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Colorimetric Determination of Some *N*-1-Substituted Nitroimidazoles

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Abstract □ A colorimetric method is presented for the determination of some *N*-1-substituted nitroimidazoles. The method utilizes diazotization of a sulfanilamide with the nitrite ions produced during the alkaline hydrolysis of the respective imidazole. Subsequent coupling is then carried out with Bratton-Marshall reagent. The sensitivity of this method of analysis is approximately 0.1 mcg./ml. This procedure can be used for the determination of one of the *N*-1-substituted nitroimidazoles, 1-methyl-2-isopropyl-5-nitroimidazole, in feed premixes containing multiple vitamins, and is herein presented.

Keyphrases □ Nitroimidazoles, *N*-1-substituted—analysis □ Sulfanilamide reagent—diazotization, imidazole hydrolysis □ Bratton-Marshall reaction—analysis □ Colorimetric analysis—spectrophotometer □ 1-Methyl-2-isopropyl-5-nitroimidazole—determination, feed premix

An important class of compounds which are effectively used as antiprotozoal agents are substituted nitroimidazoles. Nitroimidazoles can be analyzed by polarography (1, 2) and by reduction of the nitro group to the corresponding amine, which is subsequently determined by diazotization and coupling reaction (3). A rapid and sensitive colorimetric procedure based on the hydrolysis of the nitroimidazoles was developed for the quantitative determination of *N*-1-substituted nitroimidazoles and the method of determination of one of these compounds in feed premix is described.

Although quantitative spot tests have been reported (4) for the alkaline hydrolysis of aliphatic nitro and aromatic polynitro compounds, a quantitative procedure for the determination of heterocyclic nitro compounds such as the nitroimidazoles based on the alkaline hydrolysis of the molecule was not found in the literature.

In the course of these kinetic studies (5) pertaining to the hydrolysis of nitroimidazoles, it was observed that the nitrite ion was found in the reaction. Controlled conditions for the hydrolysis permitted stoichiometric liberation of nitrite ion which can be used for the determination of the corresponding nitroimidazoles. A number of procedures for the determination of nitrite ion which involve diazotization and coupling reactions have been reported (6, 7). A very convenient method, with good sensitivity, is the Bratton-Marshall color reaction (3). This procedure involves the diazotization of sulfanilamide with nitrous acid produced to form a diazonium salt in acidic medium. The diazonium salt is then treated with an aromatic coupling compound forming a colored substance suitable for spectrophotometric measurement.

The concentration of the *N*-1-substituted 5-nitroimidazoles after alkaline hydrolysis, diazotization, and coupling was found to be proportional to absorbance.

The rate of hydrolysis and the formation of nitrite ions from the substituted 5-nitroimidazoles depends upon the structural configuration. Graphic presentation of the preliminary kinetic data illustrates the substituent effect for the alkaline hydrolysis of this type of compound as shown in Fig. 1 and the corresponding structures as given in Table I. Compound I exhibited good stability and did not hydrolyze in 0.1 *N* sodium hydroxide to form nitrite ions. As is evident from Table I, it appears that

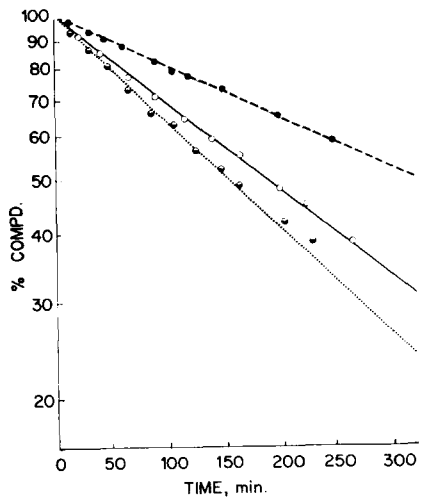


Figure 1—*Semilogarithmic plots of the hydrolysis of some* N-*1-substituted nitroimidazoles in 0.1* N *sodium hydroxide solution at 55°. Key:* ●---●, *Compound II (1-methyl-2-isopropyl-5-nitroimidazole);* ○—○, *Compound III (1,2-dimethyl-5-nitroimidazole);* ◒ . . . ◒, *Compound IV (1-β-hydroxyethyl-2-isopropyl-5-nitroimidazole).*

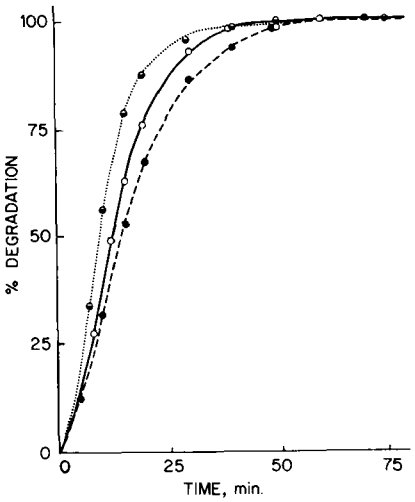


Figure 2—*Degradation of some* N-*1-substituted nitroimidazoles in 0.1* N *sodium hydroxide solution at 100° (calculated from the formation of nitrite ion as detected by Bratton-Marshall reaction). Key:* ---, *Compound II (1-methyl-2-isopropyl-5-nitroimidazole);*—, *Compound III (1,2-dimethyl-5-nitroimidazole);* . . ., *Compound IV (1-β-hydroxyethyl-2-isopropyl-5-nitroimidazole).*

substituted 5-nitroimidazoles decompose more readily when Position 1 in the heterocyclic ring is substituted. It is interesting to note that a CH_3O^- group in place of an alkyl group in Position 2 suggests an increase in the stability of the ring system which may be attributed to the inductive and inductomeric effects of the methoxy substituent.

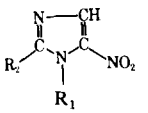
Hydrolysis of the compound in 0.1 *N* sodium hydroxide solution in a boiling-water bath appeared to be the method of choice because of the simplicity and the convenience of the procedure. A plot of percent degradation calculated as nitrite ion formed *versus* time is shown in Fig. 2. It can be seen from data presented in Fig. 2, that 2 hr. heating time is adequate for the quantitative hydrolysis of the compounds studied.

A series of 10 samples of 1-methyl-2-isopropyl-5-nitroimidazole was analyzed by this method. The recovery obtained utilizing the described assay method (Procedure I) listed in Table II, demonstrates the accuracy and precision of this assay procedure. These data were obtained by different analysts at different times.

The method outlined in this report has rather high sensitivity and a sample containing as little as 0.1 mcg./ml. of solution can be determined. The operational parameters of the method proved to be rapid, simple, and selective.

In the analysis of 1-methyl-2-isopropyl-5-nitroimidazole in a feed premix containing vitamins, acetone was found to be the most suitable solvent for extraction. Satisfactory results were not obtained by either shaking the feed premix with cold solvent or by refluxing the sample for a long period of time. However, extraction in a continuous extraction apparatus with acetone for a period of 4 hr. did give satisfactory recovery. Although some of the other materials, mostly vitamins present in the premix, were extracted along with the active component by the acetone, these were removed by precipitation in acid solution and subsequent filtration prior to analysis of the active compound. Recoveries of nitroimidazoles of varying concentrations in the premix are shown in Table III. The kinetic evaluation of this reaction is currently being investigated in this laboratory and will be reported subsequently.

Table I—Structure and Stability of Some Nitroimidazoles



Compd.	R_1	R_2	Hydrolysis[a]
I	H	$CH(CH_3)_2$	Essentially none
II	CH_3	$CH(CH_3)_2$	Complete
III	CH_3	CH_3	Complete
IV	CH_2CH_2OH	$CH(CH_3)_2$	Complete
V	CH_2CH_2OH	OCH_3	Essentially none

[a] Sample dissolved in 0.1 *N* NaOH and heated at 100° for 2 hr.

EXPERIMENTAL

Apparatus—Absorbance measurements were made on either of two spectrophotometers[1] with 1-cm. matched silica cells.

Reagents—Sulfanilamide[2] and *N*-(1-naphthyl)-ethylenediamine dihydrochloride[3] were used without further purification. All other chemicals used were of analytical reagent grade.

Sulfanilamide Reagent—Dissolve 0.1 g. of sulfanilamide in 70 ml. of hot distilled water, cool the solution. Add 5 ml. of glacial acetic acid, dilute to 100 ml. with distilled water, and mix thoroughly.

Bratton-Marshall Reagent—Dissolve 0.1 g. of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 100 ml. of distilled water.

Standard Nitrite Solution—Dissolve 6.90 g. of reagent grade sodium nitrite in distilled water and dilute to 1 l. Standardize this 0.1 *N* sodium nitrite solution by titration against sulfanilamide.

[1] Spectrophotometers: Cary model 14, Beckman model DU-2.
[2] Matheson, Coleman and Bell.
[3] Fisher Scientific Co.

Table II—Determination of 1-Methyl-2-isopropyl-5-nitroimidazole

Amount Added, mcg.	Found, mcg.	% Recovery
81	81	100
101	101	100
106	104	98
111	112	101
121	121	100
151	151	100
201	198	99
206	208	101
223	218	98
312	303	97
Av.		99
SD		±1.4

Table III—Recovery of 1-Methyl-2-isopropyl-5-nitroimidazole in Premix[a]

Amt. in 1 g. of Premix		
Added, mcg.	Found, mcg.	% Recovery
58	56	97
61	60	98
63	62	98
88	88	100
143	144	101
159	154	97
173	178	103
243	236	97
289	289	100.2
Av.		99
SD		±1.8

[a] Typical premix containing meat and bone meal, fish meal, dehydrated alfalfa meal, and other adjuvants including vitamins and minerals.

PROCEDURES

Determination of N-Substituted Nitroimidazoles, Compounds II, III, and IV (Table I)—Dissolve with 0.1 *N* sodium hydroxide about 0.1 g. of sample, containing approximately 100 mcg. of nitroimidazole, accurately weighed, in a 500-ml. volumetric flask. Dilute to volume with 0.1 *N* sodium hydroxide and mix well. Transfer a 10-ml. aliquot of this solution to a 200-ml. volumetric flask. Immerse the flask in a boiling-water bath up to the level of its contents, and heat for 2 hr. Remove the flask from the boiling-water bath, allow the solution to cool, dilute to volume with distilled water, and mix thoroughly. Transfer a 20-ml. aliquot of the sample to a 100-ml. volumetric flask. Add 3 ml. of the sulfanilamide reagent, 4 ml. of 4 *N* sulfuric acid, and 2 ml. of Bratton-Marshall reagent, respectively. Dilute to volume with distilled water and allow to stand for 15 min. at room temperature. Read the absorbance in a 1-cm. cell at 550 mμ against a reagent blank prepared as above but containing no nitrite ion. Prepare a standard curve, using the standard nitrite solution containing 50, 100, 150, 200, and 250 mcg. of sodium nitrite/100 ml. Carry out the colorimetric procedure of the standard nitrite solution as outlined for the sample. The amount of the compound can be determined directly from the standard curve or by calculation in the following equation, taking into account the number of equivalents of sodium nitrite formed in the reaction:

$$\frac{A \times W_n \times \text{mol. wt.}}{A_s \times 69 \times 2} = G$$

where: A is the absorbance of the solution; A_s is the absorbance of the standard nitrite solution; W_n is the weight of sodium nitrite in mcg./100 ml.; mol. wt. is the molecular weight of the compound; G is the amount of the compound found (in mg.); 69 is the molecular weight of sodium nitrite; 2 is the dilution and conversion factor for the described procedure.

Determination of 1-methyl-2-isopropyl-5-nitroimidazole in Premixes—*Sample Preparation*—Accurately weigh 1 g. of ground feed equivalent to 60–250 mcg. of the compound into a paper extraction thimble (10 × 50 mm.) and extract with acetone for 4 hr. in a micro-continuous extraction apparatus placed in a water bath at about 80°. Quantitatively transfer the extract to a 50-ml. volumetric flask, rinsing the extraction container with a small portion of acetone, and add the washing into the flask. Remove the solvent by evaporation with a stream of dry nitrogen until only about 3 to 4 ml. of solution remains. Dilute the sample to volume with 2 *N* sulfuric acid. The solution turns cloudy and the yellow color fades. Transfer about 35 ml. of this solution into a 50-ml. centrifuge tube and centrifuge for 10 min. Filter the centrifuged solution through a 0.45-mμ silver filter membrane and pipet a 25-ml. aliquot into a 50-ml. volumetric flask, add 9 ml. of 25% sodium hydroxide solution, and hydrolyze the solution in a boiling-water bath for 2 hr. Cool the solution to room temperature.

Colorimetric Procedure—To the cooled hydrolyzed sample solution add 3 ml. of the sulfanilamide reagent, 6 ml. of 6 *N* sulfuric acid, and 2 ml. of Bratton-Marshall reagent, respectively. Dilute to volume with distilled water and allow to stand for 15 min. at room temperature. Read the absorbance of the solution in a 1-cm. cell at 550 mμ against a reagent blank. Prepare a standard curve, using the standard nitrite solution containing 50, 100, 150, 200, and 250 mcg. of sodium nitrite/100 ml. Carry out the colorimetric procedure of the standard nitrite solution as outlined for the sample. The amount of the compound can be read directly from the standard curve or calculated by the following equation:

$$\frac{A_2 \times W_1 \times 169.2}{A_1 \times 69 \times W_2} = W$$

where: A_2 is the absorbance of the solution; A_1 is the absorbance of the standard sodium nitrite solution; W_2 is the weight of premix (in g.); W_1 is the weight of sodium nitrite in mcg./100 ml.; 169.2 is the molecular weight of the compound; W is the amount of the compound found (in mcg.)/g. of feed premix; 69 is the molecular weight of sodium nitrite.

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