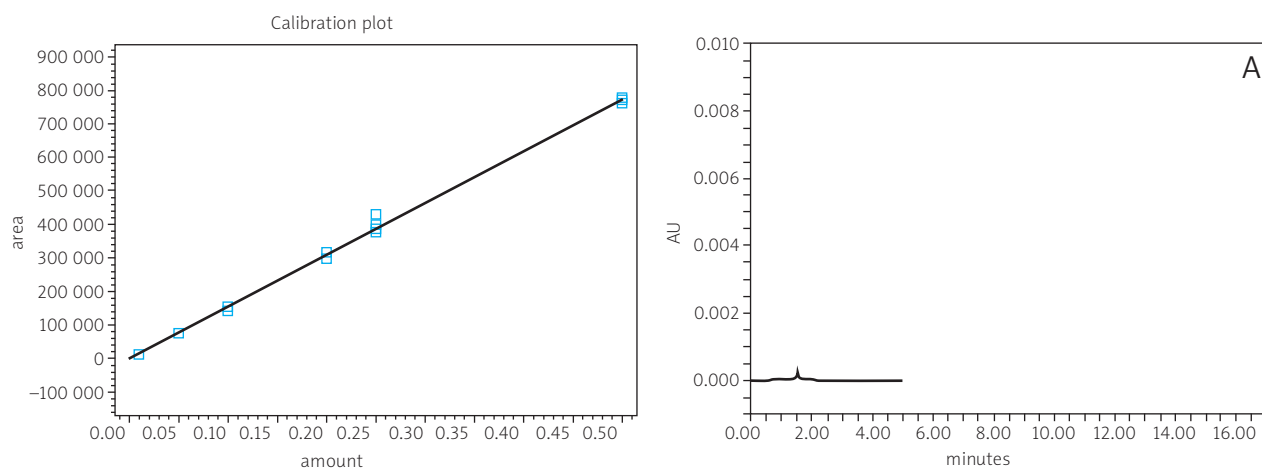


Fig. 2. Standard calibration curves for fludarabine phosphate ($n = 3$). Each curve is based on 6 calibration standards with triplicate injections

Sample preparation

Six concentrates of Fludarabine Teva® (25 mg/ml) were opened and three of them were stored at refrigerator temperature (2-8°C) and three at room temperature (15-25°C). Six fresh solutions of Fludarabine Teva® were prepared by dilution of 1700 μ l of concentrate with 100 ml of sodium chloride 0.9% to a final fludarabine phosphate concentration of 0.05 mg/ml. Fludarabine phosphate solutions were kept in plastic containers (PE) in triplicate in a refrigerator and also in a room. All the samples were kept away from light. Aliquots were taken at specific time intervals and the concentration of fludarabine phosphate was determined by HPLC assay as described above. The concentrations of the fludarabine phosphate in the analysed samples were calculated by using the regression equation of the straight line $y = ax + b$.

Results and discussion

The analytical method described was developed and validated to be applied to determine the stability of fludarabine phosphate solutions (concentrate and in 0.9% NaCl). For validation, a range from 0.01 to 0.50 mg/ml of fludarabine phosphate was chosen. The calibration line $y = 1.25 \cdot 10^6 (\pm 0.24 \cdot 10^6) x - 1.39 \cdot 10^6 (\pm 2.35 \cdot 10^6)$ represents the mean of the three graphs (Fig. 2). The correlation coefficient (r) for each calibration graph was greater than 0.99 and the % RSD of each concentration studied was less than 15%. Both intra- and inter-day precisions were less than 15.0% and intra- and inter-day accuracies ranged from 99.7% to 106.8%. Fig. 3 A, B and C show typical chromatograms obtained from a drug-free solution, concentrate and solution of fludarabine phosphate in 0.9% NaCl, respectively. The chromatograms show that the separation from matrix constituents is sufficient for reliable quantitation and no endogenous components interfered with the analyte peak. The retention time of fludarabine phosphate was 1.8 ± 0.1 min. The lower limit of quantification (LLOQ) and limit of detection (LOD) were 0.01 and 0.007 mg/ml, respectively.

Tables 2 and 3 list the percentage of intact drug remaining at equilibrium for all cases studied. Fludarabine phosphate appears to be stable for at least 15 days in concen-

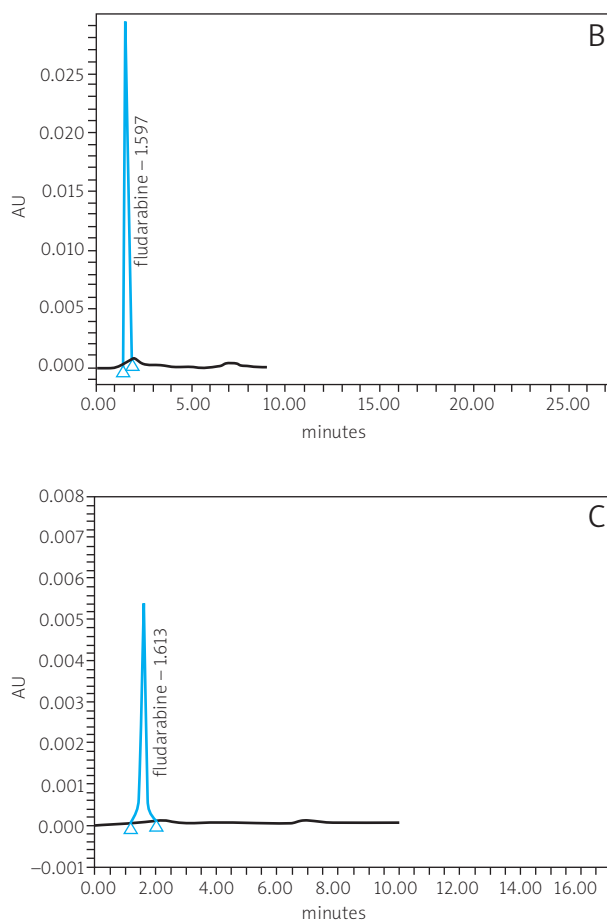


Fig. 3. The chromatogram of fludarabine phosphate: a drug-free solution (A), concentrate – 0.179 mg/ml, (B) and solution of fludarabine phosphate in 0.9% NaCl – 0.049 mg/ml (C)

trates and in a sodium chloride 0.9% solution at concentration 0.05 mg/ml. Fludarabine phosphate stability was similar both when the solutions were stored in plastic bags (PE) and in glass containers.

In clinical use it is important to establish the stability of once opened parenteral antineoplastic agents, because it is very variable. For example, the stability of mecloethamine lasts only a few minutes [12], that of pentostatin 72 hours [14], while that of fluorouracil lasts as long as 112 days [13]. The rates of drug degradation are determined by the pH of

Table 2. Fludarabine phosphate (%) at different sampling times in concentrate stored at refrigerator temperature (2-8°C) and room temperature (15-25°C)

Temperature	Percentage of initial concentration at indicated time								
	0 h	12 h	24 h	36 h	48 h	60 h	72 h	13 days	15 days
2-8°C									
Mean	100.0	100.8	101.1	100.7	99.5	100.3	98.6	98.3	97.0
SD	0.0	3.0	1.9	2.5	2.9	1.6	1.0	1.0	3.3
n	3	3	3	3	3	3	3	3	3
15-25°C									
Mean	100.0	102.0	101.5	99.6	98.2	98.2	98.0	98.0	95.6
SD	0.0	1.2	1.9	2.0	0.8	0.3	0.6	0.6	1.4
n	3	3	3	3	3	3	3	3	3

Table 3. Fludarabine phosphate (%) at different sampling times in sodium chloride 0.9% stored at refrigerator temperature (2-8°C) and room temperature (15-25°C)

Temperature	Percentage of initial concentration at indicated time								
	0 h	12 h	24 h	36 h	48 h	60 h	72 h	13 days	15 days
2-8°C									
Mean	100.0	100.0	98.7	100.0	99.3	100.0	95.7	94.7	93.3
SD	0.0	2.0	1.2	0.0	1.2	0.0	1.5	1.2	4.2
n	3	3	3	3	3	3	3	3	3
25°C									
Mean	100.0	100.7	99.3	100.0	100.7	100.0	96.3	94.0	98.0
SD	0.0	1.2	1.2	0.0	1.2	0.0	0.6	0.0	2.0
n	3	3	3	3	3	3	3	3	3

the final infusion, buffer, buffer concentration, and temperature. Protection from light is also important for many drugs, e.g., solutions of methotrexate (MTX) and leucovorin (LV) should be protected during preparation and storage from direct sunlight and room light. Additional factors which may affect the stability of the drug are diluents (e.g. dextrose for injection, sodium chloride for injection, sterile water for injection, and lactated Ringer's injection) [12]. The degradation process may also be caused by drug-drug interactions. Several drugs are visually incompatible with fludarabine phosphate, e.g., acyclovir sodium (darker colour forms in four hours), daunorubicin hydrochloride (initially clear red-orange solution develops a very slight haze in four hours), and amphotericin B (small amount of particulate matter develops in four hours) [15]. Thus, from the clinical point of view the stability of the drug is very important, especially after its opening. The conducted research confirmed the stability of fludarabine phosphate in concentrate and after dilution in NaCl 0.9% for Fludarabine Teva®. However, it is important to pay special attention to the aseptic conditions necessary to prepare the solution of the drug for parenteral administration.

Conclusion

Fludarabine phosphate appears to be stable (<10% degradation) for at least 15 days in concentrate in glass containers or diluted with NaCl 0.9% in PE bags at a concentration of 0.05 mg/ml, at refrigerator temperature (2-8°C) and room temperature (15-25°C).

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