# Determination of Diphenoxylate Hydrochloride and Atropine Sulfate in Solutions and Tablets

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**Abstract** □ Methods for the determination of diphenoxylate hydrochloride and atropine sulfate combinations in solutions and powdered tablet composites are presented. A semiautomated assay for diphenoxylate hydrochloride in individual tablets (content uniformity) also is presented. The USP XIX assays for these products are cumbersome and, in the case of solutions, inaccurate due to spectral interferences; the proposed methods offer substantial improvements in sensitivity, specificity, and speed. Results obtained by the USP and proposed methods are compared for several lots of commercial products. The accuracy and precision of the proposed methods are shown by standard recovery studies.

**Keyphrases** □ Diphenoxylate hydrochloride—spectrophotometric analysis in solutions and tablets containing atropine sulfate □ Atropine sulfate—GLC analysis in solutions and tablets containing diphenoxylate hydrochloride □ Spectrophotometry—analysis, diphenoxylate hydrochloride in solutions and tablets containing atropine sulfate □ GLC—analysis, atropine sulfate in solutions and tablets containing diphenoxylate hydrochloride □ Antiperistaltic agents—diphenoxylate hydrochloride, spectrophotometric analysis in solutions and tablets containing atropine sulfate □ Anticholinergic agents—atropine sulfate, GLC analysis in solutions and tablets containing diphenoxylate hydrochloride

The official USP assays (1) for diphenoxylate hydrochloride and atropine sulfate combinations in tablets and solutions have several disadvantages. The titrimetric assay for diphenoxylate hydrochloride requires the extraction of a large sample portion, which presents troublesome emulsion problems. The colorimetric assay for atropine sulfate in solution gave inaccurately high results when applied to commercial products because of appreciable background interference. The procedures are also time consuming since separate sample portions must be weighed or measured and extracted for each component.

The USP assay for diphenoxylate hydrochloride is an application of the commonly used nitrogenous organic base determination by nonaqueous titration with perchloric acid. Alternative analytical methods for this drug have not been found in literature reviews.

The *p*-dimethylaminobenzaldehyde reaction in the USP colorimetric assay for atropine sulfate also was used for the determination of tropic acid (2) and other tropane alkaloids (3). Various other reagents were reported for the colorimetric determination of atropine and related alkaloids (4–7). IR (8) and fluorometric (9) methods also were developed.

The USP methods for atropine sulfate in single-ingredient dosage forms (10) are a result of the collaborative study of a GLC method (11). Since this approach offered the sensitivity and specificity desired, its adaptation to the assay of atropine sulfate in diphenoxylate hydrochloride–atropine sulfate combination dosage forms was investigated and found suitable.

A UV spectrophotometric determination is used in the USP content uniformity analysis for diphenoxylate hydrochloride in tablets. When combined with a preliminary column chromatographic separation, this procedure was suitable for the assay of diphenoxylate hydrochloride in composited diphenoxylate hydrochloride–atropine sulfate tablets. However, UV spectrophotometry was unsuitable for the assay of diphenoxylate hydrochloride in commercial solutions. The relatively low absorptivity of the drug necessitated the extraction of a volume of solution too large for practical column chromatography. Separator extraction resulted in the coextraction of UV-absorbing interferences.

Sensitive methods for tertiary amines based on dye complex formation with various sulfonphthalein dyes were reported (12–15). This approach was subsequently investigated for the determination of diphenoxylate hydrochloride in commercial solutions. Bromphenol blue was investigated and provided good sensitivity. Reagent blanks showed baseline absorbance relative to the dye complex extraction solvent. This dye also was suitable for use in a semiautomated system for the content uniformity analysis of diphenoxylate hydrochloride in tablets.

## EXPERIMENTAL

**Bromphenol Blue Dye Solution**—Dissolve 400 mg of bromphenol blue in 20.0 ml of 0.1 *N* NaOH in a 1-liter volumetric flask. Add 800 ml of water and 10.0 g of potassium acid phthalate. Mix to dissolve. Add 125 ml of 0.1 *N* HCl, dilute to volume with water, and mix. Prepare fresh daily.

**Diphenoxylate Hydrochloride Standard Solution**—Dissolve about 50 mg of USP diphenoxylate hydrochloride reference standard, accurately weighed, in chloroform contained in a 100-ml volumetric flask. Dilute to volume with chloroform and mix.

**Atropine Sulfate Standard Solution**—Dissolve about 15 mg of USP atropine sulfate reference standard, accurately weighed, in 0.05 *N* HCl contained in a 100-ml volumetric flask. Dilute to volume with 0.05 *N* HCl and mix.

**Homatropine Hydrobromide GLC Internal Standard Solution**—Dissolve about 20 mg of USP homatropine hydrobromide reference standard, accurately weighed, in 0.05 *N* HCl contained in a 100-ml volumetric flask. Dilute to volume with 0.05 *N* HCl and mix.

**Solutions**—*Determination of Diphenoxylate Hydrochloride*—Transfer an accurately measured volume of solution, equivalent to about 25 mg of diphenoxylate hydrochloride, to a 250-ml separator. Add sufficient water to give a total volume of 100 ml; then add exactly 1.0 ml of homatropine hydrobromide standard solution and 5 ml of 1 *N* HCl. Extract with seven 30-ml portions of chloroform. After each extraction, transfer the chloroform layer to a second separator containing 10 ml of water and reextract.

Use the same separator and 10 ml of water for each reextraction. Transfer the reextracted chloroform solutions to a 250-ml volumetric flask and dilute to volume with chloroform. Combine the aqueous wash with the original sample solution and save it for the atropine determination.

Transfer 10.0 ml of the chloroform extract, 2.0 ml of diphenoxylate hydrochloride standard solution plus 8 ml of chloroform, and 10 ml of chloroform (to serve as a blank) to three separate 125-ml separators. Add 30.0 ml of dye solution to each separator and shake vigorously for 1 min. Let the solutions stand for 5 min and then transfer the chloroform layers to separate 100-ml volumetric flasks. Reextract the aqueous layers with 10-ml portions of chloroform, adding the extracts to the respective volumetric flasks. Continue extractions until no yellow color is apparent in chloroform and then extract with one additional 10-ml portion of chloroform.

Dilute combined extracts in separate flasks to volume with chloroform. Concomitantly determine the absorbances of the solutions in 1-cm cells, at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate

**Table I—Comparison of Results Obtained by USP and Proposed Assay Procedures for Commercial Solutions Labeled to Contain 2.5 mg of Diphenoxylate Hydrochloride (I) and 25 μg of Atropine Sulfate (II)/5 ml**

| Sample (Lot) | Component | Percent of Label Declaration | |
|---|---|---|---|
| | | USP | Proposed |
| 1 | I | 99.2 | — |
| | II | 126 | 102.8 |
| 2 | I | 100.4 | — |
| | II | 128 | 105.0 |
| 3 | I | 101.8 | 99.1, 101.3, 100.5 (ave. = 100.3) |
| | II | 115.6 | 96.4 |
| 4 | I | 97.4 | 97.2, 97.9, 97.9 (ave. = 97.7) |
| | II | — | 101.2, 99.6 (ave. = 99.9) |

the milligrams of diphenoxylate hydrochloride in the volume of solution taken as follows: milligrams = $50C(A_u/A_s)$, where $C$ is the concentration, in milligrams per milliliter, of the diphenoxylate hydrochloride standard solution; and $A_u$ and $A_s$ are the absorbances of the final sample and standard solutions, respectively, both being corrected for the reagent blank absorbance.

*Determination of Atropine Sulfate*—Make the aqueous solution saved from the diphenoxylate determination alkaline with 6 *N* $NH_4OH$. Extract with six 10-ml portions of chloroform, filtering each extract through a single 5-g portion of anhydrous sodium sulfate supported by a small pledget of glass wool in a funnel. Evaporate the solution to about 0.3 ml using a suitable evaporation device[1]. (If evaporated from an open container using positive pressure, a current of dry nitrogen is recommended to aid the evaporation.)

Pipet 1-, 2-, and 3-ml aliquots of atropine sulfate standard solution into three separate 250-ml separators. Add 100 ml of water and exactly 1.0 ml of homatropine hydrobromide standard solution to each separator, make alkaline with 6 *N* $NH_4OH$, and proceed as directed for the sample assay preparation beginning with: "Extract with six 10-ml portions of chloroform . . . ." Inject the solutions into a GLC system[2] as described in the USP XIX monograph for atropine sulfate injection and plot a standard curve as specified in the monograph. Read from the standard curve the quantity of atropine sulfate in the volume of solution taken.

**Tablets**—*Determination of Diphenoxylate Hydrochloride*—Insert a small pledget of glass wool above the stem constriction of a 20 × 2.5-cm chromatographic tube and uniformly pack with a mixture of about 2 g of chromatographic siliceous earth[3] and 1 ml of 0.05 *N* HCl. To an accurately weighed quantity of finely ground powdered tablets, equivalent to about 30 mg of diphenoxylate hydrochloride, add 0.5 ml of dimethyl sulfoxide and mix to a uniform paste. Add about 3 g of chromatographic siliceous earth, exactly 1.0 ml of homatropine hydrobromide standard solution, and 1 ml of 0.05 *N* HCl. Mix and add to the column.

Scrub the sample container with about 1 g of chromatographic siliceous earth and add the latter to the column. Place a pledget of glass wool on top and uniformly pack the sample mixture onto the column. Elute the diphenoxylate hydrochloride with 200 ml of water-saturated chloroform, added in several portions, into a 200-ml volumetric flask[4]. Then dilute to volume with chloroform. Pipet 50 ml of this solution and 15 ml of diphenoxylate hydrochloride standard solution into separate glass-stoppered erlenmeyer flasks and evaporate the solutions to dryness on a steam bath.

Dissolve each residue in 15 ml of a 1:100 solution of hydrochloric acid in methanol and concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance (about 257 nm) and at 280 nm (background absorbance) with a suitable spectrophotometer[5], using a 1:100 solution of hydrochloric acid in methanol as the blank. Calculate the milligrams of diphenoxylate hydrochloride in the quantity of tablets taken as follows: milligrams = $60C[A_u(257) - A_u(280)/A_s(257) - A_s(280)]$, where $C$ is the concentration, in milligrams per milliliter, of the diphenoxylate hydrochloride standard solution; and $A_u(257)$, $A_u(280)$, $A_s(257)$, and $A_s(280)$ are the absorbances of the final sample ($A_u$) and standard ($A_s$) solutions at 257 and 280 nm, respectively.

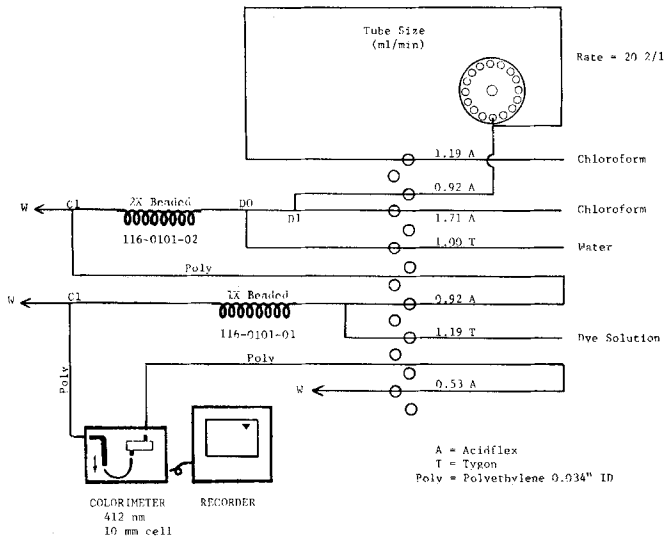


**Figure 1**—*Flow diagram for automated colorimetric determination of diphenoxylate hydrochloride in tablets. Both C1 fittings are silanized and oriented as shown.*

*Determination of Atropine Sulfate*—Insert a small pledget of glass wool above the stem constriction of a 20 × 2.5-cm chromatographic tube and place 10 g of anhydrous sodium sulfate in the tube. Place the tube under the column prepared for the determination of diphenoxylate hydrochloride tablets. Then elute atropine from the column and through the sodium sulfate with 50 ml of ammonia-saturated chloroform (prepared by mixing 4 volumes of chloroform with 1 volume of ammonium hydroxide in a separator), followed by 50 ml of water-saturated chloroform. Evaporate the combined eluates using a suitable evaporation device and proceed with the GLC determination as directed in the *Determination of Atropine Sulfate* under *Solutions*. Read from the standard curve the amount of atropine sulfate in the quantity of tablets taken.

**Tablet Content Uniformity: Semiautomated Determination of Diphenoxylate Hydrochloride in Single Tablets**—To each tablet and 2.50 mg of USP diphenoxylate hydrochloride reference standard contained in glass-stoppered erlenmeyer flasks, add 0.5 ml of water and disperse by placing the flasks in an ultrasonic bath. Then add 50.0 ml of chloroform and shake vigorously to dissolve the drug. Add 5 g of anhydrous sodium sulfate granules and shake. Allow the solutions to stand until the chloroform is clear, decant about 3 ml of each solution into 4-ml polyethylene sample cups, and overlay each with 10 drops of water to prevent evaporation.

The automated system[6] is assembled as shown in Fig. 1. The tablet and standard solutions are sampled at a rate of 20 cups/hr with a sample-to-wash ratio of 2:1. A sampling pattern of two standards, five samples, one standard . . . five samples, one standard is used. The first standard peak is ignored in the calculations.

The sample stream is diluted with chloroform and washed with water. The chloroform layer is resampled and mixed with the aqueous bromphenol blue dye solution. The chloroform layer, containing the diphenoxylate–dye complex, is pumped through a flowcell where the intensity of the color is measured at 412 nm[7]. The tablet content is calculated from the absorbance values.

**Identification Tests**—IR identification of diphenoxylate hydrochloride in solutions and tablets was done as follows. A portion of the chloroform extract prepared for the assay, equivalent to about 2.5 mg of diphenoxylate hydrochloride, was evaporated to dryness. The residue was crystallized by adding several drops of hydrochloric acid-saturated ether and again evaporated to dryness. A potassium bromide disk was prepared from a mixture of the crystalline residue with 200 mg of potassium bromide, and the IR spectrum was then compared with a reference standard prepared similarly. This technique was preferred to the USP XIX identification test since less drug was required, which eliminated the need for additional sample extraction, and the spectra obtained were of better quality.

[1] Kuderna Danish evaporative concentrator, catalog No. K-570000 (125-ml size), with graduated concentrator tube, catalog No. K-570050 (1-ml capacity), Kontes Glass Co., Vineland, NJ 08360.

[2] Perkin-Elmer model 3920.

[3] See USP XIX, p. 750.

[4] At this point, it is preferable to proceed with the elution of atropine from the column as described under *Determination of Atropine Sulfate*.

[5] Cary model 118.

[6] Technicon AutoAnalyzer system with sampler II and proportioning pump I.

[7] Beckman DB-GT spectrophotometer equipped with a 1-cm flowcell.

**Table II—Comparison of Results Obtained by USP and Proposed Manual and Semiautomated Procedures for Composites of Commercial Tablets Labeled to Contain 2.5 mg of Diphenoxylate Hydrochloride (I) and 25 μg of Atropine Sulfate (II)/Tablet**

| Sample (Lot) | Component | Percent of Label Declaration | | |
|---|---|---|---|---|
| | | USP | Proposed Manual | Proposed Semiautomated |
| 1 | I | 100.8 | 98.8 | 101.3 |
| | II | — | 110.8, 109.6 (ave. = 110.2) | — |
| 2 | I | — | 100.0 | 100.0 |
| | II | 96.0, 99.2 (ave. = 97.6) | 103.2, 102.8 (ave. = 103.0) | — |
| 3 | I | 92.0 | 96.8 | 99.9 |
| | II | — | 100.8 | — |
| 4 | I | — | 100.4 | 99.1, 100.9, 102.4 (ave. = 100.8) |
| | II | 107.2 | 116.0, 116.4 (ave. = 116.2) | — |
| 5 | I | 99.2 | 94.8 | 95.7 |
| | II | 109.2 | 105.6 | — |
| 6 | I | 101.2 | 99.6 | 101.5 |
| | II | — | 106.4, 103.2 (ave. = 104.8) | — |

Atropine was identified in the samples by comparison of the GLC retention time with that of the standard.

## RESULTS AND DISCUSSION

**Solution Assay Results**—A comparison of results obtained using the USP and proposed assay procedures for several lots of commercial diphenoxylate hydrochloride and atropine sulfate solutions is shown in Table I. Due to obvious background interference in the spectra of the sample solutions, the USP results for atropine sulfate were considerably higher than the proposed procedure results. Samples 1 and 2 exceeded the USP upper potency limit on the basis of the USP assays.

The results for diphenoxylate hydrochloride in solutions show close agreement between the two procedures. However, the sensitivity of the proposed procedure required 1 mg of the drug for the determinative step as compared with the 100 mg required for the USP determination.

Repetitive assay results obtained for Samples 3 and 4 suggest that good precision can be expected with the proposed procedure.

**Tablet Assay Results**—A comparison of the results obtained for several lots of commercial tablets using the USP and proposed manual and semiautomated assays is shown in Table II. (Semiautomated assays were done on portions of powdered composites equivalent to single tablets.) The results of the proposed manual and semiautomated assays for diphenoxylate hydrochloride were in close agreement. The USP results for both diphenoxylate hydrochloride and atropine sulfate differed in some cases, primarily because of an apparent loss of the drugs as a result of emulsion problems. The lack of sensitivity of the USP titrimetric method for diphenoxylate hydrochloride is also likely to introduce error.

The semiautomated method permits rapid, unattended, multiple-tablet analysis. The proposed manual procedures require less sample and less time and are less painstaking than the USP procedures.

**Table III—Recoveries from Fortified Commercial Solutions[a]**

| Drug | Fortified Sample | | Recovery of Added Drug, % |
|---|---|---|---|
| Diphenoxylate hydrochloride, 12.5 mg added | 1 | | 101.3 |
| | 2 | | 100.5 |
| | 3 | | 99.8 |
| | 4 | | 98.1 |
| | 5 | | 100.2 |
| | | Average | 100.0 |
| | | *SD* | 1.186 |
| Atropine sulfate, 0.1 mg added | 1 | | 98.8 |
| | 2 | | 100.6 |
| | 3 | | 99.3 |
| | 4 | | 97.4 |
| | 5 | | 103.3 |
| | | Average | 99.9 |
| | | *SD* | 2.229 |

[a] The amount of solution taken for fortification was one-half the amount specified in the procedure so as not to exceed the optimum detection levels of the procedure.

**Standard Recovery Results**—Recovery studies, using the respective proposed procedures, were made on fortified samples of commercial solutions (Table III) and on simulated mixtures of the drugs with tablet excipients based on manufacturing formulations (Table IV). Essentially 100% recoveries were achieved in all cases.

**Extraction of Drugs from Commercial Products**—The extraction of atropine from samples of commercial diphenoxylate hydrochloride and atropine sulfate solutions was initially attempted as in the USP assay of atropine sulfate injection (10); that is, a single separator extraction was made of each standard and sample solution. However, extraction of the drug from sample solutions was apparently suppressed by the sample matrix, resulting in considerable loss relative to the amounts extracted from standard solution. Therefore, it was necessary to conduct multiple shakeouts to assure complete and uniform extraction of the drug from all solutions.

Drying the chloroform extracts containing atropine by eluting through anhydrous sodium sulfate, prior to concentration and GLC analysis, was essential; elimination of this step resulted in low recoveries of atropine. While not thoroughly investigated, it was theorized that hydrolysis of the drug could occur while concentrating the undried chloroform extracts with heat.

The column chromatographic extraction of the drugs from commercial tablets was initially attempted without the addition of dimethyl sulfoxide to the powdered tablet samples. The results of diphenoxylate hydrochloride assays thus performed were consistently lower than the results obtained by the semiautomated method. Investigation revealed a "tailing" retention of the drug on the column, resulting in incomplete elution using a practical volume of solvent. Since this problem was not evident when eluting diphenoxylate hydrochloride standard material from the column, it was assumed that the tablet matrix was retaining the drug through interactive "binding." Preliminary trituration of the tablet material with dimethyl sulfoxide, which has been used fairly extensively as a drug solubilizing agent, eliminated this problem.

Addition of a small volume of water to individual tablets and subse-

**Table IV—Analysis of Simulated Tablet Mixtures**

| Drug | Simulated Mixture | | Recovery, % | |
|---|---|---|---|---|
| | | | Proposed Manual | Proposed Semi-automated |
| Diphenoxylate hydrochloride | 1 | | 99.1 | 101.1 |
| | 2 | | 98.9 | 100.4 |
| | 3 | | 99.9 | 103.1 |
| | 4 | | 100.3 | 100.8 |
| | 5 | | 100.4 | 99.7 |
| | | Average | 99.7 | 101.1 |
| | | *SD* | 0.687 | 1.276 |
| Atropine sulfate | 1 | | 97.0 | — |
| | 2 | | 99.5 | — |
| | 3 | | 103.0 | — |
| | 4 | | 99.3 | — |
| | 5 | | 101.0 | — |
| | | Average | 100.0 | |
| | | *SD* | 2.221 | |

quent ultrasonication were necessary to effect complete dissolution of diphenoxylate hydrochloride in chloroform prior to automated analysis. In addition to hastening tablet dispersion, the water retains atropine sulfate and water-soluble excipients, which are subsequently separated from the chloroform solution.

**Bromphenol Blue Dye Solution**—The pH of the dye solution prepared as specified is approximately 3.4. The effect of the pH of the dye solution on the color intensity of the resultant diphenoxylate–dye complex was investigated. The intensity was fairly constant between pH 2 and 4, with a maximum at pH 3–3.5. Above pH 4, the intensity decreased rapidly.

The concentration of the dye required in the solution was similarly determined by incrementing the weight ratio of dye to diphenoxylate hydrochloride until a constant color intensity was attained for the resultant complex. The amount of dye specified in the solution prepared for the assays is an excess of about twice that needed to react completely with the anticipated concentration of drug.

**GLC**—The collaborative study of the GLC method for atropine (11) cited references to reports of injection site decomposition. However, the authors of the collaborative study did not observe decomposition, which they attributed to the use of commercial silanized glass wool. GLC decomposition of the atropine was observed in the present study, even when using silanized glass wool, if an excessive amount of wool was placed in the injection site end of the column. The decomposition was evidenced by the appearance of one or more small peaks preceding the atropine and homatropine peaks. By using very little glass wool or none at all, the decomposition problem can be avoided.

**Linearity Studies**—The standard curve prepared for the GLC determination of atropine was linear over the range of standard concentrations specified.

Conformity to Beer's law was observed when concentrations of diphenoxylate hydrochloride introduced into the automated system were varied from 70 to 150% of the tablet dosage level. The manual dye complexation procedure also provided linear absorbance readings when the drug concentrations were varied from 50 to 150% of the level anticipated in solution sample extracts.

## REFERENCES


(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 158.

(2) K. R. Gottleib, *Dan. Tidsskr. Farm.*, **24,** 40 (1950).

(3) O. A. Akopyan, *Aptechn. Delo,* **7** (2), 19 (1958); *cf., ibid.*, **4,** 431 (1955).

(4) V. D. Gupta and N. M. Ferguson, *Am. J. Hosp. Pharm.*, **26,** 168 (1969).

(5) S. El-Masry and S. A. H. Khalil, *J. Pharm. Sci.*, **62,** 1332 (1973).

(6) J. Levine and J. E. Roe, *J. Assoc. Offic. Agr. Chem.*, **42,** 693 (1959).

(7) J. A. Feldman and B. J. Robb, *J. Pharm. Sci.*, **59,** 1647 (1970).

(8) "Official Methods of Analysis of the Association of Official Agricultural Chemists," 10th ed., Association of Official Agricultural Chemists, Philadelphia, Pa., 1965, p. 540 (32.035–32.036).

(9) L. A. Roberts, *J. Pharm. Sci.*, **58,** 1015 (1969).

(10) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 40, 41.

(11) L. T. Grady and R. O. Zimmerer, *J. Pharm. Sci.*, **59,** 1324 (1970).

(12) B. B. Brodie and S. Udenfriend, *J. Biol. Chem.*, **158,** 705 (1945).

(13) G. Cronheim and P. A. Ware, *J. Pharmacol. Exp. Ther.*, **92,** 98 (1948).

(14) R. A. Lehman and T. Aitken, *J. Lab. Clin. Med.*, **28,** 787 (1943).

(15) N. R. Kuzel, *J. Pharm. Sci.*, **57,** 852 (1968).


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# Synthesis and Anticancer Activity of Novel Cyclic *N*-Hydroxyureas


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**Abstract** □ To overcome the disadvantages of hydroxyurea in anticancer therapy such as fast biotransformation and low potency, five cyclic *N*-hydroxyureas were synthesized. A new reaction was developed to prepare the desired products from the appropriate alkyl ω-haloalkylcarbamates with hydroxylamine. This reaction probably involves a two-step mechanism: nucleophilic substitution and intramolecular cyclization. The anticancer screening tests of these compounds were done both *in vitro* using tissue culture and *in vivo*. One compound, 1-hydroxy-1,3-diazacyclohexan-2-one, had anticancer activity comparable to hydroxyurea both *in vivo* and *in vitro*.

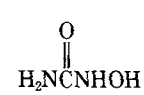


**Keyphrases** □ *N*-Hydroxyureas, cyclic—various derivatives synthesized, anticancer activity evaluated □ Anticancer activity—various cyclic *N*-hydroxyureas evaluated □ Structure-activity relationships—various cyclic *N*-hydroxyureas evaluated for anticancer activity


Hydroxyurea (I) was first synthesized in 1869 (1). Although hydroxyurea was reported not to be active against sarcoma 180 (2) and RC mouse mammary carcinoma (3) in mice, it demonstrated antitumor activities against cancers such as L-1210, Walker carcinoma, P-388 leukemia, and B16 melanoma in mice (4–6). Clinically, hydroxyurea is a simple organic compound currently used as a cancer chemotherapeutic agent in the treatment of chronic myeloid leukemia and in the management of malignant melanoma, head and neck cancers, and brain tumors concomitantly with X-ray therapy (7, 8).

Hydroxyurea immediately inhibits DNA synthesis (S phase) in various systems without any or with slight effect on the synthesis and metabolism of RNA and protein (9, 10). Hydroxyurea-induced inhibition of DNA synthesis is due primarily to interference with the biosynthetic reduction of ribonucleotides to deoxyribonucleotides by inhibition of the enzyme ribonucleotide reductase (11). This inhibition blocks the formation of deoxyribonucleotides required for *de novo* DNA synthesis.

Hydroxyurea has some advantages and disadvantages



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