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STABILIZATION OF EPINEPHRINE IN A LOCAL ANESTHETIC INJECTABLE SOLUTION USING REDUCED LEVELS OF SODIUM METABISULFITE AND EDTA

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ABSTRACT

The effect of sodium metabisulfite concentration upon the stability of epinephrine in a Lidocaine HCl with Epinephrine solution was evaluated using an elevated temperature (60°C) screen for up to 35 days. Long term room temperature data (23°C) was collected and evaluated for up to forty months and substantiated the results observed in the accelerated studies. Enhancement of the epinephrine chemical stability was predicated upon a tenfold reduction in the metabisulfite level from 0.05% to 0.005% plus the inclusion of a specific chelating agent, disodium edetate, into the formula. Protection from light is of optimum importance since more rapid photolytic degradation occurred in the experimental formula than

in the control. In two distinct packages, 20 ml glass vials and 1.8 ml glass cartridges, the epinephrine stability was found to be significantly improved ($p < 0.05$) compared to a control, suggesting the possibility of increased expiration dating.

INTRODUCTION

In aqueous solutions epinephrine decomposes by several pathways which can occur consecutively and in parallel. The principal degradation route is oxidative and is accelerated by the presence of oxygen, heavy metals, ultraviolet light, and increased pH [1-5]. Direct and indirect agents have been used to prolong the shelf-life of oxygen-sensitive products. These agents may be preferentially degraded due to their higher oxidation potential or act as free radical acceptors, chain inhibitors, or as sequestering agents [6]. In local anesthetic solutions containing epinephrine, sulfite salts have demonstrated the unique ability to reduce the rate of oxidative degradation and retard the ensuant discoloration. Levels from 0.025% to 0.1% have been included in most marketed products containing epinephrine.

The use of sulfite salts, however, presents several drawbacks which must be taken into account. Higuichi and Schroeter reported an additional route of epinephrine degradation in solutions containing bisulfite which resulted in the formation of epinephrine sulfonic acid [7]. From a pharmacological approach, Munson stated that higher local anesthetic concentrations are needed in sulfite-containing solutions. This was attributed to increased tissue buffering caused by the antioxidant's strongly acidic nature [8]. Recently, the FDA raised concern regarding allergic responses in parenteral products containing sulfite salts. They have recommended that drug manufacturers explore the feasibility of substituting other antioxidants, wherever possible, and now require labeling statements in products containing sulfite salts.

Investigations into alternative antioxidants have been initiated by several workers, but all efforts indicate that sulfite salts remain the antioxidant of choice in epinephrine solutions. Wollman and Raether screened 54 stabilizers in a 1% epinephrine solution and only the inclusion of sulfiting agents, alone or in combination with other stabilizers, provided sufficient protection to meet stability requirements[9].

In earlier publications, reduction of sulfite salt levels have been reported. Portmann showed that the chemical stability of Isoproterenol could be enhanced if sodium metabisulfite levels were decreased from 0.1% to 0.025%. The improved stability was attributed to a decreased sulfonation rate [10]. In epinephrine solutions, Lachman observed that by including a chelating agent, such as disodium edetate, and lowering the sodium metabisulfite content from 0.1% to 0.05%, comparable or enhanced color stability could be effected [11]. Similar results were found by Roscoe and Hall when disodium edetate was combined with sodium metabisulfite [12].

The objective of this investigation was to increase the epinephrine stability in a local anesthetic injectable solution by including a chelating agent and by reducing the metabisulfite concentration to below 0.025%.

EXPERIMENTAL DESIGN

Preformulation Screen

Control solutions of a local anesthetic with epinephrine packaged in 20 ml molded glass vials were commercially obtained. The formulation consisted of 2% Lidocaine Hydrochloride, 0.001% Epinephrine, 0.05%

TABLE 1

Preformulation Screen of Test Solutions:
Percent Epinephrine Remaining After Storage at 60°C
in 20 ml Vials

TEST FORMULATIONS[1]				TIME (days)			
Code	Meta-bisulfite (%)	Na2 EDTA (%)	Citric Acid (%)	7	14	21	35
Control	0.050	-	-	93.5%	86.9%	77.8%	62.6%
A	0.025	-	-	93.7%	92.6%	89.5%	77.9%
B	0.010	-	-	82.1%	62.0%	58.8%[2]	
C	0.005	-	-	48.5%[2]	-	-	-
D	0.010		0.1	91.6%	82.1%	62.1%[2]	-
E	0.010	0.01	-	89.5%	90.5%	86.3%	84.7%
F	0.0075	0.01	-	93.7%	92.6%	90.4%	84.0%
G	0.005	0.01	-	91.5%	93.7%	91.5%	89.4%
H	-	0.01	-	92.4%	88.6%	86.7%	83.4%

[1] All formulations contained lidocaine HCl (2%), epinephrine (0.001%), methylparaben (0.1%) and sodium chloride (0.6%), pH - 3.8-4.0.
[2] Discolored yellow.

Sodium Metabisulfite, 0.1% Methylparaben and 0.6% Sodium Chloride in sterile water for injection. Solutions under investigation were prepared with identical concentrations of Lidocaine, Epinephrine, methylparaben and sodium chloride. The levels of sodium metabisulfite and the inclusion of citric acid or disodium edetate in the formulation were the variables investigated.

During manufacture, prior to the addition of metabisulfite and epinephrine, the bulk solution was degassed for two hours with nitrogen. The pH of the finished solution was adjusted to 3.3 to 4.0 with 0.1N HCl. Both the bulk solution and the vials were purged with a continuous stream of nitrogen throughout the filling process. The solution was then filled into a 20 ml molded glass vial and sealed using a chlorobutyl rubber closure with an aluminum overcap. Samples were stored at 60°C for 35 days. The epinephrine content was determined at several intervals using a stability indicating HPLC procedure [13].

Long Term Stability Study

The prototype formulation which exhibited a stability profile equal to or better than the control based on the results from the 60°C study, was selected for long term evaluation at room temperature (23°C). Two types of packaging were utilized in this study; 20 ml molded glass vials with a chlorobutyl rubber closure, and 2 ml glass cartridges sealed with a natural rubber plunger and a chlorobutyl I-cap closure. The solution selected contained sodium metabisulfite reduced to 0.005% with 0.01% disodium edetate as a stabilizer. This formulation was degassed for two hours with nitrogen prior to the

inclusion of the metabisulfite and epinephrine. Automated equipment was used for filling product into vials and cartridges. All packaging was purged with nitrogen prior to filling, during filling, and while the closure and overcap were applied. Again, commercial product was obtained as a control for both packages being evaluated. All samples were maintained at 23°C and protected from light.

In a second study the control, consisting of the identical formulation previously described, and the test formulation were placed in a light cabinet (1000 ft. candles) for one month. The samples were assayed for epinephrine content and observed for discoloration.

RESULTS

The results obtained for the 60°C preformulation screen revealed that lowering the metabisulfite concentration from 0.05% to 0.025% (Table 1, Formulation A) improved the epinephrine chemical stability without resulting in discoloration of the solution. Reducing the metabisulfite concentration to 0.01%, and 0.005% (Formulations B & C) resulted in severe degradation (> 40%) and discoloration within 14 days. When citric acid (0.1%) was incorporated as a

chelating agent along with a metabisulfite level of 0.01% (Formulation D), approximately 40% degradation occurred within 21 days and the solution discolored. Replacing citric acid with 0.01% disodium edetate and lowering the metabisulfite levels to 0.01%, 0.0075%, and 0.005% (Formulations E, F, & G) significantly retarded epinephrine degradation (< 15%) compared to the control (>40%) for 35 days at 60°C. No discoloration in these solutions was observed. Formulation H contained disodium edetate (0.01%) but did not contain any metabisulfite. The epinephrine stability for this solution was comparable to formulations E, F, and G, however, physical discoloration was observed after 35 days at 60°C.

Results from the study conducted in the light cabinet indicated that the test solution containing 0.005% metabisulfite and 0.01% disodium edetate was both degraded (> 50%) and discolored, while the control remained chemically and physically unchanged after one month.

Table 2 summarizes the results obtained for the analysis of epinephrine in vials and cartridges after storage for 40 months at room temperature in the dark. Graphical scatter plots for the controls and test formulation suggest a linear relationship between the

TABLE 2

Long Term Room Temperature Study of Prototype Formulation
- Percent Epinephrine Remaining Over Time -

PACKAGE	TIME (mths)	FORMULATION		
		Control[1] (0.05% Meta)	Test Formulation (0.005% Meta + 0.01% EDTA)	
20 ml Vial	3	100.0	100.3	98.9
	6	96.8	97.1	96.3
	11	94.5	96.2	96.4
	29	91.5	93.7	93.9
	41	85.0	91.1	91.4
2 ml Cartridge	1	98.2	100.8	
	11	94.1	96.0	
	24	86.8	93.2	
	34	84.3	90.0	

[1] All formulations contained lidocaine HCl (2%), epinephrine (0.001%), methylparaben (0.1%), and sodium chloride (0.6%), pH - 3.3-4.0.

percent epinephrine remaining and time (Figures 1 and 2). The data was fitted to fit a zero-order kinetic model using least squares regression analysis, and the results were found to support the model ($p<0.05$).

Correlation coefficients ranged from -0.953 to -0.992. The regression coefficients for the slopes were significantly different from zero ($p<0.05$). The regression lines for the control and test formula in both vials and cartridges were compared. A statistically significant difference was found between

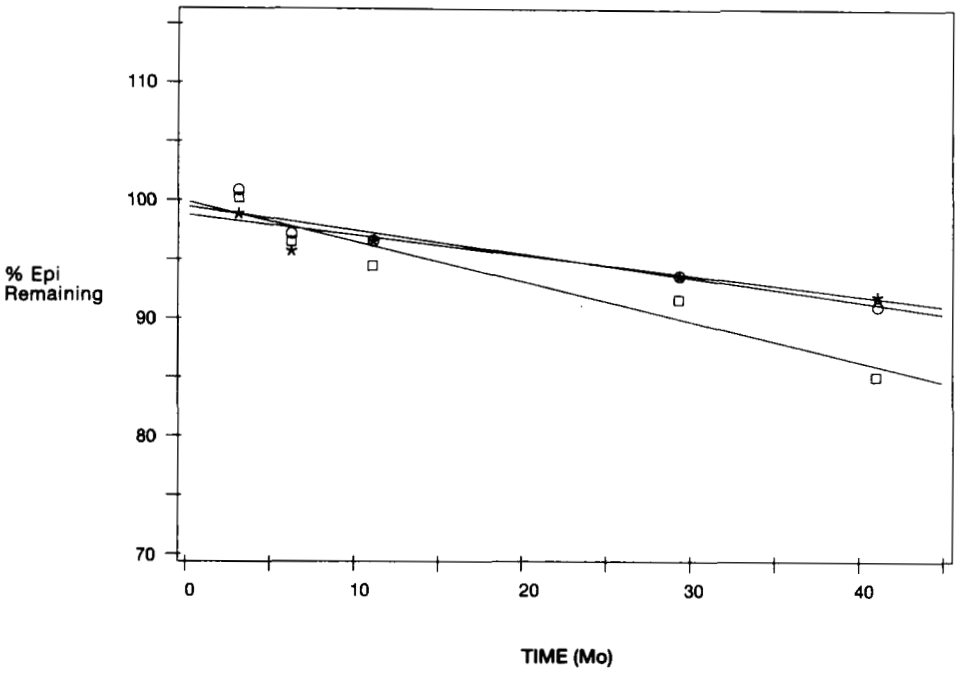

FIGURE 1:

Scatter Plot of Epinephrine Degradation in Vials at 23°C versus Time

KEY: , Control containing 0.05% Metabisulfite; X, O, Test Formulations (Lot I and II) Containing 0.005% Metabisulfite and 0.01% Na_2EDTA

the control and test formula in vials, as well as in cartridges. The $t_{90\%}$ values and 95% confidence limits were calculated for all five solutions.

The results indicate that epinephrine is more stable in vials than in cartridges and that a longer shelf-life may be obtained using the test formulation than the control.

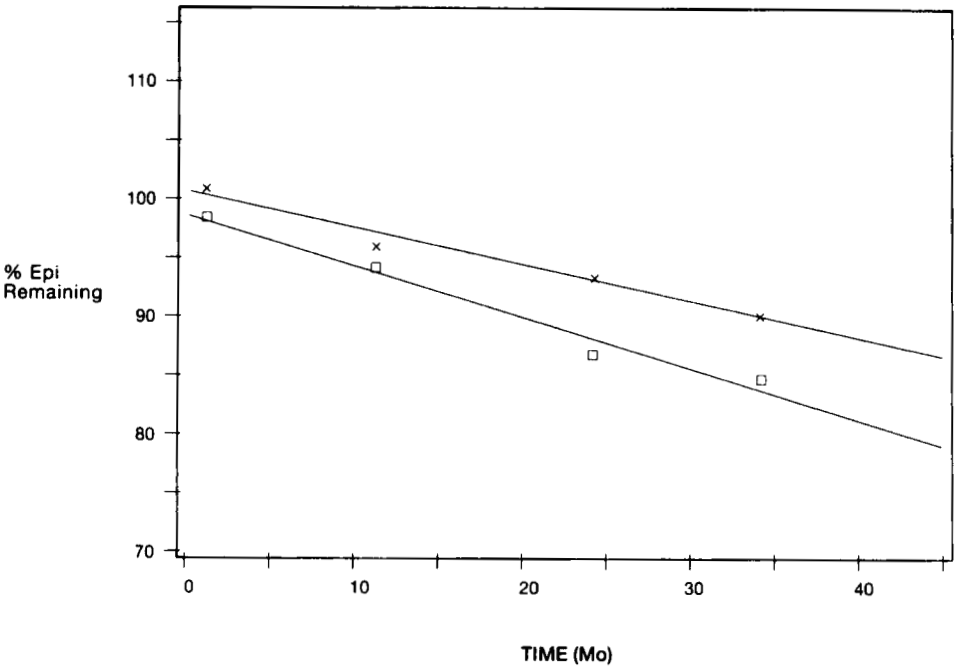

FIGURE 2:

Scatter Plot of Epinephrine Degradation in Cartridges at 23°C versus Time

KEY: □ , Control containing 0.05% Metabisulfite; X Test Formulation Containing 0.005% Metabisulfite and 0.01% Na_2EDTA

The HPLC chromatograms obtained for the control solutions stored for approximately forty months revealed the presence of epinephrine sulfonate, a degradation product resulting from bisulfite addition (Figure 3A, 3B). The concentration of this decomposition product was determined using the HPLC procedure previously described [13]. Standards were

TABLE 3

Statistical Summary of Least Squares Analysis
For Vials and Cartridges

FORMULATION	PACKAGE	b_o***	b_1	r	F (d.f.)
Control*	20 ml Vial	99.6	-0.339	-0.969	46.24 (1,3) p<0.01
Test Lot I**	20 ml Vial	99.3	-0.203	-0.953	30.11 (1,3) p<0.05
Test Lot II	20 ml Vial	98.4	-0.167	-0.963	37.83 (1,3) p<0.01
Control	2 ml Cart.	98.6	-0.440	-0.992	117.1 (1,2) p<0.01
Test Lot I	2 ml Cart.	100.5	-0.312	-0.988	78.8 (1,3) p<0.05

* Control - Consists of lidocaine HCl (2%), epinephrine (0.001%), methylparaben (0.1%), sodium chloride (0.6%), and sodium metabisulfite (0.05%), pH = 3.3 - 4.0.

** Test Formulation - Consists of the above with the following modification; sodium metabisulfite (0.005%), sodium edetate (0.01%).

*** b_o = y-intercept, b_1 = slope, r = correlation coefficient, d.f. = degrees of freedom.

TABLE 4

Calculated $t_{90\%}$ Values and 95% Confidence Limits Using Zero-Order Regression Coefficients: Epinephrine Remaining Over Time

FORMULATION	PREDICTED $\hat{y}$ AT 24 MTHS	95% POPULATION C.L.	$t_{90\%}$ (MTHS)
Control - Vial	91.5%	89.0% - 94.0%	28.4
Test I - Vial	94.5%	92.6% - 96.3%	45.9
Test II - Vial	94.4%	93.0% - 95.7%	50.1
Control - Cart.	88.0%	85.5% - 90.5%	19.4
Test I - Cart.	93.0%	90.8% - 95.1%	33.5

$\hat{y}$ = Percent Epinephrine Remaining

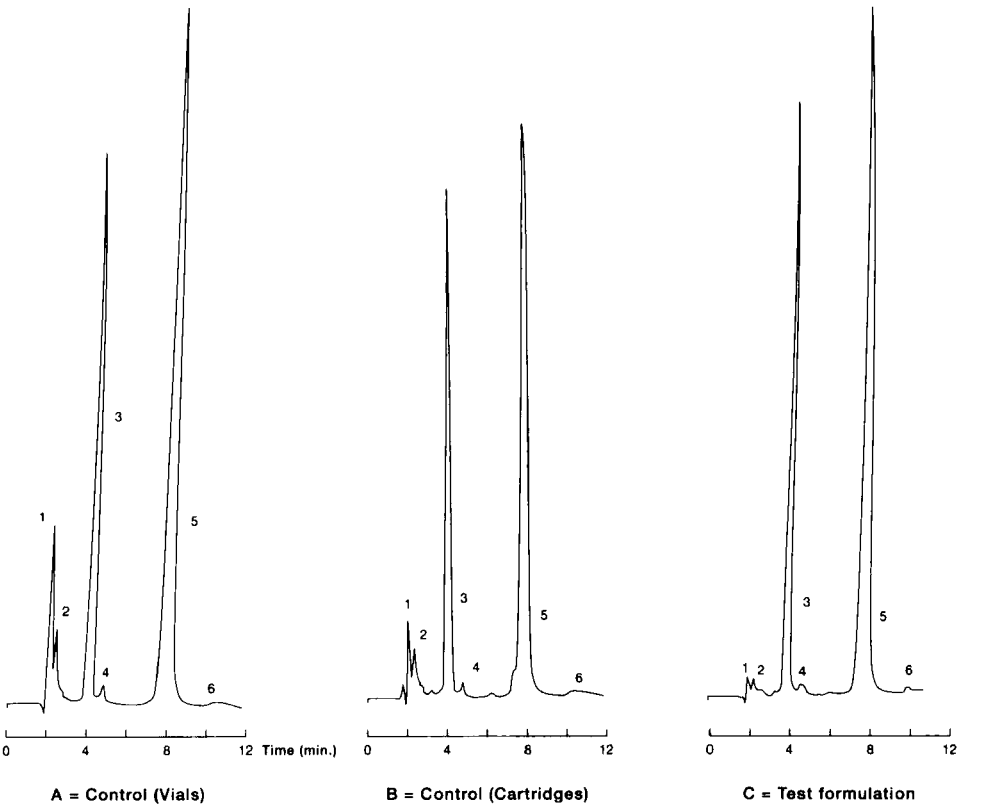

FIGURE 3:

HPLC Chromatograms for (A) Control Solution in Vials, (B) Control Solution in Cartridges, and (C) the Test Formulation in Vials or Cartridges

KEY: (1) Metabisulfite
(2) Epinephrine Sulfonate
(3) Epinephrine
(4) p-hydroxybenzoic Acid
(5) Methylparaben
(6) Lidocaine HCl

prepared using known quantities of epinephrine sulfonate prepared by the method of Schroeter and Higuchi [14] and the electrochemical response for standards and samples compared.

The results of this analysis indicated that in control solutions the bisulfite addition reaction accounted for 88.8% of the epinephrine degradation observed in vials and 48.3% of that observed in cartridges. Comparatively the test formulation exhibited no evidence of the bisulfite addition reaction since epinephrine sulfonate was not observed (Figure 3C).

Oxidative degradation of epinephrine could not be determined directly from the chromatograms since the oxidative products are not electrochemically active under the conditions of the analysis. However, the effects of increased oxygen concentration in the solution may be estimated by assaying metabisulfite. The HPLC procedure previously described was again utilized [13]. In control solutions metabisulfite was significantly more degraded in cartridges than in vials (63.2% vs 30.0%).

The increase in the decomposition of metabisulfite in cartridges may be due to increased permeation of atmospheric oxygen at both the I-cap and plunger seals.

The chromatographic results obtained indicate that in the control solution both bisulfite addition and oxidation contribute to the loss of epinephrine potency. In the test formulation the bisulfite addition reaction is minimized by reducing the initial concentration of metabisulfite.

SUMMARY AND CONCLUSIONS

To increase the rate of epinephrine degradation and to allow for short term formulation evaluations, 60°C was chosen for the accelerated studies. Akers[6] reported that high temperature stability studies are not reliable predictors of an antioxidant's efficiency because oxygen solubility in solution is lower at higher temperatures than at room temperature. Although Arrhenius kinetics were not performed, the high temperature screen provided a means to evaluate the physical and chemical stability of epinephrine from a number of formulations over a relatively short period of time. The results observed in the preformulation screen at 60°C were found to be indicative of those obtained for 40 months at room temperature.

Based on the data presented in Tables 2 and 3, it is apparent that in the test formulation the rate of

epinephrine degradation was significantly reduced by lowering the level of metabisulfite and including a specific sequestering agent, disodium edetate. Rapid metal-catalyzed oxidation of epinephrine was expected since trace metals contributed by the constituents of the formula and the package are known to be present. Disodium edetate was found to inhibit this reaction by chelating those metals catalyzing the free radical oxidative process.

In the control solution chromatographic evidence indicates that bisulfite addition is a major route of epinephrine decomposition. Reducing the ratio of metabisulfite to epinephrine from 50:1 to 5:1 in the test formulation was shown to eliminate this degradation route.

The data also shows a specific package-related effect; the epinephrine degradation rate was more rapid in cartridges than in vials. This difference has been attributed to the higher permeability of oxygen through the cartridge seals. Although the rate of oxidative decomposition was greater for both formulations in this package, the test solution still exhibited improved epinephrine stability compared to the control.

The findings of this investigation suggest that metabisulfite levels may be reduced and that with the inclusion of disodium edetate and protection from light, increased expiration dating may be realized for a lidocaine hydrochloride with epinephrine solution.

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