Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 339–343
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Shelf lives of aseptically prepared medicines—Stability of piperacillin/tazobactam in PVC and non-PVC bags

Diane Clare Rigge *, Martin Frederick Jones

Quality Control North West, Stepping Hill Hospital, Poplar Grove, Stockport, Cheshire SK2 7JE, UK

Received 23 January 2005; received in revised form 19 March 2005; accepted 19 March 2005
Available online 26 April 2005

Abstract

Parenteral aseptic preparations of piperacillin/tazobactam are used frequently in hospitals, but there is limited published information on their stability in polyvinyl chloride (PVC) and polyolefine laminate (non-PVC). The purpose of this study was to evaluate the stability in these containers and to determine the optimum validated shelf life so that the formulations may be prepared in bulk in appropriately licensed facilities.

In the first study, the stability of piperacillin/tazobactam 45 mg/ml was determined in polyvinyl chloride and polyolefine laminate bags in 0.9% (w/v) sodium chloride at 7 °C, 25 °C/60% relative humidity (RH) and room temperature in the light (RTL) with storage up to 41 days for PVC bags and 98 days for non-PVC bags. In the second study, the stability of piperacillin/tazobactam 45 mg/ml was determined in non-PVC bags in a buffered sodium chloride formulation at 7 °C, 25 °C/60% RH and RTL with storage up to 201 days.

Samples from each admixture were analysed for piperacillin concentration, tazobactam concentration and appearance of decomposition products by stability indicating high-performance liquid chromatography (HPLC). The pH and appearance of solution and container were also monitored.

Shelf lives were calculated using the maximum rate method. Tazocin™ was found to be stable in 0.9% (w/v) sodium chloride in PVC bags for up to 5 days at 7 °C and 4 days at both 25 °C and RTL. In non-PVC bags, it was stable for 17, 4 and 3 days, respectively. It was stable in the buffered sodium chloride formulation in non-PVC bags for up to 58 days at 7 °C, 10 days at 25 °C and 7 days at RTL.

Keywords: Piperacillin; Tazobactam; Tazocin; Stability; Shelf life

1. Introduction

Piperacillin (Fig. 1) is broad-spectrum semi-synthetic penicillin active against Gram positive and Gram negative bacteria. It is susceptible to hydrolysis by a range of β-lactamases. These enzymes inactivate β-lactam antibiotics by opening the β-lactam ring. Piperacillin is usually administered with tazobactam (Fig. 2), a penicillanic acid sulphone derivative that is a potent β-lactamase inhibitor.

Piperacillin/tazobactam combinations are used in the treatment of infections of the urinary tract, intra-abdominal infection, skin infections and bacterial septicaemia. It is given intra-venously in a ratio of piperacillin (as sodium salt) 8:1 tazobactam (as sodium salt), and is manufactured as Tazocin™ (4 g piperacillin, 0.5 g tazobactam).

Tazocin™ is frequently administered by intra-venous injection or infusion, usually after dilution in 0.9% (w/v) sodium chloride. In order to avoid the dose mismanagement and microbiological hazard of preparation in clinical areas, these solutions can be prepared in validated licensed aseptic facilities under the control of a pharmacist. For this to be viable, batch production needs to be adopted, which in turn relies on a long shelf life being available.

Mathew et al. [1] showed that piperacillin 60 mg/ml with tazobactam 7.5 mg/ml in 50 ml PVC bags, in both 0.9% (w/v) sodium chloride and 5% dextrose was stable for 2 days at 25 °C and 28 days at 5 °C.

* Corresponding author. Tel.: +44 161 4195011; fax: +44 161 4195394.
E-mail address: Diane.Rigge@stockport-tr.nwest.nhs.uk (D.C. Rigge).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.03.013

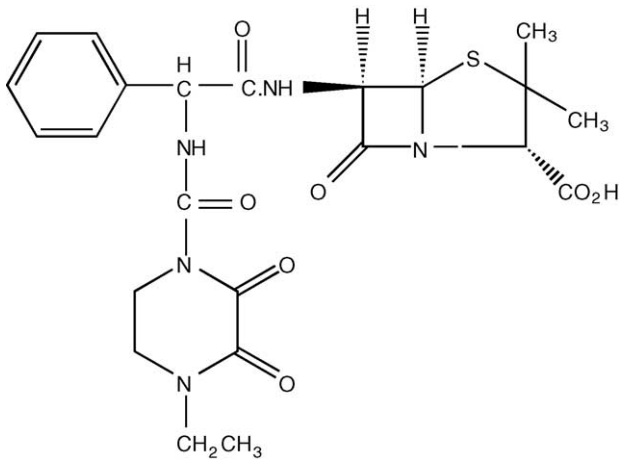

Fig. 1. Structure of piperacillin.

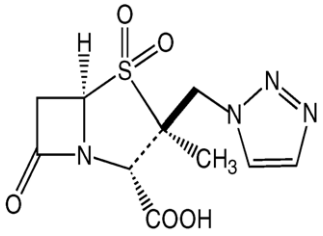

Fig. 2. Structure of tazobactam.

Das Gupta et al. [2] showed that piperacillin sodium 10 mg/ml in both 0.9% (w/v) sodium chloride and 5% dextrose in PVC bags was stable for 2 days at 25 °C, 28 days at 5 °C and at least 71 days at −10 °C.

Moon et al. [3] showed that piperacillin 150 mg/ml and 200 mg/ml with tazobactam 18.75 mg/ml and 25 mg/ml in polypropylene syringes in both 0.9% (w/v) sodium chloride and 5% dextrose were stable for 1 day at 25 °C, 7 days at 4 °C and 30 days at −15 °C. They also showed that piperacillin 80 mg/ml with tazobactam 10 mg/ml in PVC minibags was stable in both diluents for 30 days at −15 °C.

There was a need for a more prolonged and detailed study to allocate a maximum shelf life appropriate for the infusion formulations.

2. Experimental

2.1. Materials and reagents

All commercial reagents and materials were obtained from VWR International Ltd. (Lutterworth, England). Piperacillin analytical standard was purchased from Sigma–Aldrich Company Ltd. (Poole, England). Tazocin™ 4.5 g vials were kindly donated by Wyeth Pharmaceuticals (Maidenhead, England). Polyvinyl chloride (PVC) 100 ml bags and polyolefine laminate (non-PVC) 100 ml bags containing 0.9% (w/v) sodium chloride were kindly supplied by Baxter Healthcare Ltd. (Thetford, England). Non-PVC 100 ml bags were purchased from Sengewald GmbH (Halle, Germany) and filled with an isotonic mixture of a citrate buffer and 0.72% sodium chloride with a nominal pH of 7.0 (buffered sodium chloride) by Preston Pharmaceuticals (Preston, England).

2.2. Apparatus and chromatographic conditions

The high-performance liquid chromatographic (HPLC) system (Thermo Electron, Hemel Hempstead, England) consisted of a vacuum degasser, binary gradient pump (P200), autosampler fitted with sample preparation (AS3000) and a UV–vis detector (UV150). Chromatographic results were collected by data handling software (Scientific Software Inc., EzChrom Elite Version 2.61, Aston Scientific Ltd., Stoke Mandeville, England). Measurements of pH were carried out using a combination electrode pH meter (Corning, Model 120, Halstead, England). The chromatographic separation was performed at ambient temperature on a reversed phase Platinum EPS C18 column (Alltech Associates Inc., Carnforth, England) 250 mm × 4.66 mm i.d., 5 μm particle size. Elution was established with a mobile phase composition of acetonitrile and water (35:65, v/v) containing 0.4% tetrabutylammonium hydroxide pH 3.5 at a flow rate of 1.0 ml/min. The chromatographic signal was monitored at 230 nm. The injection volume was 10 μl.

2.3. Standard and sample solutions for HPLC analysis

For the assay of piperacillin, piperacillin sodium in water at a nominal concentration of 1 mg/ml (as piperacillin free acid) was further diluted in water to give a final injection concentration of 0.1 mg/ml. For the assay of tazobactam, a specified and reserved batch of Tazocin™ powder for injection was used. Tazocin™ in water at a concentration equivalent to 0.13 mg/ml tazobactam free acid was further diluted in water to give a final injection concentration of 0.013 mg/ml tazobactam.

Samples were diluted in water to give a final injection concentration of 0.08 mg/ml piperacillin and 0.01 mg/ml tazobactam.

All dilutions were carried out using the autodilution and sample preparation function of the autosampler.

2.4. Preparation of admixtures

Tazocin™ 4.5 g was reconstituted with 20 ml of 0.9% (w/v) sodium chloride or buffered sodium chloride taken from a 100 ml i.v. infusion bag. After reconstitution, the solution was replaced in the appropriate bag giving final concentrations of 40 mg/ml piperacillin and 5 mg/ml tazobactam.

2.5. Stability study protocol

Two PVC and two non-PVC bags each containing Tazocin™ in 0.9% (w/v) sodium chloride, and two non-

PVC bags containing Tazocin™ in buffered sodium chloride were stored at each of 7 ± 1 °C, 25 ± 2 °C and 60% relative humidity (RH) ±5% and at room temperature in the light (RTL).

Seven degree celsius was chosen to reflect the highest temperature the bags were likely to be exposed to in a hospital refrigerator conforming to the ICH guideline condition for refrigerated storage [4]. 25 °C/60% RH is the ICH guideline condition for long term stability testing and is representative of room temperature storage (we note however that the current requirement is for presentations in semi-permeable containers to be stored at 25 °C/40% RH). Room temperature exposed to continuous irradiation from daylight fluorescent tubes was chosen to reflect the conditions the bags may be exposed to on a hospital ward.

At each time point, duplicate samples from each bag were tested for piperacillin content, tazobactam content and pH. The presence of decomposition products, and the appearance of solution and container were also monitored.

2.6. Calculation of shelf life

The shelf life was calculated using the confidence bound, or maximum rate method [5].

The slope of the regression line of ln(concentration) versus time represents the rate constant (k) of the decomposition. The regression line was constructed using all the individual assay values.

The maximum rate method calculates the upper confidence bound of this rate of decomposition, which thus corresponds to the maximum rate of decomposition represented by the analytical data.

A validated Excel™ spreadsheet was used to calculate the shelf lives based on an acceptable loss of 10% of the initial concentration of the least stable constituent of the mixture (t_{90}).

3. Results

3.1. Validation of the analytical method

Four peaks were observed in Tazocin™ sample chromatograms (Fig. 3). Piperacillin was tentatively identified as the largest peak at 12.13 min by retention time comparison with authentic piperacillin. The two small peaks at 6.36 and 9.01 min were also present in the piperacillin raw material chromatogram and were assumed to be piperacillin related compounds. The peak at 5.95 min was assumed to be tazobactam, the only other component of Tazocin™.

All peaks were base line separated and had resolutions (Rs) greater than 1.

In order to determine whether the proposed method could separate piperacillin and tazobactam degradation products, samples containing 0.15 mg/ml of Tazocin™ were heated at

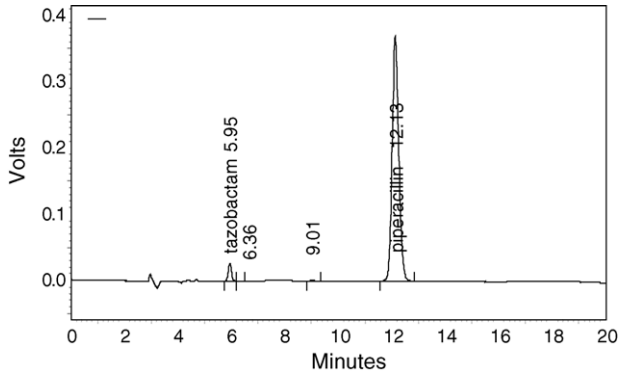

Fig. 3. Chromatogram of sample.

60 °C for up to 26 h. These showed a decrease in tazobactam and piperacillin peak areas. The additional peaks observed did not interfere with either analyte peak (Fig. 4). Accelerated decomposition of piperacillin solutions not containing tazobactam confirmed that these peaks seen in the Tazocin™ decomposed sample chromatogram were due to decomposition products of piperacillin.

The most likely decomposition route in aqueous solution is cleavage of the β-lactam moiety [6]. Penicillins in general have been shown to decompose in this way (Fig. 5). The bicyclic β-lactam-thiazolidine structure is more sensitive than simple β-lactam structures to decomposition by nucleophiles, electrophiles, oxidising agents and water molecules. The initial β-lactam cleavage is responsible for many succeeding degradation reactions.

The antibacterial activity of piperacillin is related to the fused β-lactam-thiazolidine ring, and breakage of this results in loss of activity [7].

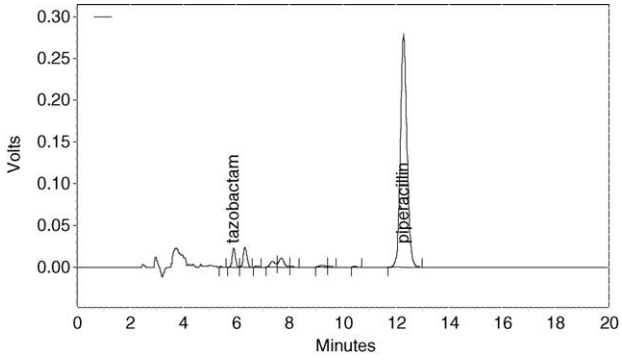

Fig. 4. Chromatogram of decomposed sample.

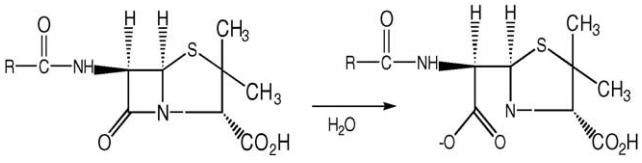

Fig. 5. Cleavage of β-lactam moiety.

3.1.1. Precision, accuracy, recovery and linearity

Precision was established by assaying six replicate sample preparations. Intermediate precision was carried out using a different HPLC instrument on a different day. The overall precision for piperacillin and tazobactam was 0.85% R.S.D. and 0.68% R.S.D. ($n = 12$), respectively.

The accuracy of the assay system was assessed by spiking a solution of 0.9% (w/v) sodium chloride with 20%, 50%, 75%, 100% and 125% of the label claim (45 mg/ml) with Tazocin™ powder. The average recovery at the 100% level was 98.3% piperacillin and 101.0% tazobactam, calculated using the equation:

$$\left[\frac{\text{actual amount found (mg/ml)}}{\text{theoretical amount (mg/ml)}}\right] \times 100$$

The linear regression analysis of the dependence of the amount found in mg/ml (y) on the amount added in mg/ml (x) in the accuracy determination gave the relationships $y = 0.9659x + 0.596$, with a correlation coefficient of 0.9995 for piperacillin and $y = 0.9797x + 0.1502$, with a correlation coefficient of 0.9994 for tazobactam.

3.2. Results and discussion of stability study

For PVC and non-PVC bags containing 0.9% (w/v) sodium chloride, the rate of piperacillin decomposition, reflected in the rate constants, shows negligible difference between 25 °C/60% RH and RTL storage, or between the bag types. At 7 °C, however, a significantly slower rate of decomposition was noted for non-PVC bags as opposed to PVC, resulting in shelf lives of 17 and 5 days, respectively (Table 1). The tazobactam concentrations also decreased but at a slower rate (Table 2). One explanation of these differences could be that at the higher temperature, the reaction rate is so fast that the nature of the storage container becomes insignificant. At refrigerated temperature, however, the rate of decomposition is much slower and the time spent in the container allows sorption to the container wall to occur.

Samples in buffered sodium chloride showed greater stability under all temperature conditions. RTL showed the fastest rate of decomposition and 7 °C showed the slowest (Table 3).

The pH decreased marginally at all temperature conditions in both diluents and bag types. In 0.9% (w/v) sodium chloride, the pH fell from 5.3 to 4.5 and in buffered sodium chloride, the pH fell from 6.6 to 5.2. The appearance of the solutions in both diluents and bag types remained clear and colourless at 7 °C. At 25 °C/60% RH in 0.9% (w/v) sodium chloride, a slight yellow colour was noted after 21 days storage in both bag types. At RTL, non-PVC bags showed colouration sooner than the PVC bags. The appearance of the solutions in buffered sodium chloride at 25 °C showed a very slight yellow colouration after 65 days. At RTL, colour appeared at 21 days turning to a pale yellow colour after 65 days.

Tazocin™ was considerably more stable in buffered sodium chloride than unbuffered sodium chloride, suggesting that pH has a large influence on stability. The optimum pH of stability for the β-lactam antibiotics in general, is between 6 and 7 [7], this strongly supports our findings as the initial pH of the buffered sodium chloride solution was 6.6.

Table 1
Percent of initial piperacillin remaining (PVC and non-PVC bags containing 0.9% (w/v) sodium chloride)

Time point (days)	PVC bags percent of initial remaining			Non-PVC bags percent of initial remaining		
	7 °C	25 °C	RTL	7 °C	25 °C	RTL
0	100.0	100.0	100.0	100.0	100.0	100.0
2	105.0	101.3	102.3	–	–	–
4	–	–	–	104.4	92.6	84.2
6	96.1	87.7	88.9	–	–	–
7	–	–	–	100.7	85.4	78.1
8	100.5	86.8	89.5	–	–	–
10	100.1	80.4	83.4	–	–	–
14	–	–	–	94.0	66.7	54.9
15	94.8	73.6	78.4	–	–	–
21	–	–	–	92.8	56.4	44.9
23	62.0	58.9	62.2	–	–	–
27	–	–	–	85.4	46.8	36.1
29	59.3	49.1	50.4	–	–	–
35	–	–	–	82.0	38.7	28.9
41	67.0	36.6	35.5	–	–	–
43	–	–	–	82.0	–	–
68	–	–	–	72.4	–	–
98	–	–	–	58.0	–	–
Rate constant (k) days^{-1}	−0.0148	−0.0253	−0.0257	−0.00563	−0.0281	−0.0360
Calculated shelf life (days)	5.8	4.0	3.8	17.7	3.6	2.8

Table 2
Percent of initial tazobactam remaining (PVC and non-PVC bags containing 0.9% (w/v) sodium chloride)

Time point (days)	PVC bags percent of initial remaining			Non-PVC bags percent of initial remaining		
	7 °C	25 °C	RTL	7 °C	25 °C	RTL
0	100.0	100.0	100.0	100.0	100.0	100.0
2	103.4	101.6	102.8	–	–	–
4	–	–	–	104.2	94.0	95.6
6	104.6	103.0	103.1	–	–	–
7	–	–	–	103.0	95.3	94.5
8	108.0	103.2	105.7	–	–	–
10	106.2	101.2	104.2	–	–	–
14	–	–	–	98.4	88.4	84.4
15	101.8	95.6	98.7	–	–	–
21	–	–	–	94.0	79.9	74.3
23	96.8	93.5	97.2	–	–	–
27	–	–	–	92.6	78.3	73.3
29	91.3	81.3	82.3	–	–	–
35	–	–	–	97.0	77.6	71.5
41	92.2	75.8	76.4	–	–	–
43	–	–	–	93.3	–	–
68	–	–	–	94.0	–	–
98	–	–	–	86.9	–	–
Rate constant (k) days^{-1}	−0.0036	−0.0077	−0.0076	−0.00137	−0.0079	−0.0107

Table 3
Percent of initial piperacillin and tazobactam remaining (non-PVC bags containing buffered sodium chloride)

Time point (days)	Piperacillin percent of initial remaining			Tazobactam percent of initial remaining		
	7 °C	25 °C	RTL	7 °C	25 °C	RTL
0	100.0	100.0	100.0	100.0	100.0	100.0
2	98.0	97.7	97.7	100.6	99.2	98.7
7	97.6	95.4	92.6	103.3	100.0	95.8
16	99.3	92.8	88.4	99.0	92.6	89.3
21	99.7	91.1	84.8	100.8	93.4	87.6
28	93.8	84.7	75.2	95.6	85.6	77.5
41	96.1	72.6	63.6	97.7	79.5	73.7
65	93.0	54.3	41.9	94.6	63.2	51.7
100	88.6	–	–	86.8	–	–
147	78.5	–	–	79.9	–	–
201	70.7	–	–	73.4	–	–
Rate constant (k) days^{-1}	−0.00166	−0.0091	−0.0130	−0.00164	−0.0070	−0.0098
Calculated shelf life (days)	58.4	10.6	7.5	–	–	–

4. Conclusion

Results of the study concluded that Tazocin™ 45 mg/ml in 0.9% (w/v) sodium chloride in PVC bags is stable for up to 5 days at 7 °C, 4 days at 25 °C and 4 days at RTL. In non-PVC bags, it is stable for up to 17, 4 and 3 days, respectively.

Tazocin™ 45 mg/ml has been shown to be considerably more stable when diluted in the buffered sodium chloride vehicle and stored in non-PVC bags. It is stable for up to 58 days at 7 °C, 10 days at 25 °C and 7 days at RTL.

Acknowledgements

The authors wish to acknowledge the kind donation of Tazocin® from Wyeth Pharmaceuticals, and the intra-venous infusion bags from Baxter Healthcare Ltd. We also thank the NHS Executive North West R&D Directorate for the grant, which made the study possible.

References

[1] M. Mathew, V. Das Gupta, C. Bethea, J. Clin. Pharm. Ther. 19 (1994) 397–399.
[2] V. Das Gupta, D.D. Davis, K.R. Stewart, Am. J. Intra. Ther. Clin. Nutr. 11 (1984) 14–19.
[3] Y.S.K. Moon, K.C. Chung, A. Chin, M.A. Gill, Am. J. Health Syst. Pharm. 52 (1995) 999–1001.
[4] ICH QIE Evaluation of stability data, The European Agency for the Evaluation of Medicinal Products, London, 2002.
[5] T.E. Norwood, Drug Dev. Ind. Pharm. 12 (1986) 553–560.
[6] T. Yamana, et al., Int. J. Pharm. 11 (1982) 71–80.
[7] J.P. Hou, J.W. Poole, J. Pharm. Sci. 60 (1971) 503–531.