ion as a participant in a charge-transfer interaction and points to the possibility that it can interact similarly with enzyme or coenzyme systems in the body. It is conceivable that such an occurrence might be involved in metabolic processes which are dependent on this vitamin.

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Determination of Ephedrine Salts in Liquid Dosage Forms

By MARTIN I. BLAKE and DANIEL A. NONA

Analytical procedures are presented for the determination of ephedrine salts in syrups, elixirs, solutions, and injections. The ephedrine is extracted with the strong cation exchange resin, Dowex 50 X-8, and is subsequently eluted with 2 normal hydrochloric acid. After evaporation of the eluate to dryness, the residue is titrated nonaqueously with perchloric acid in dioxane in the presence of mercuric acetate. In formulations containing sodium chloride and other salts, the ephedrine is eluted with an alcoholic solution of ammonia. The eluate is aerated until all the ammonia is expelled. The ephedrine is determined by titration with hydrochloric acid. Comparison is made with the official assay for ephedrine sulfate solution.

EPHEDRINE, ephedrine salts, and their dosage forms have been official since U.S.P. XI and N.F. VI. The official assay procedure has usually involved ether extraction of the alkaloid liberated from the salt combination by addition of base. The ephedrine is then determined by residual alkalimetry. Ephedrine sulfate and phenobarbital capsules (1) are analyzed for ephedrine by a distillation method developed by Hilty (2) and further applied by Hilty and Wilson (3). Until recently ephedrine sulfate capsules (4) were also analyzed by this method. There is no official assay for N.F. XI ephedrine sulfate syrup.

Colorimetric procedures have been developed for the estimation of ephedrine. These have been reviewed by Higuchi and Bodin (5) and Snell and Snell (6).

Ephedrine and ephedrine salts have been determined by nonaqueous titration. Auerbach (7) titrated ephedrine in acetic acid with acetous perchloric acid. Salts of organic bases were titrated with perchloric acid in dioxane by Pifer and Wollish (8). Salts of alkaloids including ephedrine were studied. Titrations were effected visually and potentiometrically. Since chloride ion is too weakly basic to react quantitatively with the perchloric acid, the authors found that by adding mercuric acetate to the titration mixture, the chloride is tied up as undissociated mercuric chloride. The liberated acetate ion is then titratable with perchloric acid. Mercuric acetate itself is essentially undissociated in acetic acid and, therefore, does not titrate as a base. Rink and Lux (9) and Ekeblad (10) determined ephedrine hydrochloride with acetous perchloric acid by applying the method of Pifer and Wollish. "The British Pharmacopoeia" (11) recognizes this procedure for the assay of ephedrine hydrochloride tablets. Chatten and Pernarowski (12) analyzed for ephedrine in oily nasal sprays with acetous perchloric acid. Aqueous sprays were extracted with chloroform prior to nonaqueous titration.

Ion-exchange resins have been employed in the determination of alkaloidal salts including ephedrine hydrochloride and ephedrine sulfate. Amberlite IR-4B, a weak anion-exchange resin, was used by Jindra (13) for ephedrine sulfate but was found unsuitable (14) for ephedrine hydrochloride. The resin removes the acid component of the sul-

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fate salt while the alkaloidal base appears in the eluate which is titrated visually with standard hydrochloric acid. This resin apparently does not split the ephedrine hydrochloride quantitatively. Samuelson (15) has noted that with weakly basic anion exchangers incomplete conversion may occur when salts of fairly strong bases such as ephedrine are involved. Saunders, *et al.* (16), used a strong anion-exchange resin to liberate the free base from ephedrine hydrochloride. Since this resin is a salt splitter, inorganic salts interfere. These authors also noted that weak anion exchangers are unsuitable for salts of strong bases as ephedrine. However, Vincent, *et al.* (17), found Amberlite IR-45 (a weakly basic anion-exchange resin) suitable for the estimation of ephedrine sulfate in liquid dosage forms. The column was washed with water to remove sodium and other interfering components. The ephedrine was removed subsequently by elution with 75% ethanol.

Huyck (18) found that Amberlite IRC-50 was a good adsorbent for ephedrine alkaloid. Blaug and Zopf (19) used this resin to extract the ephedrine from an elixir which also contained an antihistamine salt. The ephedrine was retained by the resin while the antihistamine salt appeared in the eluate. Ephedrine salts were not employed in their study.

The present paper describes procedures for determining ephedrine salts in liquid dosage forms by ion-exchange chromatography and nonaqueous titrimetry. A modification is presented which eliminates interference by inorganic salts and certain synthetic sweetening agents.

EXPERIMENTAL

Preparation of Exchange Column

The strongly acidic cation-exchange resin Dowex 50 X-8 (200–400 mesh) was used in this study. A Mohr buret cut to a length of 30 cm. served as the chromatographic tube. The end of the tube was joined to a glass tip by a 5-cm. piece of rubber tubing. A Hoffman clamp was fixed to the rubber tubing midway between the buret and the glass tip. A plug of glass wool was inserted into the bottom of the buret to support the resin column. Ten grams of resin was added to the tube in the form of a suspension in water. When the resin settled completely, the column was washed with 200 ml. of distilled water, 100 ml. of 1 *N* hydrochloric acid, and finally with distilled water until the eluate was neutral. The column was ready for use. A layer of solvent was maintained above the resin column at all times.

Assay Procedure

Method A.—A 100-mg. sample of ephedrine sulfate or ephedrine hydrochloride, accurately weighed, was dissolved in 10 ml. of distilled water. The solution was added to the resin column. The column was washed with at least 100 ml. of distilled water. The ephedrine was then eluted from the column with 2 *N* hydrochloric acid. One-hundred milliliters of eluate was collected in a 150-ml. beaker. The solution was evaporated to dryness on a steam plate. Ten milliliters of distilled water was added; the solution was again evaporated to dryness. This was repeated once. The residue was analyzed for ephedrine content by nonaqueous titration.

Ten milliliters of glacial acetic acid, 20 ml. of dioxane, and 10 ml. of 6% mercuric acetate in glacial acetic acid were added to the beaker containing the ephedrine hydrochloride residue. The solution, magnetically stirred, was titrated with 0.1 *N* perchloric acid in dioxane using a Fisher titrimeter equipped with a calomel and glass electrode system.

Titration was also effected visually by adding two drops of a 0.2% methyl violet solution in glacial acetic acid to the titration beaker. The proper indicator color change was noted by using indicator solution in conjunction with a potentiometric titration. The color change corresponding to the graphic end point was from a violet to an intense blue coloration and was readily detectable with the addition of one drop of titrant at the end point.

Method B.—A sample of ephedrine hydrochloride or ephedrine sulfate was applied to the resin column as described under *Method A*. The ephedrine was eluted with 5% ammonium hydroxide in 95% alcohol prepared by diluting stronger ammonia water with ethanol. A total of 60 ml. of eluate was collected in a 150-ml. beaker. The solution was gently aerated by passing a stream of air over the beaker until the ammonia was completely expelled from the solution. A study, reported later in this paper, indicated that at least 3 or 4 hours were necessary for this purpose. The liquid level in the beaker was kept constant by adding alcohol from time to time. The solution was then titrated visually with 0.1 *N* hydrochloric acid using three drops of methyl red T.S. as the indicator.

Method C.—After titration of the eluate as de-

Table I.—Analysis of Ephedrine Salts, Free and in the Presence of Sodium Chloride

Ephedrine salt	Weighed, mg.	Sodium Chloride, mg.	Recovery, %	Analysis, Method
Ephedrine HCl	122.0	...	98.7	A
	123.4	...	99.7	A
	124.7	...	100.2	A
	126.2	...	99.2	A
	104.6	50.2	98.7	B
	...	...	99.2	C
	113.2	49.6	100.3	B
	...	...	98.2	C
	122.5	51.5	100.2	B
	...	...	100.7	C
Ephedrine sulfate	96.4	...	99.9	A
	102.7	...	99.2	A
	106.3	...	99.6	A
	110.0	...	99.7	A
	103.7	57.1	98.7	B
	...	...	98.2	C
	105.5	52.1	99.9	B
	...	...	100.1	C
	109.3	74.5	100.8	B
	...	...	102.1	C

TABLE II.—ANALYSIS OF LIQUID DOSAGE FORMS CONTAINING EPHEDRINE SULFATE

Dosage Form	Sample	Labeled Amount	Recovery, %	Method
Ephedrine sulfate solution	I (N.F.)	3 Gm./100 ml.	99.51 ± 1.05[a]	B
			100.37 ± 1.68	C
			99.00[b]	D
	II (N.F.)	3 Gm./100 ml.	101.46 ± 1.21	B
			100.00 ± 1.06	C
			103.33	D
	II	1 Gm./100 ml.	100.52 ± 0.33	B
			100.31 ± 0.47	C
			99.40	D
Ephedrine sulfate elixir	I	0.440 Gm./100 ml.	100.49 ± 0.24	B
			101.41 ± 1.01	C
			99.55	D
Ephedrine sulfate syrup	I	0.220 Gm./100 ml.	99.88 ± 1.02	A
			100.70 ± 0.84	B
			100.61 ± 1.17	C
			102.35	D
	I (N.F.)	0.400 Gm./100 ml.	99.60 ± 0.93	A
			97.96 ± 1.06	B
			98.35 ± 0.81	C
			98.79	D
	II	0.400 Gm./100 ml.	100.20 ± 0.94	B
			100.35 ± 0.55	C
			98.50	D
Ephedrine sulfate injection	I	50 mg./ml.	97.65 ± 0.41	B
			97.55 ± 1.25	C

[a] Standard deviation; based on at least five determinations. [b] Results of official assay are averages of duplicate runs.

scribed under *Method B*, the solution (now containing ephedrine hydrochloride) was evaporated to dryness and the residue analyzed by nonaqueous titration as described under *Method A*.

Method D.—Certain samples (indicated in Table II) were analyzed for comparative purposes by the procedure described for N.F. XI ephedrine sulfate solution.

For *Methods A*, *B*, and *C* blank runs were conducted with each series of four determinations, and the necessary corrections were made in the calculations. The data for the analysis of ephedrine salts, free and in the presence of sodium chloride, are shown in Table I.

Aeration Time

The time of aeration necessary for complete removal of ammonia from eluate obtained in *Method B* was determined in the following manner. A series of pure ephedrine samples was prepared by weighing accurately 75 to 100 mg. of pure alkaloid into 100-ml. beakers. Sixty milliliters of 5% ammonia in alcohol was added to each beaker. Each solution was subjected to gentle aeration by directing a fine stream of air above the surface of the liquid for varying intervals of time. The solutions were analyzed for ephedrine content by titration with 0.1 *N* hydrochloric acid using methyl red T.S. as the indicator.

Analysis of Liquid Dosage Forms

A number of dosage forms containing ephedrine salts were analyzed by the procedures previously described. For viscous solutions, as the syrup and elixir, an aliquot containing about 100 mg. of ephedrine salt was transferred by pipet to a suitable beaker. The solution was diluted with an equal volume of 50% alcohol and added to the ion-exchange column. When the last of the solution passed below the surface of the resin, the column was washed with 150–200 ml. of distilled water. The ephedrine was eluted from the column as described for *Methods A* or *B*. For the ephedrine sulfate solutions and the injection, a suitable aliquot was added directly to the column and treated as above. The data for the analysis of liquid dosage forms are shown in Table II. Samples designated as I are commercial preparations, those listed as II are preparations routinely prepared in the manufacturing pharmacy of our pharmacy department.

DISCUSSION

The official assay for ephedrine salts, free and in dosage forms, is based on an extraction procedure with ether. It is tedious and time consuming. While no assay is recognized for the syrup, application of the official assay for the solution to the syrup did yield favorable results (Table II).

The strong cation-exchange resin, Dowex 50 X-8, is an effective salt splitter and removes the ephedrine quantitatively from its salt form. Excellent results were obtained by *Method A* for both the hydrochloride and the sulfate. The data are reported in Table I. Since this resin is a salt splitter, sodium chloride interferes. Both sodium ion and ephedrine are retained by the column. Elution with 5% ammonium hydroxide in alcohol (*Method B*) displaces the ephedrine from the column but not the sodium. This proved to be an effective desalting technique. The eluate was aerated by passing a stream of air over the solution until all the ammonia was removed. The effect of aeration time on ephedrine recovery was noted experimentally. Under the conditions used, high results (122.6%), due to the presence of ammonia calculated as ephedrine, were still obtained after aeration for 2.5 hours. All the ammonia was removed by aeration for 3 hours, and there was no appreciable loss of ephedrine after aeration for 8 hours. Results obtained by *Method B* for ephedrine

salts in the presence of sodium chloride are recorded in Table I. The ephedrine hydrochloride formed in *Method B* was then determined by nonaqueous titration (*Method C*) as a confirmatory procedure.

The proposed methods were applied to liquid dosage forms including solutions, an elixir, syrups, and an injection. The data are reported in Table II. Since the solutions contain sodium chloride, *Method A* could not be used. Favorable results were obtained with *Methods B*, *C*, and *D*.

The elixir reported in Table II was a commercial sample and when analyzed by *Method A* gave very high results. The manufacturer upon request was kind enough to supply the formula of the elixir which did contain appreciable amounts of sodium saccharin and sodium chloride. Quantitative results were obtained by *Methods B* and *C*. The elixir responded to the official assay for ephedrine sulfate solution.

The syrups yielded good results by the proposed methods as well as by the official assay procedure for the solution. Since the injection contains sodium chloride, *Methods B* and *C* were used.

Coloring agents found in the elixir and syrup do not interfere. They are readily washed from the column with water. Nonionic agents and acidic components in general, are not adsorbed by the column. Where synthetic sweeteners such as sodium saccharin are used in the formulation, *Method B* must be employed. Other organic bases and their salts will interefere with the proposed methods. However, if the base strength differs significantly from that of ephedrine, a nonaqueous differential titration may be possible.

Since four assays may be conveniently conducted at the same time, the proposed methods are less time consuming and less tedious than the official assay. The procedures are simple, accurate, and applicable to all commonly available liquid dosage forms of ephedrine salts. They should also be applicable to solid dosage forms and to combinations of ephedrine with other therapeutic agents such as barbiturates.

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Rate of Anaerobic Degradation of Ascorbic Acid in Aqueous Solution

By PER FINHOLT, ROLF B. PAULSSEN, and TAKERU HIGUCHI

The pH rate profile of the rate of disappearance of ascorbic acid from aqueous solution under anaerobic conditions has been determined at 96°. The dependency on pH is surprisingly low over pH range of 1–11. The profile shows a small but apparently real maximum at pH = pK_{a_1}, an effect which can be rationalized by assuming formation of a salt-acid complex in solutions. The anaerobic rate shows buffer dependency but relatively small ionic strength effect.

ALTHOUGH the oxidative route of degradation of ascorbic acid has been extremely well studied, relatively few papers have appeared dealing with degradative loss of ascorbic acid under anaerobic conditions. Since in practice reduction in the concentration of this vitamin in liquid pharmaceutical preparation appears to follow largely the latter route, serious study of the factors influencing its rate was felt needed. Even in instances where losses in the ascorbic acid concentration are of no major concern, the gas produced in the process often poses a problem.

Previous studies have been largely of qualitative nature. Reichstein and Grüssner (1), for example, showed that when ascorbic acid was heated with 0.2 *N* hydrochloric acid a decrease in the iodine consumption and furfural was formed. Their observation was part of a work on the synthesis of ascorbic acid from 2-keto-L-gulonic acid.

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