Epinephrine Stability in Plastic Syringes and Glass Vials

Ronald F. Donnelly and Margaret Yen

ABSTRACT

As a result of a prospective, double-blind, randomized, controlled study to determine the optimal dose of epinephrine during cardiac arrest, the stability of diluted epinephrine stored in pre-loaded syringes and glass vials was investigated. The commercial epinephrine solution (1 mg/mL) was diluted with sterile water to either 1 mg or 7 mg/10 mL and packaged in glass vials or plastic syringes with 18G needles attached. No attempts were made to adjust pH nor were solutions packaged under nitrogen or additional antioxidant added. The containers were stored at room temperature and protected from light for the duration of the study. Analysis of the samples using a stability-indicating HPLC assay determined a significant decrease (> 10%) in epinephrine concentration in the 1 mg/10 mL solution stored in vials or syringes after 14 days. Solutions containing 7 mg/10 mL of epinephrine and packaged in syringes or vials were stable for 56 days. Therefore, this study indicates that when epinephrine 1 mg/10 mL is prepared extemporaneously in glass vials or as pre-loaded syringes and optimum packaging conditions are not incorporated, the solutions have only a short shelf-life. Solutions containing epinephrine 7 mg/10 mL and packaged in glass vials or pre-loaded syringes are stable for at least 56 days.

Key Words: *Stability, Epinephrine, Extemporaneous*

RÉSUMÉ

Suite aux résultats d'une étude prospective contrôlée qui a été menée à double insu et avec répartition aléatoire dans le but de déterminer la dose optimale d'épinéphrine dans les cas d'arrêt cardiaque, on a étudié la stabilité de l'épinéphrine diluée et conservée dans des seringues préremplies et des flacons de verre. Les solutions commerciales d'épinéphrine (1 mg/mL) ont été diluées avec de l'eau stérile à raison de 1 ou 7 mg/mL, puis conditionnées dans des flacons de verre ou des seringues de plastique munies d'aiguilles de calibre 18. Aucune tentative pour ajuster le pH ou conditionner les solutions avec de l'azote ou l'ajout d'un antioxydant n'a été faite. Les conditionnements ont été entreposés à la température ambiante et à l'abri de la lumière pour toute la durée de l'étude. Des analyses subséquentes des échantillons effectuées au moyen d'une épreuve de stabilité par chromatographie liquide à haute pression ont révélé une diminution de la concentration d'épinéphrine dans les dilutions de 1 mg/10 mL conditionnées dans des flacons ou des seringues, après 14 jours. Cependant, les dilutions d'épinéphrine de 7 mg/10 mL qui ont été conditionnées dans des seringues ou des flacons étaient stables durant 56 jours. Par conséquent, cette étude révèle que les préparations extemporanées d'épiniphrine de 7 mg/10 mL conditionnées dans des flacons de verres ou des seringues préchargées ont une durée de conservation brève. Par contraste, les solutions d'épinéphrine de 7 mg/10 mL conditionnées dans des flacons de verre ou des seringues préchargées sont stables durant au moins 56 jours.

Mots clés : *stabilité, épinéphrine, extemporané*

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INTRODUCTION

Epinephrine has been the most thoroughly evaluated drug used in advanced cardiac life support (ACLS). Yet, the optimal epinephrine dose for cardiac arrest therapy is an unresolved key issue. Redding and Pearson's work[1,2] in the 1960s found that administration of 1 mg of epinephrine hydrochloride was effective in 10 kg dogs (average weight interpolated). This is equal to 0.1 mg/kg. The current ACLS recommended adult dose is 0.5 to 1 mg IV every five minutes, without variation for body weight.[3] In a 70 kg patient, this is equal to 0.007 to 0.014 mg/kg. It is confusing that this dose is considerably lower than the mg/kg dose used in the experiment by Redding and Pearson in dogs, which is provided as a major reference in the ACLS guidelines.[1,2] No published human dose-response curves or volume of distribution during arrest seemed to exist as a basis for this difference.

An enlarging body of research[4,5,6] suggests that the currently recommended standard dose (1 mg every five minutes) may be too low. Therefore, a prospective, double-blind, randomized, controlled study involving 650 patients from the Ottawa Civic Hospital and the Ottawa General Hospital, two tertiary teaching institutions, was undertaken to compare the standard dose

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(1 mg every five minutes) with the high dose (7 mg [0.1 mg/kg] every five minutes) of epinephrine. Since epinephrine is supplied by the manufacturer as a 1 mg/mL ampoule, our pharmacy department was approached by the investigators to prepare the study drugs so that they would be identical in appearance, easy to administer during cardiac arrest, and reasonably stable to facilitate delivery and dispensing of the study drugs.

METHODS

Liquid Chromatographic Analysis (HPLC)

The chromatographic system consisted of an isocratic solvent pump (Model LC-10AS; Shimadzu Corpration; Kyoto, Japan), a photodiode array detector (Model SPD M6A; Shimadzu Corporation; Kyoto, Japan) set at 280 nm, a manual injector (Model 7010; Rheodyne Incorporated; Cotati, California) with a 20 µL sample loop and a 5 micron C^{18} column (Model LC-18-DB; Supelco Canada; Mississauga, Ontario). The mobile phase was prepared by mixing 85 volumes of buffer solution and 15 volumes of methanol (cat.# B90234; BDH Incorporated; Toronto, Ontario). The buffer solution consisted of 6.0 g of monobasic sodium phosphate (cat.# ACS 795; BDH Incorporated; Toronto, Ontario), 520 mg of 1-heptanesulfonic acid, sodium salt (cat.# B15278; BDH Incorporated; Toronto, Ontario) and 45 mg of disodium ethylenediaminetetraacetate (cat.# ACS 342; BDH Incorporated; Toronto, Ontario) dissolved in 1 litre of water. The pH was adjusted to 3.8 with phosphoric acid (cat.# ACS 591; BDH Incorporated; Toronto, Ontario). The internal standard used was 3, 4-dihydroxybenzylamine HBr (cat.# 85,878-1; Aldrich Chemical Company; St. Louis, MO).

Assay Validation

To determine the suitability of our HPLC method as a stability-indicating assay, a stock solution of epinephrine 1 mg/mL (cat # 02260; Fluka Chemical Corporation; Ronkonkoma, New York, NY) was diluted (1:10) with distilled water. The pH of 10 mL aliquots of the resulting solution was then adjusted to approximately one (with concentrated hydrochloric acid) and 12 (with 5 N sodium hydroxide). A third 10 mL aliquot had 30% hydrogen peroxide (1 mL) added to it. Samples from these degraded solutions were then analyzed by HPLC, over several days, and the chromatograms were inspected for the appearance of additional peaks. Both fresh and degraded samples were compared for a change in concentration, retention time, and peak shape. The UV spectra created by the photodiode detector for epinephrine samples, authentic epinephrine and all degradation products was compared for similarities by spectral overlay. Multi-channel analysis was also preformed to confirm peak purity.

To assess the linearity of the detector response for the samples tested, a 5-point standard curve from duplicate injections was prepared with solutions ranging from 0.05 to 0.13 mg/mL (50 to 134% of expected values). The sensitivity of the assay was also assessed to determine the minimum amount of epinephrine that could be measured by the assay. Reproducibility for intra-day and inter-day samples was determined by coefficient of variation (CV) which was defined as standard deviation divided by mean. A recovery study was conducted to measure the accuracy of the assay. Solutions, prepared from weighed samples, were analyzed and then compared to theoretical values.

Stability Study

Ampoules of epinephrine hydrochloride (1 mg/mL;cat.# 00155357; Parke-Davis Limited; Scarborough, Ontario) were diluted with sterile water for injection (cat.# 7990; Abbott Laboratories Limited; Montreal, Quebec) to make stock solutions of 1 mg and 7 mg per 10 mL. Aliquots (10 mL) of each solution were then packaged into either 10 mL glass vials (cat.# 7515ZA; Miles Incorporated; Spokane, Washington) or plastic syringes with 18G needles attached (cat.# 309604; Becton Dickinson & Company; Franklin Lakes, New Jersey). Samples were then stored at room temperature and protected from light. On days 0, 3, 7, 14, 28 and 56, the contents of two syringes from each concentration were transferred to separate empty glass vials and then all samples were stored at -70°C for analysis at a later date. The pH of the solutions was monitored over the course of the study.

Samples were thawed and brought to room temperature. An aliquot of 3 mL was then removed from the storage container. Each solution (750 µL) was combined with the internal standard (200 µL of 1 mg/mL solution). Samples were diluted either 1:2 (1 mg/10 mL) or 1:20 (7 mg/10 mL) with mobile phase and then analyzed in duplicate.

Means (± standard deviation) were calculated from samples prepared and analyzed in duplicate. An endpoint of no greater than 10% decrease from the initial concentration was chosen to indicate stability.

RESULTS

Assay Validation

The solutions of epinephrine that had the pH adjusted to one and 12, turned a dark brown colour indicating that degradation was occurring. The solution containing the hydrogen peroxide initially turned a pinkish colour but then changed to a dark brown colour. Analysis of

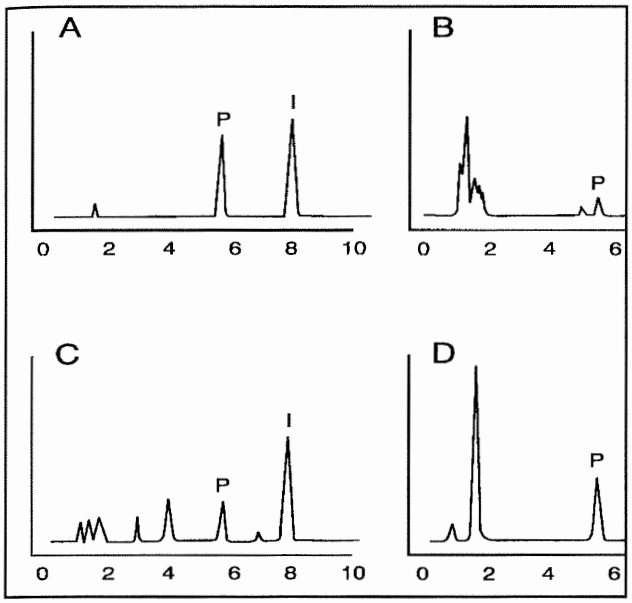

Figure 1: Sample chromatographs of epinephrine in sterile water:
A) fresh solution,
B) pH adjusted to 12 with 5 N sodium hydroxide,
C) pH adjusted to one with concentrated hydrochloric acid and
D) 1 mL of hydrogen peroxide 30 % added.
P = epinephrine, I = internal standard.

these solutions resulted in the detection of at least one additional peak in the chromatogram, which was not present in the fresh stock solution (Figure 1). These additional peaks did not interfere with either the epinephrine or internal standard peaks. Spectral and multichannel analysis of the epinephrine peaks from the various degradation samples showed no overlapping peaks. Spectral overlays of the epinephrine and degradation peaks showed limited similarities. Similar overlays between epinephrine and authentic material indicated good correlation.

The detector response curve was linear over the range studied (r^2=0.9988). The sensitivity of the assay was 20 ng. The intra-day and inter-day variances were 1.67% and 2.50%, respectively. The average recovery value was 101.1 ± 2.1%.

Stability Study

Over the course of the study, the solutions remained clear and colourless. However, there was a trend towards a decrease in concentration. At the 14-day point, the concentration of the 1 mg/10 mL solution stored in vials and syringes was below 90% of the original concentration. At 56 days, the high concentration solutions, 7 mg/10 mL, packaged in vials and syringes were still above the end-point. These data are summarized in Table I. The pH of freshly prepared 1 mg/10 mL and 7 mg/10 mL solutions was 4.99 and 3.99, respectively. However, this declined over the course of the study for both solutions.

DISCUSSION

There have been several reports on the stability of epinephrine in various containers and under different conditions.[7,8,9] However, no information could be found for solutions packaged under sub-optimum conditions.

Epinephrine is oxidized to adrenochrome and then to melanin.[10] Factors that influence this degradative process are atmospheric oxygen, light, pH, alkalies, oxidizing agents, and inorganic impurities. Oxygen content and solution pH seem to be the most important factors. The effect of high and low oxygen concentrations and high and low epinephrine concentrations on the initial rate of epinephrine degradation has been studied by Sokoloski and Higuchi.[11] They determined that the degradation reaction was extremely complex and that the rate of degradation was always less than first order when oxygen tension was high. The oxygen tension was not measured during our study so it is not known how much oxygen was introduced into the solutions during the manufacturing and packaging process. The amount of atmospheric oxygen that the epinephrine was exposed to during the study was not controlled. As well, the antioxidant present in the

Table I: Stability of Epinephrine Diluted in Sterile Water

Storage in Glass vials					
Initial Concentration (mg/10 mL)	% Initial Concentration Remaining*				
	Day 3	Day 7	Day 14	Day 28	Day 56
1.1 ±0.02	98.5 ±1.31	94.2 ±2.60	87.4 ±1.36	83.6 ±1.22	76.7 ±1.95
7.7 ±0.08	99.7 ±1.82	99.2 ±0.27	98.3 ±0.55	98.3 ±1.06	97.8 ±1.42
Storage in Plastic Syringes with an 18G Needle Attached					
Initial Concentration (mg/10 mL)	% Initial Concentration Remaining*				
	Day 3	Day 7	Day 14	Day 28	Day 56
1.1 ±0.02	100.1 ±1.49	96.0 ±1.01	88.5 ±1.29	86.2 ±1.40	79.5 ±2.06
7.1 ±0.09	96.6 ±1.81	98.5 ±1.98	96.8 ±1.38	94.0 ±3.69	94.6 ±6.47

* Mean ± S.D. of four determinations.

manufacturer's formulation was diluted 1:10 and 7:10 for this study. This would decrease the effectiveness of the agent in the more dilute solution and may account for the longer shelf-life of the more concentrated product. The vials contained approximately 2-3 mL of air above the solution and the pre-loaded syringes had 18G needles attached. This would increase the amount of atmospheric oxygen the solutions were exposed to during the study period. The final pH of the solution was not adjusted to the optimum range of 3 to 4[10] which most likely would increase the degradation rate.

This study demonstrated that solutions of commercial epinephrine 1 mg/mL diluted to 1 mg/10 mL with sterile water and packaged in glass vials and plastic pre-loaded syringes under less than optimum storage conditions (e.g., the appropriate concentration of antioxidant, packaged under nitrogen and pH adjusted) are stable for only seven days when stored at room temperature and protected from light. However, solutions diluted to 7 mg/10 mL and packaged similarly are stable for at least 56 days.

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