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Improved, Rapid Spectrophotofluorometric Method for Assay of Isoproterenol Hydrochloride Injections: A Comparative Study

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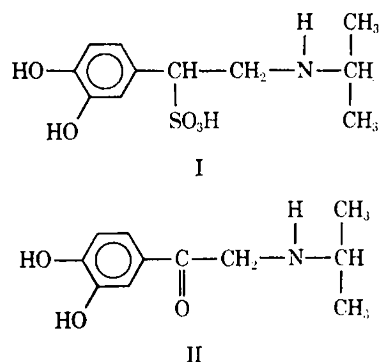
Abstract ☐ A spectrophotofluorometric method for the determination of isoproterenol hydrochloride in parenteral formulations is described. This method is quite simple, precise, and extremely specific for unchanged isoproterenol in decomposed formulations. The method depends on the initial oxidation of isoproterenol hydrochloride with iodine-potassium iodide solution in pH 4.0 (McIlvaine's citrate-phosphate) buffer. The partially oxidized isoproterenol "chrome" derivative is cyclized to the fluorescent "lutin" derivative with strong alkali. The fluorescence is stabilized with alkaline ascorbate solution, which is subsequently determined. The influence of the various decomposition products of isoproterenol, such as isoproterenol sulfonic acid and isoproterenone, on the fluorometric procedure was investigated with concurrent determination of the same solutions by the USP XVIII method. The usefulness of the fluorometric procedure to analyze fresh as well as partially decomposed isoproterenol solutions, stabilized with other antioxidants, is evaluated. The inapplicability of the existing methods to analyze very dilute (1:50,000) solutions of isoproterenol is also described. The specific advantages of the fluorometric procedure in the routine analysis of a large number of samples with speed and accuracy are discussed. Statistical evaluation of the fluorometric method is also reported.

Keyphrases ☐ Isoproterenol hydrochloride solutions—spectrophotofluorometric analysis, compared to compendial method ☐ Spectrophotofluorometry—analysis, isoproterenol hydrochloride solutions

The official USP XVIII method (1) for the analysis of isoproterenol hydrochloride injection in specific formulations and isoproterenol hydrochloride in other formulations involves the column chromatographic separation of unchanged isoproterenol from its degradation products. This is achieved by ion-pair extraction of the intact isoproterenol with bis(2-ethylhexyl)phosphoric acid in ether solution, leaving decomposition products behind on the column (2). The major product of decomposition of isoproterenol injections containing the most commonly used antioxidant sodium bisulfite is isoproterenol sulfonic acid (I). The formation of this derivative is pH dependent, and the reaction is favored at higher pH values. This fact was demonstrated by Higuchi and Schroeter (3). However, when an insufficient quantity of sodium bisulfite is used or when other antioxidants are employed, complications arise due to the presence of other isoproterenol decomposition products such as isoproterenone (II). In such instances, the USP XVIII method may not work satisfactorily. This fact was investigated in depth, and the data are presented in this paper.

Another major disadvantage with the USP XVIII method is that it cannot be used to assay isoproterenol injections in very dilute solutions such as 1:50,000 concentrations. The official method does not work when large volumes of sample have to be eluted from the column. Other disadvantages are: (*a*) the method is lengthy and time consuming; (*b*) the method cannot be successfully used to assay several samples at a time; and (*c*) it is extremely difficult to reproduce results, since the USP XVIII does not describe any of the column chromatographic variables such as tightness of the



packing of the stationary phase in the column, the rate of elution, and other pertinent factors.

Welsh and Sammul (2) reported on the analysis of aged isoproterenol solutions using a modification of Hellberg's (4) fluorometric assay for epinephrine. However, no details of the procedure were given. The purpose of this report is to present a simple, rapid, accurate, and selective fluorometric procedure for the quantitative determination of unchanged isoproterenol in aged formulations. The fluorometric method is based on the fact that isoproterenol in an acidic buffer solution can be selectively oxidized to its "chrome" derivative, which subsequently is cyclized in strong alkaline solution to the fluorescent "lutin" derivative, which is stabilized with sodium ascorbate. The described method has the additional advantage that it can be used to assay dilute (1:50,000) solutions of isoproterenol even in the presence of unusually higher concentrations of its two known decomposition products: isoproterenol sulfonic acid (I) and isoproterenone (II). The merits of the fluorometric method over that of the USP XVIII method, including a statistical evaluation of the method, are reported here.

EXPERIMENTAL[1]

Preparation of Isoproterenol Sulfonic Acid (I)—Isoproterenol hydrochloride (10.0 g., 0.04 mole) and sodium bisulfite (6.78 g., 0.04 mole) were dissolved in 50 ml. of water. The mixture was heated on a steam bath under nitrogen for 2 hr. and then was stored in the dark under nitrogen for 3 days. At the end of 3 days, 25 ml. of water was removed on a flash evaporator and the residue solution was stored in the dark at room temperature under nitrogen for a further period of 6 days. At the end of 6 days, the remaining solution was concentrated on a flash evaporator. The residue was suspended in 25 ml. of ethanol and saturated with ether. The precipitate was filtered, washed with ether, and air dried. The precipitate was suspended in 150 ml. of water, stirred for 10 min., filtered, and then crystallized from water to produce a white crystalline compound. This compound was dried at 40° under vacuum overnight to give isoproterenol sulfonic acid·1/2 H_2O. On drying the sample at 80° under vacuum over phosphorus pentoxide for 48 hr., anhydrous isoproterenol sulfonic acid was obtained, 7.6 g. (68%), m.p. 185–190° (softens), 225–230° dec.; UV (95% ethanol): λ_{max} 284 (ϵ, 3593) and 232 (ϵ, 7558) nm.; IR (KBr): 1135, 1045 (—SO_2), and 650 (—S—O) cm.$^{-1}$; NMR (dimethyl sulfoxide-d_6): δ 8.70 (br, 3, —OH + NH), 6.66 (m, 3, aromatic H), 3.80 [br, t, 1, —(—SO_3H)CH—], 2.34 (m, 3, —CH + CH_2O), and 1.20 (d, 6, —CH_3).

Anal.—Calc. for $C_{11}H_{17}NO_6S$: C, 47.97; H, 6.23; N, 5.08; S, 11.64. Found: C, 47.72; H, 6.29; N, 5.08; S, 12.13.

Preparation of Isoproterenone (II)—Ten grams (0.053 mole) of 3,4-dihydroxy-α-chloroacetophenone[2] was suspended in 100 ml. of 95% ethanol. Isopropylamine, 80 ml. (an excess), was added slowly with stirring. The mixture was refluxed for 2.5 hr., and the solvent was removed under vacuum. The solid was taken up in 400 ml. of acetone, stirred for 30 min., and filtered, and the insoluble solid was washed with more acetone. The crude isoproterenone was suspended in 200 ml. of distilled water, filtered, and washed with distilled water several times. The material was recrystallized from dimethyl sulfoxide–water, resulting in a light-tan compound which, after drying at 60° under vacuum overnight, yielded the desired material, 8.6 g. (76%), m.p. 168–169° dec.; UV (95% ethanol): λ_{max} 312 (ϵ, 7555), 280 (ϵ, 8475), and 232 (ϵ, 12,555) nm.; IR (KBr): 1655 [C_6H_5—C(=O)—CH_2—], 1600, 1550, 1495 (phenyl ring), 1470 (—CH_2—, scissoring), 1345 (CH_3—), 1250 (aromatic ketone), 1125, 1105 (the —CH— out-of-plane vibrations), and 800 (adjacent 3H) cm.$^{-1}$; NMR (dimethyl sulfoxide-d_6): δ 7.10 (m, 6, aromatic H, —OH + NH), 4.02 [S, 2, —C(=O)—CH_2—NH—], 2.85 (m, 1 ϵ H), and 1.05 (d, 6, —CH_3).

Anal.—Calc. for $C_{11}H_{15}NO_3$: C, 63.12; H, 7.23; N, 6.69. Found: C, 62.48; H, 7.12; N, 6.34.

Isoproterenol Standard—Isoproterenol hydrochloride USP, 200 mg., was placed into a 1-l. flask containing 10 ml. of 0.1 *N* hydrochloric acid. The solid was dissolved and the volume was adjusted to 1 l. with nitrogen-purged distilled water. The resulting solution is stable over a period of several weeks when stored in the refrigerator.

Reagents—The preparation of McIlvaine's citrate-phosphate buffer of pH 4.0, of the iodine solution (0.005 *N*) in 1.25% (w/v) sodium iodide, and of the 0.2% ascorbic acid in 5 *N* sodium hydroxide was previously described (5).

Procedures—Isoproterenol hydrochloride injection USP (200 mcg./ml.) is assayed exactly according to the method described for the assay of 100 mcg./ml. epinephrine injection (5). For the assay of isoproterenol hydrochloride injection USP (200 mcg./ml.), 2.0 ml. is diluted to 100 ml. with buffer. To assay the more dilute 20 mcg./ml. isoproterenol hydrochloride injection, 5 ml. of the standard and the sample is diluted to 25 ml. with the citrate–phosphate buffer. Five and 15 ml. of the diluted solutions are further diluted to 50 and 100 ml., respectively, with the citrate–phosphate buffer. The procedure from here on is the same as described for 100 mcg./ml. epinephrine injection (5).

RESULTS AND DISCUSSION

The selective determinations of isoproterenol hydrochloride in the presence of its two known degradation products, isoproterenol sulfonic acid (I) and isoproterenone (II), using the fluorometric method has not been described. Numerous modifications of the fluorometric method for the analysis of other catecholamine derivatives have been published, but none of them is useful as a routine procedure because:

1. The methods described for the initial removal of the interfering material, sodium bisulfite, are cumbersome and have to be carried out in a precise fashion (4).
2. The reported methods have not checked out the fluorometric procedure to find out whether the two above-mentioned decomposition products of isoproterenol or other unknown degradation products interfere in the assay.
3. The fluorescence produced according to the reported methods is quite unstable and necessitates precise timing between the development of fluorescence and its determination on the fluorometer. For this reason, most of the published methods recommend that the fluorescence be determined immediately.
4. Finally, the number of steps involved in the actual procedure of the older methods (4) are many and cumbersome. Fewer steps are involved in the new method described in this paper.

Effect of Various Reagents on Fluorescence—In general, the effect of the concentration of the oxidizing agent, iodine, was found to be of the same nature as was described for the assay of epinephrine (5). The optimum iodine concentration that results in maximum fluorescence was 0.005 *N*. Higher concentrations resulted in decreased fluorescence and lower concentrations were not adequate to oxidize the high concentration of bisulfite used in the simulated formulations. In general, the commercial formulations tested contained 10-fold excess by weight of sodium bisulfite as compared to the isoproterenol hydrochloride. The oxidation of isoproterenol to the chrome derivative with iodine reagent was complete at the end of 3 min. after the addition of the reagent, beyond which lowering of the fluorescence was observed. The chrome derivative of isoproterenol was then cyclized by alkali to the lutin derivative, which is responsible for the fluorescence. The lutin derivative is quite unstable and can undergo further oxidation.

Several antioxidants such as sodium sulfite and ascorbic acid have been recommended not only to stop the further oxidation with iodine but also to prevent the quenching effect of oxygen on the fluorescence. Sodium sulfite was used as the antioxidant in the fluorometric determination of epinephrine at pH 5.0 in acetic acid solution (6). When employing these conditions for the assay of isoproterenol solutions, the fluorescence was found to be extremely

[1] An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) was used. For the isoproterenol concentrations described in the procedures, a Meter Multiplier of the instrument was set at 0.03, with slit width at 1.0 and sensitivity at 30. The excitation wavlength was 420 nm., and the emission wavelength was 510 nm.

[2] 3,4-Dihydroxy-α-chloroacetophenone was obtained from Winthrop Laboratories, Rensselaer, NY 12144

Table I—Comparison of Fluorometric and USP XVIII Methods for the Assay of Fresh Isoproterenol Solutions

Concentration of Isoproterenol Hydrochloride, mcg./ml.	USP XVIII Method: Amount Recovered, mcg./ml.	USP XVIII Method: % Recovered	Fluorometric Method: Amount Recovered, mcg./ml.	Fluorometric Method: % Recovered
180	175	97.3	178.2	99.0
150	155	103.3	149.6	99.7
100	102	102	100.5	100.5
50	49	98	50.0	100

unstable and readings had to be taken immediately after the addition of the glacial acetic acid (6). However, alkaline ascorbate solutions stabilize the fluorescence up to 30 min., thus permitting the reading of several samples at a time on the fluorometer (5). It was found that determination of the fluorescence between 15 and 20 min. after the addition of sodium ascorbate resulted in consistent recovery of isoproterenol from simulated formulations. The concentration of ascorbic acid used was between 1 and 3 mg./ml., with high and stable fluorescent readings resulting when 2 mg./ml. of ascorbic acid was employed. In general, the effects of different reagents on the stability and duration of maximum fluorescence were identical to those described in the assay of epinephrine by this method (5).

Comparison of USP XVIII Method with Fluorometric Method—To evaluate the official method and the fluorometric procedure, fresh solutions of isoproterenol hydrochloride as well as solutions after aging at higher temperatures for several months were employed. Thus, the effect of the interfering decomposition products could be checked, which was not done by an earlier reported fluorometric method (7).

Fresh solutions of different concentrations of isoproterenol hydrochloride containing sodium bisulfite were made and analyzed both by the USP XVIII and fluorometric methods (Table I). The USP XVIII method led to large errors with minor alterations in the technique. For example, the packing of the adsorbent into the column was critical. Best results were obtained when the initial water-saturated ether was allowed to pass through the column for 45–55 min. The results were consistent only when the column was eluted with bis(2-ethylhexyl)phosphoric acid–ether solution for about 50 min. Such details as the tightness of packing the adsorbent into the column and the elution times are not given in USP XVIII but are extremely important to get consistent results. Fresh solutions of isoproterenol were analyzed according to USP XVIII, taking all the above-mentioned precautions. It is evident from Table I that such results are in excellent agreement with the results of the fluorometric method. The USP XVIII method as well as the described fluorometric method gives excellent results when the initial concentration is 50–180 mcg./ml. The limitation of the USP XVIII method is that below 50 mcg./ml. of isoproterenol hydrochloride the final absorbance is less than 0.1, where the accuracy of the results was seriously affected. When a larger volume of the sample was applied to the column (to compensate for dilute solutions), the results were erratic, especially when partially decomposed solutions were analyzed. This was probably due to the excess moisture on the column, which seriously affected the partitioning of the decomposition product between the adsorbent and the bis(2-ethylhexyl)-phosphoric acid–ether solution. However, with the fluorometric method, the solutions can be appropriately diluted so that the final concentration of isoproterenol hydrochloride is between 0.2 and 0.3 mcg./ml. and estimated with accuracy.

Table II—Effect of Isoproterenol Sulfonic Acid on the Recovery of Isoproterenol from Simulated Formulations

Isoproterenol Hydrochloride, mcg./ml.	Isoproterenol Sulfonic Acid, mcg./ml.	Isoproterenol Recovered, %: USP XVIII Method	Isoproterenol Recovered, %: Fluorometric Method
180	20	95.2	101.6
150	50	94.5	101.3
100	100	94.7	101.5
50	150	97.5	100.3
0	200	—	0

Table III—Effect of Isoproterenone on the Recovery of Isoproterenol Hydrochloride from Simulated Formulations Analyzed by USP XVIII and Fluorometric Methods

Isoproterenol Hydrochloride, mcg./ml.	Isoproterenone, mcg./ml.	Recovery of Isoproterenol Hydrochloride, %: USP XVIII Method	Recovery of Isoproterenol Hydrochloride, %: Fluorometric Method
195	5	100	—
190	10	102.5	—
180	20	115.2	—
170	30	124.7	101.1
150	50	152.9	100.5
100	100	—	101.2
50	150	—	100.5
0	200[a]	—	0

[a] This sample consisted of isoproterenone only.

Comparison of USP XVIII and Fluorometric Methods for Assay of Simulated Isoproterenol Formulations—Two known decomposition products of isoproterenol are the sulfonic acid derivative (I), which is formed in solutions containing sodium bisulfite, and the oxidation product isoproterenone (II). Simulated formulations consisting of isoproterenol and its decomposition products were made to determine their effects on the recovery of isoproterenol. To evaluate the merits of the fluorometric method, the simulated formulations were analyzed by the USP XVIII and the fluorometric methods. The effect of each of the degradation products on the recovery of isoproterenol is described below.

Influence of Isoproterenol Sulfonic Acid—Results of the effect of isoproterenol sulfonic acid on the recovery of simulated isoproterenol hydrochloride formulations are shown in Table II. For the simulated formulation containing 75% isoproterenol sulfonic acid, the recovery of isoproterenol according to the USP XVIII method was always low. However, in the concentration range tested, the fluorometric method produced excellent agreement between the actual amount of isoproterenol used in the simulated formulation and its percent recovery. Even 200-mcg./ml. concentrations of isoproterenol sulfonic acid did not result in any fluorescence. Kaistha (8) mentioned that the column chromatographic method always resulted in a positive bias with formulations containing isoproterenol sulfonic acid, so he recommended a preliminary washing of the ether–bis(2-ethylhexyl)phosphoric acid layer containing the unchanged isoproterenol with the phosphate buffer solution to remove any isoproterenol sulfonic acid.

A pertinent observation made in the present study was that if any sodium bisulfite was eluted from the column with the bis(2-ethylhexyl)phosphoric acid–ether solution, the absorbance reading was higher than expected. Perhaps Kaistha's recommendation for washing the eluate initially with phosphate buffer might result in the effective removal of bisulfite or other bisulfite-derived products. In spite of this, Kaistha (8) mentioned higher recovery due to some additionally encountered decomposition products. The metaperiodate method (8) has the particular disadvantage in that higher concentrations of isoproterenol sulfonic acid, when present, adversely affect the assay method. Therefore, Kaistha recommended that the official compendia set upper and lower limits for isoproterenol sulfonic acid when the formulations contain sodium bisulfite as an antioxidant. The particular advantage with this new fluorometric method is that isoproterenol sulfonic acid, even if present in high concentrations, has no effect on the recovery of isoproterenol from formulations stabilized with sodium bisulfite or metabisulfite. When the fluorometric method is used, there is no necessity for stipulating any limits on the isoproterenol sulfonic acid concentration.

Effect of Isoproterenone on Recovery of Isoproterenol—USP XVIII describes a limit test for isoproterenone (λ_{max} 310) in isoproterenol hydrochloride. Since isoproterenone can be formed as one decomposition product of isoproterenol, the possible interference of isoproterenone on the fluorometric method was studied in detail. Simulated formulations consisting of isoproterenone and isoproterenol hydrochloride were made and analyzed both by the USP XVIII and fluorometric methods (Table III). The UV spectra of isoproterenol hydrochloride and isoproterenone when present as a mixture are

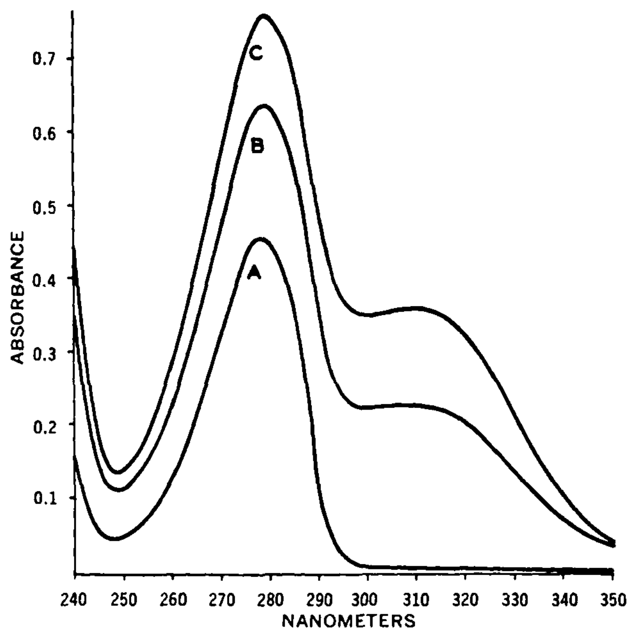


Figure 1—*UV spectra showing the effect of different amounts of isoproterenone on the USP XVIII method for determination of isoproterenol hydrochloride solutions. Key: A, isoproterenol hydrochloride, 200 mcg./ml.; B isoproterenol hydrochloride, 170 mcg./ml., and isoproterenone, 30 mcg./ml.; and C, isoproterenol hydrochloride, 150 mcg./ml., and isoproterenone, 50 mcg./ml.*

shown in Fig. 1. Isoproterenone (molar absorptivity ϵ_{280}^{25} = 8053 and ϵ_{310}^{25} = 6737), when present in low concentrations, does interfere in the assay of isoproterenol (molar absorptivity ϵ_{280}^{25} = 2695) when determined by the USP XVIII method. This is obvious because of the approximately threefold difference in the molar absorptivities of isoproterenone and isoproterenol at the 280-nm. absorption maximum. According to USP XVIII, the absorbance due to pure isoproterenol is determined at 278 nm. Serious errors can result if isoproterenone (λ_{max} 280 nm.) is also present in the samples of isoproterenol because both have nearly identical absorption maxima and because of the higher molar absorptivity of isoproterenone. Thus, both the USP XVIII method and the bis(2-ethylhexyl)phosphoric acid–ether extraction method (8) give false results when isoproterenone is also one decomposition product. As shown in Table III, when 200 mcg./ml. of isoproterenone was assayed by the fluorometric method, the final fluorescence was negligible. It can, therefore, be inferred that under these circumstances the new fluorometric method should be preferred over the USP XVIII method or the bis(2-ethylhexyl)phosphoric acid–ether extraction method (8) to assay isoproterenol formulations.

TLC of simulated inhalations and injections stored in the dark over a period of 4 months showed that they consisted of decomposition products other than the sulfonic acid derivative of isoproterenol (8). Although the exact nature of these additionally encountered

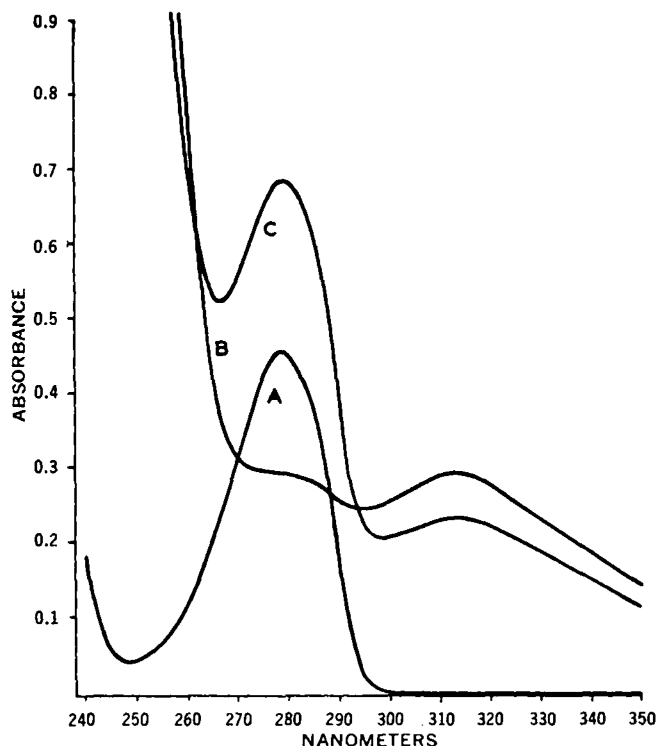


Figure 2—*UV spectra showing the effect of unknown isoproterenol decomposition products on the recovery of isoproterenol from solutions assayed by the USP XVIII method. Key: A, isoproterenol hydrochloride, 200 mcg./ml.; B, isoproterenol hydrochloride solution, 200 mcg./ml., completely decomposed in the presence of 350 mcg./ml. of sodium bisulfite; and C, mixture of A and B.*

artifacts could not be established unequivocally, it was theorized that one of them could be aminochrome–sodium bisulfite addition compound by analogy with a similar compound described by Tse and Oesterling (9) in their determination of total catecholamines in urine. According to Kaistha (8), when simulated isoproterenol formulations were analyzed by the method of Welsh and Sammul (2) (which was essentially adopted by USP XVIII), high and inconsistent values for the parent drug were obtained. This was attributed to the presence of additional decomposition products besides the sulfonic acid derivative (8). Kaistha reconciled this discrepancy by introducing a step to wash the ether effluent from the column with potassium phosphate buffer prior to acid extraction. Although this modification resulted in increased specificity for the USP XVIII method, the procedure is long and cannot be applied to very dilute solutions. The fluorometric method does not require preliminary separation of decomposition products. The fluorescence, due to the lutin derivative, results only from intact isoproterenol and not from its decomposition products.

Comparison of USP XVIII and Fluorometric Methods for Assay of Aged Commercial Isoproterenol Injections—Formulations of isoproterenol hydrochloride injection USP were made containing sodium lactate–lactic acid buffer, sodium bisulfite antioxidant, and sodium chloride for isotonicity. These were stored at room temperature for several months and analyzed both by the fluorometric and USP XVIII procedures. In addition, the amount of isoproterenol sulfonic acid formed was estimated according to the method described by Kaistha (8) (Table IV). The close agreement between the two methods indicates that the fluorometric method is comparable to the USP XVIII method when the method is exactly followed, as indicated previously in this report. The material balance between isoproterenol hydrochloride and isoproterenol sulfonic acid was in good agreement with the original isoproterenol hydrochloride concentration. A point worth noting here is that, in the presence of a large excess of sodium bisulfite, the isoproterenol solutions remained clear and colorless even though the solutions were completely decomposed. This suggests that the sulfonic acid derivative of isoproterenol is the only decomposition product for formulations containing high amounts of sodium bisulfite. However, as

Table IV—Comparison of USP XVIII and Fluorometric Methods for the Assay of Aged Isoproterenol Injections (1:5000)[a]

Sample[b]	USP XVIII Method, %	Fluorometric Method, %	Isoproterenol Sulfonic Acid[c], %
A	85.9	88.2	13.4
B	62.8	63.5	39.4
C	58.0	58.0	44.2
D	61.2	58.8	42.4

[a] These injections also contained a buffer consisting of lactic acid–sodium lactate, sodium chloride (7.0 mg./ml.), and sodium bisulfite (1.00 mg./ml.) as antioxidant, the final pH adjusted to 3.50. Celite 545 AW was used as the adsorbent in the USP XVIII method. [b] Sample A was stored for 2 months at 25°, while Samples B, C, and D were stored 10.5 months at 25°. [c] Isoproterenol sulfonic acid was estimated according to the method described by Kaistha (8).

Table V—Statistical Evaluation of Fluorometric Method to Analyze Isoproterenol Hydrochloride Solutions

	Concentration of Isoproterenol Hydrochloride, mcg./ml.	
	202.5	100.5
	200.5	100.5
	196.5	99.5
	199.5	102.0
	198.5	100.5
Mean	199.5	100.60
SD, %	1.118	0.894
SEM %	0.5	0.400
Mean error	−0.5	+0.6

pointed out earlier, if other unknown decomposition products such as isoproterenone are present, the USP XVIII method results in large errors.

Influence of Other Unknown Isoproterenol Oxidation Products on Fluorometric Method—It was stated earlier that isoproterenol decomposes to its sulfonic acid derivative in the presence of excess sodium bisulfite. However, in the presence of lower amounts of sodium bisulfite, additional decomposition products have been reported (8). The completely decomposed solutions in such cases were not clear and colorless and occasionally were accompanied with black precipitate. Therefore, it is essential to establish that the fluorometric method is specific only for the unchanged isoproterenol. Isoproterenol hydrochloride (230 mcg./ml.) in lactic acid-sodium lactate buffer (pH 3.50–3.60) stabilized with 350 mcg./ml. of sodium bisulfite was made. These solutions were stored in the dark at 56° for an extended time and were analyzed by the fluorometric method. TLC of the completely decomposed solutions indicated the presence of products other than isoproterenol sulfonic acid[3]. The completely decomposed solution was added in varying amounts to solutions of isoproterenol hydrochloride of known concentration, and the samples were analyzed by the fluorometric method. There was no fluorescence due to the completely degraded isoproterenol under the conditions tested. The results indicated that isoproterenol decomposition products other than the sulfonic acid, when present in high concentrations, do not influence the recovery of isoproterenol hydrochloride when assayed by the fluorometric method. The same decomposed isoproterenol solution was added to a known concentration of fresh isoproterenol hydrochloride solution. The decomposed isoproterenol and the mixture were separately analyzed by the USP XVIII method. The UV spectra of the results shown in Fig. 2 indicate interference from the unknown degradation products. The USP XVIII procedure requires the drawing of a baseline between the two minima (about 300 and 250 nm.) of isoproterenol hydrochloride to measure the absorbance due to unchanged isoproterenol hydrochloride. From Fig. 2, it is obvious that the minima do not occur at these prescribed wavelengths when these unknown oxidation products are present. This experiment thus proves that the fluorometric method described here is specific for the unchanged isoproterenol hydrochloride and that its decomposition products, when present, do not have any effect.

Statistical Analysis of Fluorometric Method—To check the precision and accuracy of the procedure, several samples containing isoproterenol hydrochloride of low and high concentrations were analyzed by the fluorometric method. The statistical treatment of the data is summarized in Table V. The percent standard deviation ranged from 0.89 to 1.12. However, the standard error of the mean was 0.4–0.5 at the low and high levels of isoproterenol hydrochloride. The mean error, which is the difference between the average test results and the true result, ranged from +0.6 for the lower concentration to −0.5 for the higher concentration of isoproterenol hydrochloride.

Influence of Other Antioxidants on USP XVIII and Fluorometric Methods of Isoproterenol Assay—The effect of other antioxidants, such as ascorbic acid and α-monothioglycerol, on the recovery of isoproterenol from aged solutions was investigated. Fresh solutions of isoproterenol containing ascorbic acid or α-monothioglycerol were analyzed by the fluorometric method. There was no interference from either of these two antioxidants on the fluorescence.

[3] F. Bangert, Chemical Control, Bristol Laboratories, personal communication.

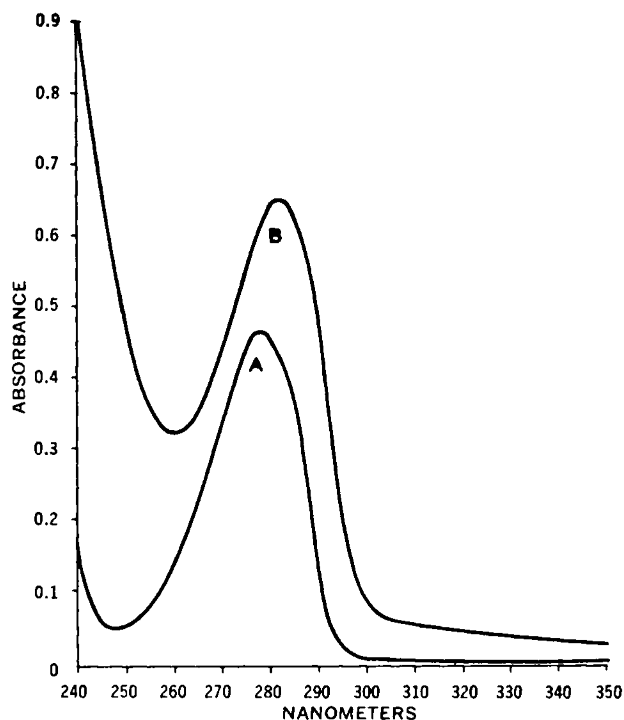


Figure 3—*UV spectra indicating the effect of isoproterenol hydrochloride decomposition products resulting from solutions stabilized with α-monothioglycerol as the antioxidant and assayed by the USP XVIII method. Key: A, isoproterenol hydrochloride, 200 mcg./ml.; and B, solution of 230 mcg./ml. isoproterenol hydrochloride reacted with 2 mg./ml. of α-monothioglycerol. This solution did not have any isoproterenol activity by the fluorometric method.*

In addition, isoproterenol formulations stabilized with either ascorbic acid or α-monothioglycerol were stored at elevated temperatures. These samples were analyzed by the fluorometric method and were shown to be completely devoid of any isoproterenol activity. The completely decomposed solution and another sample of isoproterenol were then assayed by the USP XVIII method (Fig. 3). It is obvious that the UV spectra of the intact isoproterenol hydrochloride and its degradation product in the presence of excess α-monothioglycerol are similar. It is also interesting to note that the absorbance due to this decomposition product is much higher at its maximum (about 278 nm.) when compared to the unchanged isoproterenol. Similar interferences were observed with isoproterenol solutions preserved and aged in the presence of ascorbic acid. Thus, the USP XVIII method or its modification, the bis-(2-ethylhexyl)phosphoric acid shakeout method (8), can be misleading if used to analyze solutions preserved with antioxidants other than sodium bisulfite. The reliable method in such instances is the fluorometric method, which is specific for the unchanged isoproterenol hydrochloride.

SUMMARY

The described fluorometric method is unique because of its applicability to the analysis of extremely dilute solutions of isoproterenol. The simplicity of the procedure lends itself to the assay of several samples at a time; with the official methods, it is difficult to carry out several determinations simultaneously. The fluorometric method is timesaving, whereas the USP XVIII method is lengthy and tedious. Moreover, it has been shown that the fluorometric method is unique in that it does measure only the unchanged isoproterenol; decomposition products, when present, do not interfere even in extremely high concentrations. Oxidative degradation products, if present, interfere in the USP XVIII and the bis(2-ethylhexyl)phosphoric acid shakeout methods. These latter two methods were specifically developed with the assumption that isoproterenol sulfonic acid is the only decomposition product present in the formulations.

It has been shown that the fluorometric method is the method of choice to assay isoproterenol formulations, since this procedure is based only on the intact isoproterenol and other decomposition products, even if present in significant amounts, do not influence the final fluorescence. The described fluorometric method has the potential for automation and thus can be of immense value to the pharmaceutical industry to monitor routinely the stability of isoproterenol formulations.

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Head-Space GLC Determination of Triethylamine in Pharmaceuticals

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Abstract □ A head-space GLC procedure is described for the quantitative determination of residual triethylamine in streptomycin sulfate and methacycline hydrochloride. This method is both rapid and accurate.

Keyphrases □ Streptomycin sulfate—head-space GLC analysis for residual triethylamine □ Methacycline hydrochloride—head-space GLC analysis for residual triethylamine □ Triethylamine residue in streptomycin sulfate and methacycline hydrochloride—head-space GLC analysis □ GLC, head-space—analysis, residual triethylamine in streptomycin sulfate and methacycline hydrochloride

Triethylamine has been determined qualitatively by GC (1–3). Umbreit *et al.* (4) decomposed aliphatic amine hydrochloride mixtures in the injection port of a gas chromatograph. They then analyzed 17–170 p.p.m. of each amine in the mixture. Pesez and Bartos (5) used derivatization and colorimetry to determine triethylamine at the 15-mcg. level. Kertes (6) used colorimetry to measure triethylamine at the 5% level. Derivative formation and TLC were employed by Baudot (7) at the 20-mcg. level. None of these methods involved the determination of triethylamine in pharmaceuticals.

Table I—Preparation of Standard Curve for Determination of Triethylamine in Streptomycin Sulfate

Stock Solution, ml.	1 *M* Sodium Hydroxide, ml.	Triethylamine per Serum Vial, mcg.
1.0	9.0	1090
3.0	7.0	3270
5.0	5.0	5450

Table II—Preparation of Standard Curve for Determination of Triethylamine in Methacycline Hydrochloride

Stock Solution, ml.	1 *M* Sodium Hydroxide, ml.	Triethylamine per Serum Vial, mcg.
0.5	9.5	535
1.0	9.0	1090
2.0	8.0	2180

This report describes a simple, rapid method for determining triethylamine in streptomycin sulfate[1] and in methacycline hydrochloride [4-(dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a* - octahydro - 3,5,10,12,12*a* - pentahydroxy - 6 - methylene - 1,11 - dioxo - 2 - naphthacenecarboxamide hydrochloride][1] to levels as low as 0.05%. The principle on which this method is based is that the head-space gas over a solution of a nonvolatile solute in a sealed container will contain amounts of any volatile substances present in the solution after equilibration at a constant temperature. The quantity of solvent vapors in the head-space gas will be proportional to the concentration of the solvents in the solution.

The method of Bassette *et al.* (8) was modified for this determination.

EXPERIMENTAL

Preparation of Standard Curve—Dilute 150 µl. triethylamine[2] to 100 ml. with 1 *M* sodium hydroxide solution. Transfer 1 ml. of this

[1] Pfizer Inc., New York, N. Y.
[2] Technical grade, Matheson, Coleman and Bell, East Rutherford, N. J.