262 mμ against the elution solvent blank. Compare the absorbance of the sample to that of the sulfanilamide reference standard solution.

RESULTS AND DISCUSSION

Proprietary standards of sulfacetamide and sodium sulfacetamide were analyzed by the column chromatographic procedure. A portion of the sodium sulfacetamide was recrystallized and also analyzed. All three standards were eluted with an additional 30 ml. of elution solvent after the first 50 ml. was collected. The 50-ml. fraction exhibited absorbance maxima at 262 mμ; the subsequent eluates did not show UV absorbance. Therefore, the sulfanilamide was completely eluted and no additional sulfanilamide or any sulfacetamide was present in subsequent 30-ml. eluates. However, each batch of alumina should be tested to ensure that the desired separation occurs in the first 50 ml. of eluting solvent.

Sulfanilamide was added to the free sulfacetamide and to the recrystallized sodium salt, and recoveries were obtained. Data from analyses using these standards are reported in Table I.

Two buffered ophthalmic solutions of 10 and 30% sodium sulfacetamide and a tablet form of sulfacetamide, 500-mg. label declaration, were assayed for their sulfanilamide content. Known amounts of sulfanilamide were added and percent recoveries were determined. Data from these analyses showed that the ophthalmic solutions contained a larger percentage of sulfanilamide than the tablets. This agrees with the quantitative findings of Gruber and Klein (4).

The USP XVII (6) or NF XII (7) procedure failed to detect any degradation of sulfacetamide preparations since it is a general titrimetric procedure for sulfonamides.

The residue left after evaporation of the column eluate was confirmed as sulfanilamide by IR spectroscopy, employing micro-KBr disk techniques.

All standards and samples used in this study were analyzed by the screening procedure of Gruber and Klein (4) on silica gel. Amounts were spotted to yield about 0.1 mcg. of sulfanilamide as determined by the column chromatographic procedure. Sulfanilamide was detected in all cases except in the sulfacetamide standard and the tablet form of sulfacetamide. These also gave the lowest results by the column chromatographic procedure.

The method described in this paper can also be used to determine purity of reference standards of the free sulfa or its sodium salt to meet compendial requirements. Levels of sulfanilamide below 1% can be quantitated.

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Selective Determination of Isoproterenol and Isoproterenol Sulfonic Acid in Pharmaceutical Dosage Forms

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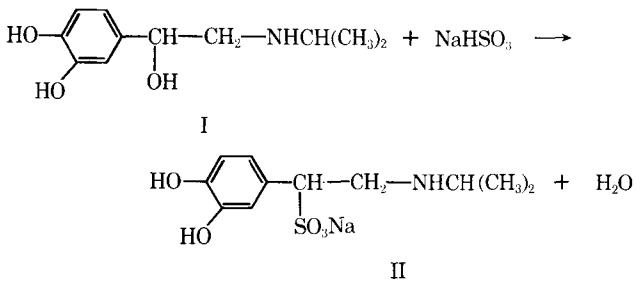
Abstract □ Two simple, precise, and specific methods for the determination of isoproterenol in decomposed formulations are described. DEHP shakeout procedure, which depends upon ion-pair formation with the unchanged drug at a suitable pH, enables the quantitative determination of isoproterenol from the DEHP–ether phase and of its sulfonic acid from the aqueous buffer phase. Isoproterenol sulfonic acid can then be determined selectively by treating the aqueous buffer phase with Doty's reagents. The sodium metaperiodate method has been developed as an alternate checking procedure to validate the results obtained by the DEHP method and involves the quantitative formation of an aryl aldehyde of the unchanged drug. The merits of both the procedures over the conventional UV and visible spectrophotometric procedures are shown by their application to the analysis of simulated decomposed formulation, aged simulated inhalations and injections, and commercial formulations. A thin-layer chromatographic procedure for the separation and detection of isoproterenol and its sulfonic acid and other artifacts is described.

Keyphrases □ Isoproterenol dosage forms—analysis □ Isoproterenol sulfonic acid formed in products—analysis □ Degradation products presence—isoproterenol determination □ Di-(2-ethylhexyl)phosphoric acid extraction method—isoproterenol determination □ Metaperiodate sodium method—isoproterenol determination □ UV spectrophotometry—analysis □ TLC—analysis

The selective determination of isoproterenol (3,4-dihydroxy-α-[(isopropylamino)methyl]-benzyl alcohol hydrochloride), in the presence of isoproterenol sulfonic acid and other decomposition products or vice versa, presents an unusually difficult analytical problem. Recently it became evident that the composition of pharmaceutical dosage forms, especially inhalations and injections containing isoproterenol hydrochloride, can change with aging. The change is attributed to the interaction of isoproterenol with the bisulfite antioxidant, by which the alcoholic hydroxyl group of the drug is replaced by a sulfonic acid group (1) as shown in



Scheme I

Scheme I. Higuchi *et al.* (2, 3) have reported a similar type of reaction between bisulfite and epinephrine and other suitably substituted benzyl alcohol derivatives. The UV spectrum of the physiologically inactive product has the same absorption maximum as that of isoproterenol. Thus the current USP XVII (4) assay procedure based on spectrophotometric measurement of the active drug at 279 mμ will not yield true values for the unchanged drug in decomposed formulations. Similarly the colorimetric procedure, as described in the BP 1968 (5) for tablets is not specific for isoproterenol, as any impurity such as isoproterenol sulfonic acid possessing at least two phenolic groups attached to adjacent carbon atoms will undergo this color reaction (6).

Recently, a notable improvement in the determination of unchanged isoproterenol in pharmaceutical dosage forms was achieved by Welsh and Sammul (1), who used the principle of ion-pair formation of the drug with di-(2-ethylhexyl)phosphoric acid (DEHP) at a suitable pH. This idea of DEHP–organic base ion-pair formation was originally contributed by Temple (7) and Temple and Gillespie (8), who reported that several phenolic phenethylamines, including isoproterenol, show partition coefficients favoring their extraction into a chloroform phase containing DEHP from an aqueous phase adjusted to a suitable pH. Levine and Doyle (9) extracted phenylephrine quantitatively as its DEHP ion-pair, using partition chromatography and an ether solution of DEHP as a mobile phase. In the method proposed by Welsh and Sammul (1), a portion of the sample buffered to pH 5.8 is used as a stationary phase supported on a diatomaceous earth[1] column. The passage of an ether solution of DEHP through the column extracts the DEHP–isoproterenol ion-pair while the sulfonic acid is retained in the stationary phase. This method, when applied to simulated preparations, gave higher values for isoproterenol. The results could not be reproduced unless a step to wash the DEHP–ether effluent (from the diatomaceous earth column) with buffer was introduced prior to the extraction with 0.1 *N* sulfuric acid. Although the method as modified was found specific in the presence of Compound II (Scheme I), the recoveries for unchanged drug in the aged simulated inhalations were found higher due to some additionally encountered decomposition products.

By modification of Hellberg's fluorimetric assay for epinephrine (10), Pratt *et al.* (referred to in *Reference 1*) were able to analyze aged isoproterenol solutions in the presence of Compound II. No published data are yet available about the variability or about the inherent instability of this lutin method when applied to aged pharmaceutical dosage forms of isoproterenol.

The purpose of this communication is to present two simple, rapid, accurate, and selective assay procedures for the quantitative determination of unchanged isoproterenol in aged formulations. The chromatographic method of Welsh and Sammul (1) based on ion-pair formation with DEHP has been modified to a simple shakeout procedure. An aliquot of the formulation (without dilution) is transferred to a separator containing potassium phosphate buffer and extracted with DEHP–ether solution. The DEHP–ether solution after washing with buffer and water, respectively, is extracted with dilute sulfuric acid to partition the unchanged isoproterenol as its sulfate into the aqueous phase. The UV absorbance of the sulfate is then determined. The aqueous-buffer phase left after DEHP–ether extraction is diluted to a definite volume. The UV absorbance is measured in order to calculate the amount of total water-soluble decomposition products. Subsequently, an aliquot of this is treated with aminoacetate buffer and ferrous sulfate–citrate reagent (Doty reaction) (6) to determine quantitatively the amount of isoproterenol sulfonic acid (Compound II) present in the formulation. The second method developed in these laboratories consists of treating a suitably diluted aliquot of the preparation with 2% aqueous solution of sodium metaperiodate, extracting the resulting aryl aldehyde of the unchanged drug with chloroform, and then measuring its UV absorption at 270 mμ. This reaction is specific to those compounds having two vicinal hydroxyl groups; thus Compound II or aminochrome-type oxidation products analogous to adrenaline (11) do not yield this aldehyde. This reagent was first used by Heimlich *et al.* for the determination of phenylpropanolamine in the urine (12) and later on by Chafetz (13) for the determination of some phenethanolamine drugs having one or no phenolic group. However, as far as the author is aware, there is no reference in the literature which describes an assay procedure for the determination of catecholamines using this reagent.

EXPERIMENTAL

Preparation of Isoproterenol Sulfonic Acid—This was prepared by the modification of the Schroeter and Higuchi method described for the preparation of 1-(3,4-dihydroxylphenyl)-2-methylaminoethane sulfonic acid from epinephrine (14). Isoproterenol sulfate, 0.018 mole (5.0 g.), and sodium bisulfite, 0.018 mole (1.869 g.), were dissolved in about 25 ml. of water (pH of the reaction solution was 5.0). The solution was heated on a steam bath while keeping it flushed with nitrogen. After 1.50 hr., the flask was removed from the steam bath (pH of the yellowish solution was 6.4) and stored in the dark after flushing with nitrogen. At the end of 3 days, the excess water (about 15 ml.) was removed with a rotary evaporator and the residual solution was stored in the dark at room temperature for a further period of 6 days. The white crystalline precipitate that separated during storage was collected on a Büchner funnel, was washed with water to remove unchanged isoproterenol sulfate, and was dried under vacuum at 60° for 2 hr. Yield: 4.90 g. (70%). The product was recrystallized once from 1 *N* HCl and twice from water to give prisms, m.p. 191–194° with decomposition (Fisher-John hot stage).

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

Anal.—Calcd. for $C_{11}H_{17}NO_5S \cdot H_2O$: C, 45.1; H, 6.18; N, 4.78; O, 32.76; S, 10.92. Found: C, 45.24; H, 6.12; N, 4.75; O, 32.75; S, 11.13. (Analysis was performed after drying the sample for 7 hr. under vacuum at 100°.)

ANALYTICAL METHODS

Reagents—*DEHP–Ether Solution*—Five milliliters DEHP in 140 ml. ether, washed with four 10-ml. portions of water (water washings discarded).

Potassium Phosphate Buffer—Two volumes of 1 *M* K_2HPO_4 (dibasic potassium phosphate) were mixed with eight volumes of 1 *M* KH_2PO_4 (monobasic potassium phosphate), and the pH was adjusted to 5.8 ± 0.05 with a pH meter.

Iron Reagents—(*a*) Ferrous sulfate heptahydrate (1.5 g.) in 200 ml. of distilled water to which 1 ml. of 1 *N* HCl had been added. (*b*) Ferrous sulfate–citrate solution prepared by adding 0.5 g. of sodium citrate to 10 ml. of ferrous solution (*a*) (freshly prepared before use).

Aminoacetate Buffer—Solution of sodium bicarbonate (42 g.) and potassium bicarbonate (50 g.) in 180 ml. of water was mixed with solution of aminoacetic acid (37.5 g.) and strong ammonia (17 ml.) in 180 ml. of water, and volume was adjusted to 500 ml. with water (6).

Sodium Metaperiodate Solution—Sodium metaperiodate (2%) (reagent grade) in water (freshly prepared).

Sodium Bisulfite Solution—Sodium bisulfite (0.024%) (reagent grade) in water.

Standard Isoproterenol HCl Solution (*A*)—Isoproterenol HCl USP reference standard (125 mg.) in water (25 ml.) (freshly prepared). For inhalations a 2-ml. aliquot was carried through the DEHP assay procedure along with the sample solution. For tablets a 2-ml. aliquot diluted with 3 ml. of water was carried through the DEHP assay procedure along with the sample solution.

Dilute Solution (*B*)—A 4-ml. aliquot of Solution A diluted to 100 ml. with water. For injections a 10-ml. aliquot of this solution was carried through the DEHP assay procedure along with the sample solution. For the $NaIO_4$ method a 4–6-ml. aliquot of this solution was carried through the assay procedure along with the sample solution.

Standard Isoproterenol Sulfonic Acid Solution—Isoproterenol sulfonic acid (30 mg.) in 30 ml. of water was shaken mechanically for 20 min. Then 120 mg. $NaHSO_3$ was added to the clear solution, which was made to 50 ml. with water and mixed.

Sample Preparation—*Inhalations* (*0.5% Isoproterenol HCl*)—A 2-ml. aliquot of the undiluted sample (equivalent to about 10 mg. of isoproterenol HCl) was used for the DEHP method (Procedure A). A 4-ml. aliquot was diluted to 100 ml. with water and a 4–6-ml. aliquot of this diluted solution (equivalent to 0.8–1.2 mg. of isoproterenol HCl) was used for the $NaIO_4$ method (Procedure B).

Injections (*0.02% Isoproterenol HCl*)—A 10-ml. aliquot of the undiluted sample (equivalent to 2.0 mg. of isoproterenol HCl) was used for the DEHP method and a 4-ml. aliquot (equivalent to 0.8 mg. of isoproterenol HCl) was used for the $NaIO_4$ method.

Tablets (*10 mg. Isoproterenol HCl per Dosage Unit*)—Twenty tablets were weighed and finely powdered. An accurately weighed aliquot containing about 50 mg. of isoproterenol HCl was quantitatively transferred to a 25-ml. volumetric flask with the aid of water (20 ml.). The mixture was shaken on a mechanical shaker for 20 min., made to volume with water, mixed, and filtered. A 5-ml. aliquot of the filtrate (equivalent to 10 mg. of isoproterenol HCl) was used for the DEHP method. A 10-ml. aliquot of the filtrate was diluted to 100 ml. with water and a 4-ml. aliquot of the diluted solution (equivalent to 0.8 mg. of isoproterenol HCl) was used for the $NaIO_4$ method.

Determination of Unchanged Isoproterenol—*DEHP Method* (*A*)—A 2-ml. (inhalations) or 10-ml. (injections) aliquot of the undiluted sample or 5-ml. aliquot of the aqueous filtrate in the case of tablets (prepared as already described) was transferred to a 250-ml. separator containing 3 ml. of potassium phosphate buffer (pH 5.8) and extracted with 50 ml. of DEHP–ether solution. The aqueous buffer phase was transferred to a 60-ml. separator and, after extracting successively with four 20-ml. portions of DEHP–ether solution, was drained into a 25-ml. volumetric flask. The DEHP–ether solutions were combined and washed first with two 1-ml. portions of potassium phosphate buffer and then with 2 ml. of water. These washings were combined with the aqueous buffer phase in the volumetric flask, made to volume with distilled water, and mixed (reserved for the determination of total water-soluble degradation products).

The combined residual DEHP–ether solutions were then extracted with five 25-ml. portions of 0.1 *N* H_2SO_4. Acid extracts were combined in a 200-ml. volumetric flask, made to volume with 0.1 *N* H_2SO_4, and mixed. The absorbance of the acid solution of the sample and that of the standard isoproterenol HCl carried simultaneously through the assay procedure were measured at 279 mμ against 0.1 *N* H_2SO_4 blank obtained by carrying a reagent blank through the assay procedure.

Calculations—*For Inhalations and Injections* (*a*)—

$$\text{mg. isoproterenol HCl/100 ml. formulation} = \frac{Au}{As} \times Cs \times 200 \times \frac{100}{Vu}$$

where Au = absorbance of acid solution of the sample after dilution to final volume, As = absorbance of acid solution of the standard after dilution to final volume, Cs = final concentration of isoproterenol HCl standard (mg./ml.) in acid solution after dilution to volume, and Vu = milliliters of the formulation taken.

For Tablets (*b*)—

$$\text{mg. isoproterenol HCl/tablet} = \frac{Au}{As} \times \frac{Ws \times Wo}{Wu}$$

where Au = absorbance of acid solution of the sample after dilution to final volume, As = absorbance of acid solution of the standard after dilution to final volume, Ws = weight of standard isoproterenol HCl (mg.), Wu = weight of sample (mg.), and Wo = tablet average weight (mg.).

Sodium Metaperiodate Method (*B*)—A 4-ml. aliquot of the diluted sample solution (for inhalations or tablets) or 4-ml. aliquot of the undiluted sample (for injections) was transferred to a 60-ml. separator, treated with 6 ml. of 2% sodium metaperiodate, mixed, stoppered, and allowed to stand overnight at room temperature. After 18 hr., the reaction solution was extracted successively with two 3-ml. and one 3.8-ml. portions of chloroform. The chloroform solutions were combined in a 10-ml. volumetric flask, made to volume with chloroform, and mixed. A 4-ml. aliquot of the dilute solution (Solution B) of isoproterenol HCl standard, after treating with appropriate volume of $NaHSO_3$ solution (containing $NaHSO_3$ equivalent to the amount present in the sample solution, *i.e.*, 2 ml. for inhalations containing 0.3% $NaHSO_3$; 1.3 ml. for inhalations containing 0.2% $NaHSO_3$; 1.0 ml. for injection containing 0.1% $NaHSO_3$; 0.67 ml. for tablets containing 2 mg. of $NaHSO_3$ per dosage unit), and 6 ml. of sodium metaperiodate solution were simultaneously carried through the assay procedure. The absorbance of the chloroform solution of the sample and that of the standard were measured concomitantly at 270 mμ after allowing the solutions to stand in the cells for 1 min. The chloroform solution used in the reference cell was obtained by carrying the reagent blank, *i.e.*, sodium metaperiodate and sodium bisulfite solutions, through the assay procedure.

Calculations—*For Inhalations* (*a*)—

$$\text{mg. isoproterenol HCl/100 ml. of formulation} = \frac{Au}{As} \times Cs \times 10 \times \frac{100}{4} \times \frac{100}{Vu}$$

where Au = absorbance of chloroform solution of the sample after dilution to final volume, As = absorbance of chloroform solution of the standard after dilution to final volume, Cs = final concentration of isoproterenol HCl standard (mg./ml.) in $CHCl_3$ after dilution to final volume, and Vu = milliliters of the formulation taken for dilution.

For Injections (*b*)—

$$\text{mg. isoproterenol HCl/100 ml. of formulation} = \frac{Au}{As} \times Cs \times 10 \times \frac{100}{Vu}$$

where Vu = milliliters of the formulation treated with $NaIO_4$ and the rest same as above.

For Tablets (*c*)—Same as given in Procedure A for tablets.

Determination of Isoproterenol Sulfonic Acid—A 2-ml. aliquot of the isoproterenol sulfonic acid standard (for inhalations) or 2-ml. aliquot diluted with 8 ml. of water (for injections) or 2-ml. aliquot diluted with 3 ml. of water (for tablets) was carried through the DEHP assay (Method A) simultaneously with the formulation. The absorbance of the aqueous buffer phase of the standard and that of the sample solution as obtained under *Determination of Unchanged Isoproterenol* (Method A) were measured at 279 mμ. The aqueous buffer solution used in the reference cell was obtained by carrying a reagent blank (potassium phosphate buffer and the appropriate volume of the distilled water containing sodium bisulfite) through the assay procedure. These absorbance values were used to deduce the amount of total water-soluble degradation products with reference to isoproterenol sulfonic acid.

Isoproterenol sulfonic acid was then determined colorimetrically by treating a 6-ml. aliquot each of the aqueous buffer solution of the sample and that of the standard (aqueous buffer solutions left after UV determination) with 3.9 ml. of aminoacetate buffer and 0.1 ml. of ferrous sulfate–citrate reagent. The purple color formed was measured after 2.50 hr. at 530 mμ against the reagent blank. Alternatively, a 2-ml. (inhalation) or 10-ml. (injection) aliquot of the undiluted sample was carried through the DEHP assay (Method A) starting at: "A 2-ml. (inhalations) or 10-ml. (injections) aliquot of the undiluted..." and ending at "these washings were combined with aqueous buffer phase." These combined aqueous buffer solutions were treated with 10 ml. of aminoacetate buffer and 0.1 ml. of ferrous sulfate–citrate reagent, made to 25 ml. with water, and mixed. The absorbance of the sample solution and that of the isoproterenol sulfonic acid standard (a 2-ml. aliquot) carried simultaneously through the assay procedure were measured at 530 mμ against the reagent blank. The amount of isoproterenol sulfonic acid (mg./100 ml. of formulation) was calculated from the respective absorbance values.

Determination of Total Catechols as Isoproterenol—*Modified Colorimetric Method*—A 3-ml. aliquot of the diluted sample solution (for inhalations or tablets) or 3-ml. aliquot of the undiluted sample solution (for injections) containing the equivalent of 0.6 mg. of isoproterenol HCl was treated with 1.9 ml. of aminoacetate buffer and 0.1 ml. of ferrous sulfate–citrate solution in a 10-ml. volumetric flask, made to volume with distilled water, and mixed. A 3-ml. aliquot of the dilute solution of isoproterenol HCl standard (Solution B), after treating with appropriate volume of $NaHSO_3$ solution (containing $NaHSO_3$ equivalent to the amount present in the sample solution, *i.e.*, 1.5 ml. for inhalations containing 0.3% $NaHSO_3$; 1.0 ml. for inhalations and injections containing 0.2% $NaHSO_3$ or 0.1% $NaHSO_3$, respectively; 0.51 ml. for tablets containing 2 mg. of $NaHSO_3$ per dosage unit) was concurrently carried through the assay procedure. The absorbance of the colored solutions of the sample and that of the standard were measured after 2.50 hr. at 530 mμ against the reagent blank.

USP Method—An aliquot of the inhalation (equivalent to 50 mg. of isoproterenol HCl) or injection (equivalent to 5.0 mg. of isoproterenol HCl) or tablet powder (equivalent to 50 mg. of isoproterenol HCl) was carried through the assay procedure as described in USP XVII, pp. 330–332. The absorbance of the sample and that of the standard solution were measured at 279 mμ.

Thin-Layer Chromatography (TLC)—*Preparation of Layers*—The chromatoplates were prepared using standard equipment (15). Cellulose MN300 and silica gel G were used as adsorbents. To obtain five plates 20 × 20 cm., 10 g. of cellulose powder containing 20 mg. of fluorescent indicator was mixed with 70 ml. of water and 10 ml. of 1% aqueous solution of sodium carboxymethylcellulose (CMC). For silica gel G, five plates were obtained from 25 g. of powder in 55 ml. of water. A layer-thickness of 250 μ was used throughout. Cellulose plates were activated by heating at 110° for 30 min. and stored in a desiccator until use; silica gel plates were air dried and used without activation.

Solvent Systems—The following solvent systems were used: (*a*) *n*-butanol–acetic acid–water (4:1:5) (16) were shaken together in a separator and set aside for 2 hr.; the upper organic layer was separated and used as the running solvent for cellulose plates. (*b*) *n*-Butanol–acetic acid–water (60:10:25) were mixed together to form a clear solution and used as the developing solvent for silica gel plates.

Detection Reagents—(*a*) Short wavelength and long wavelength UV light. (*b*) Potassium ferricyanide and ferric chloride solution: 5 ml. of 5% aqueous solution of potassium ferricyanide and 10 ml. of 10% aqueous solution of $FeCl_3$ were mixed and made to 50 ml. with water. This was used to spray cellulose plates. (*c*) Sodium metaperiodate 2% aqueous solution. (*d*) Doty's reagent: mix immediately before use equal volumes of ferrous sulfate–citrate solution and aminoacetate buffer. (For preparation of solutions, refer to *Analytical Methods* of this paper.) (*e*) Folin-Ciocalteu reagent (FCR) (17). This reagent was used for silica gel plates; the plates were first sprayed with FCR solution, air dried, and then sprayed with 10% aqueous Na_2CO_3 solution.

Chromatographic Procedure—A solution of isoproterenol HCl (230 mg.) and isoproterenol sulfonic acid (20 mg.) in about 30 ml. of water was prepared by mechanical shaking for 20 min., adding 139 mg. of sodium bisulfite to the clear solution, and making it to 50 ml. in a volumetric flask. Quantities of this standard solution (1–5 μl.) containing equivalent of 4.6–23 mcg. of isoproterenol HCl and 0.4–2.0 mcg. of isoproterenol sulfonic acid were spotted adjacent to the aqueous solutions of the simulated formulations and/or commercial formulations (5 μl.) containing equivalent to 25 mcg. of isoproterenol HCl. The plate was inserted in a solvent jar previously equilibrated with solvent [solvent system (*a*) for cellulose plates and solvent system (*b*) for silica gel plates] which was allowed to rise to a height of 10 cm. for cellulose plates and 15 cm. for silica gel plates (time about 120 min. for cellulose plates and 135 min. for silica gel plates). The plate was then air dried and examined under short and long wavelength UV light.

Examination of cellulose plates under short wavelength UV light showed a spot at the origin having a blue fluorescence (indicative of $NaHSO_3$ presence) and long wavelength UV light showed an additional spot having a strong blue fluorescence (R_f about 0.26) both for simulated and commercial formulations but not seen for the standard solution. Spraying the plate with DR (*b*) (potassium ferricyanide and $FeCl_3$ solution) caused the immediate formation of blue spots for isoproterenol HCl (R_f about 0.53) and isoproterenol sulfonic acid (R_f about 0.33). The spot seen under long wavelength UV light also stained blue after about half a minute, and thus the whole area from about 0.33 to 0.53 R_f values was seen as a single blue spot (limiting sensitivity of this spray for isoproterenol sulfonic acid 0.4 mcg.). Citric acid and sodium citrate present in a few commercial formulations also gave a blue spot of minor intensity after 2–5 min.; glycerin, another excipient, gave a minor blue spot after 5–10 min. Thus these spots due to their late formation did not interfere with the detection of the decomposition products. Spraying the second half of the plate with DR (*c*) or (*d*) caused the formation of reddish-colored spots for isoproterenol HCl and isoproterenol sulfonic acid with sodium metaperiodate spray and mauve-colored spots with Doty's reagent. The reddish-colored spot formed with $NaIO_4$ for isoproterenol sulfonic acid disappeared within 1 min. of its formation but that of Doty's reagent (mauve-colored spot) did not disappear. (Limiting sensitivity of both of these sprays was 2.0 mcg. for isoproterenol sulfonic acid.)

Examination of silica gel G plates under short wavelength UV light did not show any spot; long wavelength UV light showed two spots having blue fluorescence for inhalations containing 0.3% $NaHSO_3$ (R_f values of 0.03 and 0.1); three spots for inhalations containing 0.2% $NaHSO_3$ (R_f values 0.03, 0.1, and 0.16) and two spots for injections (depending upon the quantity spotted) at R_f of 0.1 and 0.16. Simulated formulations stored in the dark over a period of 4 months showed almost the same pattern, while a freshly prepared standard solution did not show any of these spots. Spraying the plate with DR(*e*)–FCR reagent followed by Na_2CO_3 spray caused the formation of purple spots for isoproterenol HCl (R_f about 0.4) and isoproterenol sulfonic acid (R_f about 0.26) (limiting sensitivity 0.6 mcg. for isoproterenol sulfonic acid). Among the spots seen under long wavelength UV, spots at R_f 0.1 gave coloration with this reagent for all formulations, while the spot at 0.16 R_f gave coloration for formulations having 0.2% $NaHSO_3$. An additional bluish-purple spot at R_f 0.43 was also seen in some formulations. A freshly prepared standard solution mixture showed only two purple spots at R_f 0.4 and 0.26 for isoproterenol HCl and isoproterenol sulfonic acid, respectively.

RESULTS AND DISCUSSION

The TLC of simulated inhalations and injections stored in the dark over a period of 4 months showed that in addition to Compound II (Scheme I), the drug can give rise to two to

Table I—Analysis of Isoproterenol Formulations

Formulation	$NaIO_4$ Method: Label Claim, Av., %	$NaIO_4$ Method: Anal. Range	$NaIO_4$ Method: *SD*	$NaIO_4$ Method: No. of Detn.	DEHP Method: Label Claim, Av., %	DEHP Method: Anal. Range	DEHP Method: *SD*	DEHP Method: No. of Detn.
Simulated Formulations								
Decomposed[b]	92.6	91.9–93.2	0.55	4	92.5	91.9–93.7	0.85	4
Tablets[c]	101.8	100.9–102.2	0.54	4	100.6	100.3–100.8	—	2
Inhalation[d]	98.4	97.1–99.3	1.0	4	—	—	—	—
Inhalation[e]	98.9	98.4–99.2	0.50	4	—	—	—	—
Injection[f]	86.0	85.4–86.9	0.66	4	—	—	—	—
Commercial Formulations								
Inhalation A[g]	106.8	106.3–107.4	0.65	4	110.8	110.5–111.2	—	2
Inhalation B[h]	106.1	104.9–107.7	1.23	4	106.2	105.9–106.5	—	2
Injection[i]	100.0	—	—	1	100.1	—	—	1
Tablets[j]	100.8	—	—	2	98.9	—	—	2

Formulation	Colorimetric Procedure[a]: Label Claim, Av., %	Colorimetric Procedure[a]: Anal. Range	Colorimetric Procedure[a]: *SD*	Colorimetric Procedure[a]: No. of Detn.	USP XVII Method: Label Claim, Av., %	USP XVII Method: No. of Detn.
Simulated Formulations						
Decomposed[b]	99.7	98.9–99.9	0.45	4	99.9	2
Tablets[c]	99.2	98.7–99.8	—	2	101.1	2
Inhalation[d]	—	—	—	—	102.6	2
Inhalation[e]	—	—	—	—	100.8	2
Injection[f]	—	—	—	—	101.8	2
Commercial Formulations						
Inhalation A[g]	117.5	—	—	2	116.7	2
Inhalation B[h]	109.2	—	—	2	110.8	2
Injection[i]	115.0	—	—	1	116.0	1
Tablets[j]	101.2	—	—	2	101.9	2

[a] Modified USP XVII method for the determination of epinephrine in procaine hydrochloride injection (determines total catechols as isoproterenol). [b] Isoproterenol HCl 115 mg., isoproterenol sulfonic acid 10 mg. (8.0%), and sodium bisulfite 65 mg. (0.3% w/v) in 25 ml. water. [c] Isoproterenol HCl 10 mg., sodium bisulfite 2 mg. plus excipients such as starch, lactose, saccharin, and talcum per tablet of average weight of 100 mg. [d] Isoproterenol HCl 125 mg., sodium bisulfite 75 mg. (0.3% w/v) plus excipients such as sodium chloride, citric acid, glycerin, and chlorobutanol dissolved in water to 25 ml. The pH was adjusted to 3.75 with 1 *N* sodium hydroxide. Molar ratio of sodium bisulfite to isoproterenol HCl is 1.42:1. [e] Isoproterenol HCl 125 mg., sodium bisulfite 50 mg. (0.2% w/v) plus excipients such as chlorobutanol and sodium chloride dissolved in water to 25 ml. The pH of the formulation was 2.8 (without adjustment). Molar ratio of sodium bisulfite to isoproterenol is 0.95:1. [f] Isoproterenol HCl 20 mg., sodium bisulfite 100 mg. (0.1% w/v) plus excipients such as lactic acid, sodium lactate, and sodium chloride dissolved in water to 100 ml. The pH of the formulation was 2.8 (without adjustment). Molar ratio of sodium bisulfite to isoproterenol is 11.9:1. [g,h,i,j] Labeled to contain active ingredients and sodium bisulfite in the same amounts as in simulated formulations [d,e,f], and [c], respectively. Formulations [g], [h], and [i] were found to have the pH values of 3.75, 3.35, and 3.75, respectively.

three decomposition products. The exact chemical nature of these additionally encountered artifacts could not be established but one of them can be envisaged as an aminochrome–sodium bisulfite addition compound by analogy with the similar compound described by Oesterling and Tse (18) in their procedure for the determination of total content of catecholamines in the urine. The total percent ratio of these to Compound II was found to be about 1:4 depending upon the molar ratio of sodium bisulfite to isoproterenol used in a formulation. In addition, other factors including conditions of storage and its length and the pH of the formulation may affect the relative formation of these components.

A method of analysis, to be completely reliable, therefore, should be capable of differentiating between the parent drug and the closely related sodium bisulfite addition product. Both isoproterenol and sulfonic acid II exhibit maximum absorption at 279 mμ and have similar molar absorptivity values. Although the method of Welsh and Sammul (1) based on the DEHP ion-pair and utilizing a diatomaceous earth column represents a major step in solving the problem, the technique suffers from certain disadvantages. When applied to a synthetic preparation containing known amounts of Compound II and sodium bisulfite, this method gave higher and inconsistent values for the parent drug. This discrepancy was reconciled by introducing a step to wash the ether effluent from the column with potassium phosphate buffer prior to the acid extraction. The aged simulated formulations containing artifacts in addition to Compound II (as shown by TLC) showed higher recoveries for the unchanged drug due to carrying over some of the degradation products other than sulfonic acid to the DEHP–ether phase. The above shortcomings and the cumbersome steps of column packing and its elution were overcome by modifying the column method to a simple shakeout procedure and by washing the combined DEHP–ether solutions first with potassium phosphate buffer and then with water prior to the extraction of the unchanged drug with 0.1 *N* H_2SO_4. These modifications not only resulted in the increased specificity of the method (by removing some of the water-soluble degradation products) but also permitted the quantitative determination of isoproterenol sulfonic acid which is retained in the aqueous buffer phase. Thus the Compound II (sulfonic acid) is then determined selectively by treating the aqueous phase with Doty's reagents.

Application of the modified procedure to the simulated isoproterenol tablets and to the simulated decomposed sample containing 8% Compound II and 0.3% $NaHSO_3$ gave the results shown in Table I. Analytical range and standard deviation values demonstrate the accuracy and precision of the method. A batch of commercial isoproterenol tablets was analyzed by this method and the results (Table I) appear satisfactory in comparison with those obtained from the USP analyses and with data obtained with simulated tablets.

Data in Table II show the recoveries of isoproterenol sulfonic acid II from a simulated decomposed sample by the procedure described under *Determination of Isoproterenol Sulfonic Acid.*

Table II—Amount of Degradation Products and Isoproterenol Sulfonic Acid in Simulated and Commercial Formulations

Formulation	Assay Values for Isoproterenol,[a] % [d]	Total Water Soluble Degradation Products,[b] % [d]	Isoproterenol Sulfonic Acid,[c] % [d]	Isoproterenol HCl Undergone Degradation, % [d]
Simulated Formulations				
Inhalation[e]	89.6	12.4	11.6	11.1
Inhalation[f]	88.6	12.0	9.0	10.7
Injection[g]	84.3	19.1	15.0	17.2
Decomposed[h]	92.6	8.1	8.2	—
Commercial Formulations				
Inhalation[i]	106.8	7.5	4.7	6.8
Inhalation[j]	106.1	5.5	3.4	4.9
Injection[k]	100.0	15.6	15.5	14.0

[a] Values quoted have been obtained by $NaIO_4$ method. [b] Values quoted for total water soluble degradation products have been obtained by measuring UV absorbance of the aqueous-buffer phase, and calculated as isoproterenol sulfonic acid. [c] Values quoted for isoproterenol sulfonic acid were obtained by the spectrophotometric quantitative measurement of the purple color formed by treating the aqueous phase with ferro-citrate solution and buffer. The reaction is specific for catechol nucleus only. [d] Calculated relative to the label claim. [e,f,g] Formulations same as [d,e,f] in Table I; assay values for [e] and [f] were obtained after 83 days of storage at room temperature, and for [g] after 88 days of storage. [h] Formulation same as [b] in Table I containing 8% isoproterenol sulfonic acid. [i,j,k] Commercial formulations same as [g,h,i] in Table I.

Simulated formulations which had been stored in the dark over a period of 3 months were also analyzed by this procedure. Table II also depicts the amounts of Compound II in the aged simulated formulations and in commercial formulations. One of the commercial formulations having a molar ratio of 11.9:1 of sodium bisulfite to isoproterenol was found to contain a considerable percentage of Compound II when it was analyzed 8–9 months after its procurement from the factory premises. The logic of using such a higher molar ratio of sodium bisulfite is not clearly understood, especially when the injection is to be administered by the intravenous route.

An alternate checking procedure to validate the results obtained by the DEHP method was sought. Consequently, sodium metaperiodate which is known to cleave carbon-carbon bonds of vicglycols, α-aminoalcohols where the amine is primary or secondary, and α-hydroxyketones (19), was used for the selective determination of the unchanged drug. This treatment results in the formation of an aryl aldehyde of the parent drug, and the Compound II or aminochrome-type oxidation products do not yield this aldehyde. The aryl aldehyde is extracted into chloroform and UV-determined at 270 mμ. The typical UV spectrum of the reaction product is given in Fig. 1.

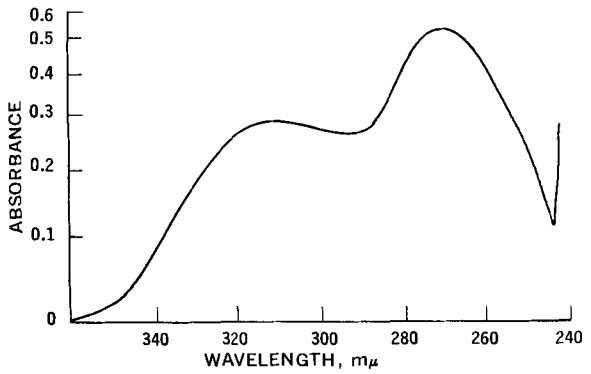


Figure 1—*Absorption spectrum of the reaction product in chloroform (concentration equivalent to 140 mg. of isoproterenol HCl per milliliter of final chloroform solution).*

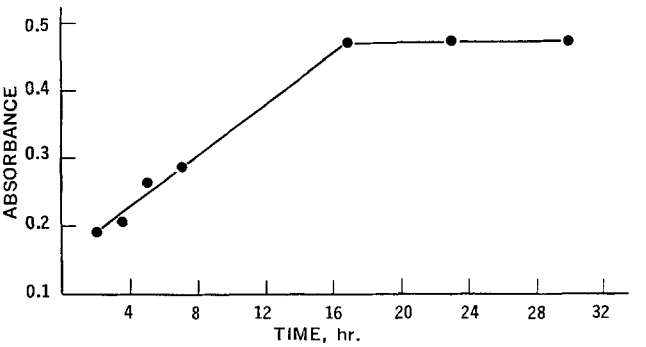


Figure 2—*Effect of time on the completion of reaction (concentration equivalent to 120 mg. of isoproterenol HCl per milliliter of final chloroform solution).*

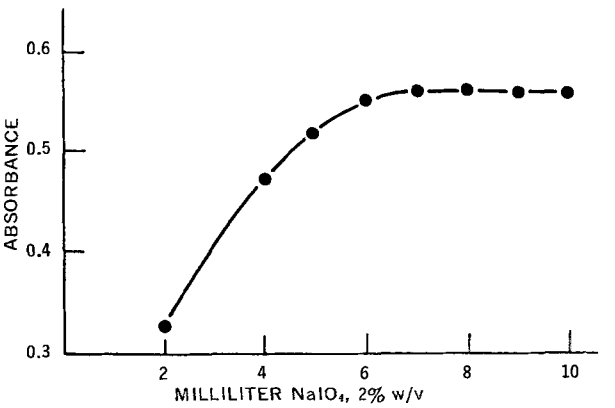


Figure 3—*Effect of varying concentration of sodium metaperiodate on the formation of reaction product.*

Experiments undertaken to obtain the quantitative yield of the reaction product indicated that (*a*) the sample and reagent be allowed to react for about 17–18 hr. (overnight) at room temperature, although periods up to 30 hr. did not adversely affect the reaction (Fig. 2); (*b*) a molar ratio, 116:1, of sodium metaperiodate to isoproterenol was satisfactory for analysis but a molar ratio of 82:1 was insufficient. On the other hand, a molar ratio as high as 165:1 did not show any untoward effect on the yield of the reaction product (Fig. 3); (*c*) the standard solution of isoproterenol HCl should contain approximately the same amount of sodium bisulfite as is present in the formulation. A 4–6-ml. aliquot of the diluted formulation used for $NaIO_4$ assay will contain about 0.48–0.72 mg. of $NaHSO_3$ and as shown in Fig. 4, the $NaHSO_3$ concentration from 0.48–0.96 mg. in the isoproterenol HCl standard did not affect the yield of the reaction product. Under these conditions, Beer's law was obeyed for a concentration of at least 140 mcg. of isoproterenol HCl per milliliter of final solution (Fig. 5). The effect of varying concentrations of Compound II on the recoveries of isoproterenol is shown in Fig. 6; the recoveries were not affected up to 28% concentration of the decomposition product.

When applied to the simulated isoproterenol tablets and to a simulated decomposed sample containing 8% Compound II and 0.3%

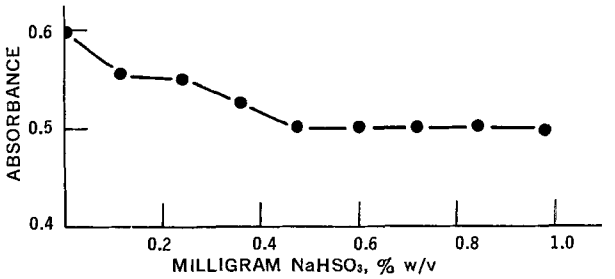


Figure 4—*Effect of varying concentration of sodium bisufilte on the formation of reaction product.*

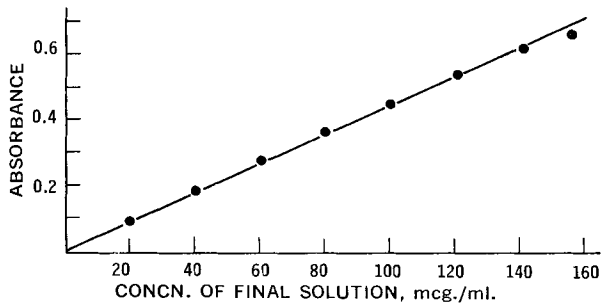


Figure 5—*Relationship between concentration and absorbance (absorbance of the reaction product in chloroform at 270 mμ).*

$NaHSO_3$, the $NaIO_4$ method gave results (Table I) virtually identical with those obtained by the DEHP method. Results obtained by applying the $NaIO_4$ method to four commercial products containing isoproterenol are shown in Table I. The comparison of the experimental data with those obtained by the DEHP method shows a good correlation between the two, with comparable precision for three formulations. Only one formulation showed higher values by the DEHP method which were explicable by additionally encountered artifacts other than Compound II. The data show that although inhalations and injections sold on the market conform to the USP limits, the formulations may contain considerably large amounts of decomposition products. The accuracy and the precision of this method are demonstrated by the analytical range and standard deviation values obtained (Table I) by its application to the freshly prepared inhalations and injections. A simulated injection containing a molar ratio of 11.9:1 of $NaHSO_3$ to isoproterenol (identical to the commercial formulation) showed only 86% of the added isoproterenol content. Virtually no further deterioration of the isoproterenol content was noticed, even when the formulation was analyzed after 88 days of storage in the dark at room temperature (Tables II and III). Lower values were ascribable to the formation of 15% isoproterenol sulfonic acid.

The isoproterenol content found by the ferro-citrate colorimetric method (6, 20) and by the USP XVII UV spectrophotometric method (4) is a measure of the isoproterenol present at the time the solutions were manufactured. Values obtained by the two methods are satisfactory and in close agreement (Table I). The colorimetric method reported in the paper is a modification of the USP XVII procedure described for the determination of epinephrine in procaine hydrochloride injection (20). It was found that the amounts of $NaHSO_3$ as specified in the USP were too high and a substantial decrease in the color intensity was noticed. The amount of $NaHSO_3$ present in the commercial formulations was found adequate for reproducible color development. The order of adding reagents was another variable considered to be important, and thus the maximum reproducibility was obtained by adding ferrous sulfate reagent to the reaction solution to which the appropriate volume of the aminoacetate buffer had already been added. Time for color development was changed from 30 min. to 2.50 hr.

Table III shows the stability data of the simulated inhalations and injections stored in the dark at room temperature over a period of 88 days. The data indicate that the inhalation having a molar ratio of 1.42:1 of $NaHSO_3$ to isoproterenol did not show further deterioration of the isoproterenol content after 39 days. The data also revealed that no consistent pattern of degradation was followed

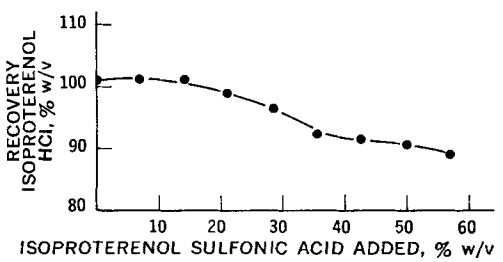


Figure 6—*Effect of varying concentration of isoproterenol sulfonic acid on the recovery of isoproterenol HCl.*

Table III—Analysis of Isoproterenol in Aged Simulated Formulations[a]

Formulations	Storage Period, days	Assay Values for Isoproterenol[b] $NaIO_4$ Method, %	DEHP Method, %	USP Method, %
Inhalation[c]	0	98.4	—	102.0
	39	89.5	—	—
	83	89.6	95.4[d]	102.6
Inhalation[e]	0	98.9	—	100.3
	39	96.7	—	—
	83	88.6	95.9[d]	100.8
Injection[f]	0	86.0	—	—
	39	86.0	—	—
	88	84.3	82.7[g]	101.8

[a] Stored in dark at room temperature. [b] Calculated relative to the label claim. [c,e,f] Formulations same as [d,e,f] in Table I. [d] Higher values for unchanged drug were obtained due to carrying over of certain decomposition products into DEHP-ether phase. [g] Values for unchanged drug in this formulation agreed very well to that of $NaIO_4$ method due to formation of isoproterenol sulfonic acid as the main degradation product. Formation of isoproterenol sulfonic acid as the main degradation product was favored due to high molar ratio of sodium bisulfite to isoproterenol, *i.e.*, 11.9:1 and DEHP method has been found to be specific if the main degradation product formed is only sulfonic acid.

in all three formulations. These findings agree well with those made by Chafetz *et al.* (21).

The investigation showed that although solvent system (*a*) on cellulose was satisfactory for resolving isoproterenol and its sulfonic acid II, the solvent system (*b*) on silica gel G was an ideal one for the complete separation and detection of isoproterenol and its artifacts. Despite the specificity of $NaIO_4$ spray (formation of reddish color for isoproterenol and transient coloration for sulfonic acid) and Doty's reagent spray (mauve coloration for isoproterenol and its sulfonic acid), these are not suitable to detect the presence of isoproterenol sulfonic acid due to their low sensitivity (2 mcg. or more is required for color formation). Potassium ferricyanide–$FeCl_3$ spray (though less specific but having a sensitivity of less than 0.4 mcg.) and FCR spray (more specific having a sensitivity of 0.6 mcg.) were found very satisfactory to detect the presence of Compound II even in minor concentrations. Under the conditions used, the problem of an apparent second front as described by Choulis (16) and Chafetz *et al.* (21) was not encountered for the standard solution containing isoproterenol and its sulfonic acid and $NaHSO_3$.

It is recommended that the DEHP shakeout method as described in the paper may be adopted as the official procedure in the USP XVIII for the analysis of isoproterenol formulations. This procedure has overcome the shortcomings of the method reported by Welsh and Sammul (1). Procedure with these modifications achieves all the essential criteria required for its inclusion in various official compendia by its simplicity, precision, accuracy, and specificity. It is further suggested that the limits (maximum and minimum) for isoproterenol sulfonic acid may be delineated for all the formulations containing sodium bisulfite as an antioxidant. TLC using silica gel G and *n*-butanol–acetic acid–water (60:10:25) as an ideal solvent may be included in the monograph to detect the presence of isoproterenol sulfonic acid and other artifacts.

The sodium metaperiodate method is also very specific and simple and can be automated very easily using the currently available Liquid Prep. Technicon Auto-Analyzer equipment. The method can be of potential value to the pharmaceutical industry to be utilized as a routine-monitoring and stability-indicating procedure for the unchanged drug in drug quality control.

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TECHNICAL ARTICLES

Construction and Operation of an Automated Dispensing Analyzer for the Assay of Individual Tablets

WILLIAM F. BEYER* and EDWIN W. SMITH†

Abstract □ An automated system of modular design has been developed for the assay of single tablets. Commercially available proportioning pumps, continuous filter, tablet homogenizer, spectrophotometer, and recorder are mated to specially designed components for unattended operation. The system is constructed to analyze automatically up to 300 identifiable tablets with standards inserted prior to tablets, after any selected number of tablets, and at the end of the particular type of tablet being assayed. Up to six different concentrations or types of tablets can be automatically processed sequentially, each with its own standard insertion. Provisions are made to alter automatically the dilution of samples as tablet strengths vary. Under normal program conditions the system can operate unattended for approximately 18 hr.

Keyphrases □ Tablets, individual—automated analysis □ Automated dispensing analyzer, tablet—construction, operation □ Diagram—automated analyzer, individual tablets □ UV spectrophotometry—analysis

The assay of single tablets and single units in other dosage forms is recognized as an important feature in the quality control of manufacturing processes. Compendia requirements for content uniformity of selected tablets containing 50 mg. or less of drug substance has placed additional burdens on the quality control chemist. To make data available in the quantity needed for statistically valid results, analytical procedures have been automated to varying degrees. The majority of automated assay systems that include sample preparation have incorporated a commercially available solid sampler,[1] as evidenced by symposia of the New York Academy of Sciences and Technicon Corp. (1–5). Papers presented at the 1967 New York Academy of Sciences included a presentation by Rehm *et al.* (2) describing an automated system for the UV analysis of single tablets.[2]

An automated system constructed in these laboratories for UV analyses of individual tablets has been in routine operation for more than a year. The system is of modular design and consists of commercially available components wherever possible. Provisions have been made for the introduction of liquid standards, dilution, and

[1] SOLIDprep, Technicon, Inc., Tarrytown, N. Y.
[2] Available commercially as the Assayomat, American Instrument Company, Inc., Silver Spring, Md.