Preformulation Studies I: Molindone Hydrochloride

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Abstract □ Preformulation studies on molindone hydrochloride, a dihydroindolone tranquilizer, are described. A TLC assay is outlined, degradation products are identified, and results of stability studies are presented. The dissociation constant is estimated using pH determinations.

Keyphrases □ Molindone hydrochloride—preformulation assay development, identification of degradation products, stability, and estimated dissociation constant □ Preformulation assay development, identification of degradation products, stability, and estimated dissociation constant—molindone hydrochloride □ Drug development, preformulation studies—assay development, identification of degradation products, stability, and estimated dissociation constant

This series of articles will present examples of preformulation studies that attain a proper balance between exhaustive quantitative experiments and those that are only semiquantitative. Studies of this type should consider the overall time-economic factors present in new drug development. This paper is concerned with assay development, identification of degradation products, stability studies, and an estimation of the dissociation constant for a dihydroindolone tranquilizer, molindone hydrochloride [3-ethyl-6,7-dihydro-2-methyl-5-(morpholinomethyl)indol-4(5*H*)-one hydrochloride].

EXPERIMENTAL

Selection of Assay—Molindone hydrochloride has UV absorption peaks at 255 and 299 nm., which can be used to determine quantitatively the pure drug substance. However, preliminary studies indicated that obvious chemical degradation (as evidenced by color and precipitate formation) was not accompanied by a concomitant decrease in UV absorption. This showed that degradation products absorb significant amounts of light at these wavelengths and necessitate a separation procedure prior to the determination of UV absorbance. TLC was selected as the separation procedure; the general technique described by Spencer and Beggs (1) was used, with some modifications.

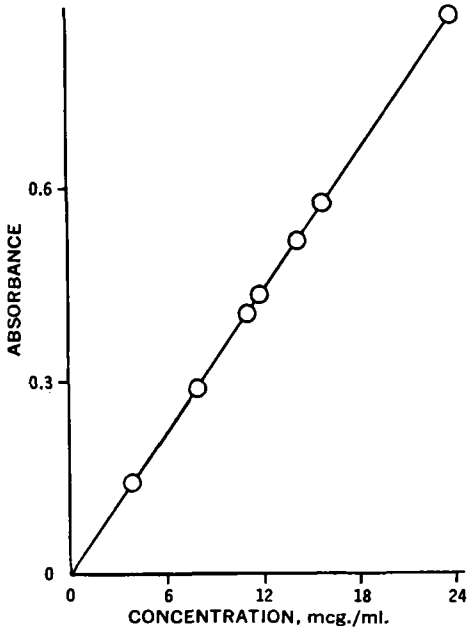


Figure 1—*Standard Beer's law plot for molindone hydrochloride at 255 nm.*

Development of TLC System—Precoated silica gel plates[1] were employed; the developing solvent system was butanol–methanol–1 *N* HCl (85:10:5). Drug solutions (either freshly prepared or degraded by heat[2] or light) were spotted with previously calibrated disposable pipets. The system was then developed for 10 cm. in a sealed glass jar lined with saturated filter paper. Inspection under shortwave UV light provided initial qualitative analysis.

Quantitative TLC Separation and UV Analysis—From 20 to 40 μl. of the solution to be assayed (representing 100–600 mcg. of drug) was applied to the plate and developed as already outlined. The plate was then dried and examined under shortwave UV light; the area containing the drug was removed from the plate with a razor blade and quantitatively transferred to a syringe fitted with a Millipore filter holder containing a 0.22-μ filter. The silica gel was washed in the syringe with four 5-ml. portions of 0.1 *N* HCl; the washings were then filtered directly into a 25-ml. volumetric flask. The solution was brought to volume with 0.1 *N* HCl (also passed through the filter) and read at 255 nm. against 0.1 *N* HCl. A 0.1 *N* HCl blank was subjected to the same treatment.

Preparation of Standard Curve—Because complete recovery of drug from silica gel is seldom accomplished, it was necessary to construct a standard curve. Solutions of molindone hydrochloride were freshly prepared in distilled water at concentrations of 0.5, 0.7, 0.9, and 1.0% and subjected to the described quantitative TLC separation and UV analysis. Each determination was done in quadruplicate.

Determination of Adsorption Isotherm—Results from the standard curve data confirmed that extraction of the drug from silica gel was not complete. Consequently, an adsorption isotherm for this system was constructed to determine whether the amount of drug lost was constant. The average amount of silica gel scraped off the plate (119.5 mg.) was added to standard solutions of molindone hydrochloride (100, 200, 300, 400, and 600 mcg./25 ml.). The flasks were then shaken for 5 min. and filtered through a 0.22-μ filter. The absorbance of each solution was determined at 255 nm. against a silica gel-treated blank. No effort was made to control temperature carefully; this procedure would correspond to the conditions present during the assay.

Identification of Degradation Products—The additional spots from the TLC treatment of degraded solutions were sprayed with test solutions (see *Results and Discussion*) to indicate the chemical nature of the degradation products. In addition, the brown precipitate found in degraded samples was collected and decolorized by dissolving in acetone–methanol (50:50) and treating with activated charcoal. After evaporating the filtered solution to dryness, the residue was recrystallized from methanol. Identification was made by comparison with a known compound and by elemental (C, H, N) analysis.

Stability Studies—The stability of molindone hydrochloride (in solution and in dosage forms) was investigated in the usual manner. Constant-temperature baths were used for studies on the drug substance, while ovens were used for dosage forms.

Determination of Dissociation Constant—The dissociation constant of molindone was estimated by the procedure described by Stern *et al.* (2). Molindone hydrochloride (0.001 or 0.0006 mole) was added to 450 ml. of recently boiled, distilled water. Two milliliters of 0.1 *N* NaOH was added, along with sufficient sodium chloride

[1] Brinkmann HF 254, 250 μ, with fluorescent indicator.
[2] All samples in this report, other than light-degraded ones, were protected from light.

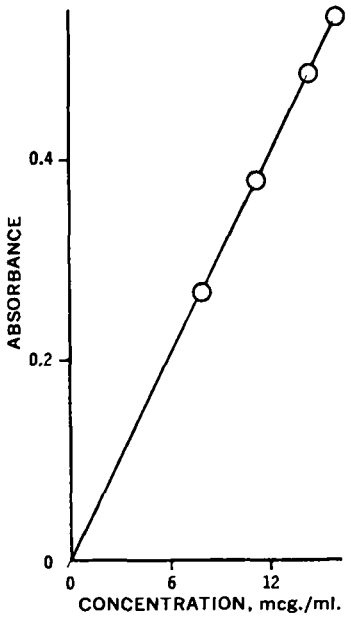


Figure 2—*Standard curve for TLC assay of molindone hydrochloride.*

to bring the ionic strength to 0.2; then the solution was made up to 500 ml. with recently boiled, distilled water. The pH of the solution at 25° was immediately measured using an expanded scale pH meter[3].

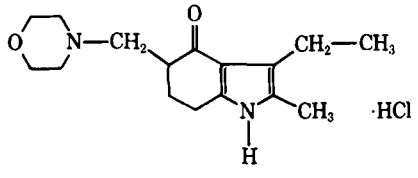
RESULTS AND DISCUSSION

TLC of Freshly Prepared Solutions—Two spots with R_f values of 0 and 0.5 are visible under shortwave UV light. The R_f 0.5 spot stains blue with iodoplatinate reagent (I, specific for heterocyclic nitrogen), stains blue with modified Ehrlich's reagent (II, specific for the indole ring), and exhibits a green fluorescence under longwave UV light after spraying with Prochazka reagent (III, specific for the indole ring); all of these results indicate the presence of molindone. The R_f 0 spot is very faint and does not respond to any of the spray treatments.

TLC of Heat-Degraded Samples—Molindone samples, whether heat degraded in the solid state or in aqueous solution, produce four spots visible under shortwave UV light. The R_f 0 spot does not stain with any of the three reagents. The R_f 0.24 spot (faint) yields a blue color with I and a faint blue color with II, and it produces a faint green fluorescence under longwave UV light in the presence of III. The R_f 0.5 spot produces a blue color with I and a strong blue color with II, and it reacts positively to III. The R_f 0.79 spot does not react with I but yields a faint blue reaction to II and III.

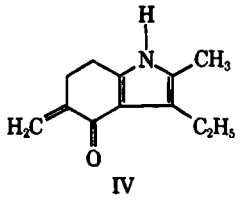
TLC of Light-Degraded Samples—Light-degraded samples of molindone, whether solid or aqueous solutions, show three spots with R_f values of 0, 0.24, and 0.5. These spots react in the same way as the corresponding spots of heat-degraded molindone; however, no R_f 0.79 spot is present in this case.

Quantitative Assay Results—A standard Beer's law plot appears in Fig. 1 (absorptivity with 1-cm. cell = 36.00 ml./mg.). The standard curve for the TLC assay is shown in Fig. 2 (slope = 34.00 ml./mg.). Based upon the slopes of the least-squares lines in Figs. 1 and 2, the average recovery in the standard curve data was 94.5%. To check the validity of the standard curve, nine determinations with known concentrations of drug were performed, reading the calculated concentrations off the standard curve. The average figure



molindone hydrochloride

[3] Corning.



IV

was 98.7% with a standard deviation of 0.23%, indicating that the assay procedure was valid.

Determination of Adsorption Isotherm—The adsorption isotherm for the silica gel–molindone hydrochloride system is shown in Fig. 3. The slope of the line is 33.50 ml./mg., which is in excellent agreement with the 34.00 value obtained for the standard curve. This indicates that a constant percentage of the amount of drug present is recovered from the silica gel; hence, it is valid to use the standard curve in assaying samples (initial and stability) of molindone hydrochloride. It should be mentioned at this point that no drug was adsorbed by the filters used in the assay procedure.

Identification of Degradation Products—Chemically, molindone hydrochloride is 3-ethyl-6,7-dihydro-2-methyl-5-(morpholinomethyl)indol-4(5*H*)-one hydrochloride. From the TLC studies, the R_f 0.5 spot was identified as molindone hydrochloride, staining with Reagent I (specific for heterocyclic nitrogen, *i.e.*, the morpholine substituent) and Reagents II and III (specific for the indole ring). The staining behavior of the R_f 0.79 spot, present in the heat-degraded samples but absent in the light-degraded material, indicated that the indole structure was present (positive II and III) but the morpholine substituent was not (negative I). This suggests an amine elimination reaction similar to that reported by Koshy and Mitchner (3), yielding morpholine and the following compound: 3-ethyl-6,7-dihydro-2-methyl-5-methyleneindol-4(5*H*)-one (IV), used in the synthesis of molindone.

Confirmation that the R_f 0.79 spot is, in fact, Compound IV was accomplished by comparing the recrystallized precipitate from heat-degraded samples with a known sample. The melting points coincided (known 217.5–219° *versus* unknown 218–219°), as did their UV and IR spectra. TLC treatment of the two yielded R_f values of 0.80 and 0.79, and the staining properties were identical with each other and with the aforementioned 0.79 spots. Finally, the elemental analysis of the precipitate yielded the following results.

Anal.—Calc.: C, 76.15; H, 7.99; N, 7.40. Found: C, 76.05; H, 8.12; N, 7.59.

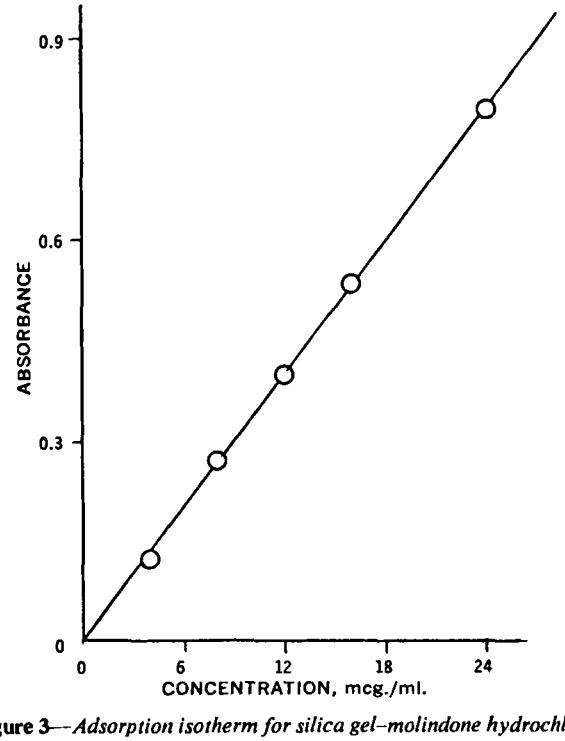


Figure 3—*Adsorption isotherm for silica gel–molindone hydrochloride at room temperature.*

Table I—Pseudo-First-Order Rate Constants and Half-Lives for Molindone Hydrochloride Degradation at Various Temperatures and pH's

pH	Temperature	k, day^{-1}	Half-Life, days
1.24	50°	0.0051	136
1.46	50°	0.0056	124
1.86	50°	0.0044	157
2.24	50°	0.0045	154
1.23	60°	0.0161	43
1.47	60°	0.0140	50
1.86	60°	0.0128	54
2.22	60°	0.0140	50

Trace amounts of this degradation product could be detected on the plates (R_f 0.79) by spraying with 0.5% potassium ferricyanide in a 10% aqueous solution of ferric chloride. While this reagent is not specific (it will react with most substances that are easily oxidized), it is very sensitive and stains the R_f 0.79 spot blue when the amount of the degradation product is too small to be detected by any of the aforementioned methods including UV light.

The R_f 0.24 spot probably represents oxidation products of molindone (4); the morpholine substituent probably hydrolyzes open and is not visible on the developed TLC plates. Because the work on the 0.79 spot indicated the nature of the primary degradation reaction, no definitive experiments were performed on the 0.24 spot.

Stability Studies—While time did not permit a complete investigation of molindone's stability to heat, light, oxygen, and pH, sufficient preformulation studies were conducted to point the way to successful formulation conditions. Fortunately, color development could be used as a semiquantitative end-point to determine the effect of light, oxygen, and pH on the drug in solution. Results from these studies indicated that molindone was susceptible to light degradation and oxidation; these conclusions were confirmed by the TLC studies. Brief semiquantitative experiments indicated that the usual protection from light (amber containers) and oxygen (inclusion of an antioxidant) was sufficient to prevent color formation; subsequent studies concentrated on the effect of pH, buffer species and concentration, and heat.

Because of various formulation restrictions, including solubility, taste (oral liquids), and tissue irritation (injectables), it was desirable to formulate solutions of the drug between pH 3 and 5. When using color formation as a rough indicator, it was found that a pH range of 2.8–3.5 was most desirable from a stability standpoint. With this preliminary information, the following quantitative experiments were performed.

To show the effect of high concentrations of hydrogen ion on stability, four different aqueous solutions (0.1%) of molindone hydrochloride (pH adjusted to 1.2–2.2 with hydrochloric acid and ionic strength held constant with sodium chloride) were stored at 50 and 60° and assayed periodically. The results (Table I) indicated that: (*a*) the reaction (first order) was not particularly sensitive to hydrogen ion, and (*b*) the drug was relatively stable, having a half-life of about 140 days at 50° in this pH range. From these and subsequent experiments, the heat of activation was estimated to be in the "normal" range of about 20 kcal./mole.

To show the effect of buffer species and concentration and to determine whether the pH 3 or 5 area was more desirable, three solutions (A, pH 5.0, 0.167 *M* citrate buffer; B, pH 5.0, 0.333 *M* citrate buffer; and C, pH 3.5, 0.333 *M* citrate buffer, all at constant ionic strength) were stored at 70° and assayed periodically. The results (Fig. 4) confirmed the first-order nature of the reaction and indicated that: (*a*) the drug was about four times more stable at pH 3.5 than at 5.0, and (*b*) the reaction was catalyzed by citrate. No additional spots appeared on the TLC plates, eliminating the possibility of a molindone–citrate reaction as might be suspected from the work of Higuchi and Miki (5). Similar results were obtained with bitartrate and phosphate buffers (still keeping ionic strength constant). Because of these results and because unbuffered solutions of molindone hydrochloride remain at a relatively constant pH with respect to time, no further attempts at buffering solutions of the drug were made.

As a result of these experiments, formulation of solution dosage forms (oral and parenteral) was relatively routine; the stability of the resulting formulations was satisfactory. Preformulation work also included testing the stability of the drug with various tableting excipients. As expected from the pH–stabilty studies in solution, basic materials (*e.g.*, dicalcium phosphate) proved to be unacceptable while acidic components (*e.g.*, calcium sulfate) were acceptable.

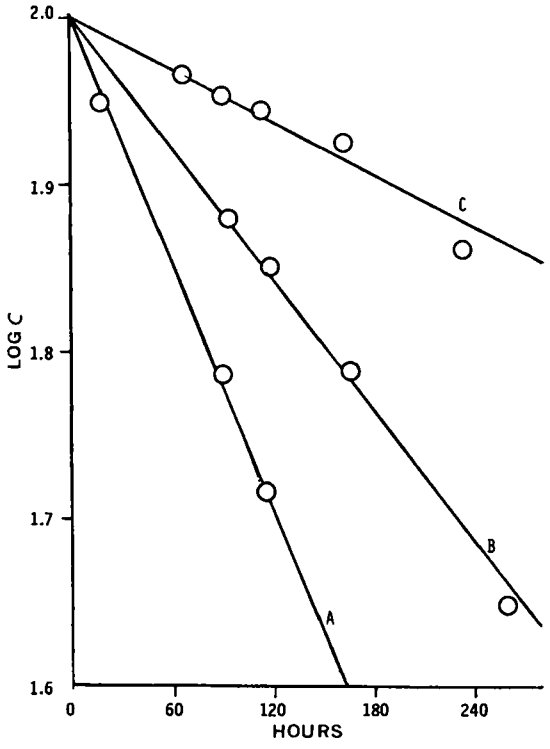


Figure 4—*First-order plot of molindone hydrochloride degradation at 70°. Key: A, pH 5.0, 0.167 M citrate buffer; B, pH 5.0, 0.333 M citrate buffer; and C, pH 3.5, 0.333 M citrate buffer.*

Determination of Dissociation Constant—The pKa of molindone was estimated to be 6.89 (using 0.001 mole of drug) and 6.76 (using 0.0006 mole of drug). As Stern *et al.* (2) pointed out, the values of the dissociation constant obtained with this method are "semi-classical," since the hydrogen ion is expressed in activity units and the other figures in concentration units. The usefulness of this method for estimating dissociation constants is shown by the fact that subsequent determination of pKa by potentiometric titration in water–dioxane mixtures (then extrapolating to zero dioxane concentration), according to the method of Van Uitert and Haas (6), yielded a value of 6.94.

CONCLUSION

The information presented provides a good example of preformulation studies that lead to meaningful semiquantitative and accurate quantitative results useful in product formulation.

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