ARTICLE

Effects of Freezing, Long-Term Storage, and Microwave Thawing on the Stability of Piperacillin Plus Tazobactam in 5% Dextrose for Infusion

Jean-Daniel Hecq, Valère Berlage, Danielle Vanbeckbergen, Jacques Jamart, and Laurence Galanti

ABSTRACT

Background: A combination of piperacillin with tazobactam is often administered to hospital patients by infusion. Advance preparation of IV solutions of these drugs could be an efficient way to improve the quality, time management, and cost associated with drug delivery, but little is known about the stability of these drugs after freezing followed by microwave thawing.

Objective: The purpose of the study was to investigate how a process of freezing, long-term storage, and microwave thawing affects the stability of piperacillin with tazobactam in 5% dextrose for infusion.

Methods: Five replicates of a mixture of 4 g piperacillin and 0.5 g tazobactam in 20 mL of water plus 100 mL of 5% dextrose were prepared and stored in polyvinyl chloride (PVC) bags. The stability of the mixtures was studied after freezing for 3 months at –20°C followed by thawing in a microwave oven and additional storage at 4°C for up to 60 days. The concentrations of piperacillin and tazobactam in each sample were measured by high-pressure liquid chromatography. The samples were inspected visually, and pH was measured.

Results: After 3 months of storage at –20°C, microwave thawing, and 35 days of storage at 4°C, more than 90% of the initial concentration of both piperacillin and tazobactam remained. At this point, the lower 95% confidence limit of the best-fit line by linear regression of percent remaining was 90.1% for piperacillin and 90.3% for tazobactam. The pH decreased by 1.16 units over 35 days of storage at 4°C, but no colour change or precipitation was observed. From 35 to 60 days, the concentrations of both piperacillin and tazobactam fell below the acceptable limit and pH values continued to decrease slightly.

Conclusions: The combination of piperacillin (4 g/120 mL) and tazobactam (0.5 g/120 mL) in 5% dextrose stored in PVC bags, frozen for 3 months at –20°C, thawed in a microwave oven, and then stored at 4°C for an additional 35 days retained more than

RÉSUMÉ

Historique : On administre souvent par perfusion de la pipéracilline en association avec du tazobactam aux patients hospitalisés. Les mélanges préalablement préparés de solutions i.v. de ces médicaments peuvent s'avérer un moyen efficace d'améliorer la qualité et de réduire le temps ainsi que les coûts associés à leur distribution, mais on ne sait que peu de choses quant à la stabilité de ces médicaments une fois congelés puis décongelés au micro-ondes.

Objectif : Le but de cette étude était d'évaluer de quelle manière le processus de congélation, d'entreposage à long terme et de décongélation au micro-ondes influe sur la stabilité de la pipéracilline mélangée à du tazobactam dans du dextrose à 5 % pour perfusion.

Méthodes : Cinq mesures de 4 g de pipéracilline mélangée à 0,5 g de tazobactam dans 20 mL de l'eau plus 100 mL de dextrose à 5 % ont été préparées et conservées dans des sacs de polychlorure de vinyle (PVC). Ces mélanges ont été étudiés pour évaluer leur stabilité après une période de congélation de trois mois à –20 °C, suivie de leur décongélation dans un micro-ondes, puis d'une période d'entreposage jusqu'à 60 jours à 4 °C. Les concentrations de pipéracilline et de tazobactam dans chaque échantillon ont été mesurées par chromatographie liquide à haute pression. Les échantillons ont été inspectés visuellement et leur pH mesuré.

Résultats : Après avoir été entreposés pendant trois mois à –20 °C, décongelés au micro-ondes, puis entreposés à nouveau pendant 35 jours à 4 °C, les mélanges ont conservé plus de 90 % de leur concentration initiale de pipéracilline et de tazobactam. À ce point, la limite inférieure de confiance à 95 % de la régression linéaire du pourcentage restant était de 90,1 % pour la pipéracilline et de 90,3 % pour le tazobactam. Le pH a diminué de 1,16 unité après 35 jours d'entreposage à 4 °C, mais aucun changement de couleur ni précipité n'a été observé. Durant la période d'entreposage de 35 à 60 jours, les concentrations de la

90% of the initial concentration of each drug. This should allow for advance preparation of this drug combination.

Key words: piperacillin, tazobactam, dextrose infusion, stability, microwave thawing

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pipéracilline et du tazobactam ont toutes deux chuté sous le seuil acceptable et les valeurs de pH ont continué à diminuer légèrement.

Conclusions : Les mélanges de pipéracilline (4 g/120 mL) et de tazobactam (0,5 g/120 mL) dans du dextrose à 5 %, entreposés dans des sacs en PVC, congelés pendant trois mois à –20 °C, décongelés dans un micro-ondes, puis entreposés de nouveau pendant 35 jours à 4 °C ont conservé plus de 90 % de leur concentration initiale de chaque médicament. Les mélanges de cette association médicamenteuse préparés à l'avance peuvent donc être recommandés.

Mots clés : pipéracilline, tazobactam, perfusion de dextrose, stabilité, décongélation au micro-ondes.

INTRODUCTION

Piperacillin, an antibiotic of the penicillin family,[1] has a broad bactericidal spectrum covering many gram-negative bacteria such as *Pseudomonas, Enterobacter, Klebsiella, Serratia*, and species of the Bacteroidaceae family, including *Bacteroides fragilis*. The combination of piperacillin with tazobactam, a ß-lactamase inhibitor, broadens the bactericidal activity.[2,3]

Because this drug combination must be administered intravenously,[3] advance centralized batch preparation of the IV solution might improve the speed and time management of drug delivery to the hospital floor. Freezing drug solutions can extend the long-term stability of ready-to-use injectable drugs. To reduce thawing time, some authors have used a microwave oven.[4,5] The stability of piperacillin alone or in combination with other antimicrobial agents has been investigated under a variety of conditions,[6-21] including different diluents (5% dextrose in water [D5W], 0.9% sodium chloride [normal saline, NS], peritoneal dialysis solution, parenteral nutrition solution, culture media), containers (glass, plastic bags, syringes, portable pumps), temperatures (37°C, 25°C, 4°C, –15°C, –20°C, –70°C), and storage periods (from 6 h[15] to 1 year[7]). Although the stability of piperacillin in NS[8] or D5W,[8,11] stored frozen at –15°C[8] or –20°C,[11] has been evaluated, the longest period of storage in either of these diluents was 30 days[8,11] followed by an additional 7 days at room temperature.[11]

The purpose of this investigation was to study the stability of piperacillin plus tazobactam in a ready-to-use 5% dextrose solution after storage for 3 months at –20°C followed by thawing in a microwave oven and final storage at 4°C.

MATERIALS AND METHODS

Preparation of Solutions

One bottle of a commercially available preparation of powdered piperacillin with tazobactam (Tazocin, 4 g piperacillin/500 mg tazobactam, Lederle, Louvain-la-Neuve, Belgium, lot 99K23A, expiry November 2002) was reconstituted with 20 mL of purified water in a laminar air flow hood. This solution was added to 100 mL of 5% dextrose in a polyvinyl chloride (PVC) bag (Baxter, Lessines, Belgium) and used to prepare an IV sample containing 4 g piperacillin and 0.5 g tazobactam per 120 mL. On each test day, a standard curve was prepared with the same commercially available powder reconstituted extemporaneously with purified water to obtain the same drug concentrations as in the test solution. Nafcillin (Sigma, St Louis, Missouri, reference N-3269), prepared by dissolving 500 mg in 10 mL of purified water, was used as an internal standard; the standard was stored in 500-µL aliquots at –20°C for a period of a few days. This stock solution was thawed and diluted in purified water before each test (100-fold dilution for tazobactam analysis and 200-fold dilution for piperacillin analysis).

Chromatographic Apparatus and Conditions

A high-performance liquid chromatographic (HPLC) separation module (Waters Alliance 2690, Waters Corporation, Milford, Massachusetts) equipped with an ultraviolet (UV) detector (model UV 481, Waters Corporation) was used. A platinum extended polar selectivity (EPS) column (Rocket C_{18} column, 3-µm, 53 mm ± 7 mm, Alltech, Lokeren, Belgium) coupled

with a platinum EPS precolumn (Rocket C_{18} column, 5-µm, 7.5 mm ± 4.6 mm, Alltech) was the stationary phase.

The mobile phase contained 45% v/v of acetonitrile (Baker, Deventer, Holland, reference 9017) in 0.05 mol/L potassium dihydrogen phosphate buffer (Merck, Darmstadt, Germany, reference 1.04873) in water, adjusted to pH 3 with phosphoric acid [H_3PO_4], Vel, Leuven, Belgium, reference 1072). All solvents were prepared from HPLC-grade solvents and purified water (Milli-Q, Millipore, Brussels, Belgium). The flow rate was 1 mL/min, and the column temperature was 30°C. The detector wavelengths were 211 nm for tazobactam, 230 nm for piperacillin, and 228 nm for nafcillin.

Determination of pH

The pH of the samples was measured with a pH meter (model pHM82, Radiometer, Copenhagen, Denmark).

Analytical Validation

Assays of control solutions of piperacillin 4 g/120 mL plus tazobactam 0.5 g/120 mL were undertaken to determine precision, as the within-day and between-day coefficients of variation.

Linearity of the analytical response was evaluated by analyzing serial dilutions of the piperacillin plus tazobactam solution with sterile water for injection.

To determine specificity, piperacillin plus tazobactam solutions were degraded by heating at 100°C for 15 and 30 min under acidic conditions (by addition of 6N H_3PO_4 to obtain a pH of 3.72), initial pH conditions (pH 5.41), and basic conditions (by addition of 10N sodium hydroxide [NaOH] to obtain a pH of 8.75).

Stability Study

Five PVC bags each containing 4 g piperacillin and 0.5 g tazobactam per 120 mL were prepared and mixed as described above, and stored for 3 months at –20°C. After storage, the bags were thawed in a microwave oven. The variable settings of the microwave oven were used to select 270-W output as a low power setting following a standard procedure currently used in the pharmacy department at the authors' institution. The bags were thawed for 13 min, shaken and then thawed for another 7 min to obtain a remaining ice volume of between 1 and 8 mL.

Immediately after preparation of the bags, after thawing (day 0), and after 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 25, 28, 35, 42, 49, 56, and 60 days of storage at 4°C, a 2-mL sample of the solution was withdrawn from each bag by means of a 2-mL polypropylene plastic syringe (Terumo, Haasrode, Belgium). Each sample was placed in a glass container for visual inspection; the pH was also measured. Samples were then diluted in purified water (10-fold for tazobactam analysis and 70-fold for piperacillin analysis). A 100-µL volume of each diluted sample was then added to 100 µL of internal standard. Ten-microlitre aliquots of the prepared mixture of samples, standard solutions, and control solutions were injected in triplicate into the chromatographic system under the conditions described previously.

Data and Statistical Analysis

Results were automatically determined by interpolation of a standard curve performed by Millennium software (Waters Corporation) on the basis of least-squares linear regression of response (peak height of drug concentration) versus nominal standard concentration.

Data are expressed as mean ± standard deviation (SD). Drug concentration and pH were followed as a function of time. The drug solutions were considered stable as long as the lower limit of the 95% confidence interval of the estimated regression line of the concentration with respect to time remained above 90% of the initial concentration, as recommended by the US Food and Drug Administration.[22]

RESULTS AND DISCUSSION

Analytical Validation

Retention times were approximately 1.50 min for tazobactam, 2.15 min for piperacillin, and 3.37 min for nafcillin under the chromatographic conditions described. No difference in the chromatograms for piperacillin and tazobactam were observed before and after freezing and microwave thawing (Figures 1A and 2A).

Degraded samples of piperacillin and tazobactam were assayed to confirm separation of the parent antibiotic from its degradation products. After heating at 100°C for 15 and 30 min in the initial pH solution and following treatment with 6N H_3PO_4 or 10N NaOH, peaks for the decomposition products could be observed on the chromatogram without interference from the peak corresponding to the intact drug. Observed overlap did not affect either peak shape or height (Figures 1B, 1C, 2B, and 2C).

The within-day and between-day coefficients of variation for replicate analysis averaged 1.0% (n = 10) and 2.4% (n = 20), respectively, for tazobactam and 2.4% (n = 10) and 4.4% (n = 20) for piperacillin, respectively.

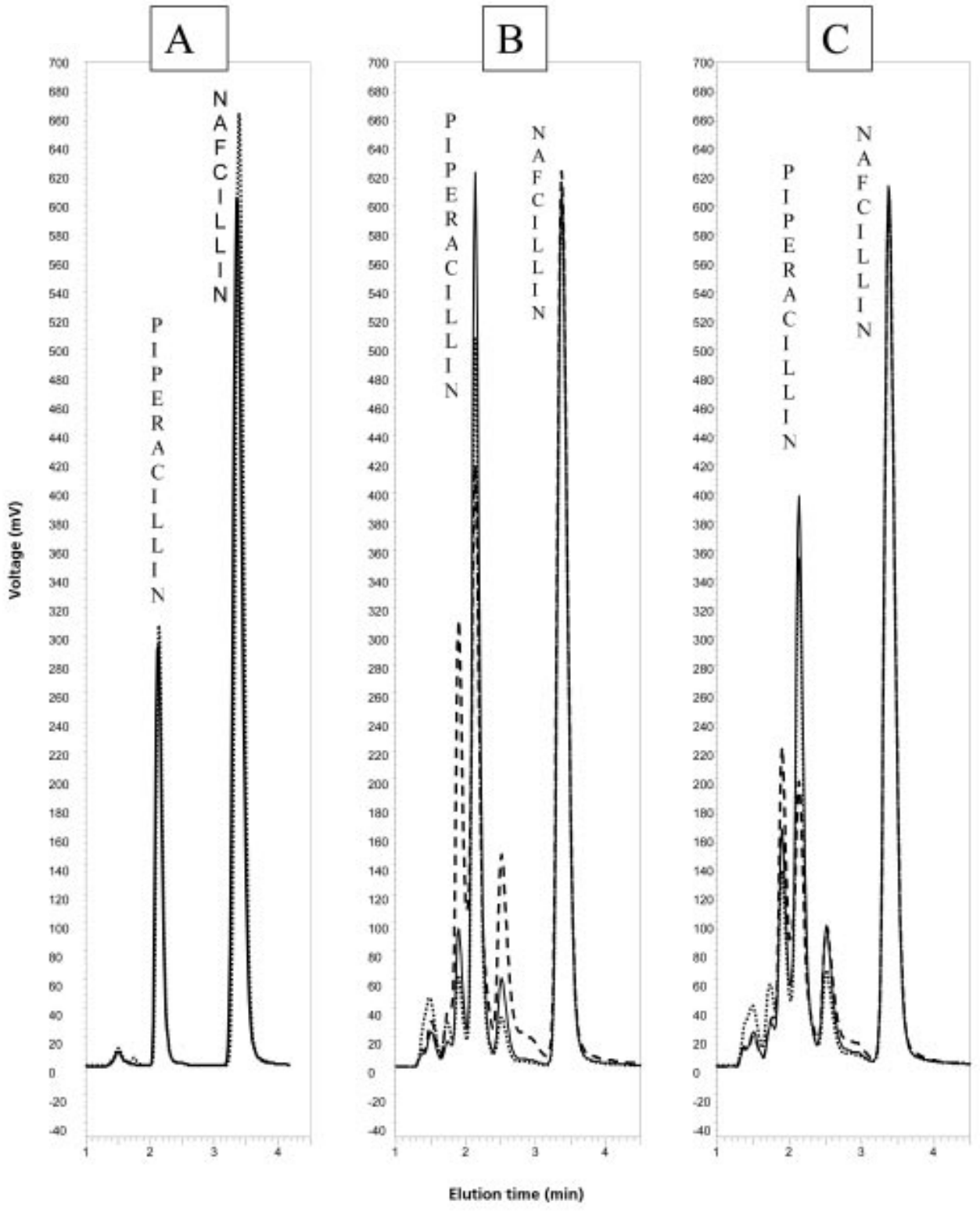

Figure 1. Chromatograms of piperacillin. Data are presented as detector outputs (subsequently converted to concentrations by system software). A: Before (dotted line) and after (solid line) freezing and microwave thawing. B: After heating at 100°C for 15 min, under initial pH conditions (solid line), under acidic conditions (pH about 3, by addition of phosphoric acid) (dashed line), and under alkaline conditions (pH about 9, by addition of sodium hydroxide) (dotted line). C: After heating at 100°C for 30 min, under initial pH conditions (solid line), under acidic conditions (pH about 3, by addition of phosphoric acid) (dashed line), and under alkaline conditions (pH about 9, by addition of sodium hydroxide) (dotted line).

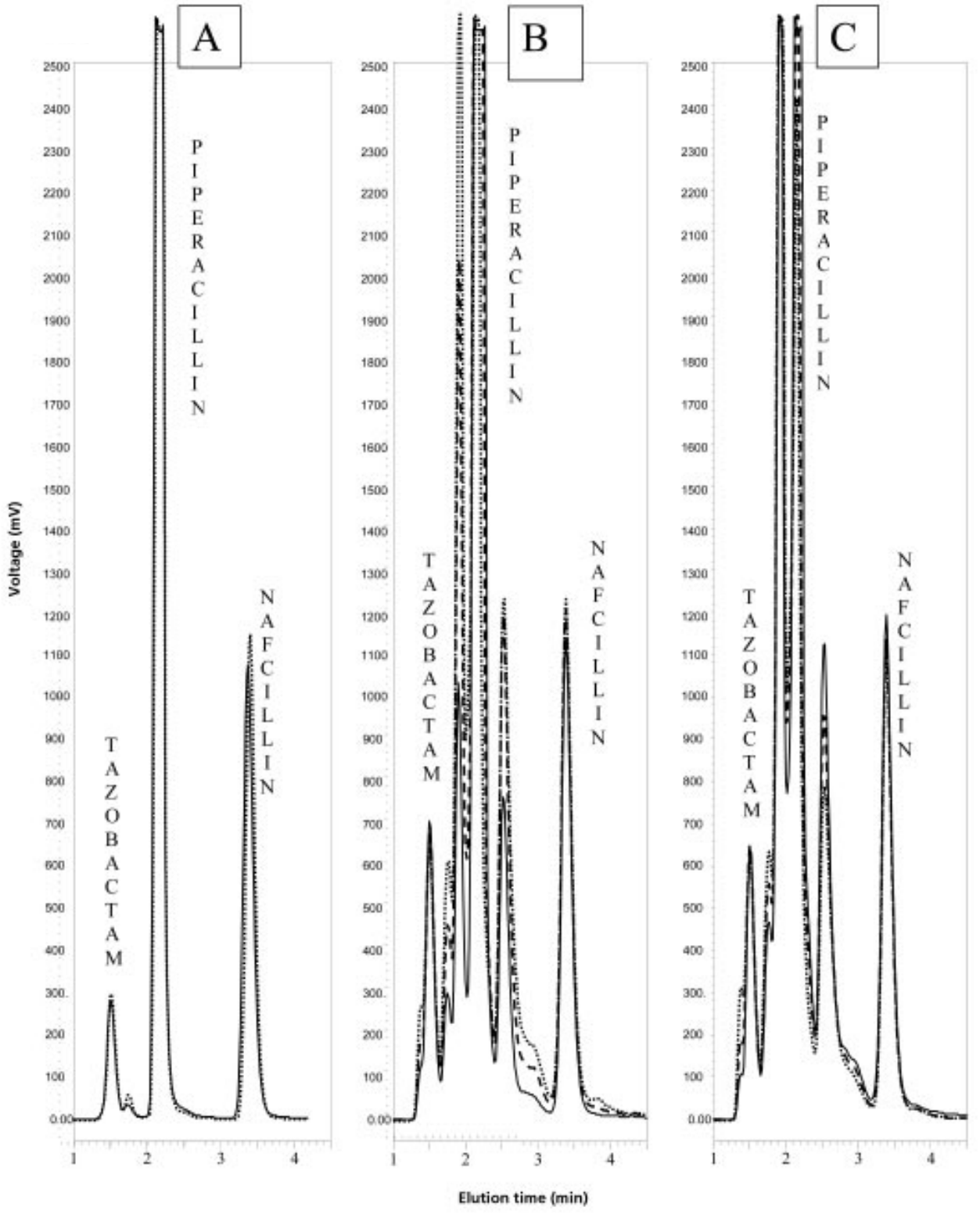

Figure 2. Chromatograms of tazobactam in combination with piperacillin. Data are presented as detector outputs (subsequently converted to concentrations by system software). A: Before (dotted line) and after (solid line) freezing and microwave thawing. B: After heating at 100°C for 15 min, under initial pH conditions (solid line), under acidic conditions (pH about 3, by addition of phosphoric acid) (dashed line), and under alkaline conditions (pH about 9, by addition of sodium hydroxide) (dotted line). C: After heating at 100°C for 30 min, under initial pH conditions (solid line), under acidic conditions (pH about 3, by addition of phosphoric acid) (dashed line), and under alkaline conditions (pH about 9, by addition of sodium hydroxide) (dotted line).

Table 1. Characteristics of piperacillin and tazobactam solutions in 5% dextrose stored in polyvinylchloride bags at 4°C (after freezing, storage, and microwave thawing)

Period of storage at 4°C (days)	pH ± SD	Mean % remaining* ± SD (and lower limit of 95% CI)†			
		Piperacillin		Tazobactam	
Before freezing	5.73 ± 0.021	NA		NA	
0	5.50 ± 0.01	101.4 ± 0.6	(97.1)	102.7 ± 1.7	(97.7)
1	5.39 ± 0.02	100.3 ± 0.1	(96.9)	104.2 ± 2.4	(97.5)
2	5.29 ± 0.01	99.7 ± 1.3	(96.7)	103.6 ± 1.6	(97.3)
3	5.23 ± 0.01	96.9 ± 1.6	(96.5)	103.8 ± 1.8	(97.1)
4	5.15 ± 0.01	97.8 ± 1.4	(96.3)	100.8 ± 0.6	(96.9)
7	5.05 ± 0.00	99.5 ± 1.3	(95.7)	102.3 ± 1.0	(96.2)
9	4.99 ± 0.01	99.3 ± 0.7	(95.3)	98.8 ± 1.4	(95.8)
11	4.93 ± 0.01	99.2 ± 1.9	(94.9)	101.1 ± 1.4	(95.4)
14	4.86 ± 0.01	98.5 ± 0.4	(94.3)	98.9 ± 1.7	(94.8)
16	4.83 ± 0.01	95.8 ± 1.3	(93.9)	96.8 ± 1.8	(94.4)
18	4.80 ± 0.01	97.4 ± 0.6	(93.5)	102.9 ± 1.3	(93.9)
21	4.76 ± 0.01	96.8 ± 1.3	(92.9)	101.0 ± 2.0	(93.3)
25	4.69 ± 0.01	96.7 ± 1.0	(92.1)	98.3 ± 1.7	(92.5)
28	4.66 ± 0.01	94.3 ± 0.9	(91.5)	99.4 ± 2.6	(91.8)
35	4.57 ± 0.00	91.5 ± 1.4	(90.1)	98.1 ± 1.6	(90.3)
42	4.55 ± 0.00	92.4 ± 0.5	(88.6)	100.2 ± 2.3	(88.8)
49	4.53 ± 0.01	89.8 ± 1.2	(87.2)	88.2 ± 1.8	(87.3)
56	4.50 ± 0.00	90.7 ± 1.1	(85.8)	89.5 ± 1.8	(85.8)
60	4.48 ± 0.01	86.8 ± 0.6	(85.0)	91.1 ± 2.1	(85.0)

SD = standard deviation, CI = confidence interval, NA = not applicable.
*Initial concentration of piperacillin was nominally 4 g/120 mL (measured concentration 3.599 ± 0.190 g per 120 mL). Initial concentration of tazobactam was nominally 0.5 g/120 mL (measured concentration 0.495 ± 0.011 g per 120 mL).
†Lower limit of the 95% confidence interval of the estimated regression line.

Linearity of Analytical Response

Linear regression analysis of the peak height of the drug concentration yielded a correlation coefficient (r) of greater than 0.99, for both tazobactam (concentration ranging from 0.1 to 10 mg/mL; slope ± percent relative standard deviation [RSD] 0.847 ± 0.637; intercept 0.03) and piperacillin (concentration ranging from 0.8 to 80 mg/mL; slope ± RSD 1.069 ± 1.887; intercept –0.22).

Stability Study

There was no evidence of haze, colour change, or precipitation in the antibiotic admixtures when frozen bags were thawed in a microwave oven under standardized conditions.

The concentration of both drugs decreased over the 60-day storage period following microwave thawing (Table 1). These decreases were statistically significant ($p < 0.001$), but the lower 95% confidence limit of the estimated regression line remained above 90% of the initial concentration until day 35.

The pH also decreased significantly during storage ($p < 0.001$). On day 35, when 90% of the initial concentration still remained, the pH had decreased by 1.16 units. However, this decrease in pH did not affect chromatographic parameters, and the pH remained in an acceptable range for perfusion.

Internal data from Baxter[23] have previously shown that the combination of piperacillin with tazobactam remained stable after 3 months of storage at –20°C. The data reported here suggest that piperacillin plus tazobactam solutions were not affected by either freezing or thawing according to a standardized protocol. Furthermore, the concentration of the thawed solutions remained acceptable with storage at 4°C until day 35. This period of stability is longer than that reported by Zhang and Trissel (21 days),[10] who studied piperacillin alone, and is approximately the same as that observed by Mathew and others (28 days),[6] who studied piperacillin plus tazobactam.

CONCLUSIONS

Piperacillin plus tazobactam solution (4 g and 0.5 g per 120 mL, respectively) in 5% dextrose stored in PVC bags may be frozen and thawed in a microwave oven without major changes affecting concentration. Subsequent storage at 4°C is possible for up to 35 days. Within these limits, piperacillin plus tazobactam may be prepared in advance by a centralized IV admixture service, frozen, thawed, and stored under refrigeration for a few weeks before use on hospital wards.

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Jean-Daniel Hecq, PharmD, is Head of Pharmacy, Hospital Pharmacy, University Hospital of Mont-Godinne, Yvoir, Belgium.

Valère Berlage, PharmD, is a Laboratory Fellow in the Medical Laboratory, University Hospital of Mont-Godinne, Yvoir, Belgium.

Danielle Vanbeckbergen, is a Biochemist in the Medical Laboratory, University Hospital of Mont-Godinne, Yvoir, Belgium.

Jacques Jamart, MD, is a Biostatistician in the Center of Biostatistics and Medical Documentation, University Hospital of Mont-Godinne, Yvoir, Belgium.

Laurence Galanti, MD, PhD, is Director of the Medical Chemistry Laboratory, Medical Laboratory, University Hospital of Mont-Godinne, Yvoir, Belgium.

Address correspondence to:
Dr Laurence Galanti
Medical Laboratory
University Hospital of Mont-Godinne
B-5530
Yvoir, Belgium

e-mail: galanti@mexp.ucl.ac.be