Stability of Nitrofurantoin in Extemporaneously Compounded Suspensions

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ABSTRACT

Background: To evaluate the stability of nitrofurantoin suspensions in a vehicle consisting of equal parts of Ora-Sweet and Ora-Plus vehicles after storage at 4°C and 25°C for up to 91 days.

Methods: Suspensions of nitrofurantoin 10 mg/mL in a 1:1 mixture of Ora-Sweet and Ora-Plus were prepared in 50-mL amber plastic prescription bottles. Three bottles of the suspension were stored at 4°C (refrigerated), and 3 were stored at 25°C (room temperature). Physical characteristics, including pH, odour, and taste, were evaluated, and visual testing of colour, viscosity, precipitation, and ease of resuspension was performed weekly up to 91 days. Samples were removed from each bottle weekly and stored at –85°C until analysis by a validated high-pressure liquid chromatography method. A suspension was considered stable if it maintained 90% or more of its initial concentration.

Results: No notable changes in pH or colour were observed in the suspensions after storage at 4°C or 25°C for 91 days. Very slight changes in odour and taste were observed between days 14 and 21, but these characteristics then remained constant until the end of the study. Viscosity was constant. Precipitates were easily resuspended, and there was no caking or clumping of material. Suspensions of nitrofurantoin maintained at least 90% of initial concentration at both temperatures throughout the 91-day period.

Conclusions: Nitrofurantoin suspensions of 10 mg/mL in a 1:1 mixture of Ora-Sweet and Ora-Plus were stable for a period of up to 91 days, with or without refrigeration. The expiry date for these products can thus be set at 91 days.

Key words: nitrofurantoin, suspension, stability

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RÉSUMÉ

Objectifs : Évaluer la stabilité de préparations de nitrofurantoïne 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 : Des suspensions de nitrofurantoïne de 10 mg/mL dans un mélange 1:1 d'Ora-Sweet et d'Ora-Plus ont été préparées dans des flacons pour médicaments d'ordonnance en plastique ambré de 50 mL. Trois flacons de préparations ont été entreposés à 4°C (réfrigérés), 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 toutes les semaines sur une période allant jusqu'à 91 jours. Des aliquotes ont été retirées de chaque flacon toutes les semaines, puis conservées à –85°C jusqu'à ce qu'elles soient soumises à une analyse validée par chromatographie liquide à haute pression. Une préparation en suspension était jugée stable lorsqu'elle avait conservé 90 % ou plus de sa concentration initiale.

Résultats : Aucun changement notable du pH ou de la couleur des préparations n'a été observé après entreposage à 4 °C ou à 25 °C pendant une période de 91 jours. Une très légère modification de l'odeur et du goût est survenue entre les jours 14 et 21, mais ces propriétés sont ensuite demeurées stables jusqu'à la fin de l'étude. 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 nitrofurantoïne en suspension ont conservé au moins 90 % de leur concentration initiale aux deux températures pendant toute la période de 91 jours.

Conclusions : Les préparations de nitrofurantoïne en suspension de 10 mg/mL dans un mélange 1:1 d'Ora-Sweet et d'Ora-Plus ont été jugées 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 : nitrofurantoïne, suspension, stabilité

INTRODUCTION

Nitrofurantoin, an antibacterial agent used in the treatment of urinary tract infections, is commonly prescribed for children and elderly adults who are unable to swallow tablets or capsules. An oral suspension is available in the United States, but only tablets and capsules are commercially available in Canada.[1-3] As such, Canadian pharmacists must prepare the suspensions extemporaneously.

At the authors' institution, nitrofurantoin suspension has traditionally been prepared in a methylcellulose vehicle. On the basis of historical procedures, this extemporaneous suspension has been given an expiration date of 8 days. The methylcellulose suspension presents some major problems including labour-intensive preparation, inability of community pharmacists to prepare (for both technical and logistic reasons), short expiration date, and inconvenience to outpatients.

Ora-Sweet sweetening agent and Ora-Plus suspending agent (Paddock Laboratories Inc, Minneapolis, Minnesota) are now commercially available and help to ease product preparation and improve palatability. Although the stability of nitrofurantoin suspensions in other vehicles has been reported,[4-7] to the authors' knowledge there is no published information on the stability of nitrofurantoin in the combination vehicle.

This study examined the physical characteristics and chemical stability (defined as maintenance of more than 90% of initial concentration) of extemporaneously prepared oral nitrofurantoin suspensions of 10 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 of Suspensions

Nitrofurantoin suspensions (10 mg/mL) were prepared by crushing commercially available nitrofurantoin 50-mg tablets (Apotex Inc, Weston, Ontario, lot GE3153) and resuspending the powder in a 1:1 mixture of Ora-Sweet and Ora-Plus (Paddock Laboratories Inc, lots 4140869 and 4040385, respectively). Six replicates were prepared in separate 50-mL amber plastic prescription bottles (Richards Packaging Inc, RIGO Products Division, Gloucester, Ontario; polyvinyl chloride [recycle code 3]). Three of the bottles were stored at 4°C (refrigerated) and 3 at 25°C (room temperature). All bottles were exposed only to fluorescent light in the laboratory.

The physical appearance of the suspensions was evaluated qualitatively at the time of preparation and at weekly intervals up to 91 days. All suspensions were tested for odour and taste and were visually examined for changes in colour (against white and black backgrounds), viscosity, formation of precipitates, and ease of resuspension as samples were collected during the 91-day study period. Immediately after the physical observations were completed, each bottle was manually shaken for 10 s, and 2 samples were drawn. One sample (1.5-mL) was stored at –85°C until day 91, when a single batch analysis was performed by a stability-indicating high-performance liquid chromatography (HPLC) method developed in the authors' laboratory. This method was similar to previously published methods.[8-11] The second sample (also 1.5-mL) was allowed to equilibrate to room temperature, and the pH was then determined. The pH meter (model 8000, VWR Canlab, Mississauga, Ontario) was calibrated at the beginning of each testing session.

Preparation of Stock and Standards

Stock solutions of nitrofurantoin at concentrations of 0.50, 1.00, 2.00, 3.00, and 4.00 mg/mL were prepared by diluting nitrofurantoin 4 mg/mL in HPLC-grade acetonitrile (Fisher Scientific, Richmond, British Columbia, lot 042332). The internal standard was furazolidone (Sigma-Aldrich Canada Ltd, Oakville, Ontario, lot 029H1117) at a concentration of 2.0 mg/mL in HPLC-grade acetonitrile. Standards were prepared by combining a 0.5-mL aliquot of each stock solution and a 0.5-mL aliquot of furazolidone 2.0 mg/mL. The final concentrations of nitrofurantoin 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 1 mg/mL. These dilutions achieved optimal chromatographic characteristics. Before injection into the chromatographic system, all standards were passed through a 0.45-µm microfilter (Acrodisc GHP syringe filter, Gelman, Ann Arbor, Michigan, lot 40530) 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 YMC ODSA 4.0 ± 20 mm guard column (Waters Ltd, lot 4044110501), a YMC Pack ODS-AP 6.0 ± 250 mm column (Waters Ltd, lot 062506286W), and an ultraviolet detector set at 250 nm (model 2487 dual-wavelength absorbance detector, Waters Ltd). The mobile phase consisted of a 28:72 (v/v) mixture of acetonitrile and 10 mmol/L KH_2PO_4 salt buffer (Sigma-Aldrich Canada Ltd, lot 101K0025) at pH 3.0. All solvents were HPLC-grade and were filtered before use. The flow rate was set at 1.75 mL/min.

A 5-point calibration curve was prepared, with a blank (acetonitrile 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 nitrofurantoin. The calibration curve was generated by least-squares regression of the peak area ratio of nitrofurantoin to furazolidone 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, and 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. The acceptable limit for the coefficients of variation was defined a priori as less than 10%.

Degradation of Nitrofurantoin

Nitrofurantoin 2.00 mg/mL was incubated overnight at 40°C, exposed to daylight at room temperature for 7 days, and then boiled for 1 min. The sample was then cooled to room temperature. A 0.5-mL aliquot was mixed with 0.5 mL furazolidone 2.00 mg/mL, the mixture was filtered, and a 10-µL sample was injected onto the column. The chromatogram obtained for the degraded preparation was compared with a chromatogram obtained from a standard (1.00 mg/mL) to determine any changes in concentration, retention time, and peak shape.

Preparation of Samples

Nitrofurantoin (10 mg/mL) study samples were thawed, and a 0.2-mL aliquot was diluted with 0.8 mL HPLC-grade water and then centrifuged for 1 min at 10 000 rpm. A 0.5-mL aliquot of nitrofurantoin was added to a 0.5-mL aliquot of internal standard in HPLC-grade acetonitrile. The final theoretical nitrofurantoin 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.

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 initial nitrofurantoin concentration remaining was calculated for each sample. The percentage of nitrofurantoin remaining on day 91 was calculated from the concentration on day 91 as determined by linear regression and the 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 nitrofurantoin concentration.

RESULTS

Regression analysis of the peak area ratio of nitrofurantoin to internal standard versus concentration demonstrated linearity over the working range of the concentrations, with coefficients of determination (r^2) greater than 0.999 (n = 4). The intraday (n = 4) and interday (n = 4) coefficients of variation for the 3 different concentrations were within acceptable limits: 0.06% and 0.30%, respectively, for the 0.25 mg/mL suspension; 0.06% and 0.13%, respectively, for the 1.00 mg/mL suspension; and 0.20% and 0.09%, respectively, for the 2.00 mg/mL suspension.

When nitrofurantoin was subjected to degradation, there was no noticeable change in its peak shape. An apparent degradation product eluted at 4.3 min and was well separated from the nitrofurantoin such that the decline in the nitrofurantoin concentration could be easily detected (Figure 1). Thus, the HPLC method was deemed capable of indicating stability.

No notable changes in physical appearance, odour, colour, or taste of the suspensions occurred over the first 14 days of the study period. Each cloudy yellow suspension had a faint sweet smell and taste. Afterward, a very slight change in taste (more bitter) and smell (less sweet) occurred, and these characteristics remained stable until the end of the study. The suspensions appeared to maintain constant viscosity (based on visual inspection only, not on measurements with a viscometer) and were easily resuspended throughout the study period. Furthermore, only minimal fluctuations of pH were observed. The mean pH (± standard deviation) was 4.45 ±0.10 when the suspension was stored at 4°C and 4.41 ± 0.12 when stored at 25°C.

The retention time for nitrofurantoin was 5.3 min, whereas the retention time for the internal standard, furazolidone, was 6.2 min (Figure 1). The HPLC analysis showed that at both storage temperatures, the 10 mg/mL suspensions maintained at least 90% of their initial concentrations on every study day (Table 1). Furthermore, more than 98% of the initial nitrofurantoin concentration remained on day 91, according to linear regression analysis of the concentration–time data. The lower limit of the 95% CI also indicated that more than 92% of the initial concentration remained on day 91 (Table 1).

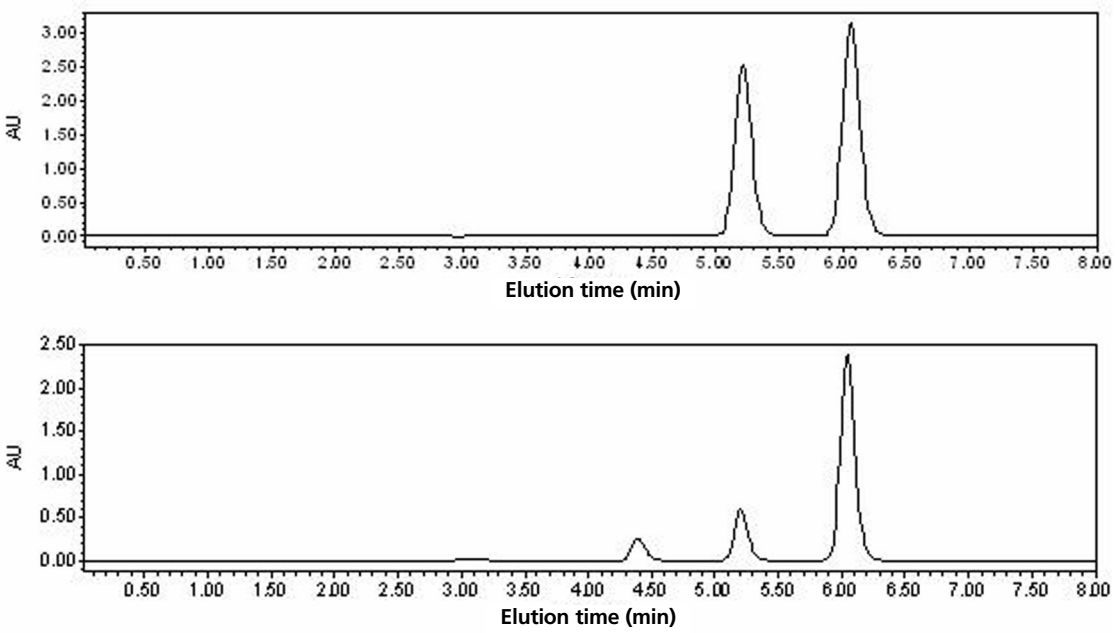

Figure 1. Upper panel: Chromatogram showing nitrofurantoin peak at 5.3 min and peak for the internal standard (IS), furazolidone, at 6.2 min. Lower panel: Chromatogram of the degradation study showing peak for presumed degradation product at 4.3 min and nitrofurantoin (26.5% remaining) and IS peaks.

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

Study Day‡	4°C		25°C	
Day 0	0.861 ± 0.034		0.859 ± 0.045	
Day 7	0.804 ± 0.115	(93.4)	0.788 ± 0.052	(91.7)
Day 14	0.862 ± 0.118	(100.1)	0.861 ± 0.056	(100.2)
Day 21	0.847 ± 0.178	(98.4)	0.904 ± 0.043	(105.2)
Day 28	0.956 ± 0.041	(111.0)	0.815 ± 0.212	(94.9)
Day 35	0.787 ± 0.057	(91.4)	0.868 ± 0.063	(101.0)
Day 42	0.939 ± 0.047	(109.1)	0.888 ± 0.071	(103.4)
Day 49	0.855 ± 0.023	(99.3)	0.792 ± 0.009	(92.2)
Day 56	0.856 ± 0.052	(99.4)	0.847 ± 0.126	(98.6)
Day 63	0.859 ± 0.074	(99.8)	0.785 ± 0.074	(91.4)
Day 70	0.877 ± 0.043	(101.9)	0.794 ± 0.129	(92.4)
Day 77	0.891 ± 0.023	(103.5)	0.810 ± 0.045	(94.3)
Day 91	0.842 ± 0.106	(97.8)	0.894 ± 0.119	(104.1)
% remaining on day 91 by linear regression§	99.43		98.58	
Lower limit of 95% CI for % remaining‖	93.13		92.63	

CI = confidence interval.
*Percentage remaining was calculated in relation to the initial concentration (day 0).
†Nominal initial concentration: l.00 mg/mL.
‡No determinations were made on day 84 (12 weeks).
§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.

DISCUSSION

The lack of a commercially available suspension of nitrofurantoin in Canada poses a problem for children and adults who are unable to swallow tablets and capsules. Until the time of this study, nitrofurantoin suspensions used at the authors' institution had been extemporaneously prepared 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 on nitrofurantoin suspensions prepared in a 1:1 mixture of Ora-Sweet and Ora-Plus.

In the weekly analysis of samples, no notable change in colour was observed in the suspensions after storage at 4°C or 25°C for up to 91 days. Very slight changes in odour and taste were observed between days 14 and 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, thus eliminating interpersonnel bias. Variation in pH was not notable.

A limitation of this study design relates to the freezing of the samples at –85°C until the time of batch analysis. It was assumed that nitrofurantoin would not degrade at this low temperature and that no volume losses would occur because of freeze-drying during storage. In addition, it was assumed that errors due to serial analysis would have been greater than errors occurring with batch analysis.

According to qualitative, pH, and HPLC analyses of weekly samples, nitrofurantoin suspensions of 10 mg/mL stored at either 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 nitrofurantoin suspensions. The expiration date has been extended from 8 days to 3 months.

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