

Effect of freezing, long-term storage and microwave thawing on the stability of sodium folinate

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ABSTRACT

Objective: To prepare intravenous solutions in advance; this would be efficient and improve quality assurance, patient safety, time management and cost-efficiency of drug delivery. The purpose of this study was to investigate how freezing, long-term storage and microwave thawing can affect the stability of sodium folinate (3.2 mg/mL in 5% dextrose) in polyolefin bags.

Methods: The stability of five polyolefin bags containing approximately 3.2 mg/mL of sodium folinate in 5% dextrose prepared under aseptic conditions was studied after freezing for three months at -20°C, thawing in a microwave oven with a validated cycle, and storage at 4°C. Sodium folinate concentrations were measured by high-performance liquid chromatography (HPLC) using a reversed-phase column—a mobile phase, consisting of 5% of methanol (v/v) in KH₂PO₄ buffer (0.01 M, pH 7.50 ± 0.05), and UV detection at 300 nm. Visual inspection, microscope observation and pH measurement were also performed.

Results: No colour change or precipitation occurred in the preparations. No micro-aggregates were observed under the microscope. Based on a shelf-life of 90% residual potency, sodium folinate solutions were stable for at least 30 days at 4°C after freezing and thawing; the 95% lower confidence limit of the concentration-time profile remained superior to 90% of the initial concentration. During this stability period, the pH values decreased slightly without affecting chromatographic parameters.

Conclusion: Within these limits, sodium folinate in 5% dextrose infusion can be prepared and frozen in advance by a centralised intravenous admixture service (CIVAS), then thawed before use in clinical units.

KEYWORDS

CIVAS, HPLC, long-term stability, sodium folinate

INTRODUCTION

Folinic acid is the 5-formyl derivative of tetrahydrofolic acid, the active form of folic acid. Sodium or calcium folinic salts are used to reduce toxicity of folic acid antagonists, such as methotrexate, in leukaemia [1, 2]. Folinic acid is also extensively used to enhance the effects of 5-fluorouracil

in various types of carcinomas, mainly colorectal [3-5]. Folinic acid can be given by mouth, by intramuscular injection, or by intravenous injection or infusion, which is the route most commonly used in clinical practice [6]. As demonstrated for several other therapeutic substances, advance preparation of intravenous solutions of folinic acid by a centralised intravenous admixture service (CIVAS) can improve quality assurance, patient safety, time management, speed and cost-efficiency of drug delivery [7-15]. Freezing the solutions can extend long-term stability of ready-to-use injectable drugs [16]. A disadvantage of such storage is the thawing time. Different investigators have used a microwave oven to reduce the time required for thawing a frozen solution, but the effect of microwave thawing on drug stability is questionable, and few data are available on the effect of freezing and microwave thawing [17-20]. Folinic acid stability in solution has been studied with the calcium [21-24] and sodium salts [25], but no data are available on its stability after freezing and microwave thawing before storage. The aim of this study was to investigate the stability of sodium folinate solutions at 3.2 mg/mL in 5% dextrose in polyolefin bags after freezing, long-term storage and microwave thawing, and then after storage at 4°C.

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MATERIALS AND METHODS

Preparation of solutions

Commercially-available vials of sodium folinate (VoriNa 500 mg/20 mL, TevaPharma, Wilrijk, Belgium, lot 05E24RB) were added, in a vertical laminar-airflow hood, to 250 mL multi-layered plastic bags with a high density polyethylene containing 5% dextrose injection (Viaflo, Baxter, Lessines, Belgium, lot 05H19E1L) to produce solutions containing approximately 3.2 mg/mL of sodium folinate [26].

On each test day, a commercial vial of sodium folinate (VoriNa 50 mg/2 mL, Teva Pharma, Wilrijk, Belgium, lot 05H300B) was used as a standard. It was extemporaneously diluted in sterile water for injection to obtain a solution containing 3.12 mg/mL of sodium folinate.

Chromatographic apparatus and conditions

The high-performance liquid chromatographic (HPLC) system (Alliance, model 2690, Waters Association, Milford, MA, USA) was used with a DAD detector (model 996, Waters Association) and a data acquisition and processing module (Empower 2 Software, Waters Association).

A reversed-phase column C18 was used with associated guard column (Hypersil ODS column C18, particle size: 3 μ m, 100 \times 4.6 mm, ref 9868 with a guard column C18, particle size: 5 μ m, 7.5 \times 4.6 mm, ref 96013; Waters Association, Milford, MA, USA).

The mobile phase comprised 5% of methanol super gradient (ref C26C11X, Lab-Scan Ltd, Dublin, Ireland) and 95% KH_2PO_4 buffer (0.01 M, pH 7.50 \pm 0.05) (KH_2PO_4 , ref 1.04873, Merck, Darmstadt, Germany) and NaOH (ref 1.06498, Merck, Darmstadt, Germany). The flow rate was 1 mL/minute, the column temperature set at 35°C and the wavelength at 300 nm.

pH determination

The pH of the solutions was measured with a pH-meter (Radiometer PHM82, Copenhagen, Denmark).

Validation of HPLC method

Precision of method

Assays of control solutions at three different concentrations of sodium folinate (1, 3.12 and 5 mg/mL) were undertaken to calculate the within-day variation.

The between-day variation was estimated on the calibration solution of sodium folinate (3.12 mg/mL).

Linearity of analytical response

Linearity was evaluated by serial dilutions of sodium folinate solution at 25 mg/mL with sterile water for injection.

Stability indication

The stability-indicating capability of the chromatographic method was assessed using partially decomposed solutions of the drug. Solutions of sodium folinate at 3.12 mg/mL at different pH were prepared: neutral (pH of 7.47), alkaline (pH of 12.12 obtained by adding NaOH 5 M) and acidic (pH of 1.21 obtained by adding HCl 12 M). These solutions were degraded by heating at 100°C for 60 minutes and then neutralised before analysis.

Stability study

Five bags each of 3.2 mg/mL sodium folinate were prepared as described above, agitated and stored at -20°C for 90 days. They were then thawed in a microwave oven with a validated cycle and finally stored at 4°C. Before freezing, immediately after defrosting and after 1, 2, 3, 4, 7, 9, 11, 14, 17, 21, 24, 27 and 30 days, we withdrew 2 mL of solution from each bag using 5 mL polypropylene plastic syringes (Terumo, Haasrode, Belgium) and placed each aliquot in a glass container.

The admixtures were visually inspected and their pH measured. They were also inspected under a microscope. The concentration of sodium folinate in each solution was determined in triplicate.

HPLC assay

A volume of 100 μ L standard solution and 100 μ L samples from bags were diluted at 1:100 with sterile water for injection. Aliquots of 10 μ L of the assay solutions were injected into the chromatograph. The injection of an identical volume of the standard solution was used to calibrate the system. Results were automatically calculated by interpolation of a single-level calibration curve (linear through zero), performed with Empower 2 software using peak areas versus standard concentrations.

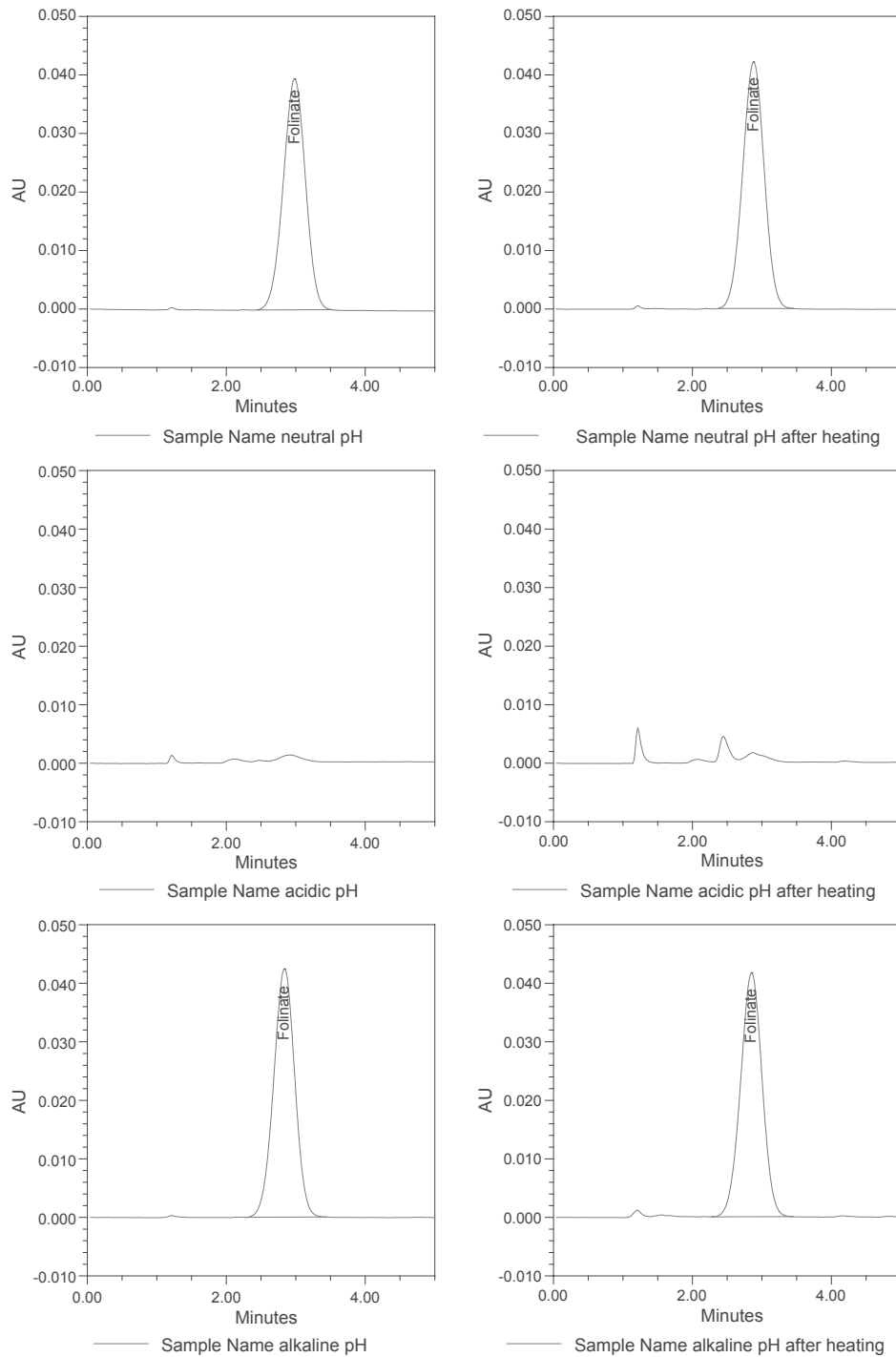
Data were expressed as mean \pm standard deviation. Drug concentrations and pH were followed at predetermined time intervals. The drug solutions were considered stable as long as the 95% lower confidence limit of the concentration-time profile remained above 90% of the initial concentration, as recommended by the FDA [27-28].

RESULTS AND DISCUSSION

Validation of HPLC method

The within-day relative standard deviation (SD) values ($n = 10$) realised with three concentrations (1, 3.12 and 5 mg/mL) were respectively 1.1%, 0.4% and 0.9%. The between-day precision ($n = 14$) estimated on the calibration solution (3.12 mg/mL) was 1.4%. Linear-regression analysis of the peak area yielded a correlation coefficient

Figure 1: Chromatograms of sodium folinate



Data are presented as detector outputs before and after heating and neutral pH (7.47), acidic pH (1.21) and alkaline pH (12.12)

Table 1: Stability of sodium folinate at 3.2 mg/mL in 5% dextrose in polyolefin bags

Initial concentration (100%) before freezing: 2.96 ± 0.02 mg/mL			
Storage time at 4°C (days)	Mean concentration ± SD (mg/mL)	Mean percentage of initial concentration ± SD	95% lower and upper confidence limit of mean percentage
0	2.99 ± 0.03	101.1 ± 1.1	100.2-102.0
1	2.91 ± 0.01	98.4 ± 1.0	97.6-99.3
2	2.99 ± 0.01	101.0 ± 0.9	100.2-101.8
3	2.93 ± 0.02	98.9 ± 0.5	98.4-99.4
4	3.00 ± 0.01	101.5 ± 0.9	100.7-102.3
7	2.97 ± 0.03	100.3 ± 0.9	99.5-101.2
9	3.02 ± 0.01	102.1 ± 0.6	101.6-102.6
11	2.98 ± 0.01	100.7 ± 0.7	100.1-101.3
14	2.96 ± 0.02	100.0 ± 0.8	99.3-100.8
17	2.96 ± 0.02	100.0 ± 0.8	99.3-100.7
21	3.00 ± 0.02	101.3 ± 0.7	100.7-101.9
24	3.00 ± 0.01	101.6 ± 0.6	101.0-102.1
27	2.99 ± 0.02	100.9 ± 0.8	100.2-101.6
30	2.95 ± 0.02	99.6 ± 1.1	98.7-100.6

SD: standard deviation. Samples were tested in triplicate

of $r^2 > 0.99$ in the range of 0.1 mg/mL to 7.5 mg/mL. Degraded samples of sodium folinate were assayed to confirm separation of the parent molecule from its degradation products. In all cases, the peaks of the decomposition products were resolved from the peak of the native drug (Figure 1).

Stability of sodium folinate infusions

There was no colour change, precipitation or micro-aggregates under the microscope in the solutions when the frozen bags were thawed in the microwave oven. Subsequent storage for 30 days at 4°C did not show changes in the same parameters. Statistical analysis demonstrated a significant decrease ($p < 0.001$) in the pH values of stored solutions ranging from 7.35 to 7.00.

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The fall in the pH of infusions could be explained by a weak degradation of the component. However, pH values remained in an acceptable range for infusion [29]. Concentrations of sodium folinate admixtures during storage expressed as percentages of the initial dose are shown in Table 1. Statistical analysis showed no significant change in sodium folinate concentrations for at least 30 days, the slope of the regression line being no different from zero. In our study, only chemical stability was evaluated and the microbiological aspects were not investigated. After preparation and dispensing of parenteral nutrition mixtures and anticancer drugs [30], the preparation of adjuvant treatments by a CIVAS contributes to the global management of cancer treatment by providing ready-to-use injectable drugs with acceptable physicochemical and bacteriological quality, and by relieving nursing staff from the tasks of infusion preparation [31].

CONCLUSION

Sodium folinate in 5% dextrose in polyolefin bags may be frozen for three months and microwave thawed without major changes that affect concentration. Subsequent storage of the bags at 4°C is possible for at least 30 days. Though compounding of this preparation is low-risk, since microbiological aspects were not investigated, the stability of sodium folinate should be 45 days in the freezer and 14 days under refrigeration, according to the USP recommendations. Within these limits, sodium folinate can be prepared in advance by a CIVAS, frozen, thawed and stored under refrigeration for a few days before use on hospital wards.

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