Fluorometric Determination of Epinephrine in Low Dosage Injections and in Lidocaine Hydrochloride–Epinephrine Combinations

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Abstract □ A procedure is reported for determining epinephrine in low dosage injections and in combination with lidocaine hydrochloride. Epinephrine is eluted from a buffer column as its ion-pair with di(2-ethylhexyl)phosphoric acid in ether, extracted into hydrochloric acid, and determined fluorometrically. The method determines unchanged epinephrine by isolating it from its sulfonic acid and other decomposition products; it is a measure of the stability of epinephrine in solution and can be modified to include an assay for lidocaine hydrochloride. When synthetic mixtures of epinephrine and epinephrine–lidocaine hydrochloride were analyzed by the proposed procedures, recoveries for epinephrine ranged from 98.5 to 101.3%, and recoveries for lidocaine hydrochloride ranged from 98.6 to 101.7%. Assay results on 23 different commercial samples of varying potencies and combinations are also reported.

Keyphrases □ Epinephrine—separation, analysis in low dosage injections and combinations with lidocaine hydrochloride □ Lidocaine hydrochloride–epinephrine mixtures—separation, analysis of epinephrine □ Partition chromatography—extraction of epinephrine as ion-pair with di(2-ethylhexyl)phosphoric acid □ Di(2-ethylhexyl)phosphoric acid—used in ion-pair partition chromatography of epinephrine □ Spectrophotofluorometry—analysis, epinephrine in low dosage injections and combinations with lidocaine hydrochloride

Recent evidence suggests that low dosage epinephrine solutions (0.01–0.1 mg./ml.) are not very stable in the presence of bisulfite, which reacts with epinephrine to produce epinephrine sulfonic acid, a physiologically inactive derivative. Conditions for this ester reaction were discussed previously (1, 2). Nevertheless, bisulfite is commonly added to this type of preparation because it is necessary to prevent adrenochrome formation.

Compounding an already complex situation, assay procedures for epinephrine are limited in scope and specificity and involve very tedious and complex manipulations. The USP XVIII (3) procedures for epinephrine injection and solution are based on its optical rotation and, as such, require at least 30 mg. of epinephrine for the assay. Obviously, this method is not applicable to low dosage epinephrine solutions.

Colorimetric methods (4, 5) can be extended to low levels of epinephrine but will not selectively determine epinephrine in the presence of epinephrine sulfonic acid and other decomposition products.

Hellberg's fluorometric procedure (6) is applicable to low levels of epinephrine and detects only the unchanged epinephrine. This method is a modification of the trihydroxyindole reaction first observed by Loew (7), and many other variations have been tried (8–17). The large number of published modifications attests to the fact that the assay is subject to many variables and interferences. Time, temperature, pH, type of oxidizing agent, presence of reducing agents, and composition of the final alkali–ascorbate mixture all affect the stability and sensitivity of the fluorescent lutine produced. Additionally, the bisulfite generally found in commercial preparations hampers the development of fluorescence (6). The USP XVIII procedure for epinephrine in lidocaine hydrochloride injections (18), still another variation of Hellberg's procedure, compensates for the presence of bisulfite by adding it to the standard.

Boon and Mace (19) reported a GC determination of epinephrine which involved ion-pair extraction and the formation of a derivative with *N,O*-bis(trimethylsilyl)acetamide. The method also included a concentration step for low dosage forms. Reproducibility was estimated to be only ±10%.

The trend now appears to be toward automated or semi-automated methods (20–22). Automated methods offer several advantages, chief of which are the reduction of human error and the normalization of errors inherent in manual procedures. However, in spite of the recent advances in automation, a reliable manual method would still be a more efficient and expedient way to analyze an occasional sample.

Levine and Doyle (23) extracted phenylephrine quantitatively by using partition chromatography with an ether solution of di(2-ethylhexyl)phosphoric acid. The same general procedure was used by Welsh and Sammul (24) to determine isoproterenol in deteriorated inhalations and injections. In their work the di(2-ethylhexyl)-phosphoric acid–ether mobile phase extracted the isoproterenol–di(2-ethylhexyl)phosphoric acid ion-pair, while the isoproterenol sulfonic acid remained in the stationary phase.

Welsh and Sammul (24) also reported standard recovery data for several substances related to isoproterenol, including epinephrine. By using their extraction procedure, a method has been developed to assay unchanged epinephrine in low dosage forms. A portion of sample is buffered to pH 5.6 and incorporated into a diatomaceous earth[1] column. Epinephrine is eluted with an ether solution of di(2-ethylhexyl)phosphoric acid, extracted into dilute hydrochloric acid, and determined fluorometrically.

This partition system, with some modification, is also effective in the analysis of epinephrine and lidocaine hydrochloride in combination. An ether wash of the sample column, prior to the elution of epinephrine, removes most of the lidocaine hydrochloride, which is subsequently trapped on a hydrochloric acid column. Chloroform removes the last traces of lidocaine hydro-

[1] Celite 545, Johns-Manville Corp., New York, N. Y.

Table I—Recovery of Epinephrine and Lidocaine Hydrochloride from Synthetic Mixtures

Sample	Epinephrine Recovery, %	Lidocaine Hydrochloride Recovery, %
I (0.01 mg./ml. epinephrine)	98.5	—
	100.2	—
	100.2	—
	100.5	—
	99.5	—
	Average = 99.8	—
	SD = 0.80%	—
II (0.01 mg./ml. epinephrine, 1% lidocaine hydrochloride)	99.9	99.4
	98.9	99.4
	100.9	101.1
	98.8	101.7
	98.9	100.2
	99.1	99.2
	Average = 99.4	Average = 100.2
	SD = 0.83%	*SD* = 1.03%
III (0.01 mg./ml. epinephrine, 2% lidocaine hydrochloride)	100.0	99.7
	101.3	99.9
	100.0	99.7
	100.0	98.6
	Average = 100.4	Average = 99.5
	SD = 0.64%	*SD* = 0.59%

chloride from the sample column, and additional chloroform elutes lidocaine hydrochloride quantitatively from the acid column.

EXPERIMENTAL

Apparatus—Fluorescence measurements were made using a recording spectrophotofluorometer[2] with 1-cm. cells. The following instrument parameters were employed: xenon lamp; meter multiplier, 0.01; sensitivity, 20–25; 1P21 photomultiplier; slit arrangement No. 4; excitation wavelength, about 281 nm.; and emission wavelength, about 334 nm.

UV spectra were obtained using a recording spectrophotometer[3].

Buffer (pH 5.6)—Mix 1.5 volumes of 1 *M* dibasic potassium phosphate with 8.5 volumes of 1 *M* monobasic potassium phosphate. Adjust to pH 5.60 ± 0.05.

Standard Solutions—The following were used: (*a*) epinephrine bitartrate, 2.16 mcg./ml. (about 1.2 mcg./ml. epinephrine base) in *ether-saturated* 0.1 *N* HCl; and (*b*) lidocaine hydrochloride monohydrate, 0.33 mg./ml. in 0.1 *N* HCl.

Sample Preparation—If necessary, dilute an accurately measured volume of sample to an epinephrine concentration of 10 mcg./ml. using pH 5.6 buffer solution.

Procedure—Use water-saturated solvents throughout.

A: Samples without Lidocaine Hydrochloride—Transfer a mixture of 1 g. of acid-washed diatomaceous earth with 1 ml. of pH 5.6 buffer to a glass chromatographic column containing a pledget of glass wool, and tamp to uniform mass. Into a beaker, pipet 3.0 ml. of sample preparation, add 3.0 ml. of pH 5.6 buffer, and swirl to mix. Add 7 g. of diatomaceous earth, mix thoroughly, and add to column in portions, tamping moderately after each addition.

Wash prepared column with 100 ml. of ether and discard wash. Place a separator containing 10 ml. of 0.1 *N* HCl as a receiver under the column. Elute epinephrine with 50 ml. of a 1.5 in 50 solution of di(2-ethylhexyl)phosphoric acid in ether, followed by 25 ml. of ether.

After all ether has passed through the column, shake separator vigorously for 2 min. Allow layers to separate and then collect the acid layer in a 25-ml. volumetric flask. Repeat extraction with another 10 ml. of 0.1 *N* HCl, again collecting the acid in the volumetric flask. Finally, dilute to volume with 0.1 *N* HCl and mix.

[2] Aminco-Bowman spectrophotofluorometer, American Instrument Co., Silver Spring, Md.

[3] Cary model 15 spectrophotometer, Applied Physics Corp., Monrovia, Calif.

Table II—Assay of Commercial Epinephrine Samples

Sample	Epinephrine Declared, mg./ml.	Epinephrine Found, mg./ml.	Percent of Declared
1	0.01	0.0062	62
2	0.01	0.0052	52
3	0.01	0.0076	76
4	0.02	0.0140	70
5	0.02	0.0182	91
6	0.1	0.078	78
7	0.1	0.100	100
8	0.1	0.107	107
9	0.1	0.108	108
10	0.1	0.088	88
11	0.1	0.111	111
12	0.1	0.110	110
13	0.1	0.113	113
14	10	8.45	85

Adjust the spectrophotofluorometer to about 70% relative fluorescence intensity at 334 nm., reading percent fluorescence at maximum. Use ether-saturated 0.1 *N* HCl as a blank.

B: Samples Containing Lidocaine Hydrochloride—Prepare the sample column as under Procedure A. Mount a second column, prepared with 3 g. of diatomaceous earth and 2 ml. of 1 *N* HCl, directly below the sample column. Pass 150 ml. of ether through both columns and discard wash. Change receivers to a 400-ml. beaker and elute through both columns with 50 ml. of chloroform. Rinse the tip of sample column with chloroform and separate columns.

Continue eluting lidocaine hydrochloride from second column with chloroform, collecting about 200 ml. Evaporate eluate to dryness, dissolve residue in 0.1 *N* HCl, and dilute to a lidocaine hydrochloride concentration of about 0.3 mg./ml. Determine the absorbance of this solution at 262 nm.

Wash sample column with 25 ml. ether and elute epinephrine as under Procedure A.

RESULTS AND DISCUSSION

The method isolates unchanged epinephrine from its sulfonic acid and from lidocaine hydrochloride by utilizing ion-pair formation and partition chromatography. The ether wash removes parabens from the buffer column, along with 90–95% of the lidocaine hydrochloride, if present. Lidocaine hydrochloride is trapped on the 1 *N* hydrochloric acid column while parabens pass through and are discarded. The lidocaine hydrochloride remaining on the buffer column is eluted with chloroform, and the total lidocaine hydrochloride is then quantitatively eluted from the acid column with additional chloroform.

The procedure for epinephrine, while not as sensitive as the USP XVIII (18) procedure, does afford more simplicity and stability. However, two precautions should be observed:

1. The pH of the sample column should not be below 5.2; otherwise, incomplete extraction might occur.
2. The final standard solution should be prepared in ether-saturated 0.1 *N* HCl. Ether-saturated hydrochloric acid apparently enhances the fluorescence of epinephrine by about 8%.

Three synthetic solutions, each containing 0.01 mg./ml. epinephrine and from 0 to 2% lidocaine hydrochloride, were prepared

Table III—Assay of Commercial Epinephrine–Lidocaine Hydrochloride Samples

Sample	Epinephrine, mg./ml. Declared	Epinephrine, mg./ml. Found	Lidocaine Hydrochloride, mg./ml. Declared	Lidocaine Hydrochloride, mg./ml. Found
1	0.01	0.0021	10	9.40
2	0.01	0.0051	10	9.59
3	0.01	0.0083	10	10.0
4	0.01	0.0061	20	19.0
5	0.01	0.0073	20	19.0
6	0.01	0.0083	20	20.4
7	0.01	0.0081	20	20.6
8	0.01	0.0081	20	20.2
9	0.01	0.0102	20	19.3

with the common excipients methyl paraben and sodium chloride in a buffer solution. Replicate analyses of these solutions are presented in Table I.

Several commercial samples were assayed by the proposed procedure (Tables II and III). Judging from the results, there appears to be a significant problem of epinephrine deterioration. Most samples over 6 months in age showed some decomposition. Samples containing 0.02 mg./ml. or less of epinephrine had higher and more severe incidences of decomposition. Additionally, samples that assayed less than 75% of the declared value often had wide variations from vial to vial; individual vial analyses of one sample ranged from 48 to 71%. This would indicate that the rate of decomposition is not constant. Several factors, including storage conditions, pH, substrate composition, and bisulfite concentration, may affect the rate of decomposition, but a discussion of these factors is not within the scope of this report. The cogent element of the method presented here is that it measures the stability of epinephrine in commercial preparations, whatever the factors affecting that stability may be.

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PHARMACEUTICAL TECHNOLOGY

Design and Evaluation of a Rotating Filter–Stationary Basket *In Vitro* Dissolution Test Apparatus I: Fixed Fluid Volume System

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Abstract □ The apparatus described in this report provides a reliable and convenient means for determining *in vitro* dissolution characteristics of tablets, capsules, powders, suspensions, and most other solid drug dosage forms. Basic components of the apparatus are a perforated stationary sample basket, a rotating filter–stirrer assembly, and a closed jacketed dissolution fluid container. Among major advantages of the apparatus are: (*a*) precision-controlled variable intensity of mild laminar liquid agitation; (*b*) continuous or intermittent filtration of representative dissolution fluid samples through a nonclogging, microporous, *in situ* filter for automated or manual dissolution rate determinations; (*c*) convenient means for introducing solid samples in a stationary basket and positioning at a set level in the fluid medium; (*d*) minimal mechanical impacts, abrasion, and wear of the solid sample, with the retainment of its microenvironment during the dissolution process; and (*e*) simultaneous determinations of disintegration–dissolution rates of tablets and capsules. Studies performed using this apparatus are described to demonstrate its reproducibility, reliability, and application versatility as a research, development, and quality control test apparatus. Dissolution rates of five different tablet lots of an antidiabetic drug evaluated by this apparatus correlated with their *in vivo* activity. A multiple-test system for the simultaneous automated determination of six dissolution rates is described.

Keyphrases □ Dissolution equipment—design and evaluation of a rotating filter–stationary basket apparatus, compared to compendial methods □ Rotating filter–stationary basket dissolution apparatus—design and evaluation, compared to compendial methods

It is now generally recognized that the dissolution rate of a drug from its solid dosage form can become the rate-limiting process in the physiological availability and *in vivo* absorption of the drug. In recent years, therefore, considerable interest has been focused on the development of a reliable *in vitro* dissolution test