

Procaine Hydrochloride Stability in St Thomas Concentrate Solution

Ronald F. Donnelly and Robert L. Thompson

ABSTRACT

The stability of procaine hydrochloride in extemporaneously prepared St Thomas concentrate solution was investigated.

A batch of St Thomas concentrate solution containing procaine hydrochloride 272.8 mg, potassium chloride 16 mmol, and magnesium chloride 16 mmol/20 mL was prepared and transferred into 10 mL glass vials. Vials were stored at either room temperature (22°C) or under refrigeration (4°C) and protected from light. Two vials from each storage condition were removed on days 0, 14, 28, 56, 84 and 168 and frozen at -72°C. The samples were analyzed in duplicate using a stability-indicating high pressure liquid chromatographic assay.

All vials remained colourless and free of precipitate over the course of the study. Greater than 90% of the original concentration remained in the St Thomas concentrate solution after 168 days at both storage temperatures. Therefore, the procaine hydrochloride in St Thomas concentrate solution is considered chemically stable for at least 168 days at both 22°C and 4°C when packaged in glass vials and protected from light.

Key Words: cardioplegic, procaine, stability

RÉSUMÉ

La stabilité du chlorhydrate de procaine dans les préparations extemporanées de solution concentrée de St. Thomas a été étudiée.

Un lot de solution concentrée de St. Thomas contenant 272,8 mg de chlorhydrate de procaine, et 16 mmol de chlorure de potassium et 16 mmol de chlorure de magnésium par 20 mL, a été préparé puis transvidée dans des flacons de verre de 10 mL. Les flacons ont ensuite été entreposés à la température ambiante (22 °C) ou au réfrigérateur (4 °C) à l'abri de la lumière. Deux flacons, chacun issu d'une des deux méthodes de conservation, ont été prélevés aux jours 0, 14, 28, 56, 84 et 168, puis congelés à -72 °C. Les échantillons ont ensuite été analysés en double par chromatographie liquide à haute pression pour en déterminer la stabilité.

Tous les échantillons sont demeurés incolores et aucun ne présentait de précipité durant toute la période de l'étude. Plus de 90 % de la concentration initiale était encore présente dans la solution concentrée de St. Thomas après 168 jours, et ce aux deux températures. On peut donc conclure que le chlorhydrate de procaine contenu dans la solution concentrée de St. Thomas est

chimiquement stable pendant au moins 168 jours aux températures de 22 °C et de 4 °C, lorsqu'il est conservé dans des flacons de verre et protégée de la lumière.

Mots clés : cardioplégique, procaine, stabilité

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INTRODUCTION

Cardioplegic solutions are used to arrest myocardial contractility and prevent cardiac tissue damage during cardiac surgery, however, the contents of the solutions vary widely.¹

At our institution, St Thomas cardioplegia solution is prepared by adding 20 mL of St Thomas concentrate solution² to a one litre bag of Ringers Lactate. St Thomas concentrate solution contains procaine hydrochloride 272.8 mg, potassium chloride 16 mmol, and magnesium chloride 16 mmol/20 mL. The pH of the final cardioplegic solution is adjusted to 7.6 by adding 10 mL of sodium bicarbonate, 1 mmol/mL, solution. This final cardioplegic solution is given an arbitrary eight-hour expiry.

The stability of the St Thomas concentrate solution which is currently found in the literature is three months; however, the storage conditions were not reported.³ This 90-day expiry date was obtained from information which determined the stability of all three ingredients.³ A modified USP assay (nitrate titration) was used to quantify the procaine.

The purpose of this project was therefore to study the stability of procaine hydrochloride in St Thomas concentrate solution using a stability-indicating high pressure

Ronald F. Donnelly, BSc(Pharm), is Product Development Pharmacist, Department of Pharmaceutical Services, Ottawa Civic Hospital, Ottawa, Ontario.

Robert L. Thompson, BScPh, is Staff Pharmacist, Department of Pharmaceutical Services, Ottawa Civic Hospital, Ottawa. At the time of the project he was a pharmacy resident at the Ottawa Civic Hospital.

Address correspondence to: Ronald Donnelly, Department of Pharmaceutical Services, Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa, Ontario, K1Y 4E9. This study was part of the submission that was awarded the 1994-5 CSHP Abbott Award for Pharmaceutical Technology.

liquid chromatography (HPLC) assay, over a six-month period while stored in glass vials at 22°C and 4°C and protected from light.

METHODS

Stability Study

St Thomas concentrate solution was prepared as previously described. Twenty-four 10 mL vials were stored at either 22°C or 4°C and protected from light. Two vials from each storage condition were collected immediately after preparation and then on days 14, 28, 56, 84 and 168. These vials were visually inspected for particulate matter and colour change and then frozen at -72°C for analysis at a later date. On the day of analysis, the vials were allowed to thaw and the contents were further diluted with mobile phase (1:200) for analysis in duplicate. Standard curves were derived for both procaine hydrochloride USP (Sel-Win Scientific Ltd.) and para-aminobenzoic acid (PABA), its major degradation product (Sigma Chemical Company). All solutions were considered stable if they retained 90% or greater of the initial concentration.

HPLC Assay

The mobile phase consisted of HPLC grade methanol (BDH Inc.) and an aqueous solution containing 1 mg/mL of 1-heptanesulfonic acid sodium (Sigma) and 0.75 mg/mL ammonium acetate (BDH) in a 50:50 (v/v) ratio. The pH of the mobile phase was adjusted to 3.5 with glacial acetic acid (BDH) using a calibrated pH meter (Orion Research; model 501) with a Ag/AgCl electrode (Orion; model 9104). Benzocaine hydrochloride (Sigma) was used as the internal standard.

The chromatographic system consisted of an isocratic solvent delivery pump (Shimadzu Corp.; model LC-10AS) and a photodiode array detector (Shimadzu; model SPD-M6A) set at 290 nm. The mobile phase was pumped through a 25 cm x 4.6 mm, 5 µm C₁₈ column (Supelco Canada Ltd; Supelcosil LC-18-DB). Injections were made using a manual injector (Rheodyne, model 7010) with a 10 µL loop. The flow rate was set at 1.5 mL/minute.

HPLC Assay Validation

To ensure the assay was stability-indicating, several attempts were made to induce degradation of procaine and compare these peaks to that of the parent compound. Solutions of St Thomas concentrate were prepared and adjusted to alkali conditions, pH 8.5, with sodium hydroxide or acid conditions, pH 1.6, with sulphuric acid in an attempt to cause hydrolysis of the procaine.

As oxidation of procaine is also possible,⁶ 0.25 mL of

30% hydrogen peroxide (Mallinckrodt Canada Inc.) was added to a third solution containing procaine and heated to 65°C for several hours in an attempt to generate the oxidation product.

Peak purity of all procaine and PABA peaks was determined using UV spectra created by the photodiode array detector and analyzing correlation coefficients from the overlaid spectra. Multi-channel analysis was also used to determine peak purity.

The linearity of the concentration vs area curve was assessed for both procaine and PABA over the concentration ranges studied. The accuracy was assessed, for both procaine and PABA, on five separate days by conducting a recovery study using solutions of known concentrations. Intra-day and inter-day variation was determined for both procaine and PABA as well as the sensitivity of the assay.

Data Analysis

The means and standard deviations were calculated on one dilution from two vials analyzed in duplicate. Linear regression was used to analyze the data from the sensitivity determination. All the samples from one storage condition were analyzed on the same day. Therefore, Z values corresponding to a one-tailed α value of 0.05 and a one-tailed β value of 0.05 were used to calculate the sample size along with an intra-day coefficient of variation (CV) of 1.26% and a detectable difference of 10%.

RESULTS

HPLC Assay Validation

The HPLC method separated procaine hydrochloride from the degradation product PABA and the internal standard benzocaine as illustrated in Figure 1. The acid and alkaline degradation of procaine produced no additional peaks other than the PABA peak. Comparison of the procaine peak which was generated from multiple samples taken during the assay validation process to procaine hydrochloride USP, PABA and aniline peaks by spectral analysis showed the peak to be pure. The peak was compared to aniline to determine if the PABA had been decarboxylated. No new peaks were formed when the procaine was mixed with the hydrogen peroxide. Procaine produced the most symmetrical peak having a tailing factor T of approximately 2.0.⁷ The resolution R between the procaine and PABA peaks was approximately 4.9.⁷

The correlation coefficients of five standard curves for procaine ranged from 0.9972 to 0.9993. The correlation coefficients of five standard curves for PABA ranged from 0.9985 to 0.9997. The average recovery over the validation period for procaine was 99.4% with a CV of 2.8. For PABA, the average recovery was 102.0% with a CV of 2.2.

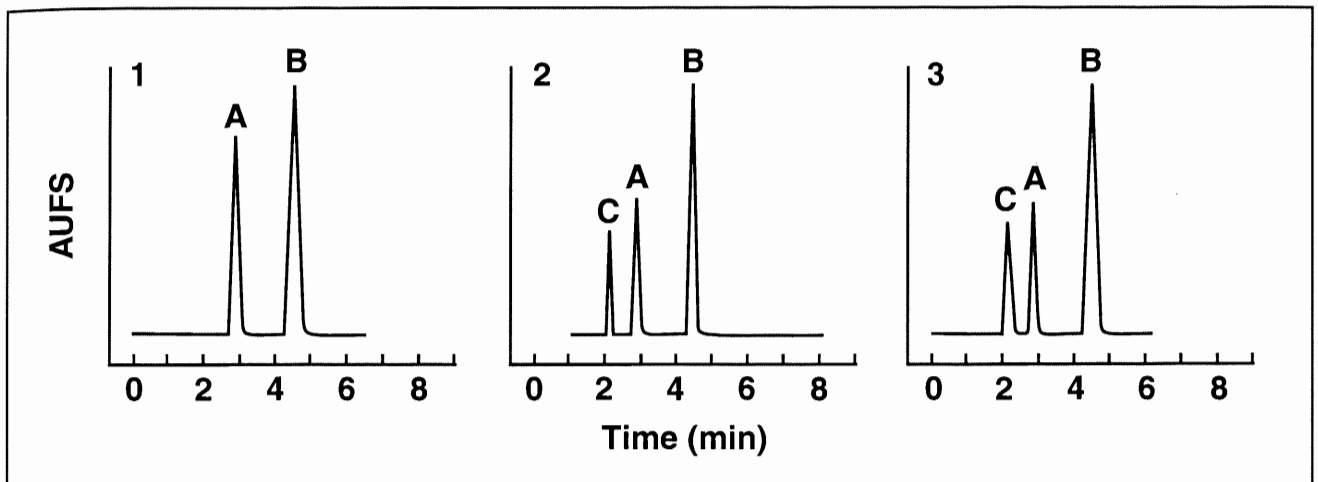


Figure 1: Sample chromatographs of procaine HCl in St Thomas concentrate solution: 1) fresh solution, 2) pH adjusted to 1.6 with 0.1 N sulfuric acid, 3) pH adjusted to 8.5 with 5 N sodium hydroxide. Peak A is PABA, peak B is procaine and peak C is benzocaine. AUFS = absorbance unit full scale.

The response was linear over the ranges of 4-28 mg/mL (28-200% of expected concentration) for procaine and 5-40 mg/mL (25-200%) for PABA. The CV for the intra-day variation test for procaine was 1.26% while that for PABA was 0.70%. The CV for the inter-day analysis for procaine was 2.16%, while that for PABA was 1.97%. The sensitivity of the assay was determined to be 2.8 ng of procaine and 0.28 ng of PABA, respectively. A sample size of 0.34 would be required to detect a difference of 10% between sample groups.

Stability Study

All the stored vials of St Thomas concentrate solution remained clear and free of precipitate over the study period. The concentration of procaine in vials stored at 22°C or 4°C and protected from light remained greater than 90% of the initial concentration over the study period of 168 days (Table I). Chromatograms of the

Table I: Stability of procaine hydrochloride in St Thomas concentrate solutions packaged in glass vials and protected from light

Stored Under Refrigeration (4°C)					
Initial Conc. (mg/20mL)	% of Initial Concentration Remaining ¹				
	Day 14	Day 28	Day 56	Day 84	Day 168
288.0±2.0	101.5±0.5	100.9±1.2	102.6±0.6	104.0±2.3	99.0±4.2
Stored at Room Temperature (22°C)					
Initial Conc. (mg/20mL)	% of Initial Concentration Remaining ¹				
	Day 14	Day 28	Day 56	Day 84	Day 168
296.0±2.0	103.7±0.8	101.6±1.2	101.1±0.5	102.6±0.6	96.7±1.7

1. Mean ± std. deviation of four determinations

samples failed to reveal the presence of any degradation peaks.

DISCUSSION

The stability of St Thomas concentrate solution is determined by the most sensitive ingredient, procaine hydrochloride. The ester linkage of the procaine can be hydrolyzed to yield diethylaminoethanol and PABA. The reaction can be catalyzed by both acid or base; however, alkali conditions are much more kinetically favoured.^{4,5}

Aqueous solutions of procaine hydrochloride show greatest stability in the pH range of 3 to 4.⁴ Thus, the St Thomas concentrate solution, with a pH of 4 to 5, should be more stable than the final, alkaline cardioplegic solution. At our institution, both the St Thomas concentrate and the Ringers Lactate solutions are cooled to 4°C before combination and final administration to the patient. The 4° and 22°C storage conditions studied were chosen to simulate what was happening pre-admixture or if the St Thomas concentrate was inadvertently stored at room temperature.

The freezing and thawing of the samples appeared to have no detrimental effect. Samples frozen at -72°C for up to 168 days did not change in content as indicated by the lack of appearance of any new peaks on the chromatograms. All samples from one storage condition were analyzed on the same day and the intra-day CV was 1.26%. Although it has been recommended that three separate samples be assayed in duplicate,⁸ the fact that our intra-day CV is less than 2% allows us to confidently detect a 10% difference in procaine concentration based on the analysis of two separate samples, assayed in duplicate.⁹

A stability-indicating HPLC assay was developed for analysis of procaine hydrochloride in St Thomas concentrate solution. Procaine hydrochloride, 272.8 mg/20 mL, is stable in St Thomas concentrate solution for at least 168 days when stored in glass vials at either 22°C or 4°C and protected from light. Proper quality control testing (sterility and pyrogen testing) would have to be conducted on the final product to determine the final expiry date in accordance with guidelines for batch production of a sterile product. ☒

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Correction

Please note that there was an error in the legend of Figure 1 in the article *Procaine Hydrochloride Stability in St Thomas Concentrate Solution* which was published in the February 1996 issue of CJHP. The legend should have read as follows:

Figure 1: Sample chromatographs of procaine HCl in St Thomas concentrate solution: 1) fresh solution, 2) pH adjusted to 1.6 with 0.1 N sulfuric acid, 3) pH adjusted to 8.5 with 5 N sodium hydroxide. Peak A is procaine, peak B is benzocaine, and peak C is PABA. AUFS = absorbance unit full scale.

We apologize for any inconvenience this may have caused.