

Penicillin stability in prefilled syringes for the purpose of skin testing for drug allergy

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Clinical Implications

- Dilutions of penicillin class drugs in syringes, as required for skin testing for drug allergy, are stable for 2 to 7 days. This study supports a change in clinical practice where reagents for penicillin skin prick tests and intradermal tests can be prepared in advance by pharmacy, improving safety and workflow.

TO THE EDITOR:

Up to 10% of the patients attending hospitals report an allergy to penicillin, though only about 6% to 10% of these have a true IgE-mediated allergy, proven by cutaneous tests and drug provocation.^{1,2} A label of penicillin allergy when a true allergy is not present often leads to lifelong avoidance of penicillin and related drugs.³ This leads to the use of less specific, more expensive antibiotics, contributing to increased adverse effects and antibiotic resistance.

The negative predictive value for penicillin skin testing for immediate reaction is close to 100%, whereas the positive predictive value is between 40% and 100%.⁴ Diagnosis is currently confirmed using cutaneous tests (skin prick testing [SPT] and intradermal testing [IDT]) with penicilloyl poly-L-lysine (PPL) as the major determinant of benzylpenicillin and minor determinant mixture (MDM) containing benzylpenicillin, benzylpenicilloate, and benzylpenilloate.^{5,6} SPT and IDT, using penicillin dilutions and PPL and MDM, are widely recommended for testing immediate hypersensitivity to penicillin class antibiotics.⁷ In Europe, both PPL and MDM are available with Diater DAP (Madrid, Spain). In the United States, only PPL is available as PRE-PEN (AllerQuest LLC, Plainville, Conn).

Traditionally, test reagents, including drug dilutions and allergic determinants, are made at the bedside by the allergist because there is no evidence of their stability in the syringes these are prepared in. This is a time-consuming, labor-intensive practice and limits the number of patients who can be tested at one time. Also, with multiple antibiotics, at multiple dilutions, errors can be made. If these dilutions could be prepared in advance in pharmacy under aseptic conditions and labeled appropriately, it would not only improve safety of testing but also contribute significantly to improving the workflow of allergy testing clinics and facilitate more patients being tested. There would be financial gains in terms of less drug wastage and time saved in preparing syringes for multiple patients together rather than one at a time. Table 1⁸ details the reagents used for SPT for penicillin class antibiotics. Other drugs can be included on the basis of individual patient requirements.

Pharmacy at Women's and Children's Hospital in Adelaide makes these dilutions within 24 hours of testing, so that patients do not have to wait while these dilutions are being made. However, there is no literature regarding the stability of these dilutions in the syringes in which they are prepared in advance. Recent information from Diater (available in Australia) confirms the stability of PPL and MDM after reconstitution to be 15 days in refrigerated conditions (Sanchez J, 2013; unpublished data).

The aim of this study was to carry out validated stability testing of penicillin class drugs commonly used for SPT and IDT in syringes to enable their preparation before the tests.

HPLC analytical methods were developed for benzylpenicillin sodium (Penicillin G sodium salt, Sigma, Sydney, Australia), amoxicillin sodium (Amoxil, GlaxoSmithKline, Uxbridge, UK), and flucloxacillin sodium (DBL Flucloxacillin sodium, Hospira Australia, Melbourne, Australia) to ensure that they were adequate and repeatable. The assay methods were validated for linearity, specificity, precision, and stability in the analytical solution.

Analysis was carried out using HPLC (Shimadzu, Kyoto, Japan) and Phenomenex Kromasil C-18 column. The mobile phases included acetonitrile, potassium dihydrogen phosphate, and sodium hydroxide for amoxicillin HPLC analysis; methanol, potassium dihydrogen phosphate, and orthophosphoric acid for benzylpenicillin HPLC analysis; and acetonitrile, potassium dihydrogen phosphate, and sodium hydroxide for flucloxacillin HPLC analysis.

Dilutions of benzylpenicillin sodium (0.6 mg/mL, pH 7.06, and 6 mg/mL, pH 6.98), amoxicillin sodium (2 mg/mL, pH 8.6, and 20 mg/mL, pH 8.66), and flucloxacillin sodium (0.5 mg/mL, pH 6.05, and 5 mg/mL, pH 5.24) were aseptically prepared in sterile water for injection and packed in 1-mL Leur-lock polycarbonate syringes with "Combi" syringe caps. This was done in a sterile pharmacy manufacturing facility by aseptically validated pharmacists. Water for injection was used to further dilute to the required concentrations. Three batches for each dilution were prepared, and 2 sample replicates were drawn from each batch for each sampling point and each temperature.

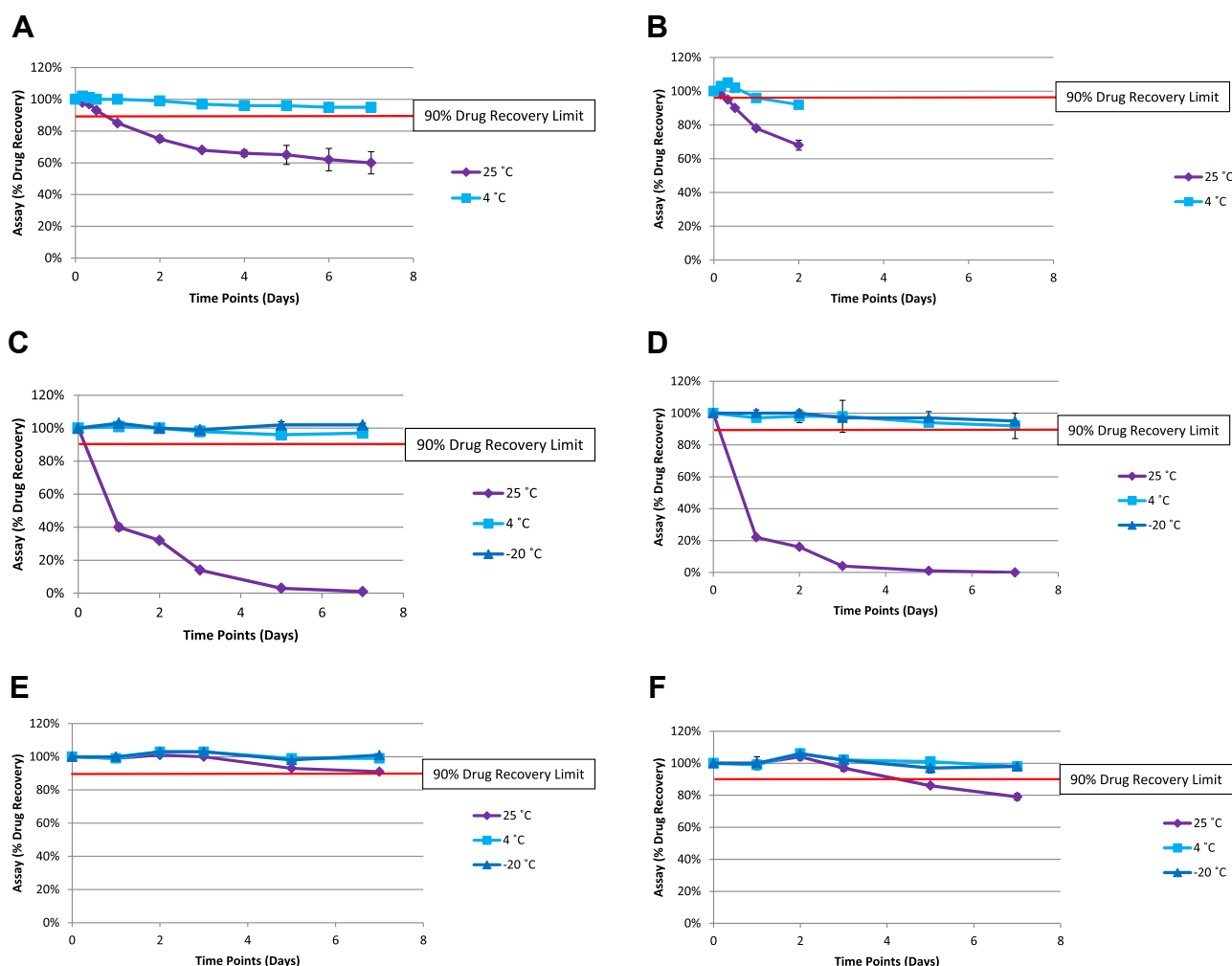
The syringes were transferred to the analytical testing laboratory immediately postproduction, maintaining the cold chain, and placed in stability chambers (Newtronic, Mumbai, India) at 25°C, 4°C, and -20°C (Refrigerator/freezer; Samsung, Seoul, South Korea). Samples were withdrawn at different time points ranging from 0 to 7 days⁹ and assessed for physical (color change, precipitation) and chemical (drug recovery $\geq 90\%$ by HPLC¹⁰) stability.

No color change or precipitation was observed for the 3 penicillins tested at all temperatures throughout the stability study. All drug dilutions were thereby deemed physically stable at all temperatures tested.

Figure 1, A to F, depicts the chemical stability of the tested penicillins (percent drug recovery with respect to time). Based on these results, new shelf life was determined for the dilutions in syringes of the penicillin class antibiotics tested, as detailed in Table II.

TABLE I. Agents for skin testing of penicillin class antibiotic allergy⁸

Drug	SPT concentration	IDT concentration 1	IDT concentration 2	IDT concentration 3
Histamine	10 mg/mL			
Amoxicillin sodium	20 mg/mL	2 mg/mL	20 mg/mL	
Benzylpenicillin sodium	6 mg/mL	0.6 mg/mL (1,000 IU)	6 mg/mL (10,000 IU)	
Flucloxacillin sodium	5 mg/mL	0.5 mg/mL	5 mg/mL	
PPL (containing 0.04 mg of benzoylpenicilloyl poly-L-lysine in 1 mL)	Full strength	1:10 dilution	Full strength	
MDM (containing 0.5 mg each of sodium benzylpenicillin, benzylpenicilloic acid, and sodium benzylpenicilloate in 1 mL)	Full strength	1:100 dilution	1:10 dilution	Full strength

**FIGURE 1.** A-F, Summary stability data of amoxicillin sodium (A and B, 2 mg/mL and 20 mg/mL, respectively), benzylpenicillin sodium (C and D, 0.6 mg/mL and 6 mg/mL, respectively), and flucloxacillin sodium (E and F, 0.5 mg/mL and 5 mg/mL, respectively).

Amoxicillin sodium exhibited concentration-dependent degradation kinetics, with higher concentration samples degrading faster. The 20 mg/mL solution reached below the threshold of 90% concentration on day 2 at 4°C and 12 hours at room temperature. At -20°C, amoxicillin sodium degraded below 90% within 4 hours. The study further to that time point was thereby suspended.

The stability studies of the penicillins for injection (amoxicillin sodium, benzylpenicillin sodium, and flucloxacillin sodium) in syringes confirmed that the formulations are physically stable throughout the study period (7 days), showing no color changes or precipitation. A shelf life of longer than 24 hours (as given currently) has been established for all formulations at refrigerated conditions, and some at room temperature. Both

TABLE II. Summary of current and proposed shelf life for the penicillin class antibiotics tested

Drug name (concentration)	Current shelf life (4°C)	Proposed shelf life		
		4°C	25°C	–20°C
Amoxicillin sodium (2 mg/mL)	24 h	7 d	Unstable*	Unstable*
Amoxicillin sodium (20 mg/mL)	24 h	2 d	Unstable*	Unstable*
Benzylpenicillin sodium (0.6 mg/mL)	24 h	7 d	Unstable*	7 d
Benzylpenicillin sodium (6 mg/mL)	24 h	7 d	Unstable*	7 d
Flucloxacillin sodium (0.5 mg/mL)	24 h	7 d	7 d	7 d
Flucloxacillin sodium (5 mg/mL)	24 h	7 d	3 d	7 d

*Stability less than 24 h.

PPL and MDM have a stability of 15 days after reconstitution as stated by Diater laboratories.

Some patients with immediate reactions to amoxicillin have IgE antibodies directed against the R group side chain. Given that benzylpenicillin sodium and amoxicillin sodium remain among the most commonly prescribed antibiotics, amoxicillin is often clinically useful in a testing algorithm. Flucloxacillin sodium appears to be more stable, but testing is usually performed only when it is the culprit drug, and should not replace amoxicillin in a testing algorithm based on better stability.^{11,12}

This study confirms that penicillin allergy SPT and IDT reagents do not have to be prepared by the bedside and supports a change in clinical practice. The syringes can be aseptically prepared in advance and remain stable for up to 2 to 7 days when stored at 4°C. Future studies in patients with confirmed antibiotic allergy and positive skin test results are recommended to validate these findings in the clinical setting.

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