Coulometric Titration of *N*-Isopropyl-α-(2-methylhydrazino)-*p*-toluamide Hydrochloride

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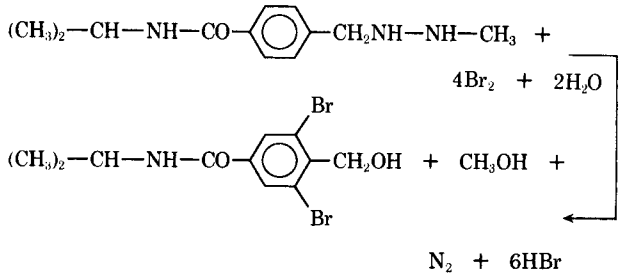
Abstract □ A coulometric titration for the determination of *N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide hydrochloride, procarbazine hydrochloride, is described. The method is specific and is based on the quantitative oxidation of procarbazine hydrochloride by internally generated iodine to the corresponding "azo" compound. The standard deviation for the method was ±0.2 and ±0.3%, while the precision *ts* at the 95% confidence level was ±0.6 and ±0.7% for the drug substance and capsules, respectively.

Keyphrases □ *N*-Isopropyl-α-(2-methylhydrazino)-*p*-toluamide hydrochloride—analysis, coulometric titration □ Procarbazine hydrochloride—analysis, coulometric titration □ Iodine—oxidation of procarbazine hydrochloride, coulometric titration □ Coulometry—analysis, procarbazine hydrochloride

Methods presently available for the determination of *N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide hydrochloride, procarbazine hydrochloride, in capsules[1] include a polarographic determination based on the oxidation of the "hydrazo" group, —N(—)—N(—)—, to the "azo" group, —N=N—[2], and a coulometric titration using internally generated bromine (1).

The polarographic procedure, while specific for the active hydrazo group, requires comparison to a reference standard for the drug substance and preliminary extraction of the active drug substance from the excipients for the dosage form. The coulometric procedure of Beral and Stoicescu (1), while less time consuming, is not specific, since electrochemically generated bromine not only oxidizes the —N(—)—N(—)— bond but also substitutes into the phenyl ring as indicated in Scheme I. Therefore, most intermediates and decomposition products are titratable with coulometrically generated bromine.

Since iodine can theoretically oxidize the hydrazo moiety to the azo group without substituting in the phenyl ring, a coulometric titration with internally generated iodine should be a more specific method for the determination of procarbazine hydrochloride in both the drug substance and the dosage form.



Scheme I

[1] Matulane, Hoffmann-La Roche Inc., Nutley, NJ 07110
[2] Quality Control Procedure, Hoffmann-La Roche Inc., Nutley, NJ 07110

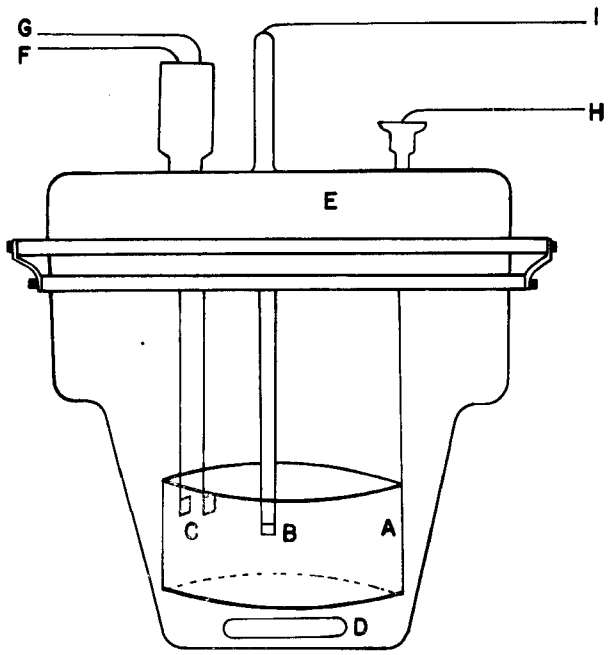


Figure 1—*Coulometric titration cell diagram. Key: A, platinum generating electrode; B, platinum reference electrode; C, platinum indicating electrodes; D, stirring bar; E, fitted top; F, to Ref position on the Orion pH meter; G, to KF position on the Orion pH meter; H, to positive terminal of constant-current source; and I, to negative terminal of constant-current source.*

EXPERIMENTAL

Reagents and Solutions—Buffered 0.5 *M* potassium iodide solution was prepared by dissolving approximately 80 g. of reagent grade potassium iodide[3] and approximately 13 g. of sodium bicarbonate[4] in demineralized water and diluting to 1 l. The pH of this solution was 8.4 ± 0.1.

Apparatus—The titrant-generating system consisted of a coulometric current source[5] in conjunction with two platinum electrodes. A platinum wire inserted in a buffered 0.5 *M* potassium iodide salt bridge was used as the cathode.

The indicating system consisted of two polarized platinum flag electrodes. A constant current of 10 μamp. was imposed on these electrodes using a digital pH meter[6] in the KF mode. The readout from the digital pH meter was recorded on an *x-y* recorder[7]. A titration vessel[8] with cover[9] was used as the cell. The arrangement of electrodes in the cell and a block circuit diagram for the combined system are given in Figs. 1 and 2, respectively. All cell connections were made as shown (Fig. 2). The current source was set to give a current of 96.5 mamp. ± 0.1%. The *x*-axis of the recorder was set at 10 sec./cm.

Procedure—The contents of 20 capsules were ground, and the equivalent of approximately 40 mg. of procarbazine hydrochloride

[3] Mallinckrodt Chemical Works.
[4] Certified ACS, Fisher Scientific Co.
[5] Sargent-Welch, model IV.
[6] Orion Research, model 801.
[7] Moseley Autograph, model 7001 AM.
[8] Metrohm EA-875-50.
[9] Metrohm EA-874.

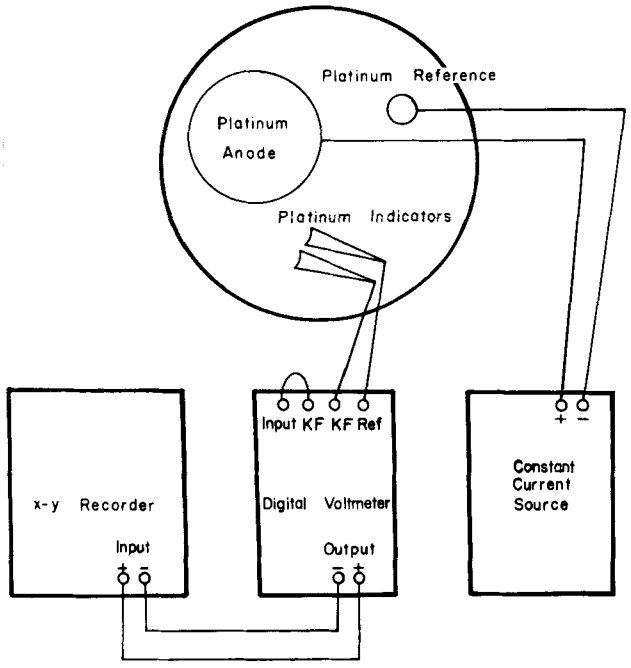


Figure 2—*Block circuit diagram for coulometric titration apparatus.*

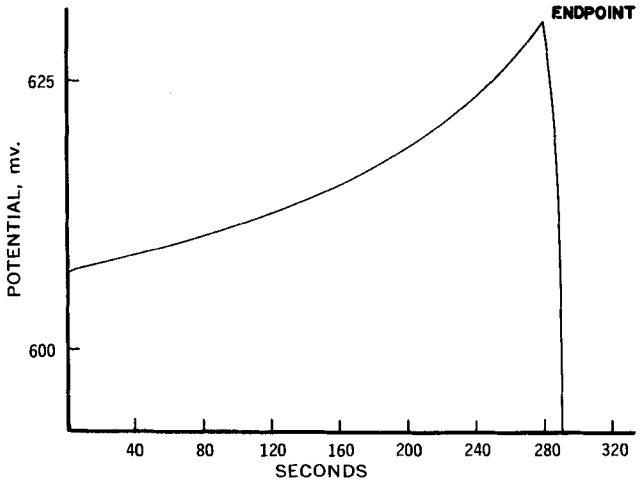


Figure 3—*Typical titration curve for procarbazine hydrochloride.*

was weighed accurately and transferred to the cell. Potassium iodide solution, approximately 140 ml., was added to the cell to cover the electrodes completely. The resulting solution was stirred using a magnetic stirrer. After the power supply of the current source had warmed for 5 min., the titration was initiated by simultaneously turning the cell current switch to On and the recorder to Start. After the end-point was reached, as noted by the peak readout potential (Fig. 3), the current switch on the current source and the recorder were turned off.

Calculations—The milligrams of procarbazine as the base was calculated from the following equation:

$$\text{mg./capsule} = \frac{(\text{number of seconds to end-point})(0.1289)(0.8585) \times (\text{average capsule fill, mg.})}{\text{weight of sample (mg.)}} \quad \text{(Eq. 1)}$$

where 0.1289 = [(96.5 mamp.) (257.8 mg./mmole)]/[(2 electrons/mmole) (96,500 coulombs/equivalent)], and 0.8585 = procarbazine hydrochloride to procarbazine base conversion factor.

RESULTS AND DISCUSSION

Determination of Procarbazine Hydrochloride Drug Substance—Initial assays were performed on 40-mg. samples of the drug substance. The average value for five determinations was 100.2%, with a standard deviation of ±0.2%. The precision *ts* for 4 degrees of freedom at the 95% confidence level was ±0.6% (Table I).

Table I—Determination of Procarbazine Hydrochloride Drug Substance

Milligrams Taken	Milligrams Found	Percent Assay
39.58	39.54	99.90
40.97	41.03	100.1
41.51	41.66	100.4
40.48	40.66	100.4
40.14	40.32	100.5
Average		100.2
Standard deviation		±0.2
Precision[a]		±0.6

[a] *ts* for 4 degrees of freedom at the 95% confidence level.

Determination of Procarbazine Hydrochloride in Dosage Forms—Determination of procarbazine hydrochloride in dosage forms was also evaluated. The average for eight determinations was 51.31 mg./capsule (102.6% of claim, based on 50 mg.). The standard deviation was ±0.17 mg./capsule or ±0.3%, and the precision *ts* for 7 degrees of freedom at the 95% confidence level was ±0.42 mg./capsule or 0.7% (Table II).

Titration Product—The nature of the product formed in the titration was investigated. The product was monitored polarographically at different stages of the titration. The diffusion current was a linear function of the generating time over the range 0–500 sec. The half-wave potential for this product, −1.08 v. *versus* Ag/AgCl, compared favorably with the half-wave potential for the azo compound [*N*-isopropyl-α-(2-methylazo)-*p*-toluamide], which was −1.02 v. The titration product obtained, using the present method, is apparently different than that obtained using classical iodometric procedures. Attempts to titrate procarbazine hydrochloride directly with 0.1 *N* iodine indicated that even after 4 equivalents of iodine was consumed, an equivalence point was not obtained. This observation emphasized the definite advantage of coulometric titrations in preventing the localization of excess titrant during analysis. Localization of excess titrant, which usually occurs during classical titrations, may lead to unfavorable stoichiometric conditions, resulting in undesirable side reactions.

Effect of Other Possible Impurities—To check for interference from possible impurities such as 4-formylbenzoic acid isopropyl amide (amide) and *N*-isopropyl-α-(methylhydrazone)-*p*-toluamide (hydrazone), procarbazine hydrochloride was titrated coulometrically in the presence of each of these compounds. A mixture of 90% procarbazine hydrochloride, 5% amide [R—C(=O)—NHR'], and 5% hydrazone (RCH=N—NHR') assayed at 90% procarbazine hydrochloride.

Table II—Determination of Procarbazine Hydrochloride in Dosage Form

Milligrams per Capsule Found	Percent Claim[a]
51.38	102.8
51.57	103.1
51.15	102.3
51.31	102.6
51.01	102.0
51.42	102.8
51.30	102.6
51.34	102.7
Average 51.31	102.6
Standard deviation ±0.17	±0.3
Precision[b] ±0.42	±0.7

[a] Based upon 50 mg./capsule. [b] *ts* for 7 degrees of freedom at the 95% confidence level.

These data indicated that quantitative conversion of procarbazine hydrochloride to the azo compound occurred during the titration. No evident substitution of iodine into the phenyl ring resulted, therefore eliminating aromatic substitution as a source of interference.

REFERENCE

(1) H. Beral and V. Stoicescu, *Pharm. Zentralh.*, **108**, 469(1969).

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Analytical Applications of *p*-Nitrosobenzoic Acid I: Specific Colorimetric Determination of *m*-Aminophenol in *p*-Aminosalicylic Acid and Sodium *p*-Aminosalicylate

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Abstract □ *p*-Nitrosobenzoic acid was used in the colorimetric analysis of *m*-aminophenol. It reacted with *m*-aminophenol in sodium bicarbonate solution, producing an intensified orange–yellow color. The reaction product was isolated and characterized as 3-hydroxyazobenzene-4′-carboxylic acid. The color produced in sodium bicarbonate was stable for at least 24 hr. and exhibited a maximum absorption peak at 440 nm. Beer's law was obeyed over a wide range of *m*-aminophenol concentrations (3.2–16.0 mcg./ml.). By applying low temperature control, the reaction specificity of *m*-aminophenol with *p*-nitrosobenzoic acid can be achieved. By using this color reaction, a new method for the direct determination of *m*-aminophenol in *p*-aminosalicylic acid and sodium *p*-aminosalicylate was developed. The method is accurate, precise, sensitive, simple, and specific. Samples of *p*-aminosalicylic acid or sodium *p*-aminosalicylate containing as little as 0.01% of *m*-aminophenol can be accurately determined. In the presence of deactivating groups such as CO_2R, SO_2R, and NO_2, this color reaction of amino compounds was inhibited.

Keyphrases □ *m*-Aminophenol—colorimetric analysis (*p*-nitrosobenzoic acid) in *p*-aminosalicylic acid and sodium *p*-aminosalicylate □ *p*-Nitrosobenzoic acid—colorimetric analysis, *m*-aminophenol □ 3-Hydroxyazobenzene-4′-carboxylic acid—formation, colorimetric analysis of *m*-aminophenol with *p*-nitrosobenzoic acid □ *p*-Aminosalicylic acid, sodium *p*-aminosalicylate—colorimetric determination of *m*-aminophenol □ Colorimetry—analysis, *m*-aminophenol

In 1904, the chemical synthesis of *p*-nitrosobenzoic acid was reported by Alway (1), but further study on this compound has not appeared in the literature. The photodegradation reactions of chloramphenicol were investigated recently, and *p*-nitrosobenzoic acid was recognized as a possible intermediate in the photodegradation reactions (2). *p*-Nitrosobenzoic acid is a stable compound, decomposing at around 250° (1, 2). It possesses two potential functional groups: carboxylic acid and nitroso. The unique physicochemical properties of this compound were applied in this study to the analytical problem of *m*-aminophenol determination in *p*-aminosalicylic acid and sodium *p*-aminosalicylate.

Both the USP (3) and BP (4) require a limit test for *m*-aminophenol in *p*-aminosalicylic acid and sodium *p*-aminosalicylate, but official methods are tedious. Although numerous analytical procedures for the direct determination of *m*-aminophenol in *p*-aminosalicylic acid and sodium *p*-aminosalicylate were proposed, the reported methods (5–11) were not reliable because of poor specificity. The complexity of this problem is reflected by Tatjana's work (11). He pointed out that due to the structural similarity of *m*-aminophenol and *p*-aminosalicylic acid, it was impossible to find a reagent that would react with *m*-aminophenol but not with *p*-aminosalicylic acid.

The present study was undertaken to solve this controversial *m*-aminophenol analysis by using *p*-nitrosobenzoic acid as a specific colorimetric reagent able to distinguish amino compounds of different chemical structures. Condensation between nitroso and amino compounds is a known reaction, giving rise to azo compounds (1, 12, 13). Experiments were designed to study the color reaction of *p*-nitrosobenzoic acid with a variety of amino compounds in sodium bicarbonate solution. By using the highly sensitive and specific color reaction of *m*-aminophenol with *p*-nitrosobenzoic acid, a simple and reliable method for the direct determination of *m*-aminophenol in *p*-aminosalicylic acid and sodium *p*-aminosalicylate was developed. *p*-Nitrosobenzoic acid was used for the first time as a potential analytical reagent.

EXPERIMENTAL

Apparatus—Recording spectrophotometers[1] were used.

Chemicals—*p*-Nitrosobenzoic acid was photosynthesized in this laboratory (2). The following chemicals were either reagent or USP grade: *m*-aminophenol[2], *o*-aminophenol[2], *p*-aminophenol[2], *p*-nitroaniline[2], sodium *p*-aminosalicylate USP[3], *p*-aminobenzoic acid[4], *p*-aminosulfonic acid[4], sodium salicylate[4], isoniazid[5], benzo-

[1] Bausch & Lomb 505 and Beckman IR-8.
[2] Eastman Organic Chemicals.
[3] May & Baker.
[4] Fisher.
[5] Hoffmann–La Roche.