ARTICLE

Stability of Sulfasalazine Oral Suspension

Karen Lingertat-Walsh, Scott E Walker, Shirley Law, Marissa Abesamis, and Pacita Sales

ABSTRACT

Background: Because no liquid formulation of sulfasalazine for oral administration is commercially available in Canada, an extemporaneous oral liquid formulation is required for administration to children and other patients who cannot swallow intact tablets.

Objective: To evaluate the stability of a 100 mg/mL suspension of sulfasalazine prepared in a mixture of 50% Ora-Sweet syrup and 50% Ora-Plus suspending vehicle and stored in 3 types of container (amber glass, amber polyethylene terephthalate [PET], and amber polyvinylchloride [PVC]) over 91 days at 4°C and 23°C.

Methods: A reverse-phase stability-indicating liquid chromatographic method was validated before the study. A sulfasalazine suspension (100 mg/mL) was prepared in a mixture of 50% Ora-Sweet and 50% Ora-Plus. Fifty-millilitre aliquots of the suspension were stored in 100-mL bottles (either amber glass, amber PET, or amber PVC). Half of the bottles of each type of container were stored at 23°C ± 2°C and the other half were stored at 4°C. On study days 0, 2, 7, 14, 21, 28, 42, and 91, the sulfasalazine concentration was determined in samples drawn from 3 bottles stored at each temperature in each type of container.

Results: The concentration of sulfasalazine in all study samples remained within 7% of the initial concentration. The concentration was significantly higher in suspensions stored in PET containers (p = 0.017). No differences in concentration related to storage temperature were observed.

Conclusion: Suspensions of sulfasalazine (100 mg/mL) in a mixture of 50% Ora-Sweet and 50% Ora-Plus retained more than 94% of the initial sulfasalazine concentration for 91 days when stored in amber glass, amber PET, or amber PVC containers at 23°C or 4°C.

Key words: sulfasalazine, drug stability, suspension

Can J Hosp Pharm 2006;59:194-200

RÉSUMÉ

Historique : Puisque la sulfasalazine sous forme liquide pour administration par voie orale n'est pas offerte dans le commerce au Canada, une telle préparation doit être effectuée extemporanément pour l'administration aux enfants et aux autres patients qui sont incapables d'avaler des comprimés intacts.

Objectif : Évaluer la stabilité d'une suspension de 100 mg/mL de sulfasalazine dans un excipient composé de parties égales d'Ora-Sweet (un édulcolorant) et d'Ora-Plus (un agent de suspension), conservées dans trois types de flacons (de verre ambré, de polyéthylène téréphtalate [PET] ambré ou de polychlorure de vinyle [PVC] ambré) pendant une période de 91 jours à une température de 4 °C et de 23 °C.

Méthodes : Une épreuve de stabilité par chromatographie liquide en phase inverse a été validée avant l'étude. On a préparé une suspension de sulfasalazine (100 mg/mL) dans un mélange 1:1 d'Ora-Sweet et d'Ora-Plus. Des aliquotes de 50 mL de la suspension ont été entreposées dans des flacons de 100 mL (de verre ambré, de PET ambré ou de PVC ambré). La moitié de chaque type de flacons a été entreposée à une température de 23°C ± 2°C et l'autre moitié, à une température de 4°C. Aux jours 0, 2, 7, 14, 21, 28, 42 et 91 de l'étude, la concentration en sulfasalazine a été déterminée à partir d'échantillons tirés de trois flacons de chaque type entreposés à chacune des températures.

Résultats : La concentration en sulfasalazine dans chacun des échantillons évalués est demeurée à près de 7 % de la concentration initiale. La concentration était significativement supérieure dans les suspensions conservées dans les flacons de PET (p = 0,017). Aucune différence de concentration liée à la température d'entreposage n'a été observée.

Conclusion : Les suspensions de sulfasalazine (100 mg/mL) préparées dans un mélange 1:1 d'Ora-Sweet et d'Ora-Plus ont conservé plus de 94 % de leur concentration initiale de sulfasalazine pendant 91 jours lorsqu'elles étaient conservées dans des flacons de verre ambré, de PET ambré ou de PVC ambré, à des températures de 23 °C ou de 4 °C.

Mots clés : sulfasalazine, stabilité des médicaments, suspension

INTRODUCTION

Sulfasalazine is used to treat ulcerative colitis. It is available in Canada as enteric-coated and film-coated 500-mg tablets.[1] Since no oral liquid is commercially available, an extemporaneous oral liquid formulation is required for administration to children and other patients who cannot swallow intact tablets. Ideally, the formulation should be acceptable to patients, easily compounded, stable for at least 60 days, and of sufficient concentration (e.g., 100 mg/mL) to avoid administration of large volumes.

The stability of sulfasalazine in liquid formulation has not previously been described. A description of a single oral liquid sulfasalazine formulation (250 mg/5 mL) has been published,[2] but the concentration of that formulation was only half of the ideal of 100 mg/mL, and the expiry date assigned was not supported by a validated stability study.

Numerous publications describe analytical methods for measuring sulfasalazine and its metabolites in biological samples[3-15] or sulfasalazine and impurities in tablets and bulk powders.[16,17] Although the last 2 cited articles reported stability-indicating methods, they used chromatographic columns that are no longer available.[16,17] Therefore, before initiating the sulfasalazine stability study, a stability-indicating analytical method for sulfasalazine had to be developed and validated.[18-20]

The objective of the stability study was to evaluate the stability of a 100 mg/mL sulfasalazine suspension prepared in a mixture of 50% Ora-Sweet syrup and 50% Ora-Plus suspending vehicle and stored in 3 different types of containers: glass, polyethylene terephthalate (PET-G) and polyvinyl chloride (PVC) at 23°C and 4°C.

METHODS

Assay Validation

Following the development of the chromatographic system for sulfasalazine, the suitability of this method for use as a stability-indicating assay was tested by analyzing samples of sulfasalazine that had been subjected to degradation under 4 different conditions. Sulfasalazine 12.5 mg (Sigma-Aldrich Co, Oakville, Ontario; lot 12K1248) was dissolved in 25 mL of a mixture of distilled water and methanol to make a 0.5 mg/mL stock solution. A 1.25-mL aliquot of this stock solution was diluted to 5 mL with distilled water to prepare a 0.125 mg/mL sample, which was placed in a glass multidose vial (5 mL total volume). To a second diluted 5-mL sample (at 0.125 mg/mL), 1 drop of 10 mmol/L sodium hydroxide was added to prepare a solution with pH 12.5. To a third diluted 5-mL sample (at 0.125 mg/mL), 1 drop of concentrated hydrochloric acid was added to prepare a solution with pH 2.5. The acidified solution exhibited gross precipitation and was not investigated further.

The other 2 multidose vials were placed in a water bath and incubated at 83°C. Samples were drawn before incubation and at 8 other times over the following 168 hours and were chromatographed directly. The remaining 0.5 mg/mL stock solution was divided into 1-mL aliquots, each of which was placed in a 5-mL glass test tube. To each sample of the 0.5 mg/mL solution, 0.1 mL of a sodium hypochlorite solution (1.00%, 0.75%, 0.50%, 0.25%, or 0.06% concentration) was added. Each mixture was combined with a vortex mixer, and chromatography was performed immediately. Chromatograms from all samples were inspected for the appearance of additional peaks, and the sulfasalazine peak was compared between samples for changes in concentration, retention time, and shape (by means of electronic overlay and numeric calculation of tailing). The ultraviolet spectral purity (200–365 nm, 6-nm bandwidth, deuterium lamp, model UV3000, Thermo Separation Products, Fremont, California) of the sulfasalazine peak in chromatograms of the degraded samples produced by sodium hypochlorite and base was compared with the spectrum of the authentic undegraded sample of sulfasalazine in water obtained at time 0.

Following this first phase of evaluation and validation, the accuracy and reproducibility of the standard curves were tested over 5 days, and system suitability criteria (theoretical plates, tailing and retention time) were developed to ensure consistent chromatographic performance on each study day.[21]

Stability Study

On study day 0, fifteen 100-mL batches of a 100 mg/mL sulfasalazine suspension were prepared in 50% Ora-Sweet (Paddock Laboratories, Minneapolis, Minnesota; distributed by Wiler PCCA, London, Ontario; lot 3137954) and 50% Ora Plus (Paddock Laboratories, distributed by Wiler PCCA; lot 321396) using film-coated sulfasalazine tablets (PharmaScience, Montréal, Quebec; lot 300344, expiry December 2004) according to the method described in Appendix 1. Each batch was prepared using a regular mortar and pestle and a 100-mL graduated cylinder. Then, 50-mL portions of the suspension were placed in either 100-mL amber glass bottles (Beatson Clark, Rotherham South Yorkshire, England; distributed by Richards Packaging, Toronto,

Ontario), 100-mL amber PET bottles (Eastman Chemical Company, Kingsport, Tennessee; distributed by Jones Packaging, Toronto, Ontario), or 100-mL amber PVC bottles (distributed by Richards Packaging, Toronto, Ontario); in total, there were 10 bottles of each type. Each bottle was half-filled, which allowed room air to be present above the suspension. These conditions simulate preparation in any pharmacy and mimic the environment likely to be encountered during use and storage of the suspension.

Half of the bottles were stored at 4°C and the other half at room temperature (23°C ± 2°C). All bottles were exposed to ambient room light. Given that the ideal expiration period for this product was at least 60 days, the stability was monitored over a 91-day period.

pH and Physical Stability

On study days 0, 42, and 91, samples were drawn for measurement of pH and physical inspection from 2 bottles of each temperature–container combination (12 bottles in total). Before sampling, the bottles were shaken and the suspensions inspected visually for caking and consistency, as well as for changes in colour, odour, and taste. The pH was determined using a pH meter (Accumet, Fisher Scientific, Nepean, Ontario) that was calibrated before use on each day with commercially purchased standard buffers of pH 4 and 7 (Fisher Scientific).

Sulfasalazine Analysis

On each study day (days 0, 2, 7, 14, 21, 28, 42, and 91), standard curves were prepared by dissolving 12.5 mg sulfasalazine (Sigma-Aldrich Co, Oakville, Ontario; lot 12K1248, certificate of analysis purity 98%) in 25 mL of methanol to make a 0.5 mg/mL (500 mg/L) stock solution. Samples of this stock solution were further diluted with distilled water to obtain standards with final concentrations of 375, 250, 187.5, 125, 93.75 and 62.5 mg/L. These standards, along with a blank, were used to construct a standard curve. One microlitre of each sample was chromatographed in duplicate. Also, 2 quality control samples of sulfasalazine (concentrations of 375 and 93.75 mg/L) were chromatographed in duplicate each day; their concentrations were determined and compared with the known concentrations. Intra-day and inter-day errors were assessed by the coefficients of variation of the peak areas of both the quality control samples and the standards.

On each study day, samples drawn from 3 containers of each container type stored at both temperatures were assayed for sulfasalazine content. Before sampling, each suspension was shaken by hand. All suspensions initially had a nominal sulfasalazine concentration of 100 mg/mL. A dilute sample with initial nominal concentration of 200 mg/L (0.2 mg/mL) was prepared from each suspension by dissolving 0.2 mL of the suspension (measured by micropipette) in 100 mL of methanol. One microlitre of each diluted suspension was injected directly onto the liquid chromatography system without further preparation, in duplicate, to ensure the ability to distinguish concentrations that differed by 10%.[22,23] The concentration of sulfasalazine in each of the replicates was determined by interpolation from a standard curve of 6 standards. These concentrations were then multiplied by the dilution factor (500) to determine the sulfasalazine concentration of the sample in milligrams per millilitre. Based on considerations of the inter-day slope and assay variability for standards observed during assay validation, the quantitative resolution of the method was determined (0.0030 mg/mL), and sulfasalazine concentrations were recorded to the nearest 0.01 mg/mL.

Chromatographic Analysis

The liquid chromatographic system consisted of a solvent delivery pump (model P4000, Thermo Separation Products, Fremont, California), which pumped a mixture of 70% acetonitrile (EM Science, distributed by BDH, Toronto, Ontario; catalogue no. AX0142-1) and 30% 0.05 mmol/L potassium phosphate monobasic at pH 4.1 (Fisher Scientific, Toronto, Ontario; catalogue no. P286). On each day the strength of the mobile phase was prepared to achieve a retention time for sulfasalazine of about 5 minutes through a 15 cm x 4.6 mm reverse-phase C_{18}, 3-µm column (Supelcosil ABZ plus, catalogue no. 59194, Supelco, Oakville, Ontario) at 1.0 mL/min. One microlitre of each prepared sample, quality control sample, and standard was injected directly onto the liquid chromatographic column in duplicate using an autoinjector (Ultra WISP 715, Waters Limited, Toronto, Ontario). This method is similar to previously published methods, which also used reverse-phase columns and acetonitrile–water mobile phases.[16,17] However, the method described here used a shorter column with a smaller particle size and a mobile phase with considerably more acetonitrile at a pH of 4.1 to obtain a similar retention time for sulfasalazine.

The column effluent was monitored with a variable-wavelength ultraviolet detector (UV 6000, Thermo Separation Products, Fremont, California) at 356 nm. Sulfasalazine has relative maxima at 200 nm, 246 nm,

and 356 nm. Some degradation products absorb strongly between 200 nm and 250 nm but have less absorbance above 300 nm. By shifting to 356 nm, interference is further reduced or eliminated. The signal from the detector was integrated and recorded with a chromatography data system (PC1000, Thermo Separation Products, Fremont, California). The area under the sulfasalazine peak at 356 nm was subjected to least-squares linear regression, and the actual sulfasalazine concentration in each sample was determined by interpolation from the standard curve and correction by the dilution factor as previously described.

Data Reduction and Statistical Analysis

After the coefficient of variation of the assay had been determined, a power calculation indicated that duplicate injection had the ability to distinguish between concentrations that differed by at least 10% within each individual container type.[22,23] However, with the study design used (3 samples stored in each of 3 container types at each of 2 different temperatures, and all samples analyzed in duplicate), the method had the power to detect a 1% change in concentration. Means calculated for replicated analyses are reported in a summary table (Table 1). Mean results from different days for each combination of container type and temperature were compared statistically to determine if there was an association between the observed result and time. Linear and multiple linear regression were used to determine if there was an association between the observed concentration and study day or temperature. A 95% confidence interval (CI) of the percent remaining on the last study day was calculated for each container and temperature combination based on the observed concentration and study day. Analysis of variance was used to test differences in concentration on different study days, at different temperatures, and in different containers. The 5% level was used as the a priori cut-off for significance. Sulfasalazine concentrations were considered acceptable or within acceptable limits if the lower limit of the 95% confidence limit of concentration remaining was greater than 90% of the initial (day 0) concentration.

RESULTS

Accelerated Degradation and Assay Validation

Degradation of a 0.125 mg/mL solution of sulfasalazine by heating at 83°C occurred very slowly,

Table 1. Observed Concentration (as Mean Percent ± Standard Deviation of Initial Concentration) of Sulfasalazine after Storage in Various Types of Container at Different Temperatures*

	Amber PVC†		Amber PET-G‡		Amber Glass	
Study Day	**23°C**	**4°C**	**23°C**	**4°C**	**23°C**	**4°C**
Initial concentration (mg/mL)	100.29±1.84	103.04±6.85§	96.93±1.43	96.64±1.71	100.34±3.95	101.50±3.82
Day 2	100.70±0.67	100.16±3.01	104.20±2.04	101.08±1.80	99.47±3.23	100.05±1.52
Day 7	101.42±2.03	100.29±1.98	100.72±0.98	100.89±2.60	100.33±0.99	100.74±3.24
Day 14	105.06±1.20	100.50±1.97	105.08±2.50	104.04±3.16	101.77±0.85	100.36±3.29
Day 21	103.89±1.32	104.69±2.84	103.77±3.91	102.05±3.60	101.78±1.76	104.37±4.94
Day 28	103.11±0.82	100.09±2.99	103.84±2.81	106.05±2.03	103.01±2.39	104.96±1.83
Day 42	105.08±3.93	103.11±1.45	105.01±4.54	106.62±2.38	104.93±1.86	105.60±2.61
Day 91	103.87±2.03	99.98±1.44	104.62±1.93	104.95±3.79	102.19±1.54	101.82±3.09
Coefficient of variation (%)‖	1.90	1.77	1.89	2.46	1.75	2.31
% remaining on day 91 estimated by regression¶	103.18	100.19	102.90	104.96	103.01	102.62
Lower limit of 95% CI for % remaining on day 91**	98.27	94.77	97.83	99.23	98.55	96.00

*Percent remaining was calculated on the basis of the concentration, determined in duplicate, of each of the 3 replicate vials of each type of container stored at each temperature relative to the concentration on study day 0.
†Amber polyvinylchloride (PVC) bottles with a number 3 recycling symbol.
‡Amber polyethylene terephthalate (PET) bottles with a number 1 recycling symbol.
§One container had an initial concentration of 110.94 mg/mL. The initial sulfasalazine concentration in all other containers was within 5% of the nominal value.
‖The variability of the estimated percent remaining over the 91-day study period is expressed as the coefficient of variation (standard deviation/mean) and is presented as a percentage.
¶Percent remaining on day 91, based on linear regression. Concentrations at time 0 (initial concentration) and 91 days were determined by linear regression. Calculation: (concentration on day 91) x 100 /(initial concentration).
**The 95% confidence interval (CI) based on percentage remaining was calculated using the lower limit of the 95% CI of the slope as calculated by linear regression and an initial concentration of 100%: {100 x [initial concentration + (slope x 91 days)]/(initial concentration)}.

and the loss was less than 4% after 7 days' storage. At pH 12.5, 33.5% remained after 7 days of incubation at 83°C. At least 6 degradation products were observed in the chromatograms (Figure 1, panels A and B). Degradation of sulfasalazine with sodium hypochlorite occurred much faster. At 23°C, a 0.5 mg/mL solution of sulfasalazine in water was degraded to 18.5% remaining within 5 minutes of adding 0.1 mL of a 1% sodium hypochlorite solution. Solutions containing lower concentrations of sodium hypochlorite degraded sulfasalazine more slowly. When 0.1 mL of a sodium hypochlorite 0.5% solution was added, 68.5% of the initial sulfasalazine concentration remained when the sample was chromatographed immediately. These solutions had different degradation products of sulfasalazine, which eluted between 2.5 and 3.5 minutes and between 7 and 10 minutes (Figure 1, panel C). None of these degradation products interfered with sulfasalazine quantification, and the ultraviolet spectrum of the sulfasalazine peak (200–365 nm) in a degraded sample was no different than the spectrum of the authentic undegraded standard. As a result of the chromatographic separation of these degradation products from sulfasalazine and the similarity of the ultraviolet spectrum between an authentic standard and sulfasalazine in a degraded sample (200–365 nm), it was concluded that this analytical method was suitable for indicating stability.[18-20]

Analysis of standard curves and quality control samples during the validation procedures indicated that the sulfasalazine concentration was measured accurately. Standards and quality control samples showed less than 5% deviation from the expected concentration over the validation period. Inter-day variation in the slope (as measured by coefficient of variation) averaged 5.2%. Intra-day analytical reproducibility (as measured by coefficient of variation) averaged less than 2.2% for each of the standards and quality control samples. This indicates that differences of 10% or more could be confidently detected within individual containers with acceptable error rates.[21,22] During the study, the intra-day analytical reproducibility (as measured by coefficient of variation) averaged 2.3% for samples and 1.4% for standards. Absolute deviation averaged less than 4% for all standards and quality control samples.

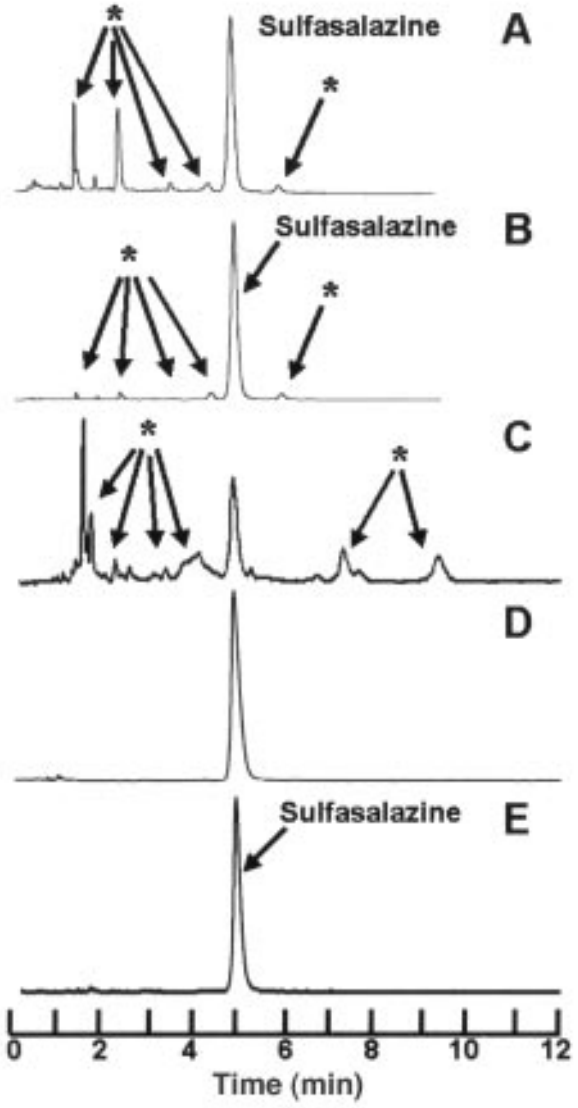

Figure 1. Representative chromatograms of sulfasalazine observed during the study. Chromatograms A and B represent a 0.125 mg/mL sulfasalazine solution at pH 12.5 following incubation at 83°C for 168 h and monitored at 226 nm and 356 nm, respectively. About a third (33.5%) of the initial sulfasalazine concentration remained and at least 6 degradation products (asterisks) were observed in this degraded sulfasalazine solution. Panel C shows a chromatogram immediately after the addition of 100 µL of 1% sodium hypochlorite; about 18.5% of the sulfasalazine remains. Degradation products eluted between 2.5 and 3.5 min and between 7 and 10 min, but none was positively identified. Chromatogram D represents a suspension drawn from the polyethylene terephthalate (PET-G) container on study day 0. Chromatogram E represents a suspension drawn from the PET-G container after storage at 23°C for 91 days. No degradation products were observed.

Stability Study

The concentrations of sulfasalazine observed in the 100 mg/mL sulfasalazine suspensions over the 91-day study period are presented in Table 1. During the study period, the concentration of sulfasalazine in all study

samples stored at both temperatures remained within 7% of the initial concentration. The inter-day variation in sulfasalazine concentration (as assessed by coefficient of variation) was less than 2.5% for each concentration–temperature–container combination.

On the basis of the 95% CI of the fastest degradation rate (percent remaining per day), all samples at both temperatures retained at least 94.77% of the initial concentration. Multiple linear regression detected a significant linear trend for a change in concentration during the 91-day study period (p = 0.004), but the change averaged only 2.85%. Significant differences in concentrations were observed between study days (p = 0.0005) and between containers, with the concentration of samples stored in glass and PVC being approximately 1% lower than that of samples stored in PET; p = 0.017). No significant differences in average concentration related to temperature were observed (difference 0.5%; p = 0.22). Inspection of chromatograms during the stability study revealed none of the degradation products that were observed during accelerated degradation with either sodium hypochlorite or alkali (Figure 1).

pH and Physical Stability

All suspensions were initially thick and opaque with a creamy brownish yellow or light orange colour and brownish yellow particles and remained so for the duration of the study. No caking of sulfasalazine was observed in any of the containers. Redispersion occurred quickly after the tubes were shaken by hand. The suspension smelled like Ora-Plus or Ora-Sweet, with a sweet taste and no bitterness. The pH was initially about 4.4 and did not change significantly during the study period at either storage temperature.

DISCUSSION

Extemporaneous oral sulfasalazine 100 mg/mL suspensions remained stable for 91 days when stored in 3 types of containers (amber glass, amber PET, and amber PVC) at 2 different temperatures (room temperature and 4°C).

During the study period, the concentration of sulfasalazine observed in all study samples at both temperatures remained within 7% of the initial concentration. The concentration on day 91 estimated by linear regression was within 5% of the initial concentration, and the lower limit of the 95% CI of the percent remaining on day 91 was within 6% of the initial concentration. Demonstration of a trend or a consistent decline in concentration during the study was considered more important than demonstrating a statistically significant difference in concentration between any 2 days. In fact, the fluctuations in concentration around the initial concentration are not of practical importance and should be considered "noise" or experimental error. Although sulfasalazine concentrations were higher for samples stored in amber PET containers, these statistically significant differences are not of practical importance since the concentration of all samples in all containers remained within acceptable limits for the entire 91-day study period.

Because only small changes in sulfasalazine concentration could be detected under these storage conditions, assurance of the specificity of the analytical method is very important. In particular, the separation and detection of intact drug in the presence of degradation compounds must be assured before the method can be considered stability-indicating.[18-20] The specificity of the analytical method was demonstrated during the accelerated degradation studies (Figure 1). In these studies, the concentration of sulfasalazine declined as the concentration of apparent degradation products increased.

No samples of the suspensions were cultured for microbiologic organisms during this study. However, both Ora-Sweet and Ora-Plus contain preservatives (methylparaben and potassium sorbate), which should be sufficient to inhibit bacterial growth. Nevertheless, expiry dates applied to this product might be constrained by individual departmental or institutional policies on expiry dates for products manufactured in pharmacies.

In conclusion, sulfasalazine suspensions of 100 mg/mL stored in amber glass, amber PET, or amber PVC plastic containers at 4°C or 23°C for 91 days were stable and retained more than 90% of the initial sulfasalazine concentration.

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Acknowledgements
This study was jointly funded by the Departments of Pharmacy at The Hospital for Sick Children, Toronto, Ontario, and Sunnybrook Health Sciences Centre, Toronto, Ontario.

Appendix 1. Method of Preparing Sulfasalazine 100 mg/mL in Ora-Plus and Ora-Sweet

To make 100 mL:

1. Count out 20 film-coated sulfasalazine 500-mg tablets and place in mortar.
2. Measure and mix together 50 mL of Ora-Plus and 50 mL of Ora-Sweet vehicles.
3. Pour some of the vehicle on top of the tablets and let soak until tablets are softened (20-30 min).
4. Levigate the tablets until a smooth paste is formed. Add more vehicle to the mixture until a liquid is formed.
5. Transfer the contents to the graduated cylinder. Use additional vehicle to rinse the remaining drug from the mortar and add to the graduated cylinder.
6. Add additional vehicle to make up to the final volume of 100 mL. Stir well.
7. Transfer suspension to a glass, polyvinylchloride (PVC), or PET-G bottle.
8. Label and indicate the expiry date. Put a "shake well" label on the bottle.
9. This suspension can be stored either at 23°C or 4°C.