Stability of Trimethoprim in Admixtures of Trimethoprim–Sulfamethoxazole Prepared in Polyvinylchloride Bags and Glass Bottles

James M. Curtis and David J. Edwards

ABSTRACT

Objective: To compare the stability of various concentrations of trimethoprim in admixtures of trimethoprim–sulfamethoxazole prepared in polyvinylchloride (PVC) bags and glass bottles.

Methods: Concentrated trimethoprim–sulfamethoxazole was added to 250-mL evacuated glass containers and 100-mL PVC bags containing 5% dextrose in water (D5W) or normal saline (NS) to produce a final trimethoprim concentration of 1.08 or 1.60 mg/mL. Samples were stored at room temperature under ambient light. Chemical stability was assessed at 12 and 24 h by a stability-indicating high-performance liquid chromatography assay. Physical stability was based on visual inspection of the samples at 12 and 24 h. Selected samples were filtered or examined microscopically to confirm the results of visual inspection. The pH of all samples was measured at 0 and 24 h.

Results: The concentration of trimethoprim at 24 h averaged more than 95% of the starting concentration irrespective of container, concentration, or IV solution. No visible precipitate was observed in any admixture prepared in PVC bags. Admixtures prepared with D5W in glass bottles at a concentration of 1.08 mg/mL were clear at 12 h, but a precipitate was present in 5 of the 20 samples at 24 h. Several samples prepared with NS in glass at 1.60 mg/mL precipitated within 12 h, and 14 of the 20 had precipitated by 24 h.

Conclusions: Admixtures containing trimethoprim exhibit chemical and physical stability over a 24-h period when prepared in PVC bags. Physical stability in PVC bags is superior to that in glass bottles, particularly at higher concentrations; use of PVC bags would allow the shelf life of admixtures to be extended to 24 h. D5W is the preferred IV solution for preparation of such admixtures.

Key words: trimethoprim, sulfamethoxazole, stability, polyvinylchloride, glass

Can J Hosp Pharm 2002;55:207-11

RÉSUMÉ

Objectif : Comparer la stabilité de diverses concentrations de triméthoprime dans des mélanges triméthoprime–sulfaméthoxazole préparés dans des sacs en polychlorure de vinyle (PVC) et des bouteilles de verre.

Méthodes : L'association triméthoprime–sulfaméthoxazole a été ajoutée à des flacons sous vide de 250 mL et des sacs en PVC de 100 mL contenant du dextrose à 5 % dans l'eau (D5W) ou une solution physiologique (NS), pour obtenir une concentration finale de triméthoprime de 1,08 ou 1,60 mg/mL. Les échantillons ont ensuite été entreposés à la température et à la lumière ambiantes. La stabilité chimique a été évaluée à 12 et à 24 heures à l'aide d'une épreuve de stabilité par chromatographie liquide à haute pression. La stabilité physique a été évaluée par l'inspection visuelle des échantillons à 12 et à 24 heures. Des échantillons choisis ont été filtrés et examinés au microscope pour confirmer les résultats de l'inspection visuelle. Le pH de tous les échantillons a été mesuré à 0 et à 24 heures.

Résultats : La moyenne des concentrations en triméthoprime à 24 heures était de 95 % de la concentration initiale, indépendamment du contenant, de la concentration ou de la solution IV. Aucun précipité visible n'a été observé dans les mélanges préparés dans des sacs en PVC. Les mélanges préparés dans des bouteilles de verre avec du D5W, à des concentrations de 1,08 mg/mL, étaient clairs à 12 heures, mais 5 des 20 échantillons présentaient un précipité à 24 heures. Plusieurs échantillons préparés dans des bouteilles de verre avec du NS, à des concentrations de 1,60 mg/mL, ont formé un précipité après 12 heures et 14 des 20 échantillons ont formé un précipité après 24 heures.

Conclusions : Les mélanges contenant de la triméthoprime sont chimiquement et physiquement stables sur une période de 24 heures, lorsqu'ils sont préparés dans des sacs en PVC. La stabilité physique des mélanges est supérieure dans les sacs en PVC que dans les bouteilles de verre, particulièrement à de fortes concentrations ; le recours à des sacs en PVC permettrait de prolonger la durée de conservation des mélanges jusqu'à 24 heures. Le D5W constitue la solution IV de choix pour les préparations de tels mélanges.

Mots clés : triméthoprime, sulfaméthoxazole, stabilité, polychlorure de vinyle, verre

INTRODUCTION

Trimethoprim–sulfamethoxazole is a clinically useful drug combination for the prophylaxis and treatment of serious infections such as *Pneumocystis carinii* pneumonia, an opportunistic infection that occurs in patients with acquired immunodeficiency syndrome. Stability guidelines for admixtures containing trimethoprim–sulfamethoxazole range from 2 to 6 h depending upon the concentration and the manufacturer.[1] This short shelf life creates logistic, administrative, and delivery problems for hospital pharmacy and nursing departments and can result in waste of the admixture and unnecessarily high costs.

The primary factor limiting the shelf life of admixtures of trimethoprim–sulfamethoxazole is the concentration-dependent solubility of trimethoprim, which precipitates over time. Sulfamethoxazole has a much higher solubility than trimethoprim in the pH range of common IV solutions, and previous studies have documented that no significant loss of this drug occurs over a 24- to 48-h storage period.[2-4] Trimethoprim is a weak base with reduced solubility at higher pH. Because the pH of 5% dextrose in water (D5W) is lower than that of 0.9% sodium chloride (NS), D5W is preferred for preparation of trimethoprim–sulfamethoxazole admixtures.

The literature regarding the stability of trimethoprim–sulfamethoxazole in various solutions and containers for IV administration is conflicting.[2-7] These studies examined storage in syringes, buretrols, and glass bottles, but there are no published papers examining stability in polyvinylchloride (PVC), even though PVC bags are widely used for the IV administration of trimethoprim–sulfamethoxazole. The purpose of this study was to compare the stability of trimethoprim in PVC bags and glass bottles at concentrations that would be suitable for patients with fluid restrictions. Stability was assessed in both D5W and NS.

METHODS

Trimethoprim Analysis and Assay Validation

Samples were assayed by means of a stability-indicating high performance liquid chromatography (HPLC) method based on that described by Kaufman and colleagues[5] and DeAngelis and colleagues.[6] A 5-µm C18 reverse-phase analytical column (Partisil 5 ODS-3, 4.6 mm x 25 cm, Whatman Inc., Clifton, New Jersey) was used, with a mobile phase consisting of 13% acetonitrile, 1% acetic acid, and 0.1% triethylamine (all HPLC grade) pumped at a flow rate of 1.7 mL/min. The eluent was monitored with a variable-wavelength ultraviolet detector set at 254 nm (model 490E, Waters Associates, Milford, Massachusetts). The analysis was conducted at room temperature (21°C to 25°C). The retention times were approximately 5 min for trimethoprim and approximately 9 min for sulfamethoxazole. Linearity of the assay was demonstrated for trimethoprim concentrations ranging from 0.15 to 2.40 mg/mL ($r > 0.9999$). Standard curves were prepared daily, and the assay was demonstrated to be both accurate and reproducible. At a trimethoprim concentration of 1.60 mg/mL, the measured concentration was within 2% of the expected value; between-day and within-day variability averaged 3.1% and 2.0%, respectively.

The stability-indicating nature of the assay was demonstrated according to the methods described by Kaufman and colleagues.[5] Briefly, samples were stressed by addition of hydrochloric acid and hydrogen peroxide with heating at temperatures up to 100°C. The peak for the stressed trimethoprim sample was smaller than that of the unstressed sample, and additional peaks, which could be differentiated from trimethoprim on the chromatogram, were apparent. In addition, admixtures of trimethoprim–sulfamethoxazole were allowed to degrade for 5 months at room temperature, and the resulting samples were compared with a fresh reference solution. A distinct degradation product not present in the reference solution and clearly separated from the trimethoprim peak was observed at 4.32 min (Figure 1).

Formulation and Sample Preparation

Sulfamethoxazole and trimethoprim for injection concentrate (Elkins-Sinn, Inc., Cherry Hill, New Jersey) was used. This product contained 16 mg trimethoprim and 80 mg sulfamethoxazole per milliliter, along with propylene glycol 400 mg, alcohol 10% w/v, diethanolamine 3 mg, benzyl alcohol 10 mg, and sodium metabisulfite 1 mg in water for injection at a final pH of 9.5 to 10.5. An appropriate volume of concentrate was added to 250-mL evacuated glass containers and 100-mL PVC bags (Baxter Healthcare Corp., Deerfield, Illinois) containing D5W or NS to produce a final trimethoprim concentration of 1.08 or 1.60 mg/mL. The samples were stored at room temperature (21°C to 25°C) under ambient light.

Assessment of Chemical Stability

The chemical stability of trimethoprim was determined by assaying 80 samples of the admixture, 10 for each container type (PVC and glass), IV solution (NS and D5W), and concentration (1.08 and 1.60 mg/mL trimethoprim). Aliquots (0.1 mL each) were removed at 0, 12, and 24 h. Each aliquot was diluted with 0.9 mL distilled water, and a 100-µL sample was injected into the chromatograph. Standards were also diluted 1:10

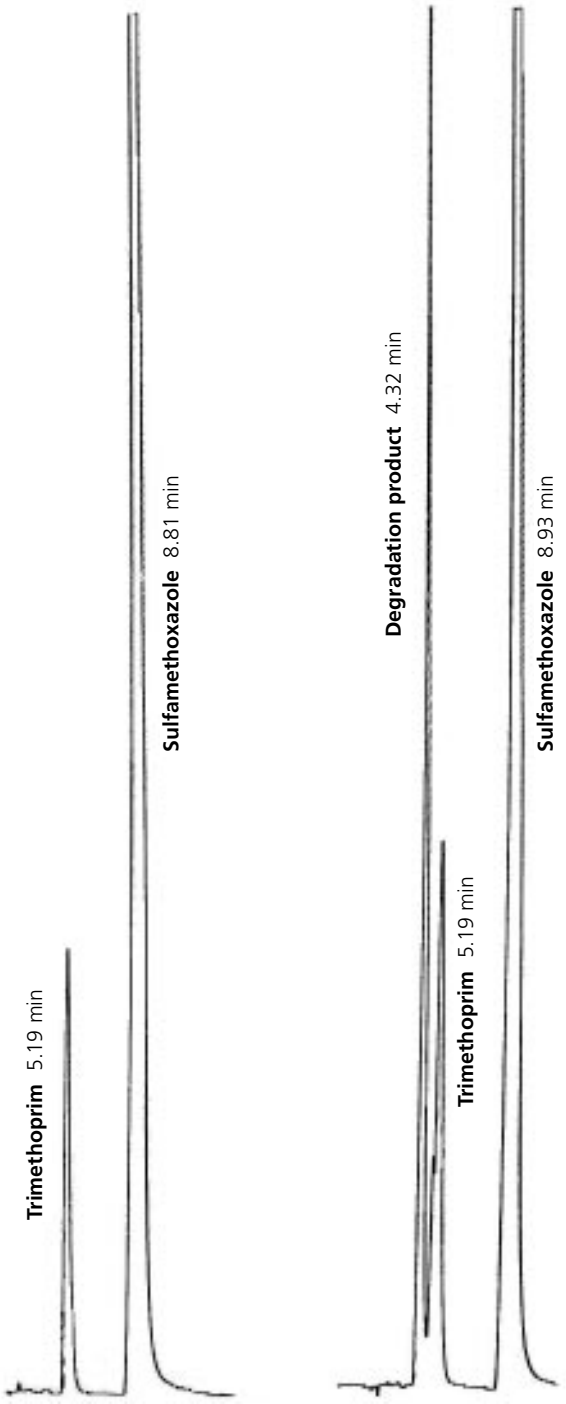

Figure 1. Left: Chromatogram of a freshly prepared admixture of trimethoprim (retention time 5.19 min) and sulfamethoxazole (8.81 min). Right: After storage for 5 months at room temperature, a degradation product with a retention time of 4.32 min became apparent.

with water before injection, and each sample was assayed in duplicate. Chemical stability was defined as a decrease in trimethoprim concentration of less than 10% over the 24-h study period. Mean percentage trimethoprim remaining and 95% confidence limits were calculated.

Assessment of Physical Stability

Twenty samples of the admixture for each container type, IV solution, and nominal trimethoprim concentration were visually inspected at 0, 12, and 24 h for evidence of particulate matter, haze, or colour change. The pH was measured at 0 and 24 h. To confirm the results of the visual inspection, selected samples of the admixture were examined microscopically or filtered with a 0.45-µm filter (Pall Gelman Sciences, Ann Arbor, Michigan). The filters were examined for the presence of solid material and, when present, the precipitate was dissolved in mobile phase and injected into the chromatograph to confirm its identity.

RESULTS

All samples exhibited chemical stability for trimethoprim (Table 1). The mean trimethoprim concentration at 24 h was more than 95% of the initial value, irrespective of container type, IV solution, or concentration. The lower limit of the 95% confidence interval was greater than 90% in all cases. At 24 h, the 95% confidence interval included the value of 100% (indicating no degradation) for all samples. Of the 80 samples assayed, only 3 exhibited more than a 10% loss of trimethoprim over 24 hours. These 3 samples were each stored in glass bottles, and in each case the decrease in trimethoprim concentration was less than 15%. In all but one of the samples containing a visible precipitate, trimethoprim concentration remained above 90% of the initial value.

No precipitate was observed in any sample prepared in a PVC bag at either concentration (Table 2). For samples stored for 12 h in glass bottles there was no precipitate in any sample prepared with D5W or in samples prepared with NS at a nominal concentration of 1.08 mg/mL. However, 4 of 20 samples prepared with NS at 1.60 mg/mL and stored in glass showed a precipitate by 12 hours. By 24 hours, a precipitate was observed in numerous of the samples prepared and stored in glass (Table 2). Precipitation was most frequent in NS at the higher concentration of trimethoprim (1.60 mg/mL). The retention time of material recovered from the filter paper, redissolved in mobile phase, and injected into the chromatograph matched that of trimethoprim. No crystals were detected under the microscope or on the filter paper in any admixture that was visually free of precipitate.

Table 1. Chemical Stability of Trimethoprim in Admixtures with Sulfamethoxazole Stored at Room Temperature over 24 h

Solvent and Storage Container	Mean Concentration and SD (mg/mL)			Mean % Trimethoprim Remaining and 95% CI			
	Nominal	Actual Initial		At 12 h		At 24 h	
D5W							
PVC	1.08	1.00	(0.05)	99.2	(97.3–101.01)	98.7	(95.8–101.6)
	1.60	1.39	(0.06)	101.2	(98.0–104.4)	101.0	(97.6–04.4)
Glass	1.08	1.05	(0.03)	99.7	(97.1–102.3)	98.5	(94.6–102.4)
	1.60	1.62	(0.04)	97.8	(94.3–101.3)	100.7	(98.6–102.8)
NS							
PVC	1.08	1.03	(0.03)	99.2	(97.7–100.7)	100.0	(98.4–101.6)
	1.60	1.36	(0.05)	99.4	(96.1–102.7)	98.3	(94.6–102.0)
Glass	1.08	1.07	(0.03)	97.0	(95.4–98.6)	96.0	(91.9–100.1)
	1.60	1.58	(0.04)	99.2	(96.5–101.9)	99.9	(97.4–102.4)

SD = standard deviation, CI = confidence interval, D5W = 5% dextrose in water, PVC = polyvinylchloride, NS = normal saline.

Table 2. Presence of Precipitate* in Admixtures of Trimethoprim–Sulfamethoxazole at 24 h

Nominal Concentration of Trimethoprim (mg/mL)	Glass		PVC Bags	
	NS	D5W	NS	D5W
1.08	2/20	4/20	0/20	0/20
1.60	14/20	5/20	0/20	0/20

PVC = polyvinylchloride, NS = normal saline, D5W = 5% dextrose in water.
*Visible to unaided eye. Data presented as number of samples showing precipitate/total number of samples.

The actual concentration of trimethoprim in PVC bags was consistently 5% to 10% below that in glass (Table 1). To ensure that lower concentrations were not a confounding factor in the superior performance of the bags, 10 admixtures were prepared at a trimethoprim concentration of 2.00 mg/mL in D5W and stored in PVC bags. A precipitate was observed in only 1 of the 10 samples at 24 h.

No changes in pH occurred over the 24-h observation period. In addition, there were no differences in the pH of admixtures prepared and stored in glass and PVC. As expected, the pH of the NS samples was higher than that of the D5W samples (9.81 and 8.94, respectively). The pH of samples containing 1.60 mg/mL trimethoprim was approximately 0.1 to 0.2 higher than that of samples containing 1.08 mg/mL trimethoprim.

DISCUSSION

Several studies have examined the stability of trimethoprim–sulfamethoxazole over the past 20 years, with conflicting results. Lesko and colleagues[2] found that the trimethoprim component was stable for only 2 h in D5W at a concentration of 1.6 mg/mL in buretrols made of cellulose proprionate. The relevance of these data to current practice is limited, because buretrols are not commonly used for administration of trimethoprim–sulfamethoxazole. Two studies have examined the stability of this admixture in glass containers. Deans and colleagues[3] reported that the solution was stable for up to 24 h in both D5W and NS at concentrations ranging from 0.64 to 1.6 mg/mL trimethoprim. In contrast, Jarosinski and colleagues[4] found that the stability in glass was markedly dependent on concentration. For trimethoprim 0.8 mg/mL in D5W, the admixture was stable for 24 h. However, the period of stability was reduced to 4 h at 1.07 mg/mL and to 1 h at 1.6 mg/mL. In addition, both Lesko and colleagues[2] and Jarosinski and colleagues[4] concluded that solubility was higher in D5W than NS. Admixtures prepared in D5W have a lower pH than those prepared in NS (8.94 and 9.81, respectively, in this study), which would favour the aqueous solubility of a weak base such as trimethoprim. Although the results of these studies differ significantly, current recommendations favour the more conservative stability times suggested by Jarosinski and colleagues.[4] However, neither of these studies[2,4] examined stability in PVC minibags, the most common container in current clinical use for admixtures of trimethoprim–sulfamethoxazole.

The results of the current study suggest that the chemical stability of trimethoprim is maintained for 24 h in both glass and PVC (Table 1). The HPLC assay separated trimethoprim from sulfamethoxazole in the admixture (no other peaks were observed) and was capable of indicating stability, as evidenced by the appearance of new peaks that could be readily distinguished from trimethoprim in a sample acutely stressed or degraded for 5 months at room temperature

(Figure 1). This result is not particularly surprising, since it is physical stability rather than chemical stability that is the primary factor affecting the shelf life of this product. It is interesting that several samples with a precipitate visible to the naked eye maintained a trimethoprim concentration greater than 90% of the initial value. Admixtures at a concentration of 1.60 mg/mL in 250-mL glass bottles contain 400 mg trimethoprim. Precipitation of 5% of the trimethoprim, for example, would represent 20 mg of compound, a quantity that can easily be detected by visual inspection of the IV container.

Although chemical stability was acceptable in both types of container, there were significant differences in physical stability between glass and PVC containers. No precipitate was observed in any admixture prepared in PVC minibags at either nominal concentration. To confirm that the visual inspection was accurate, selected samples were subjected to microscopic examination. No evidence of crystal formation could be detected under the microscope. In addition, admixtures that appeared to be free of precipitate were filtered, and the filter paper was examined for evidence of particulate matter. No precipitated material was recovered from the filter paper in any such case. When samples with precipitate visible to the naked eye were filtered, the precipitate was recovered and identified by HPLC as trimethoprim.

The physical stability of trimethoprim in glass containers was much less than that in PVC minibags, with stability times longer than reported by Jarosinski and colleagues[4] but shorter than those suggested by Deans and colleagues.[3] Significant evidence of precipitation was present at 24 h, particularly in NS and at the higher concentration of trimethoprim (Table 2). However, no physical instability was observed in the D5W samples at 12 h, which suggests that even in glass, admixtures prepared in D5W can be safely stored for up to 12 h.

The concentrations of trimethoprim in samples prepared in PVC bags were 5% to 10% lower than those observed in glass bottles. Whether this difference was related to the overfill volume of PVC bags, adsorption of drug to the bag, or some other aspect of the preparation of the admixtures requires further investigation and confirmation. However, the slightly lower concentration of trimethoprim in minibags at a nominal concentration of 1.60 mg/mL could in theory contribute to the less frequent occurrence of precipitation in these samples than in glass. To rule out this possibility, 10 admixtures were prepared in PVC bags at a trimethoprim concentration above that normally used in clinical practice (2.00 mg/mL). The frequency of precipitation (1/10) was much lower than observed with glass bottles at either 1.08 or 1.60 mg/mL (Table 2).

The results of this study indicate that trimethoprim in admixtures with sulfamethoxazole is physically stable for a longer period in PVC minibags than in glass bottles. Admixtures prepared in PVC minibags at concentrations up to 1.60 mg/mL can be stored for up to 24 h at room temperature. Although D5W is the recommended IV solution, NS can be used for patients in whom use of D5W is not desirable; stability will not be significantly compromised if such admixtures are prepared in PVC containers. If glass bottles are used, trimethoprim–sulfamethoxazole should be prepared in D5W and stored for no longer than 12 h.

References

1. Sulfamethoxazole and trimethoprim, concentrate for injection package insert. Cherry Hill (NJ): Elkin-Sinn Inc; 1990.
2. Lesko LJ, Marion A, Ericson J, Siber GR. Stability of trimethoprim–sulfamethoxazole injection in two infusion fluids. *Am J Hosp Pharm* 1981;38:1004-6.
3. Deans KW, Lang JR, Smith DE. Stability of trimethoprim–sulfamethoxazole injection in five infusion fluids. *Am J Hosp Pharm* 1982;39:1681-4.
4. Jarosinski PF, Kennedy PE, Gallelli JF. Stability of concentrated trimethoprim–sulfamethoxazole admixtures. *Am J Hosp Pharm* 1989;46:732-7.
5. Kaufman MB, Scavone JM, Foley JJ. Stability of undiluted trimethoprim–sulfamethoxazole for injection in plastic syringes. *Am J Hosp Pharm* 1992;49:2782-3.
6. DeAngelis DV, Woolley JL, Sigel CW. High-performance liquid chromatographic assay for the simultaneous measurement of trimethoprim and sulfamethoxazole in plasma and urine. *Ther Drug Monit* 1990;12:382-92.
7. McDonald C, Faridah J. Solubilities of trimethoprim and sulfamethoxazole at various pH values and crystallization of trimethoprim from infusion fluids. *J Parenter Sci Technol* 1991;45:147-51.

James M. Curtis, PharmD, is Clinical Pharmacy Specialist, Pharmacy Department, Borgess Medical Center, Kalamazoo, Michigan.

David J. Edwards, PharmD, is Professor, Department of Pharmacy Practice, College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, Michigan.

Address correspondence to:
Dr David J. Edwards
Department of Pharmacy Practice
Eugene Applebaum College of Pharmacy and Health Sciences
Wayne State University
Detroit MI
48202

e-mail: dje@wizard.pharm.wayne.edu

Acknowledgements
This study was supported by an Undergraduate Research Award from Wayne State University. Portions of this work were presented at the American Society of Health-System Pharmacists (ASHP) Mid-Year Clinical Meeting, December 1998, Las Vegas, Nevada, where Dr Curtis received the Student Research Award of the ASHP Research and Education Foundation.