Spectrophotometric Analysis of Primary Aliphatic Amines with Dichlone

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Abstract □ A spectrophotometric method is described for the determination of some primary aliphatic amines of pharmaceutical interest *via* reaction with dichlone (2,3-dichloro-1,4-naphthoquinone). A mixture of an ethanolic solution of the amine, or amine salt and sodium bicarbonate, with the chromogenic reagent was heated at 65° for 25 min. After acidification to pH 4, the majority of amines yielded an orange-colored product, λ_{max} 475 nm. Some compounds reacted without heat, yielding other colors such as rose (500 nm. with piperazine), yellow (420 nm. with cysteine), and blue (630 nm. with isoniazid) suggesting different products. Molar absorptivities ranged from 900 to 5000. Under the described assay conditions, only primary aliphatic and cyclic secondary amines reacted. Primary aromatic (except some readily oxidizable ones), secondary or tertiary aliphatic, and/or aromatic amines were found not to interfere. The pharmaceutical compounds assayed included histamine, norepinephrine, serotonin, neomycin, leucine, cysteine, aminocaproic acid, ethanolamine, aminophylline, piperazine, and isoniazid. The amines are assayed in the 0.005–0.15-mg./ml. range with a relative standard deviation of 0.02–0.03. The possible composition of the colored products is discussed.

Keyphrases □ Amines, primary aliphatic—colorimetric analysis with dichlone □ Dichlone—used in colorimetric analysis of primary aliphatic amines □ Colorimetry—analysis, primary aliphatic amines using dichlone

The reaction of dichlone (2,3-dichloro-1,4-naphthoquinone) with a number of aliphatic and aromatic amines to yield colored products was reported (1–4) in the course of synthesis of new compounds and the chromatographic separation of amines. Dichlone, a potent fungicide (5), is also a minor dye intermediate (6). There are, however, no reported examples of the quantitative determination of amines using dichlone.

In this work, a spectrophotometric procedure is proposed to assay primary aliphatic amines *via* reaction with dichlone in ethanolic solution. The selectivity, sensitivity, and precision of the proposed method were determined. The scope and limitations relative to pharmaceutical preparations were explored.

EXPERIMENTAL

Equipment—Spectra and absorbance measurements were made using two spectrophotometers[1]. A constant-temperature bath maintained at 65 ± 2° was utilized for accelerating color development.

Reagents and Chemicals—Dichlone was obtained commercially[2]. All other chemicals used were analytical reagent or pharmaceutical grade obtained from various manufacturers and were purified, when necessary, by redistillation, recrystallization, or chromatography before use.

Dichlone Reagent—The desired concentration was 1×10^{-6} mole/ml. (0.227 mg./ml.). The reagent (22.7 mg.) was dissolved in warm ethanol, cooled, and diluted to 100 ml. with ethanol. This solution was stable for 4 weeks.

Amines—Fresh solutions of the amines or amine salts (0.025 mg./ml.) were prepared daily in ethanol and kept at 4°.

[1] Unicam SP 8000 and Beckman DB.
[2] Eastman Organic Chemicals.

Table I—Influence of Chemical Structure

Amine[a]	Color	λ_{max}, nm.
Primary aliphatic:		
Ammonia[b]	Orange	465
Methylamine	Orange	475
Ethanolamine	Orange	472
Ethylenediamine[b]	Yellow	425
n-Butylamine	Orange	475
Benzylamine	Orange	478
Primary aromatic:		
Aniline	Red	495
o-Toluidine	No color	—
1-Naphthylamine	No color	—
o-Aminophenol	Yellow	420
p-Aminophenol	Pink	505
p-Nitroaniline	No color	—
p-Anisidine	No color	—
p-Phenylenediamine	Pink	505
Benzidine	Purple	510
o-Tolidine	No color	—
p-Dimethylaminoaniline	Purple	515
Secondary amines:		
Diethylamine[c]	No color	—
Diethanolamine[c]	No color	—
N-Methylaniline	No color	—
Piperidine[b]	Rose	500
Morpholine[b]	Rose	500
Piperazine[b]	Rose	500
Tertiary amines:		
Triethylamine[c]	No color	—
Triethanolamine[c]	No color	—
N,N-Diethylaniline	No color	—
p-Dimethylaminobenzaldehyde	No color	—
Aromatic heterocycles:		
Pyridine	No color	—
Quinoline	No color	—
Quaternary ammonium salts:		
Choline citrate	No color	—
Quinaldine ethiodide	No color	—

[a] Using 1 mg./ml. in ethanol. [b] Reacted in the cold. [c] Purified by chromatography.

Bicarbonate Solution—Sodium bicarbonate (840 mg.) was dissolved in glass-distilled water and diluted to 100 ml. with the same water; the final concentration was 8.4 mg./ml.

Procedure—One milliliter of the amine solution was mixed with 1.0 ml. of the reagent solution. With amine salts, 1.0 ml. of the solution of the amine hydrochloride or sulfate was mixed with 1.0 ml. of sodium bicarbonate solution and 1.0 ml. of the reagent solution. In either case, the mixture was maintained at 65 ± 2° for 25 min. Then the solution was cooled to room temperature, the pH was adjusted to 3–4 with 10% hydrochloric acid, and the solution was diluted to 10 ml. with ethanol in a volumetric flask. The absorbance of this solution was measured at 475 nm., or at the λ_{max} if different, against a reagent blank similarly treated.

RESULTS

Influence of Chemical Structure—The colors produced on reacting dichlone with various amines are indicated in Table I. The colors obtained when extending the reaction to selected pharmaceutical amines are given in Table II.

Quantitative Determination of Amines—Table III summarizes the data of the quantitative analysis of selected amines of biological and pharmaceutical interest by the proposed spectrophotometric method. These amines assayed at least 100% by established and

Table II—Application to Pharmaceuticals

Amine[a]	Color	λ_{max}, nm.	ϵ_{max}
Histamine dihydrochloride	Orange	475	1400
Norepinephrine tartrate	Orange	478	1800
Epinephrine tartrate	No color	—	—
Serotonin creatinine sulfate	Orange	478	1600
Amphetamine sulfate	Orange	475	900
Phenylephrine hydrochloride	No color	—	—
Glucosamine	Orange	475	1500
Neomycin sulfate	Orange	472	1800
Kanamycin sulfate	Orange	472	1300
Streptomycin sulfate	No color	—	—
Glycine	Orange	472	900
Leucine	Orange	475	1200
Phenylalanine	Orange	478	1800
Cysteine[b]	Yellow	420	1300
Aminocaproic acid	Orange	475	1800
Aminophylline[b]	Yellow	425	1600
Theophylline	No color	—	—
Isoniazid[b]	Blue	630	5000
Phenyltoloxamine dihydrogen citrate	No color	—	—
Chloroquin phosphate	No color	—	—
Benzocaine	No color	—	—
Sulfanilamide	No color	—	—

[a] Using 1 mg./ml. solution. [b] Reacted without heat.

compendial methods of analysis as follows: histamine, reaction with 2,4-dinitrofluorobenzene (7); serotonin, colorimetric analysis with 1-nitroso-2-naphthol (8); neomycin, the BP microbiological method using *Bacillus pumilus* (9); piperazine citrate and leucine, nonaqueous titrimetry (USP procedure) (10); aminophylline (for ethylenediamine content), titration with 0.1 *N* sulfuric acid using bromcresol green as indicator (11); and isoniazid, iodometric analysis (12).

Standard Curves—Standard curves can be constructed by plotting observed absorbance readings *versus* the volume taken of equimolar concentrations of various amines in the range 0.005–0.150 mg./ml. (1×10^{-5}–1×10^{-7} mole/ml.). In all cases, Beer's law held for the system.

Analysis of Mixtures—Table IV illustrates the analytical data obtained for various mixtures of amines of biological and pharmaceutical interest analyzed for the primary aliphatic amine component.

DISCUSSION

Reaction Involved and Influence of Chemical Structure—In the analytical procedure presented, dichlone (I) reacts with a primary aliphatic amine to yield an orange color. Chemically, the colored product is most likely a 2-amino-3-chloro-1,4-naphthoquinone derivative (II), formed by 1,4-addition to the quinone followed by reoxidation with excess reagent (13). This is in agreement with published data (14) concerning the reaction of dichlone with some aliphatic amines. Further verification of this assumption was obtained by comparing the spectrum of a typical orange product (dichlone with *n*-butylamine) with that of 2-amino-1,4-naphthoquinone (III) (Fig. 1). The aminonaphthoquinone was synthesized by an independent route (15) from 1,4-naphthoquinone and sodium azide in acetic acid. The two spectra are practically superimposable, suggesting the same basic chromophore.

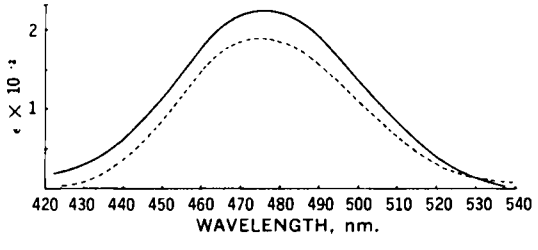


Figure 1—*Spectra of 2-amino-1,4-naphthoquinone and reaction product of dichlone with* n-*butylamine. Key:* ——, *2-amino-1,4-naphthoquinone; and* - - -, *reaction product.*

Table III—Quantitative Application

Compound	Amount, mg. Taken	Amount, mg. Found[a]	Error, %	Standard Deviation, %
Histamine	0.180	0.1811	+0.60	±2.61
Serotonin	0.200	0.2015	+0.75	±2.69
Neomycin	0.250	0.2480	−0.80	±1.9
	0.750	0.7540	+0.53	±2.2
Piperazine	0.200	0.2025	+1.25	±3.1
Leucine	0.400	0.3974	−0.65	±2.8
Aminocaproic acid	0.350	0.3445	−1.05	±2.65
Aminophylline	0.600	0.6055	+0.90	±2.68
Isoniazid	0.150	0.1512	+0.80	±3.2

[a] Mean value of five determinations.

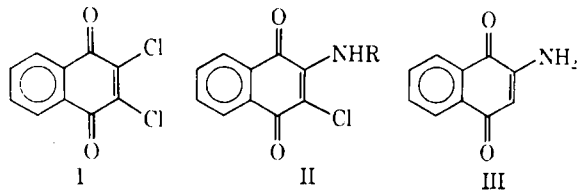
Table IV—Analysis of Mixtures

Mixture Composition	Milligrams per Milliliter	Primary Aliphatic Amine Found[a], mg./ml.	Theory, %
Norepinephrine	0.050	0.0495	99.0
Epinephrine	0.050	—	—
Histamine	0.025	0.0251	100.4
Epinephrine	0.050	—	—
Neomycin[b]	0.100	0.100	100.0
Streptomycin	0.100	—	—
Isoniazid	0.005	0.0049	98.0
Sodium *p*-aminosalicylate	0.050	—	—
Norepinephrine	0.050	0.0498	99.6
Benzocaine	0.050	—	—
Phenyltoloxamine	0.050	—	—

[a] Mean value of three determinations. [b] Mixture of commercial neomycins (A, B, and C).

Dichlone was reported to react with primary aromatic amines under certain conditions of synthesis (1, 16, 17). Under the analytical conditions described, however, only primary aliphatic amines reacted to a measurable extent. Some primary aromatic amines with sensitive nuclei (*e.g.*, *p*-aminophenol, *p*-phenylenediamine, and *p*-dimethylaminoaniline) gave red colors (Table I). This was probably due to oxidation by the quinone reagent rather than reaction by 1,4-addition, since identical colors were obtained when these compounds were treated with inorganic oxidizers such as potassium persulfate. Failure of sulfanilamide, 1-naphthylamine, *o*-toluidine, and benzocaine to yield colors (Tables I and II) supports the view that, under the mild conditions of the procedure, primary aromatic amines do not interfere. Similarly, secondary and tertiary aliphatic or aromatic amines failed to yield measurable colors (Tables I and II).

Some compounds (*e.g.*, cyclic secondary aliphatic amines, ethylenediamine, cysteine, and isoniazid) reacted rapidly without heat to yield colors different from the orange color obtained with the majority of amines (Fig. 2). This finding strongly suggests different types of products. In the case of cysteine, the product is probably a thioether at the 3-position of the quinone, in line with the analogous reaction of 2-methyl-1,4-naphthoquinone (K_3) with sulfhydryl compounds (thioglycollic acid and cysteine) to form a thioether linkage with a characteristic absorption at 420–430 nm. (18, 19). With the present data alone, however, it is difficult to reach a conclusion concerning the true nature of these colored products. After heating at 65° for 20–30 min., these different colors changed to orange, λ_{max} 475 nm.



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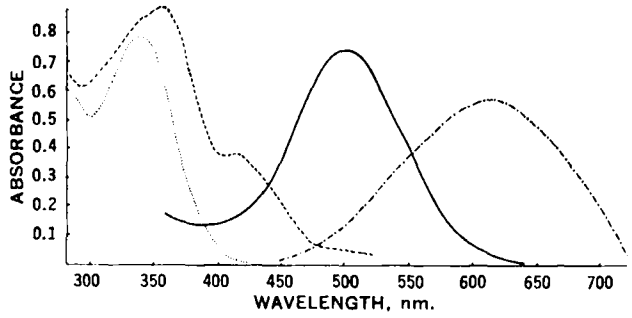

Figure 2—*Spectra of dichlone and its reaction products. Key: · · ·, dichlone (1 × 10⁻⁶ mole/ml.); - - -, with cysteine (3 × 10⁻⁶ mole/ml.); – – –, with piperazine (5 × 10⁻⁶ mole/ml.); and –·–, with isoniazid (4 × 10⁻⁷ mole/ml.).*

Quantitative Applications—Data in Tables III and IV reveal the suitability of the proposed method for the determination of pharmaceutical primary amines in the presence of primary aromatic and/or secondary and tertiary aliphatic amines in amounts of 0.005–0.150 mg./ml. with a standard deviation of ±2–3%.

Reagent blank readings at wavelengths of maximum absorbance are very low (Fig. 2). The main absorbance of the reagent is at 345 nm. No interference by excess unreacted reagent was noticed. This was further verified by the complete extraction of unreacted dichlone with ethylene chloride from the acidified (pH 3–4) aqueous colored solution. The color remained in the acidified aqueous layer because of protonation of the amino group (Structure II). No difference in absorbance readings between extracted and unextracted samples was observed.

Color Development and Stability—Color development at room temperature was too slow, but formation of the color was greatly accelerated at 60–70°. Maximum color intensity was obtained after 25 min. (Fig. 3) and was unchanged after 24 hr. at room temperature.

Limitations—The proposed method is fairly sensitive and selective for primary aliphatic amines. The procedure, however, must be considered nonspecific with regard to degradation products (unless degradation involves loss of the primary amine function) as well as other potential interfering substances.

Although cyclic secondary amines reacted, the probability of their presence with primary aliphatic amines in the same biomedia or dosage form is slim. Furthermore, these cyclic amines exhibited an absorbance maximum different from 475 nm. (at 500 nm.), so any problems arising from overlap of the spectra for the two species may be overcome by the use of differential analysis.

CONCLUSION

The analytical method presented is essentially a microprocedure with fair sensitivity (0.005–0.150 mg./ml.), good accuracy (from −0.9 to +1.3% error), and precision (0.02–0.03 *RSD*). The method offers a relatively simple and rapid means of analysis of some pharmaceutical primary aliphatic amines in admixture with secondary or tertiary aliphatic or primary aromatic amines.

The reagent is available commercially or easily synthesized (20), and its solutions are stable at room temperature for convenient periods. The colors formed are also stable.

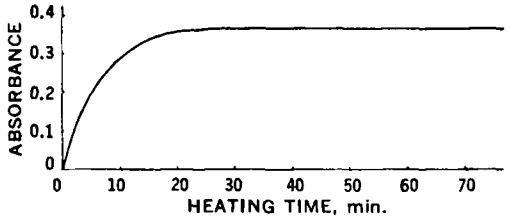

Figure 3—*Rate of color development. Histamine (5 × 10⁻⁶ mole/ml.) with dichlone (1 × 10⁻⁶ mole/ml.).*

REFERENCES

(1) A. Inove and T. Nomura, *Yuki Gasei Kagaki Shi*, **16**, 536 (1958).

(2) I. N. Nikolenko, E. V. Degeterev, N. S. Tolmachera, and A. A. Manik, *Zh. Anal. Khim.*, **21**(3), 382(1966); through *Chem. Abstr.*, **65**, 1354e(1966).

(3) J. E. Barnes, S. R. Harvey, and T. B. Herman, *J. Chromatogr.*, **45**, 82(1969).

(4) M. B. Henfest and P. J. Slade, *J. Chem. Soc.*, **1960**, 1555.

(5) Ter Horst, *Ind. Eng. Chem.*, **35**, 1255(1943).

(6) K. Venkataraman, "The Chemistry of Synthetic Dyes," vol. 1, Academic, New York, N. Y., 1952, p. 156.

(7) H. M. Kalckar, "Methods of Biochemical Analysis," vol. 3, Interscience, New York, N. Y., 1956, p. 49.

(8) S. Udenfriend, H. Weissbach, and B. B. Brodie, "Methods of Biochemical Analysis," vol. 6, Interscience, New York, N. Y., 1958, p. 95.

(9) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1968, p. 1313.

(10) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 508.

(11) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1968, p. 85.

(12) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 349.

(13) L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold, New York, N. Y., 1961, pp. 853–858.

(14) E. P. Elslager, L. M. Werbel, and D. F. Worth, *J. Med. Chem.*, **13**, 104(1970).

(15) L. F. Fieser and J. L. Hartwell, *J. Amer. Chem. Soc.*, **57**, 1482(1935).

(16) T. A. Van Allan and G. A. Reynolds, *J. Org. Chem.*, **28**, 1019(1963).

(17) W. L. Mosby and R. J. Boyle, *ibid.*, **24**, 374(1959).

(18) N. Nakai and J. Hase, *Chem. Pharm. Bull.*, **16**, 2334(1968).

(19) *Ibid.*, **19**, 460(1971).

(20) Ruetgerswerke und Toerver wertung, A. G., German pat. 1,194,392 (June 10, 1965); through *Chem. Abstr.*, **63**, 9893d(1965).

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