# Physicochemical and Microbiological Stability of Pyrimethamine in the Paraben-Free PCCA Base, SuspendIt™

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## OBJECTIVE

Pyrimethamine is commercially available as 25-mg tablets that contain corn, potato starch, lactose, and magnesium stearate as the excipients. No commercial liquid dosage form of pyrimethamine currently exists. An extemporaneously compounded suspension from pure drug powder or commercial tablets/capsules would provide an alternative option to meet unique patient needs. The purpose of this study was to determine the physicochemical and microbiological stability of extemporaneously compounded pyrimethamine suspensions in the PCCA base SuspendIt™. This base is a sugar-free, paraben-free, dye-free, and gluten-free thixotropic vehicle containing a natural sweetener obtained from the monk fruit. It thickens upon standing to minimize settling of any insoluble drug particles and becomes fluid upon shaking to allow convenient pouring during administration to the patient. Given the lack of options for patients sensitive to common excipients, there is a need for documented stability information of pyrimethamine compounded in a paraben-free, sugar-free and corn-free vehicle to create a hypoallergenic option for such patients.

A robust stability-indicating HPLC assay for the determination of the chemical stability of pyrimethamine in SuspendIt™ was developed and validated. A suspension of pyrimethamine was prepared in PCCA SuspendIt™ at a 2-mg/mL concentration, selected to provide flexibility in customizing individual doses. Samples were stored in plastic amber prescription bottles at two temperature conditions (5°C and 25°C) and assayed over an extended period of time. Physical data such as pH, viscosity, and appearance were also noted. Samples were also tested for microbiological stability. The goal was to provide a viable, compounded alternative for pyrimethamine in a thixotropic, paraben-free liquid dosage form, with an extended BUD to meet patient needs.

## METHODS

*Development of a stability-indicating HPLC assay method for Pyrimethamine*

The HPLC analytical method developed was demonstrated to be stability indicating by subjecting pyrimethamine samples to accelerated degradation. A forced degradation study was performed to determine if any degradants interfered with the analytical peak for pyrimethamine. These forced degradations included caustic, acidic, peroxide, and ultraviolet light degradation. For the caustic degradation, 0.5 mL of 5N NaOH was added to a 10-mL volumetric flask containing either 40 µL of 1-mg/mL stock solution of pyrimethamine in mobile phase, or approximately 20 mg of a test formulation containing 2-mg/mL pyrimethamine in PCCA SuspendIt™. The sample was heated to 60°C for 1 hour. For the acidic degradation, the stock solution or formulation was mixed with 0.5 mL of a 1N HCl solution and stored at room temperature for 1 hour. The peroxide degradation was accomplished in an analogous manner by mixing the sample or stock solution with 483.3 µL of deionized water and 16.7 µL of a 30% hydrogen peroxide solution, resulting in a 1% peroxide solution. Forced degradation by UV light was achieved by placing either 1-mg/mL stock solution, or a small amount of the 2-mg/mL formulation in 10-mL volumetric flasks for an hour in a Millipore UV sterilizer (Catalog No. XX6370000, Billerica, Massachusetts).

*Preparation of Pyrimethamine Suspension in SuspendIt™*

A suspension containing 2-mg/mL of pyrimethamine in PCCA SuspendIt™ was prepared by first weighing out 2.0 grams of pyrimethamine and placing the powder in a mortar. The powder was triturated to reduce the particle size, and levigated to a smooth paste using a small amount of PCCA SuspendIt™. Additional PCCA SuspendIt™ was added to the mortar and the contents transferred into a 1000-mL volumetric flask using a rubber spatula. The volumetric flask was filled to the mark with additional PCCA SuspendIt™, vortexed on a vortex mixer, and sonicated in an ultrasonic bath for 5 minutes to remove any air bubbles. The volumetric flask was again filled to the mark with PCCA SuspendIt™ and placed on a magnetic stirrer.

Twelve 4-oz. amber plastic prescription bottles were filled with 80 mL of the prepared suspension, retaining the remainder for initial (zero day) analysis. The bottles were sealed and wrapped in parafilm. Three of the bottles were stored at room temperature (25°C) in a desiccator, and three bottles were stored under refrigerated conditions (5°C). The temperature at each storage location was monitored throughout the study. The specific gravity was determined to be 1.0. Samples from each temperature and concentration were analyzed and characterized initially on day zero, and subsequently after 7, 14, 28, and 42 days of storage. The remaining six bottles were tested for microbiological stability.

*Analysis and Characterization of Pyrimethamine Suspension*

Samples of pyrimethamine in SuspendIt™ were analyzed on a Waters chromatographic system (Waters Corporation, Milford, Massachusetts) using a 717 autosampler, a 600-quaternary pump, and a 996-photodiode array detector set at 274 nm for the detection of the pyrimethamine. An isocratic mobile phase containing 85% v/v of a 50-mM potassium phosphate solution adjusted to pH 3.0 using phosphoric acid and 15% v/v acetonitrile was used at a flow rate of 1.0 mL/min. The injection volume was 10.0 microliters. An Xbridge C18 2.1 X 100-mm 5-µm particle size column was used for the separation.

A series of standards ranging from 1-µg/mL to 8-µg/mL were prepared in mobile phase from a 0.5-mg/mL stock solution of pyrimethamine in mobile phase prepared fresh for each sampling period. The chromatograms were acquired for the standards and samples. A least squares analysis was performed on the calibration curve using the peak areas from the pyrimethamine peaks at 274 nm to determine the pyrimethamine concentrations. Using the initial specific gravity measurements of the formulations, the weight/weight measurements were converted to weight/volume units. The suspensions were also analyzed for pH, appearance, and intrinsic viscosity. The pH of each sample was measured on a VWR Scientific pH meter using an Ag/AgCl combination electrode, calibrated prior to analysis. The viscosity was determined using a Brookfield DV-III Ultra programmable cone/plate rheometer fitted with a cpe-40 spindle.

## RESULTS

The HPLC method utilized in the study clearly separated any peaks associated with the SuspendIt™ from the analytical peak for the pyrimethamine (Figure 1). The method also displayed good linearity over the observed concentration range (Figure 2). Forced degradation studies revealed that peaks associated with the degradants had much shorter retention times than the pyrimethamine peak and showed no interference with its analytical peak (Figure 3).

Pyrimethamine formed a translucent suspension in PCCA SuspendIt. The pH of the samples displayed only a slight increase for the refrigerated and room temperature samples (Table 1). The initial suspension viscosity was 40.5 ± 1.3 cPs (Table 2). The viscosity decreased slightly over the test period to 39.0 ± 2.6 cPs and 35.3 ± 0.8 cPs for the refrigerated and room temperature samples, respectively. Nevertheless, the viscosity was sufficient to avoid caking and obtain a uniform drug concentration while sampling.

Using a ±10% criterion as a means of determining drug degradation, no significant degradation of pyrimethamine was found over the 42-day test period under refrigerated and room temperature conditions (Tables 3, 4; Figure 4). Drug concentrations were above 95% of initial values.

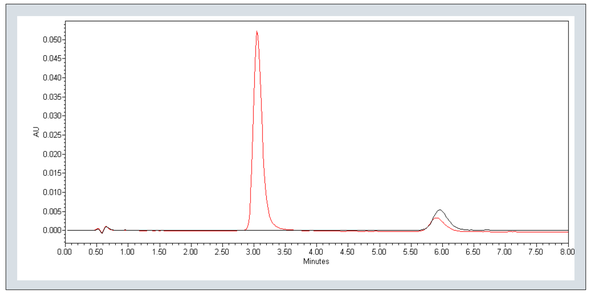


Figure 1. Sample chromatographic run of pyrimethamine standard (black) and pyrimethamine in PCCA SuspendIt™ (red) using an analysis wavelength of 274 nm.

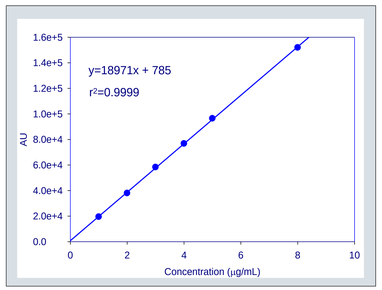


Figure 2. Calibration curve for high-performance liquid chromatographic analysis of pyrimethamine (Range:1-µg/mL to 8-µg/mL).

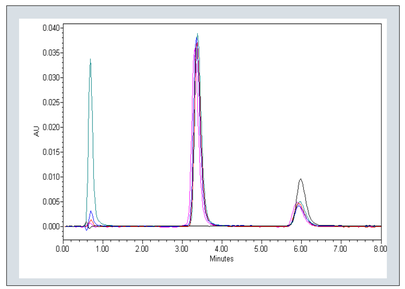


Figure 3. Chromatographic runs of pyrimethamine in PCCA SuspendIt; standard (black), with caustic degradation (blue), acidic degradation (red), oxidative degradation (green), and ultraviolet light degradation (violet).

Table 1. Measurements of pH of Pyrimethamine (2 mg/mL) in PCCA SuspendIt™

| Time | 5°C | 25°C |
|---|---|---|
| Day 0 | 5.51 ± 0.03 | 5.51 ± 0.03 |
| Day 7 | 5.49 ± 0.01 | 5.48 ± 0.01 |
| Day 14 | 5.51 ± 0.02 | 5.52 ± 0.04 |
| Day 28 | 5.52 ± 0.03 | 5.54 ± 0.02 |
| Day 42 | 5.64 ± 0.02 | 5.57 ± 0.03 |

Table 2. Viscosity (cP) measurements of Pyrimethamine (2 mg/mL) in PCCA SuspendIt™

| Time | 5°C | 25°C |
|---|---|---|
| Day 0 | 40.5 ± 1.3 | 40.5 ± 1.3 |
| Day 7 | 41.4 ± 1.0 | 38.3 ± 0.6 |
| Day 14 | 38.6 ± 2.1 | 38.3 ± 1.1 |
| Day 28 | 33.3 ± 1.6 | 30.8 ± 3.7 |
| Day 42 | 39.0 ± 2.6 | 35.3 ± 0.8 |

Table 3. Pyrimethamine Concentration (mg/mL) in PCCA SuspendIt™

| Time | 5°C | 25°C |
|---|---|---|
| Day 0 | 2.007 ± 0.024 | 2.007 ± 0.024 |
| Day 7 | 2.016 ± 0.004 | 2.028 ± 0.009 |
| Day 14 | 2.022 ± 0.036 | 1.987 ± 0.039 |
| Day 28 | 1.996 ± 0.027 | 2.012 ± 0.035 |
| Day 42 | 1.912 ± 0.281 | 1.942 ± 0.036 |

Table 4. Percent of Pyrimethamine (2 mg/mL) in PCCA SuspendIt™ relative to Day-Zero concentration

| Time | 5°C | 25°C |
|---|---|---|
| Day 0 | 100.0 ± 1.7 | 100.0 ± 1.7 |
| Day 7 | 100.4 ± 1.2 | 101.0 ± 1.3 |
| Day 14 | 100.7 ± 2.2 | 99.0 ± 2.3 |
| Day 28 | 99.5 ± 1.8 | 100.2 ± 2.1 |
| Day 42 | 95.3 ± 4.6 | 97.1 ± 2.1 |

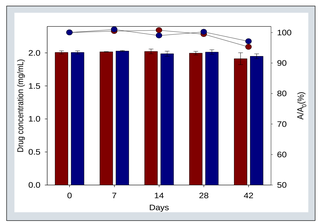


Figure 4. Change in drug concentration over 42 days on left axis for the 2-mg/mL samples of pyrimethamine in PCCA SuspendIt™ stored at 5°C (red) and 25°C (blue); and relative change in percent on right axis as compared to initial concentration [A/Ao = drug content at time t (A) over initial drug content (Ao) x100] .

## CONCLUSIONS

A robust stability-indicating HPLC assay method for the determination of pyrimethamine in PCCA SuspendIt™ was developed and validated. This assay was used to determine the chemical stability of the 2-mg/mL samples of pyrimethamine in PCCA SuspendIt™ at 5˚C and 25˚C. Drug concentrations did not go below 95% of the label claim (initial drug concentration) at both temperature conditions studied. The pH values did not change significantly. The viscosity of the suspensions was sufficient to allow easy re-dispersal of the drug particles upon shaking. Content uniformity was maintained, and no caking was observed. The preservative system in PCCA SuspendIt™ successfully protected the suspension from growth of challenge microorganisms per the *USP* Chapter <51> AME Test. This study demonstrates that pyrimethamine is physically, chemically, and microbiologically stable in PCCA SuspendIt™ for 42 days at both refrigerated and room temperatures, thus providing a viable, compounded alternative for pyrimethamine in a paraben-free liquid dosage form with an extended BUD to meet patient needs.

## ACKNOWLEDGMENTS

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