ARTICLE

Stability of Aseptically Prepared Tazocin Solutions in Polyvinyl Chloride Bags

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ABSTRACT

Background: Tazocin, a mixture of piperacillin and tazobactam, has recently been reformulated to include edetate disodium (EDTA) and citric acid. Since the introduction of this new formulation, there have been no studies of stability in polyvinylchloride (PVC) bags.

Objective: To complete a physical compatibility and chemical stability study of the new formulation of Tazocin, prepared at 2 concentrations in each of 2 diluents and stored in PVC bags.

Methods: Tazocin, at 22.5 or 90 mg/mL, was compounded in dextrose 5% in water (D5W) or 0.9% sodium chloride (normal saline [NS]) in PVC bags. The bags were stored at 5°C with protection from light for 14, 21, or 28 days, followed in each case by storage at 23°C with exposure to light for 72 h. Triplicate samples collected at each of the 7 time points were analyzed in duplicate using a stability-indicating high-performance liquid chromatography method. Physical compatibility was determined by monitoring the solutions for changes in colour, clarity, and pH.

Results: The amount of each drug remaining for each concentration in each diluent was above 95% of the initial concentration after storage at 5°C with protection from light and above 94% of the initial concentration after an additional 72 h at 23°C with exposure to light. The pH of the solutions changed only slightly over the course of the study, and all solutions remained clear and colourless.

Conclusions: Tazocin solutions at 22.5 and 90 mg/mL, prepared in PVC bags of either D5W or NS, were chemically stable after storage for up to 28 days at 5°C with protection from light followed by 72 h at 23°C with exposure to light.

Key words: tazobactam, piperacillin, Tazocin, high-performance liquid chromatography, stability, polyvinylchloride bags

RÉSUMÉ

Contexte : La Tazocine, un mélange de pipéracilline et de tazobactame, a récemment été modifiée pour inclure dans sa composition de l'acide éthylène diamine tétra acétique (EDTA) et de l'acide citrique. Depuis l'introduction de cette nouvelle composition, aucune étude de stabilité du mélange dans des sacs de polychlorure de vinyle (PVC) n'a été effectuée.

Objectif : Mener une étude de compatibilité physique et de stabilité chimique de solutions de la nouvelle composition de Tazocine préparées selon deux concentrations avec deux diluants et entreposées dans des sacs de PVC.

Méthodes : Des solutions de Tazocine à 22,5 at à 90 mg/mL ont été obtenues en la diluant dans du dextrose à 5 % dans l'eau ou dans du chlorure de sodium à 0,9 % (solution physiologique salée) dans des sacs de PVC. Les sacs ont été entreposés à une température de 5 °C, protégés de la lumière, pendant 14, 21 ou 28 jours, puis conservés à une température de 23 °C et exposés à la lumière pendant 72 heures. Les échantillons recueillis en triple à chacun des 7 points dans le temps ont été analysés en double à l'aide d'une épreuve validée par chromatographie liquide haute performance mesurant la stabilité. La compatibilité physique était évaluée en contrôlant tout changement dans la couleur, la limpidité et le pH des solutions.

Résultats : Les solutions aux deux concentrations dans les deux diluants ont retenu plus de 95 % de leur concentration initiale de chaque médicament après avoir été conservées à une température de 5 °C, protégées de la lumière, et plus de 94 % de leurs concentrations initiales des deux médicaments après avoir été conservées pendant une période supplémentaire de 72 heures à une température de 23 °C et exposées à la lumière. Le pH des solutions n'a que très peu changé au cours de l'étude, et toutes les solutions sont demeurées limpides et incolores.

Conclusions : Les solutions de Tazocine à 22,5 et à 90 mg/mL, préparées dans des sacs de PVC et diluées dans du dextrose à 5% dans l'eau ou une solution physiologique salée, étaient chimiquement stables après avoir été entreposées pendant une période allant jusqu'à 28 jours à une température de 5 °C et protégées de la lumière, et conservées pendant une période additionnelle de 72 heures à une température de 23 °C et exposées à la lumière.

Mots clés : tazobactame, pipéracilline, Tazocine, chromatographie liquide haute performance, stabilité, sacs de polychlorure de vinyle

[Traduction par l'éditeur]

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INTRODUCTION

Tazocin (Wyeth Canada) is a combination of a semisynthetic penicillin derivative, piperacillin sodium, and a ß-lactamase inhibitor, tazobactam sodium, at a ratio of 8:1. This combination product has enhanced antibacterial activity against ß-lactamase-producing gram-positive and gram-negative aerobic and anaerobic bacteria.[1]

The stability of Tazocin has been studied for solutions in dextrose 5% in water (D5W) and normal saline (NS; 0.9% sodium chloride) in polyvinylchloride (PVC) bags. Moon and others[2] reported that solutions of tazobactam (10 mg/mL) and piperacillin (80 mg/mL) in both D5W and NS were stable for 30 days when stored at –15°C. Hecq and others[3] found that solutions of tazobactam (4.4 mg/mL) and piperacillin (33.3 mg/mL) in D5W retained above 90% of the initial concentration for 35 days when stored at 4°C after freezing and microwave thawing. Rigge and Jones[4] reported that the combination of tazobactam (5 mg/mL) and piperacillin (40 mg/mL) in NS stored in PVC bags was stable for 5 days at 7°C and 4 days at 25°C, irrespective of exposure to light. Stored in non-PVC bags, the NS solution was stable for 17 days at 7°C and 4 days at 25°C with protection from light. The duration of stability was further increased to 58 days at 7°C and 10 days at 25°C with protection from light when the Tazocin was prepared in a buffered NS solution and stored in non-PVC bags. The effect of tazobactam on the stability of piperacillin was studied by Mathew and others,[5] who reported increased degradation of piperacillin when tazobactam was present in the formulation.

Tazocin has recently been reformulated to meet current USP (United States Pharmacopeia) standards for particulate matter. The formulation now includes edetate disodium (EDTA, a chelating agent) and sodium citrate (a buffer). The addition of these excipients lessens the possibility that particulate matter will accumulate during storage or upon reconstitution with commonly used diluents. It has also expanded the Y-site compatibility profile of Tazocin.[6]

Since the reformulation of Tazocin there have been no stability studies conducted in PVC bags. This study was therefore undertaken to provide physical compatibility and chemical stability data for solutions containing the reformulated Tazocin when diluted in either D5W or NS, packaged in PVC bags, and stored at 5°C with protection from light for 14, 21, and 28 days, with each of these storage periods followed by 72 h at 23°C with exposure to light.

METHODS

Assay Validation

The stability-indicating capability of the assay was validated for both tazobactam and piperacillin by chromatography of samples that had been forcibly degraded by the addition of an acidic or oxidizing agent at an elevated temperature. The acidic sample was prepared by adjusting the pH of 0.1 mL of Tazocin stock solution (2.25 g per 10 mL), diluted to 10 mL with high-performance liquid chromatography (HPLC)-grade water, to about 2.0 with concentrated hydrochloric acid (BDH Inc, Toronto, Ontario; lot 120834-78180) and storing the solution at 23°C. An oxidized sample was prepared by adding 0.15 mL of stabilized sodium hypochlorite (1% available chlorine, pH about 12) to 0.15 mL of Tazocin stock solution plus 9.7 mL of HPLC-grade water and was kept at 23°C. A third sample of the solution, without addition of any other agent, was stored at 23°C. A similar fourth sample was exposed to an elevated temperature (40°C) in a hot water bath for 96 h. Samples were taken from each solution periodically (at 5 time points) over 96 h and subjected to chromatography, to determine if there were any degradation peaks that would interfere with the parent peaks.

The linearity of the standard curve was determined over the concentration ranges of 0.625 to 3.75 mg/mL for tazobactam and 5.0 to 30.0 mg/mL for piperacillin. A stock solution of Tazocin was prepared by dissolving 119.0 mg of lyophilized powder (Wyeth Canada Ltd, Saint Laurent, Quebec; 3.375-g vial, lot B83227, expiry October 2008) in 100 mL of HPLC-grade water and then further diluting to create the standard solutions. The reproducibility of the chromatographic method was measured by completing intraday (5 replicate injections at 3 different times) and interday analyses for each drug. Interday variance was determined by comparing slopes calculated from standard curves and the average area ratio (area of drug/area of internal standard) of the recovery samples on 5 separate days. The accuracy of the method was determined by analysis of recovery samples for each drug on 5 separate days. The sensitivity of the assay for each compound was also investigated. Peak purity analysis and chemical identification of both parent peaks were completed by ultraviolet (UV) multiwavelength analysis (at 220 and 210 nm) and spectral overlay (at 200–350 nm). Relative standard deviation, resolution, and tailing factors were also determined.

Stability Study

To prepare a 100-mL bag of Tazocin, either one 2.25-g vial (22.5 mg/mL, Wyeth Canada, Saint-Laurent, Quebec; lot B68332, expiry May 2008) or two 4.5-g vials (90 mg/mL, Wyeth Canada; lot B68627, expiry May 2008) were reconstituted according to the manufacturer's guidelines and shaken to dissolve. Contents of appropriate vial(s) were transferred into empty Viaflex PVC bags (Baxter Corp; lot ST06C48). Enough D5W (Baxter Corp, Mississauga, Ontario; lot W6K01A0, expiry May 2008) or NS (Baxter Corp; lot W6L22M1, expiry June 2008) was added to each bag to make up a volume of

exactly 100 mL at a concentration of either 22.5 mg/mL (from a 2.25-g vial) or 90 mg/mL (from two 4.5-g vials). Four bags were prepared for each concentration– diluent combination, for a total of 16 bags.

Sample Collection and Preparation

Three samples (5 mL each) taken from one bag of each concentration–diluent combination were designated as day 0 samples. The rest of the bags were then stored at 5°C with protection from light. On days 14, 21, and 28, 3 additional samples of 5 mL each were removed from one bag of each concentration–diluent combination and analyzed. The bags from which the samples had been removed were then stored at 23°C with exposure to light for an additional 72 h, after which an additional 3 samples were collected and analyzed. Samples containing 22.5 mg/mL of Tazocin were diluted 1:100 with mobile phase, whereas samples containing 90 mg/mL of Tazocin were diluted 1:400 with mobile phase; the samples were analyzed in duplicate for total $n = 6$.

Chromatographic Analysis

Chemical assays were conducted using a modification of an unpublished HPLC method (Wyeth test method: Zosyn [EDTA formulation], method no. MZOSP026.00). The modifications were use of a 3-µm, 4.6 × 150 mm phenyl-hexyl column (Luna, Phenomenex Inc, Torrance, California; catalogue no. 00F-4256-E0, lot 365391-1) and adjustment of the pH of the mobile phase to 2.0 instead of 3.8. The HPLC system consisted of an isocratic pump (model LC-10AT, Shimadzu Corp, Tokyo, Japan), a photodiode array detector (model SDP-M6A, Shimadzu Corp) set at 220 nm, and an autoinjector (model SIL-10AXL, Shimadzu Corp) that injected 20-µL samples. The flow was set at 1.0 mL/min. Class-VP software (version 4.2, Shimadzu Corp, Columbia, Maryland) was used for data collection and analysis.

On each day of analysis, enough Tazocin lyophilized powder (Wyeth Canada; 3.375-g vial, lot B83227, expiry October 2008) was accurately weighed out to prepare a solution with concentration of about 1.15 mg/mL in HPLC-grade water.

Physical Tests

The pH of all samples was recorded on days 0, 14, 17, 21, 24, 28, and 31 using a calibrated pH meter. The instrument was calibrated on each day, before the pH measurements were obtained, using buffers with known pH of 4.00 and 7.00. To be within equipment specifications, the efficiency of the meter had to be 100% ± 5%.

Colour and clarity were monitored at each time point by viewing a sample from each bag under 4× magnification with illumination. These samples were observed against a black background for particulate matter and against a white background for colour change.

Data and Statistical Analysis

Relative standard deviation, resolution factors, and tailing factors were calculated using formulas taken from USP (United States Pharmacopia) 29.[7] The linearity of the standard curve (R^2) was assessed using the least mean-squared method. The concentrations of both tazobactam and piperacillin were calculated using the equation for each drug derived from the standard curve performed on the day of analysis.

RESULTS

Degradation and Assay Validation

Analysis of the acidic, oxidized, and heated samples showed no signs of interference of degradation products with either the tazobactam or piperacillin peaks. The tazobactam had degraded to 33% and the piperacillin to 44% of the original concentrations in the acidic sample; however, none of the new peaks that appeared between the tazobactam and piperacillin parent peaks interfered with analysis. Over the

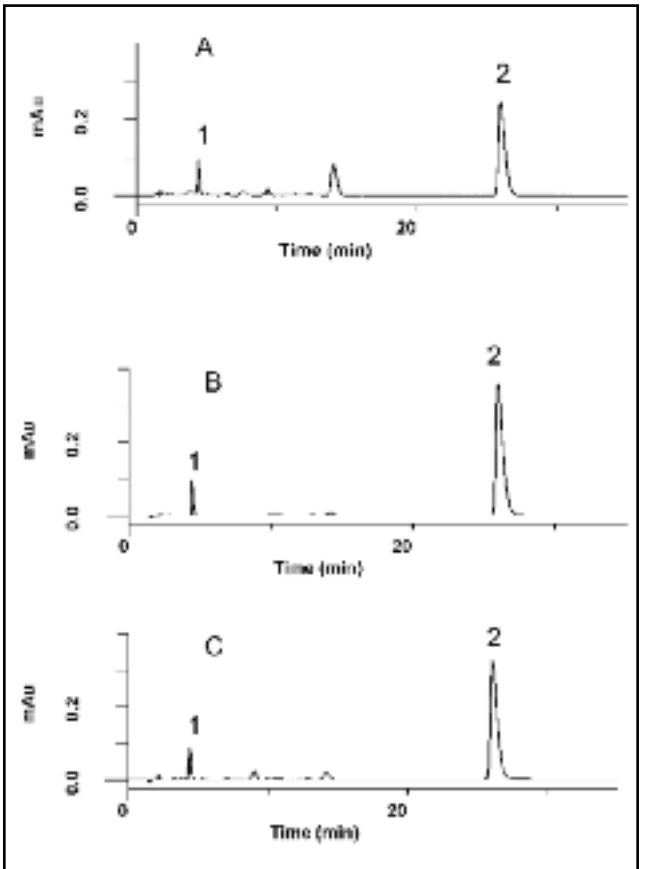

Figure 1. Sample chromatograms of Tazocin and degradation products. A: Tazocin sample after 96 h of exposure to hypochlorite at 23°C. B: Tazocin sample after storage at 23°C for 96 h. C: Tazocin sample after storage for 96 h at 40°C. Peak 1 = tazobactam, peak 2 = piperacillin.

96-h study period, the oxidized sample also produced several peaks that eluted between the tazobactam and piperacillin peaks, but none of these interfered with the parent peaks (Figure 1A). There was little change in the concentrations of tazobactam and piperacillin after storage at 23°C for 96 h (Figure 1B). The heat-degraded sample produced some degradation peaks similar to those of the oxidized sample and some different ones, but in different concentrations, over 96 h of heating (Figure 1C). None of the degradation peaks interfered with either parent compound.

Standard curves for tazobactam and piperacillin were linear over the study concentrations. The reproducibility of the assay was measured as intraday coefficients of variance of 0.77% for tazobactam and 1.20% for piperacillin, whereas the interday coefficients of variance were 1.44% (slope) and 3.02% (average area ratios) for tazobactam and 1.22% (slope) and 2.89% (average area ratios) for piperacillin. The accuracy was 100.5% ± 1.00% for tazobactam and 100.3% ± 1.08% for piperacillin. The tazobactam sensitivity was 26 ng, and the piperacillin sensitivity was 200 ng.

All parent peaks from the degradation study were pure, as determined by spectral overlays and UV multiwavelength analysis. Relative standard deviation values were less than 2.0%. Resolution and tailing factors were determined before the samples were run and were greater than 6.0 and less than 2.0, respectively.

Chemical Stability

The average percentage of initial concentration remaining for all time points for the 4 combinations of drug concentration and diluent are summarized in Tables 1 to 4. For both initial concentrations of the drug (22.5 and 90 mg/mL), prepared in either diluent (D5W or NS), the measured concentration remained above 95% after 14, 21, or 28 days of storage at 5°C with protection from light. After an additional 72 h at 23°C with exposure to light (after each refrigerated storage period), the concentrations in all concentration–diluent combinations remained above 94%. For each concentration–diluent combination, there was a slight decrease (by 3%–4%) in concentration after the additional 72 h storage

Table 1. Stability of Tazocin 22.5 mg/mL* in D5W in PVC bags

	Drug; % of Initial Concentration Remaining†‡	
Conditions and Study Day	**Tazobactam**	**Piperacillin**
Initial concentration (mg/mL)	2.7 ± 0.05	21.4 ± 0.40
Day 14§	100.3 ± 2.9	102.3 ± 3.0
Day 17‖	97.5 ± 1.9	99.1 ± 2.2
Day 21§	99.5 ± 1.5	100.9 ± 1.6
Day 24‖	96.4 ± 2.7	100.0 ± 2.3
Day 28§	99.4 ± 1.1	101.9 ± 1.0
Day 31‖	97.6 ± 1.4	100.7 ± 1.5

D5W = 5% dextrose in water, PVC = polyvinylchloride.
*Tazobactam 2.5 mg/mL and piperacillin 20.0 mg/mL.
†Mean ± standard deviation (n = 6).
‡Except where indicated otherwise
§Samples stored at 5°C ± 3°C with protection from light.
‖Samples stored at 23°C ± 3°C for an additional 72 h with exposure to light.

Table 2. Stability of Tazocin 90 mg/mL* in D5W in PVC bags

	Drug; % of Initial Concentration Remaining†‡	
Conditions and Study Day	**Tazobactam**	**Piperacillin**
Initial concentration (mg/mL)	10.9 ± 0.20	86.4 ± 1.52
Day 14§	100.5 ± 1.1	102.5 ± 2.1
Day 17‖	100.2 ± 1.1	101.5 ± 1.8
Day 21§	102.5 ± 1.8	103.8 ± 1.6
Day 24‖	99.7 ± 1.8	99.2 ± 1.8
Day 28§	100.9 ± 1.6	101.7 ± 1.4
Day 31‖	98.8 ± 1.7	99.6 ± 1.9

D5W = 5% dextrose in water, PVC = polyvinylchloride.
*Tazobactam 10.0 mg/mL and piperacillin 80.0 mg/mL.
†Mean ± standard deviation (n = 6).
‡Except where indicated otherwise
§Samples stored at 5°C ± 3°C with protection from light.
‖Samples stored at 23°C ± 3°C for an additional 72 h with exposure to light.

Table 3. Stability of Tazocin 22.5 mg/mL* in NS in PVC bags

Conditions and Study Day	Drug; % of Initial Concentration Remaining†‡	
	Tazobactam	Piperacillin
Initial concentration (mg/mL)	2.6 ± 0.02	20.7 ± 0.19
Day 14§	104.2 ± 0.8	106.7 ± 2.4
Day 17‖	104.8 ± 1.0	107.5 ± 1.1
Day 21§	105.5 ± 1.7	107.5 ± 1.7
Day 24‖	102.7 ± 2.0	102.1 ± 1.9
Day 28§	104.8 ± 1.5	106.2 ± 1.0
Day 31‖	99.5 ± 0.5	102.2 ± 0.5

NS = normal saline (0.9% NaCl), PVC = polyvinylchloride.
*Tazobactam 2.5 mg/mL and piperacillin 20.0 mg/mL.
†Mean ± standard deviation (n = 6).
‡Except where indicated otherwise
§Samples stored at 5°C ± 3°C with protection from light.
‖Samples stored at 23°C ± 3°C for an additional 72 h with exposure to light.

Table 4. Stability of Tazocin 90 mg/mL* in NS in PVC bags

Conditions and study day	Drug; % of Initial Concentration Remaining†‡	
	Tazobactam	Piperacillin
Initial concentration (mg/mL)	11.6 ± 0.13	89.9 ± 1.40
Day 14§	97.3 ± 1.2	100.1 ± 1.3
Day 17‖	97.2 ± 1.9	99.7 ± 1.5
Day 21§	95.8 ± 1.2	98.7 ± 1.2
Day 24‖	96.4 ± 1.6	99.3 ± 1.7
Day 28§	96.3 ± 1.5	99.0 ± 1.4
Day 31‖	94.6 ± 1.6	98.4 ± 1.8

NS = normal saline (0.9% NaCl),, PVC = polyvinylchloride.
*Tazobactam 10.0 mg/mL and piperacillin 80.0 mg/mL.
†Mean ± standard deviation (n = 6).
‡Except where indicated otherwise
§Samples stored at 5°C ± 3°C with protection from light.
‖Samples stored at 23°C ± 3°C for an additional 72 h with exposure to light.

at 23°C with exposure to light. There appeared to be no difference in stability related to the diluent used.

Physical Compatibility

The pH of all solutions remained within the range of 5.50 to 6.08 over the course of the study. All solutions remained clear and colourless throughout the study.

DISCUSSION

The measured concentration of both drugs in either D5W or NS remained above 95% of the initial concentration after 28 days of storage at 5°C with protection from light. The difference in the concentrations (22.5 mg/mL versus 90 mg/mL) seemed to have no effect on the shelf life. Storage at 23°C for an additional 72 h caused a 3%-4% decrease in the concentrations of both tazobactam and piperacillin.

It has previously been reported[4] that ß-lactam antibiotics are generally most stable between pH 6 and 7. Rigge and Jones[4] found that unbuffered solutions in NS had an initial pH of 5.3, with a subsequent decrease in pH to 4.5 over time, which led these authors to suggest a shorter expiry date. Inclusion of EDTA and sodium citrate in the reformulated Tazocin might explain why, in the present study, the pH never dropped below 5.50; this in turn might contribute to the longer shelf life.

Tazocin solutions at initial concentration of 22.5 or 90 mg/mL in either D5W or NS, prepared and stored in PVC bags, were chemically stable for up to 28 days at 5°C with protection from light followed by 72 h at 23°C with exposure to light.

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