

The Stability and Sterility of Epinephrine Prefilled Syringe

Saowanee Kerddonfak, Wiparat Manuyakorn, Wasu Kamchaisatian, Cherapat Sasisakulporn, Wanlapa Teawsomboonkit and Suwat Benjaponpitak

SUMMARY The commercially available auto-injector epinephrine is considerable expensive. Epinephrine pre-filled syringe is an alternative treatment for anaphylaxis patients. The objective of the present study was to evaluate the stability and sterility of epinephrine prefilled syringe. Epinephrine prefilled syringe was kept in the pencil box to prevent from light exposure. The active ingredients, integrity and level of potency were measured by high-performance liquid chromatography (HPLC). The sterility was accessed by aerobic bacteria and fungi culture. The epinephrine concentration at 1, 2 and 3 months after the preparation was 101.36, 99.31 and 101.09%, respectively (acceptable range 90 - 110%). The pH was 3.17 - 3.23 (acceptable range 2.8 - 3.6). Nor-epinephrine was undetected. The cultures for bacteria and fungus were both negative. Consequently, epinephrine prefilled syringe was stable and sterile at least three month after preparation. Epinephrine prefilled syringe is an alternative low cost treatment for anaphylaxis patient.

Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death. It is commonly triggered by food, insect sting, medications or natural rubber latex. Epinephrine (adrenaline) is widely advocated as the main drug in the treatment of anaphylaxis. There are no other medications with similar efficacy on the body systems that are potentially involved by anaphylaxis.¹

Epinephrine is an endogenous catecholamine produced primarily in the adrenal medulla with a wide variety of clinical uses. Epinephrine is poorly soluble in water, but readily forms water-soluble salts in the presence of acid. Injectable solution is buffered to maintain a pH at 2.2 to 5.0. Epinephrine solution is colorless. However, oxidation of the catechol nucleus will impart a pink color then changes to a pink-brown color.^{2,3}

In anaphylaxis, the vasopressive effect of epinephrine, along with its effects in preventing and relieving laryngeal edema and bronchoconstriction is life saving. Epinephrine also generates an inotropic effect, which increases in heart rate, contraction of the heart and vasoconstriction, thereby increasing the blood pressure.

The efficacy of epinephrine in suppression of mast cells and basophils inflammatory mediators' release is also considerably importance.⁴ The fatality during witnessed anaphylaxis, most of which occurs outside of medical facility, usually results from de-

From the Division of Allergy and Immunology, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand 10400
Correspondence: Suwat Benjaponpitak
E-mail: rasbj@mahidol.ac.th

layed administration of epinephrine.⁵ For out of hospital emergency treatment, epinephrine auto injector such as EpiPen[®] is prescribed. However, self-injectable epinephrine is underused when anaphylaxis occurs.⁶ The drawback of epinephrine autoinjector includes high cost which limits affordability and availability worldwide.⁷ Moreover, it is impossible to give an accurate dosage for infant and many children by using currently available autoinjector fixed epinephrine dose 0.15 or 0.3 mg.⁸

In Thailand, the prefilled syringe with an appropriate dose of epinephrine is prescribed to patients and parents of children with history of anaphylaxis, those currently on immunotherapy treatment and patients with history of severe asthma with food allergy. Serious concern arises, particularly in hot climates, from possible partial solution contaminations and degradation of the drug.^{9, 10}

The aim of this study was to determine the physical, chemical stability and sterility of epinephrine prefilled syringe comparing between drawing up from ampules into disposable 1-ml syringes under laminar flow hood (sterile technique) and open air.

MATERIAL AND METHODS

Materials

Epinephrine 1 mg/ml from the same batch were purchased from the Government Pharmaceutical Organization (GPO, Bangkok, Thailand). Disposable plastic 1-ml syringes and 23-gauges needles were purchased from Nipro corporation (Osaka, Japan).

Preparation and storage of prefilled epinephrine syringes

We evaluated the effect of different mode of preparation (under laminar flow hood and open air) on chemical, physical stability and sterility of a 1.0 mg of epinephrine dose loaded in 1 ml disposable syringe overtime up to 3 months after preparing and storing in the container under room temperature.

One hundred and forty syringes were loaded with 1 ml of a 1-mg/ml epinephrine solution under laminar flow hood or open air. All of the epinephrine

doses were drawn up by the same person and on the same day. Air bubbles were removed to reduce oxygen exposure during storage. Needle were left attached and recapped.

Seventy prefilled epinephrine syringes in each group were randomly allocated to 3 time points (1 month, 2 months and 3 months). All of the syringes were kept in the pencil boxes and left in room temperature. Room temperature was recorded everyday at 8.00 - 9.00 am.

Physical and chemical stability evaluation

At each time point, 20 prefilled syringes were randomly selected for evaluation. Initially, a visual inspection was made and any observation was noted (dirt, deterioration, cracks, etc.). The pH of each sample was determined using a pH meter. Epinephrine and nor-epinephrine, which is the product of epinephrine degradation in solution, concentrations were measured by high-performance liquid chromatography (HPLC). Analysis was performed on a reverse-phase Inertsil ODs 3 C18 HPLC column (4.6 mm x 150 mm) equilibrated with mobile phase at a flow rate of 1.0 ml/minute. Ultraviolet absorbance was monitored at 205 nm. Using a variable wavelength ultraviolet detector (Dionex UVD 340 u) an injection valve was configured with a 20 μ l sample loop. Epinephrine concentration was determined by comparing the peak area ratio (the ratio of the area under the epinephrine peak to that of the area under the internal standard peak) of sample solution to a known standard concentration of epinephrine. Standard solution of epinephrine bitartrate (0.02% w/v), sample solution (0.012% w/v) and the internal standard paracetamol (0.08% w/v) were prepared in mobile phase. The mobile phase was prepared by combining methanol and 10 mM sodium heptane sulfonate in water pH 3.5 containing 0.0002 M Disodium Edentate (23:77).

Standard solution of nor-epinephrine bitartrate (0.0018% w/v) was prepared in mobile phase. Sample solution was the epinephrine injection (non-diluted). The peak area of nor-epinephrine obtained from the epinephrine injection does not exceed the normalized peak area of nor-epinephrine bitartrate (0.0018% w/v).¹¹

Bacterial and fungal culture for sterility evaluation

At each time point 2 prefilled syringes were randomly selected for evaluation. Each specimen of epinephrine (1 ml) was inoculated into a blood agar and incubated at 35°C in air. Each inoculate was examined for growth every 24 hour for 3 days. This microbiological method supported the growth of gram-negative and gram-positive bacteria. For fungal cultures, inoculation was made into brain heart blood agar at 37 °C for 3 days and inoculation was into sabouraud dextrose agar at 37 °C for 1 month.

Statistical analyses

Data obtain from this study, including general appearance, epinephrine and nor-epinephrine concentration, pH values and presence or absence of microbial growth from each epinephrine syringe, and were described as descriptive data.

RESULTS

A total of 140 of epinephrine prefilled syringes were prepared and stored at room temperature (26 ± 3 °C) in the pencil boxes for light protection. All samples were clear in appearance at 1, 2 and 3 months from preparation time, respectively. Epinephrine concentration was 99.31 - 102.68% (acceptable range 90 - 110%). The pH was 3.17 - 3.23 (acceptable range 2.8 - 3.6) as shown in Table 1. No-repinephrine, which is a degradation product of epinephrine, was not detected in any sample. There were no aerobic bacterial or fungal growths in any sample after 1, 2 and 3 month from preparation time.

However, we found some brown particles at the needle cap in some syringes. These brown par-

ticles were sent for bacterial and fungal culture with negative result. We hypothesized that the brown particles were from the reaction between epinephrine and the environment.

DISCUSSION

In life-threatening emergencies, it is essential to immediately inject epinephrine to the anaphylactic patient. In many countries, autoinjector epinephrine is unaffordable or unavailable, anaphylaxis patients usually are prescribed with an ampule of epinephrine and a 1-ml syringe and needle. However, when anaphylaxis occurs in the community, patients or caregivers of children have to draw up epinephrine from an ampule at a time of crisis. A previous study has demonstrated that most parents are unable to draw up an infant epinephrine dose rapidly and/or accurately. The parents had a significantly longer time to draw up an epinephrine dose (142 ± 13 seconds) compared to emergency department nurses (29 ± 0.09 seconds).⁹ Consequently, it is much better to have syringes already prepared rather than drawing up from the ampoules at the emergency time.¹² Prefilled epinephrine syringes have become commonly used in unaffordable or unavailable country such as developing country. The reason for the widespread use of prefilled epinephrine syringes over EpiPen® is their much lower price.¹³ Moreover, EpiPen® is only available in a fix dosage which may cause problem with infant and young children of overdose of epinephrine and under dose obese.

According to the United States Pharmacopoeia (USP) stability guidelines, a product has chemical stability if each of the active ingredients retains its integrity and labeled potency overtime and microbiological stability of the product remains sterile and resistant to microbial growth. The USP/National

Table 1 The epinephrine concentration and pH of the solution in prefilled syringes left over 1, 2 and 3 months

Time	1 month		2 months		3 months		
	Laminar flow hood	Open air	Laminar flow hood	Open air	Laminar flow hood	Open air	
Epinephrine concentration	90-110%	101.40	101.36	102.68	99.31	101.19	101.09
pH	2.8-3.6	3.22	3.23	3.22	3.22	3.17	3.17

Formulary monograph requires the product to contain $100 \pm 10\%$ of label potency. Therefore, the t90% of product represents the effective shelf-life of product, otherwise known as the expiry time. The shelf-life stability of epinephrine injection stored in US hospitals was analyzed as part of the FDA-ASHP voluntary drug stability program. USP requirements are as follow: epinephrine injection is formulated to contain 90 - 115% of the labeled amount of epinephrine, the pH of the injections should be in the range of 2.5 - 5.0 There are no compound requirements for degradation products (such as nor-epinephrine).¹¹

The epinephrine ampoule is recommended to be stored between 15 to 30 °C by the manufacturer in order to keep its stability. In Thailand, however, the temperature is between 23.9 °C to 34.9 °C. Kelly, et al¹⁴ found that epinephrine stability decreased by exposure to elevated temperature. Storage at 37 °C for 6 months decreased epinephrine concentration by 50%. Fry, et al¹⁵ also reported that the rate of epinephrine degradation will increase if the temperature increases. Storage at 50 °C causes a 25% loss within 2 months. Grant, et al² determined the biological consequence of temperature induced epinephrine degradation and discovered that the environmental temperature variation causes degradation in epinephrine concentration and biological activity. The degradation of epinephrine ampoule (1:1000) was not significant even after 12 weeks of heat exposure. No change was noted from control¹⁶. It may be concluded that the epinephrine prefilled syringe should not be kept more than 3 months. Up to our knowledge, there are a few studies elucidating the stability of prefilled epinephrine syringes. A previous study has found that the stability of prefilled epinephrine solutions in unsealed syringes was 2 months and 3 months under 38°C with 15% and 95% humidity, respectively.¹⁷ Nevertheless, there is no earlier study has attempted to evaluate the sterility of prefilled epinephrine syringes before. In this current study, we have demonstrated that prefilled epinephrine syringe is stable 3 months either preparing in laminar flow hood or in open air. However, we have found the brown particle at the needle cap of some epinephrine prefilled syringes when we kept them up to 3 months. After test with bacterial and fungal culture, they all were negative. We hypothesized that the brown particle at the tip of the needle cap was caused by degradation of adrenaline via oxidation to adre-

nochrome, which turns pink first, then pink-brown oxidative product.¹⁸ Therefore, we recommended changing the needle after drawing up the epinephrine and do not push epinephrine back through the needle to keep it away from the air. Finally by lengthen time of observation, we found that the color of most of the 4-month prefilled epinephrine syringes was changed to pink-brown solution. On the basis of these result, we concluded that epinephrine prefilled syringe preparing in either laminar flow hood or open air was stable up to 3 months without loss of chemical stability or sterility.

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