Automated Fluorometric Analysis of Epinephrine in Lidocaine Hydrochloride Injection

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Abstract □ A rapid automated method for determining epinephrine in lidocaine hydrochloride injection is presented. The method is an adaptation of the official USP XIX fluorometric procedure and yields equivalent results. The linearity, accuracy, precision, excipient/background effect, and carryover characteristics of the automated system are described. A recovery study indicated that intact epinephrine is measured accurately in the presence of its degradation products. Analysis of several commercial preparations is reported.

Keyphrases □ Epinephrine—automated fluorometric analysis in lidocaine hydrochloride injection □ Fluorometry—analysis, automated, epinephrine in lidocaine hydrochloride injection □ Automated analysis, fluorometric—epinephrine in lidocaine hydrochloride injection □ Lidocaine hydrochloride—injection solution, automated fluorometric analysis of epinephrine content □ Adrenergic agents—epinephrine, automated fluorometric analysis in lidocaine hydrochloride injection

The USP XIX (1) method for assaying epinephrine in lidocaine hydrochloride injection is a costly, time-consuming procedure, particularly when used to analyze large numbers of solutions. A heating step, requiring approximately 30 min, is followed by the addition of a buffer and oxidizing agent and two accurately timed operations.

In the automated system, sodium metabisulfite, which interferes with the oxidation of epinephrine (2, 3), is destroyed at 90° in a flow-through heating bath. The automated system eliminates the errors associated with accurate timing of the addition of reagents and transferring and diluting solutions and has an analysis rate of 30 samples/hr.

EXPERIMENTAL[1]

Reagents—All reagents were ACS or USP grade. Standard epinephrine solutions were prepared from USP epinephrine bitartrate reference standard. The standard solution used with the automated system consists of 10 μg of epinephrine/ml, 0.5 mg of sodium metabisulfite/ml, and 20 mg of lidocaine hydrochloride/ml.

All reagents for the manual procedure are prepared as directed in USP XIX (1). The reagents for the automated procedure are prepared as follows.

Distilled Water—Water distilled from glass was used.

Diluent Water—Add 1.0 ml of wetting agent[2] to 1 liter of distilled water and mix thoroughly.

Hydrochloric Acid, 0.5%—Add 5.0 ml of concentrated hydrochloric acid to approximately 800 ml of distilled water. Add 1.0 ml of wetting agent, dilute to 1 liter with distilled water, and mix thoroughly.

Sodium Acetate, 2%—Dissolve 20 g of sodium acetate in approximately 800 ml of distilled water. Dilute to 1 liter with distilled water and mix thoroughly.

Potassium Ferricyanide, Stock—Dissolve 6.3 g of potassium ferricyanide in approximately 800 ml of distilled water. Dilute to 1 liter with distilled water and mix thoroughly. This reagent should be made *fresh weekly*.

Potassium Ferricyanide, Working—Pipet 1.0 ml of stock potassium ferricyanide into a 100-ml volumetric flask. Dilute to volume with distilled water and mix thoroughly. This reagent should be made *fresh daily*.

Ascorbic Acid, Stock—Dissolve 1.0 g of ascorbic acid in approximately 80 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly. This reagent should be made *fresh weekly* and kept *refrigerated*.

Ascorbic Acid, Working—Pipet 1.0 ml of stock ascorbic acid into a 100-ml volumetric flask. Dilute to volume with distilled water and mix thoroughly. This reagent should be made *fresh daily* and kept in an ice bath.

Sodium Hydroxide, 15%—Dissolve 150 g of sodium hydroxide in approximately 800 ml of distilled water. After cooling, dilute to 1 liter with distilled water and mix thoroughly.

All reagents should be in glass containers.

Manual Fluorometric Method—Follow the procedure in USP XIX (1).

Automated Fluorometric Method—A flow diagram of the automated system appears in Fig. 1. Add lidocaine hydrochloride injection, containing 2–14 μg of epinephrine/ml, to the sampling cup. Place the 10-μg/ml epinephrine standard solution in sample cups 1, 10, 20, 30, 38, 39, and 40 and optimize the fluoronephelometer using the instruction manual provided with the instrument[3]. Check for proper bubble patterns and heating bath temperature (90°).

Set the instrument baseline as soon as the reagents reach the detector. Set the recorder to a fluorescence intensity of 50 when the 10-μg/ml epinephrine standard reaches the detector. Calculate as follows:

$$\mu g \text{ of epinephrine/ml of injection solution} = \frac{\mu g \text{ of epinephrine/ml in standard}}{\text{average fluorescence intensity of standards}} \times \text{fluorescence intensity of sample} \quad \text{(Eq. 1)}$$

Standard Curve—Standards containing 2, 6, 10, 14, and 18 μg of epinephrine/ml were prepared and assayed on the automated system.

Accuracy—Standards containing variable amounts of epinephrine were prepared and analyzed over 1 year by the automated and USP XIX (1) procedures.

Precision—Thirty-nine replicate samples of a 10-μg/ml epinephrine standard were analyzed on the automated system.

Excipient/Background Effect—Solutions were prepared containing 20 mg of lidocaine hydrochloride/ml, 10 μg of epinephrine/ml, and the labeled amount of excipients found in commercial preparations of lidocaine hydrochloride injection[4]. For each solution analyzed, the concentration of one excipient was varied between zero and two or four times the labeled concentration. Solutions were also prepared in which the lidocaine hydrochloride was varied from 0 to 50 mg/ml.

Carryover—Standards containing 2, 10, and 18 μg of epinephrine/ml were prepared and analyzed in specific sample sequence to determine if there was any epinephrine carryover between samples.

Recovery of Epinephrine in Presence of Degradation Products—Varying amounts of a 50-μg/ml standard epinephrine solution were added to 20-ml aliquots of a commercial preparation of lidocaine hydrochloride in which 42% of the epinephrine was degraded. The samples were analyzed on the automated system.

[1] The automated analytical system consisted of the following Technicon (Technicon Industrial Systems, Tarrytown, N.Y.) equipment: liquid sampler IV; proportioning pump III; fluoronephelometer III fitted with an 85-w mercury lamp, a Pyrex (Corning) 2-mm i.d. flowcell, and a 420-nm primary and 520-nm secondary filter; and autoanalyzer II recorder with a chart speed of 155 cm/hr. The equipment used for the manual procedure consisted of an Aminco SPF-125 spectrophotofluorometer (American Instrument Co., Silver Spring, Md.).

[2] Brij-35, Atlas Chemical Industries, Wilmington, Del.

[3] See Technicon Publication No. TA1-0220-10, rev. July 1972.

[4] A typical formulation consists of the following excipients: 6 mg of sodium chloride/ml, 0.5 mg of sodium metabisulfite/ml, and 1.0 mg of methylparaben/ml.

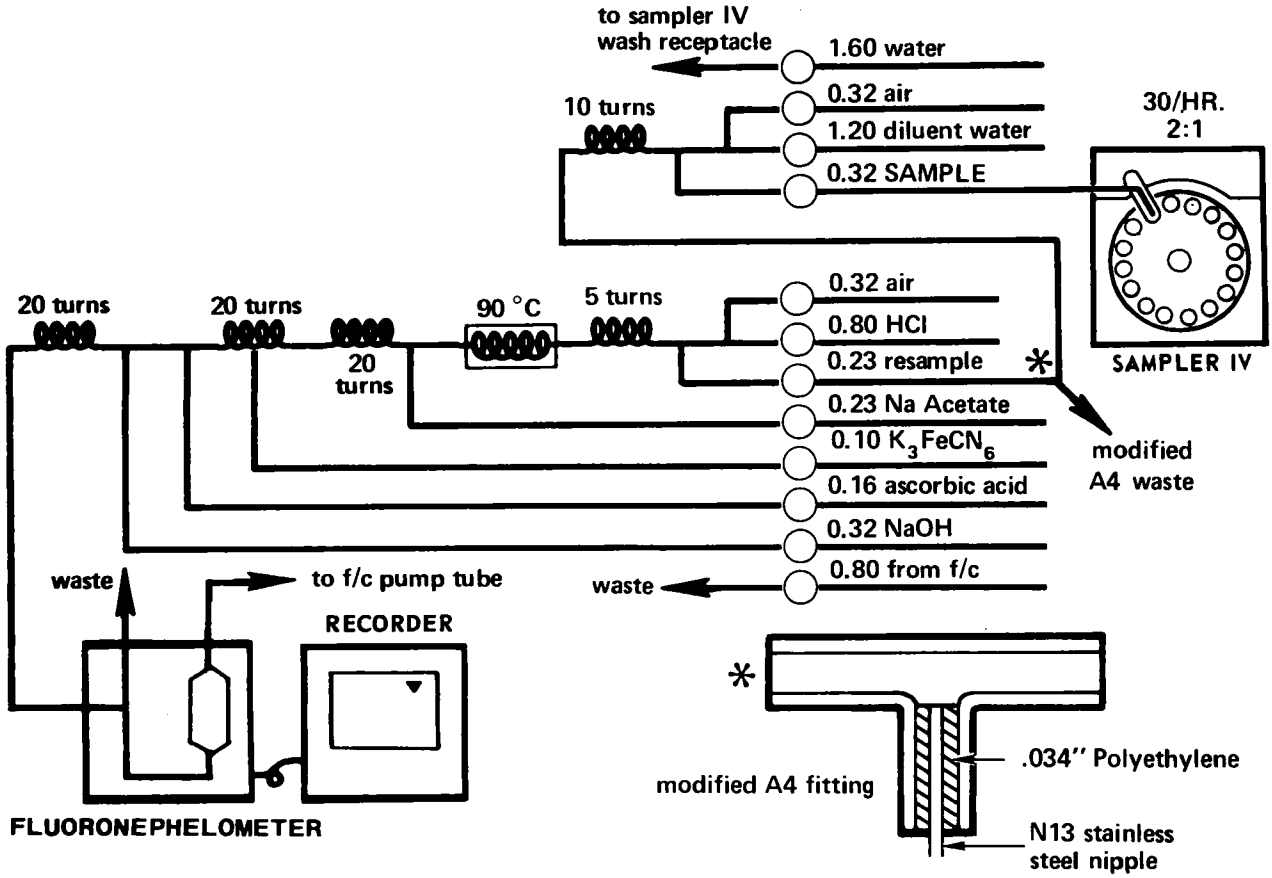

Figure 1—*Flow diagram for the automated fluorometric analysis of epinephrine. Pump tubing is given with the flow rate in milliliters per minute. All tubing is standard Tygon except on the top of the flowcell (f/c), which is 0.2-cm (0.090-in.) i.d. Acidflex.*

Analysis of Commercial Preparations—Several commercial preparations of lidocaine hydrochloride were analyzed by the automated and USP XIX (1) procedures.

RESULTS

Standard Curve—A plot of epinephrine concentration *versus* fluorescence intensity indicates that the fluorescence is linear in the concentration range of 2–18 μg of epinephrine/ml.

Table I—Recovery of Epinephrine: Comparison of Automated and Manual Procedures

Epinephrine, μg/ml		
Theoretical	Recovered by Automated Procedure[a]	Recovery by USP XIX Procedure[b]
4.0	3.9	4.0
4.5	4.4	4.6
5.0	5.0	5.0
5.4	5.5	5.4
5.5	5.3	5.5
5.6	5.5	5.7
5.8	5.6	5.7
5.8	5.6	5.9
8.0	8.1	8.0
10.0	9.9	10.0
10.0	9.9	9.9
11.0	11.2	11.1
11.0	10.9	10.9
11.0	10.9	11.0
11.0	11.2	11.1
12.0	12.1	12.2
17.0	17.0	17.2

[a] Each assay value is an average of two analyses. [b] Each value is based on one assay.

Accuracy—Table I is a summary of the recovery of epinephrine by the automated and USP XIX (1) procedures. Neither procedure yielded results that varied by more than 0.2 μg of epinephrine/ml from the theoretical concentration. The difference between the two methods never exceeded 0.3 μg/ml; the average difference was 0.12 μg/ml.

Precision—Table II shows the frequency distribution of 39 replicate samples containing 10 μg of epinephrine/ml. The average epinephrine recovery was 9.9 μg/ml ± 0.10 (*SD*), and the recovery range was 9.7–10 μg/ml.

Excipient/Background Effect—None of the excipients studied interfered with the automated epinephrine assay procedure (Table III). The fluorescence intensity was suppressed in the absence of lidocaine hydrochloride but was constant in the presence of 5–50 mg of lidocaine hydrochloride/ml.

Carryover—Table IV shows the sample sequence used to determine the effect of the samples upon one another. The fluorescence intensity of the samples indicated that the system was free from intersample carryover.

Recovery of Epinephrine in Presence of Degradation Products—Table V illustrates that intact epinephrine was recovered in the presence of epinephrine degradation products.

Analysis of Commercial Samples—Table VI is a summary of the

Table II—Automated Analysis of 39 Samples Containing 10 μg of Epinephrine/ml

	Concentration of Recovered Epinephrine, μg/ml	Frequency
	10.0	14
	9.9	9
	9.8	14
	9.7	2
Average	9.9	
Range	9.7–10.0	
SD	±0.10	

Table III—Effect of Excipients and Lidocaine Hydrochloride Concentration on Fluorescence Intensity

Compound	Concentration, mg/ml	Fluorescence Intensity[a]
Sodium metabisulfite	0.0	51, 49.5
	0.5	50.5, 50
	0.6	50.5, 50
	1.0	51, 50
	1.5	50, 49.5
	2.0	49.5, 50
Sodium chloride	0.0	51, 50
	4.0	49.5, 50
	8.0	50.5, 50
	12.0	49.5, 49
Methylparaben	0.0	50, 50.5
	1.0	49, 49.5
	2.0	50, 50
Lidocaine hydrochloride	0.0	47.5, 41
	5.0	50, 50
	10.0	51, 50.5
	20.0	51, 51
	30.0	50, 50
	40.0	50, 50
	50.0	50.5, 50.5

[a] Fluorescence intensity is for duplicate samples.

analysis of several commercial samples of lidocaine hydrochloride. The differences between the automated and USP XIX (1) methods never exceeded 0.3 μg of epinephrine/ml; the average difference was 0.18 μg of epinephrine/ml.

DISCUSSION

An accurate and precise direct method for automatically analyzing epinephrine in the presence of common excipients found in lidocaine hydrochloride injection is presented. *Both* the automated and USP XIX (1) fluorometric assay procedures yield equivalent results, since a paired t test applied to the data from Tables I and VI yielded a calculated t value of 1.430 at 28 degrees of freedom. A t value of 2.048 is required to indicate a difference between the methods at a 95% confidence level. Since fluorescence intensity is suppressed in the absence of lidocaine hydrochloride, it is essential that lidocaine hydrochloride be present in the standard.

Approximately 200 samples containing up to 14 μg of epinephrine/ml may be assayed directly in 8 hr. Samples containing more than 14 μg of epinephrine/ml may be diluted to between 2 and 14 μg of epinephrine/ml and then quantitated.

Table IV—Carryover Study: Determination of Intersample Effects

Sample[a]	Fluorescence Intensity
AAA	50, 50.5, 50.5
CCC	10, 10, 10
BBB	93.5, 94, 93.5
AAA	51, 51, 50.5
BBB	93.5, 94, 93.5
CCC	10, 10, 10
AAA	50.5, 50.5, 51
BCB	93.5, 10, 93.5
CBC	10, 93, 10
AAA	50, 50, 50
CBA	10, 93, 50
ABC	50, 92, 9.5
BBAA	92, 92.5, 49.5, 49.5

[a] Samples are shown in the order that they were assayed. Epinephrine concentrations were: A, 10 μg/ml; B, 18 μg/ml; and C, 2 μg/ml.

Table V—Recovery of Epinephrine in the Presence of Degradation Products

Total Epinephrine, μg		Total Volume of Solution Assayed, ml	Epinephrine, μg/ml	
Degraded Solution	Standard Solution		Theoretical	Recovered[a]
130	25	20.5	7.6	7.4
130	50	21	8.6	8.4
130	100	22	10.5	10.5
130	150	23	12.2	12.2

[a] Each result is an average of five determinations.

Table VI—Assay of Commercial Preparations by Automated and Manual Procedures

Dilution Range	Recovered Epinephrine, μg/ml	
	Automated[a]	Manual[b]
1:100,000	10.9	10.6
	11.0	10.8
	10.5	10.6
	10.5	10.6
	10.0	10.3
	11.0	10.7
	11.2	11.3
1:200,000	5.0	5.2
	5.0	5.2
	5.4	5.4
	5.2	5.0
	5.2	5.0

[a] Each assay value is an average of two analyses. [b] Each value is based on one analysis.

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