ARTICLE

Stability of Thiamine in Extemporaneously Compounded Suspensions

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ABSTRACT

Objective: To evaluate the stability of thiamine suspensions in a vehicle consisting of equal parts Ora-Sweet (a sweetening agent) and Ora-Plus (a suspending agent) after storage at 4°C and 25°C for up to 91 days.

Methods: Suspensions of thiamine 100 mg/mL were prepared from powder in 50-mL amber plastic prescription bottles. Three bottles of the suspension were stored at 4°C (refrigerated), and 3 bottles were stored at 25°C (room temperature). Physical characteristics, including pH, odour, taste, colour, viscosity, precipitation, and ease of resuspension (the latter 4 by visual inspection), were evaluated weekly for 91 days. Aliquots were removed from each bottle weekly for 91 days and stored at –85°C until analysis by a validated high-performance liquid chromatography method. A suspension was considered stable if it maintained 90% of its initial concentration.

Results: Minimal change in the pH of samples was observed after storage at 4°C or 25°C for 91 days. Very slight changes in odour, taste, and colour occurred between days 14 and 21, but these characteristics remained generally stable for the 91-day period. Viscosity was constant. Precipitates were easily resuspended, and there was no caking or clumping of material. Suspensions of thiamine maintained at least 90% of initial concentration at both temperatures throughout the 91-day period.

Conclusions: Thiamine suspensions of 100 mg/mL in a 1:1 mixture of Ora-Sweet and Ora-Plus were considered physically and chemically stable for a period of up to 91 days, with or without refrigeration. The expiration date for these products can therefore be set at 91 days.

Key words: thiamine, stability, Ora-Sweet, Ora-Plus, suspensions, high-performance liquid chromatography

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RÉSUMÉ

Objectif : Évaluer la stabilité de préparations de thiamine en suspension dans un excipient composé de parties égales d'Ora-Sweet (édulcorant) et d'Ora-Plus (agent de suspension) entreposées à 4 °C et à 25 °C pendant une période allant jusqu'à 91 jours.

Méthodes : Les préparations de thiamine en suspension à 100 mg/mL ont été préparées à partir de poudre dans des flacons de médicaments d'ordonnance en plastique de couleur ambre de 50 mL. Trois flacons de préparations en suspension ont été entreposées à 4 °C (réfrigérées), et trois autres à 25 °C (température ambiante). Les propriétés physiques, notamment : pH, odeur, goût, couleur, viscosité, présence de précipité et facilité de remise en suspension, ont été évaluées (les quatre dernières par inspection visuelle) toutes les semaines sur une période de 91 jours. Des aliquotes ont été retirées de chaque flacon toutes les semaines pendant une période de 91 jours, puis conservées à -85°C, jusqu'à ce qu'elles soient soumises à une analyse validée par chromatographie liquide à haute pression. La préparation en suspension était jugée stable lorsqu'elle avait conservé 90 % de sa concentration initiale.

Résultats : On a observé un changement mineur du pH des échantillons après leur entreposage à 4 °C ou à 25 °C pendant 91 jours. Une très légère modification de l'odeur, du goût et de la couleur est survenue entre les jours 14 et 21, mais ces propriétés sont demeurées généralement stables pendant la période de 91 jours. La viscosité n'a pas été modifiée. Les précipités ont été facilement remis en suspension et aucune agglutination ou agglomération de matériel n'a été notée. Les préparations de thiamine en suspension ont conservé au moins 90 % de leur concentration initiale aux deux températures pendant la période de 91 jours.

Conclusions : Les préparations de thiamine en suspension de 100 mg/mL dans un mélange 1:1 d'Ora-Sweet et d'Ora-Plus ont été jugées physiquement et chimiquement stables pendant une période allant jusqu'à 91 jours, avec ou sans réfrigération. La durée de conservation de ces préparations peut donc être établie à 91 jours.

Mots clés : thiamine, stabilité, Ora-Sweet, Ora-Plus, préparations en suspension, chromatographie liquide à haute pression

INTRODUCTION

Pharmacists prepare "homemade" suspensions for many medications that are not available in dosage forms suitable for children (and adults) who are unable to swallow tablets or capsules. Thiamine (vitamin B_1), used in the treatment of metabolic diseases, is one such extemporaneously compounded suspension. Ora-Sweet (a sweetening agent) and Ora-Plus (a suspending agent) are now commercially available (Paddock Laboratories Inc, Minneapolis, Minnesota); these products ease preparation of the suspension and improve palatability. Although the stability of thiamine in other vehicles and dosage forms has been widely reported,[1-8] there is no published information on the stability of thiamine in 1:1 Ora-Sweet:Ora-Plus.

This study examined the physical characteristics and chemical stability (defined as maintenance of more than 90% of initial concentration) of extemporaneously prepared oral thiamine suspensions of 100 mg/mL in a 1:1 mixture of Ora-Sweet and Ora-Plus, stored at 4°C and 25°C throughout a 91-day study period.

METHODS

Preparation and Evaluation of Suspensions

Thiamine suspensions (100 mg/mL) were prepared from commercially available thiamine powder (Xenex Laboratories Inc, Coquitlam, British Columbia, lot 17695), in a 1:1 mixture of Ora-Sweet and Ora-Plus (Paddock Laboratories Inc, lots 2336330 and 2296122, respectively). Six replicates were prepared in separate 50-mL amber plastic prescription bottles; 3 bottles were stored at 4°C (refrigerated, with no exposure to light except during assays), and 3 were stored at 25°C (room temperature, with exposure to light). Exposure to light was limited to fluorescent light in the laboratory.

The physical characteristics of the suspensions were evaluated at the time of preparation and at weekly intervals up to 91 days. All suspensions were tested for odour, pH, and taste and were visually examined for changes in colour (against white and black backgrounds), viscosity, formation of precipitates, and ease of resuspension. All samples were allowed to equilibrate to room temperature before measurement of pH, and the pH meter (model 8000, VWR Canlab, Mississauga, Ontario) was calibrated at the beginning of each testing period. Immediately after the physical observations were completed, each bottle was shaken manually for 10 s, pH was determined, and 1 mL of the suspension was removed. These samples were stored at –85°C until batch analysis by a stability-indicating high-performance liquid chromatography (HPLC) method developed in the authors' laboratory.

Preparation of Stock and Standards

Stock solutions of thiamine at 0.50, 1.00, 2.00, 3.00, and 4.00 mg/mL were prepared by diluting thiamine 100 mg/mL in HPLC-grade water (Fisher Scientific, Richmond, British Columbia, lot 034609). The internal standard was vitamin E (Aquasol E, Novartis Consumer Health Canada, Mississauga, Ontario, lot 1C046A) at a concentration of 10.00 mg/mL in HPLC-grade water. Standard solutions were prepared by combining a 0.5-mL aliquot of each stock solution and a 0.5-mL aliquot of Aquasol E. The final concentrations of thiamine in the samples injected onto the chromatograph were 0.25, 0.50, 1.00, 1.50, and 2.00 mg/mL. The final concentration of the internal standard was 5.00 mg/mL. These dilutions achieved optimal chromatographic characteristics. Before injection, all standard solutions were passed through a 0.45-µm microfilter (Acrodisc GHP syringe filter, Gelman, Ann Arbor, Michigan, lot 2864) to prevent injection of impurities onto the column.

The HPLC instrumentation (model 2690, Waters Alliance Systems, Waters Ltd, Mississauga, Ontario) consisted of a delivery pump, an automatic injector equipped with a 200-µL injector, a Symmetry 3.9 x 20 mm guard column (Waters Ltd, lot W40441), a Symmetry C_{18} 3.9 x 150 mm column (Waters Ltd, lot T90851T02), and an ultraviolet detector set at 258 nm (model 2487 dual-wavelength absorbance detector, Waters Ltd). The mobile phase was developed in the authors' laboratory and consisted of a 72:28 (v/v) mixture of acetonitrile (Fisher Scientific, lot H2832) and 10 mmol/L formic acid, with ammonium salt buffer (Sigma Aldrich, lot 52K1270; pH 3.0). All solvents were HPLC-grade and had been filtered before use. The flow rate was set at 1.0 mL/min.

A 5-point calibration curve was prepared, with a blank (water only) at the beginning of each run, to ensure that there was no carry-over from one run to the next. The range of this calibration curve (0.25 to 2.00 mg/mL) encompassed the diluted (1.00 mg/mL) test concentration of thiamine 100 mg/mL. The calibration curve was generated by least-squares regression of the peak area ratio of thiamine to

Aquasol E and the concentration of each standard. The precision of the assay was evaluated by intraday and interday validation methods. Intraday variability was determined by running 0.50, 2.00, and 3.00 mg/mL stock solutions (diluted to standards of 0.25, 1.00, and 1.50 mg/mL) in quadruplicate throughout a single day, whereas interday variability was determined by running the same concentrations (as in the testing for intraday variability) in quadruplicate daily for 4 days. The means, standard deviations, and coefficients of variation were then calculated. Acceptable limits for the coefficients of variation were defined a priori as less than 10%.

Degradation of Thiamine

Thiamine 1.0 mg/mL was incubated overnight (about 16 h) at 100°C, made basic (to pH 12.89) with sodium hydroxide 10N solution 30% w/v (Fisher Scientific, lot SC9105042). The preparation was brought back to pH 3.9 with hydrochloric acid (Fisher Scientific, lot 29067), adjusted to a concentration of 0.3 mg/mL, and filtered. The chromatogram obtained for the degraded preparation was compared with a chromatogram obtained from a standard (0.3 mg/mL) to determine any changes in concentration, retention time, and peak shape.

Preparation of Samples

Thiamine study samples (100 mg/mL) were thawed, and a 0.1 mL aliquot from each bottle and each temperature was diluted with 0.9 mL HPLC-grade water, centrifuged for 2 min at 10 000 rpm, and further diluted to a concentration of 2.00 mg/mL in HPLC-grade water. A 0.5-mL aliquot of thiamine was added to a 0.5-mL aliquot of internal standard in HPLC-grade water. The final theoretical thiamine concentration was 1.00 mg/mL. Each sample was passed through a 0.45-µm microfilter before a 10-µL sample was withdrawn and injected onto the column.

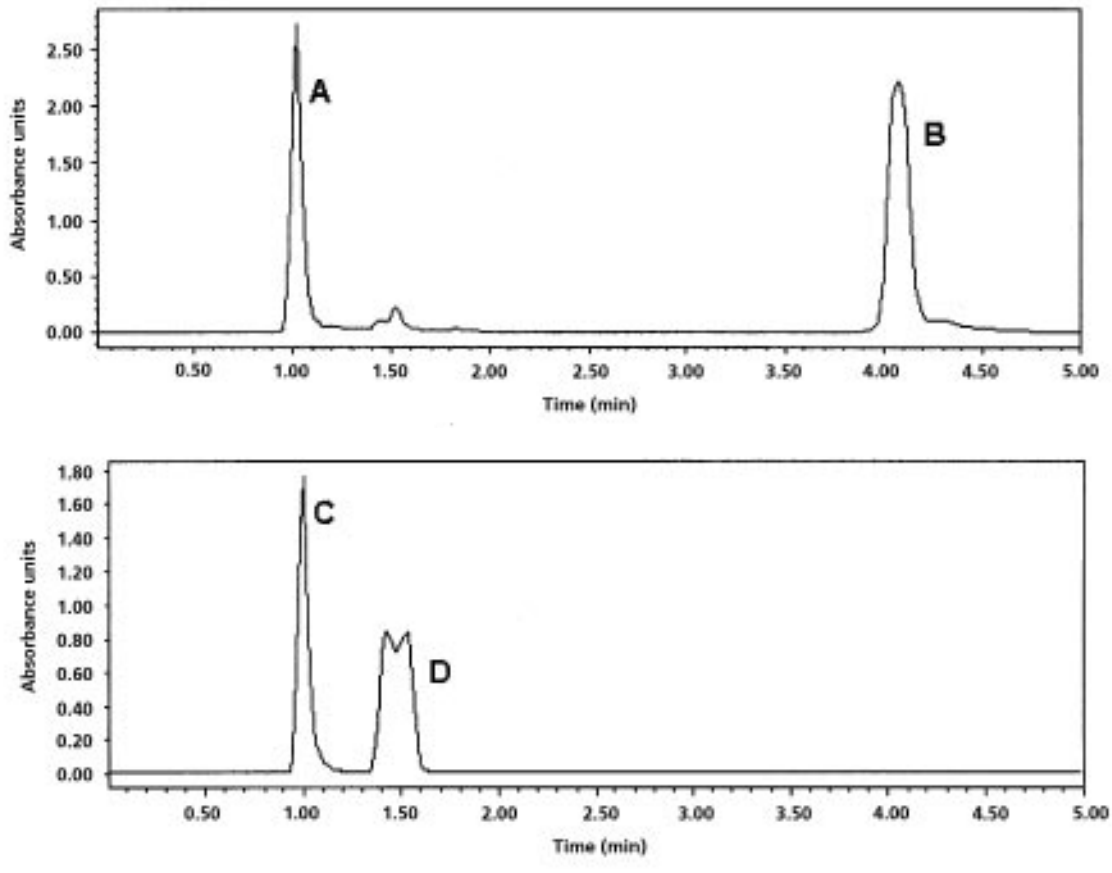

Figure 1. Upper panel: Chromatogram showing thiamine peak (A) at 1.05 min and Aquasol E peak (B) at 4.14 min. Lower panel: Chromatogram of the degradation study showing thiamine standard (C) and degradation product (D).

Table 1. Mean Thiamine Concentration ± Standard Deviation (and Mean Percentage Remaining*) during 91 Days of Storage at 4°C and 25°C

Study Day	4°C		25°C	
0	1.040 ± 0.082		1.036 ± 0.139	
7	1.036 ± 0.179	(99.6)	1.136 ± 0.117	(109.6)
14	1.120 ± 0.093	(101.7)	1.050 ± 0.161	(101.3)
21	1.124 ± 0.410	(108.0)	1.123 ± 0.197	(108.4)
28	1.100 ± 0.193	(105.7)	1.078 ± 0.071	(104.0)
35	1.096 ± 0.094	(105.4)	1.088 ± 0.076	(105.0)
42	1.019 ± 0.229	(98.0)	1.046 ± 0.020	(101.0)
49	1.164 ± 0.258	(111.9)	1.121 ± 0.093	(108.2)
56	1.001 ± 0.114	(96.2)	1.147 ± 0.071	(110.7)
63	1.005 ± 0.160	(96.6)	0.941 ± 0.115	(90.8)
70	0.979 ± 0.187	(94.1)	1.048 ± 0.179	(101.1)
77	0.945 ± 0.090	(90.8)	1.051 ± 0.239	(101.4)
84	1.173 ± 0.173	(112.8)	1.017 ± 0.181	(98.1)
91	0.997 ± 0.056	(95.9)	0.986 ± 0.056	(95.1)
% remaining on day 91 by linear regression†	102.54		106.51	
Lower limit of 95% CI for % remaining‡	95.05		100.71	

CI = confidence interval.
*Percent remaining was calculated in relation to the initial concentration (day 0). Original nominal concentration: 1.00 mg/mL.
†Calculated from concentration on day 91, as determined by linear regression and concentration observed on day 0, according to the following formula: concentration on day 91/concentration on day 0 x 100.
‡Calculated from lower limit of 95% CI of the slope of the curve relating concentration to time, determined by linear regression, according to the following formula: lower limit of 95% CI of concentration on day 91 / concentration on day 0 x 100.

Statistical Analysis

The means, standard deviations, and coefficients of variation were calculated for samples analyzed in triplicate and quadruplicate. For each study day, the percentage of the initial thiamine concentration remaining was calculated for each sample. The percentage of thiamine remaining on day 91 was calculated from the concentration on day 91 as determined by linear regression and concentration observed on day 0, according to the following formula: concentration on day 91/concentration on day 0 x 100%. The 95% confidence interval (CI) of the amount remaining on the last study day was calculated from the lower limit of the 95% CI of the slope of the curve relating concentration to time, determined by linear regression, according to the following formula: lower limit of the 95% CI of the concentration on day 91/concentration on day 0 x 100%. Stability was defined as maintenance of at least 90% of the initial thiamine concentration.

RESULTS

Regression analysis of the peak area ratio of thiamine to internal standard versus concentration demonstrated linearity over the working range of the concentrations, with coefficients of determination (r^2) greater than 0.997 (n = 4). The intraday (n = 4) and interday (n = 4) coefficients of variation for the 3 different concentrations were within acceptable limits: 2.84% and 4.82%, respectively, for the 0.25 mg/mL suspension; 0.91% and 1.47%, respectively, for the 1.00 mg/mL suspension; and 1.27% and 1.19%, respectively, for the 1.50 mg/mL suspension.

The thiamine sample was very resistant to degradation. However, a peak distinct from the thiamine peak appeared following the degradation process (Figure 1). Thus, the HPLC method was deemed capable of indicating stability.

No noticeable changes in physical appearance, odour, colour, or taste of the suspensions occurred over a period of 14 days. Each cloudy, white suspension

had a faint sweet smell and taste. By day 21, very slight changes in colour (more yellow), odour, and taste (less sweet and more "medicinal-tasting") were detected, but after that, these characteristics remained stable until the end of the study. The suspensions maintained constant viscosity and were easily resuspended throughout the study period. Furthermore, minimal fluctuations in pH were observed. The mean pH (± standard deviation) was 3.00 ± 0.16 when the suspension was stored at 4°C and 2.90 ± 0.20 when stored at 25°C.

The retention time for thiamine was 1.05 min, whereas the retention time for the internal standard, Aquasol E, was 4.14 min (Figure 1). The HPLC analysis showed that, at both storage temperatures, the suspensions maintained at least 90% of their initial concentrations on every study day (Table 1). Furthermore, about 100% of the initial thiamine concentration remained on day 91, according to linear regression analysis of the concentration–time data, and the lower limit of the 95% confidence interval indicated that more than 95% of the initial concentration remained on day 91 (Table 1).

DISCUSSION

Until the time of this study, thiamine suspensions had been prepared at the authors' institution in a methylcellulose vehicle. This product had an expiration date of only 8 days when kept refrigerated. To the authors' knowledge, there are no published stability studies for thiamine suspensions prepared in a 1:1 mixture of Ora-Sweet and Ora-Plus.

The stability of thiamine has been evaluated in other dosage forms, including oral solutions containing sucrose, dextrose, fructose, and dextrose–fructose[2]; parenteral nutrition solutions[1,4-8]; and extemporaneously prepared preloaded syringes.[3] Interestingly, although Ora-Sweet and Ora-Plus do not contain metabisulfites, studies of other formulations have found thiamine to be relatively unstable in sulfite-containing vehicles.[4,5,7] Reduction of thiamine by metabisulfites is reported to be the reason for the chemical instability.[4,5,7]

In the weekly analysis of samples, very slight changes in odour, taste, and colour occurred between days 14 to 21, but these characteristics remained stable for the rest of the 91-day period. Viscosity was constant. Precipitates were easily resuspended, and there was no caking or clumping of material. Although these measures are qualitative, observations were documented by the same individual throughout the 91 days, which eliminated interobserver bias. Variation in pH was not notable.

According to qualitative, pH, and HPLC analyses of weekly samples, thiamine suspensions of 100 mg/mL stored at either or 4°C or 25°C remained stable and maintained at least 90% of their original concentrations for up to 91 days. These results led to changes at the authors' hospital for extemporaneous compounding of thiamine suspensions, and the expiration date has been extended from 8 days to 3 months.

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