Determination of a Benzothiazepin-2-one in Blood by GLC–Electron-Capture Detection

J. ARTHUR F. de SILVA▲, NANCY MUNNO, and ROBERT E. WEINFELD

Abstract □ A sensitive and specific blood level method was developed for the determination of 8-chloro-1-(3-dimethylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one (I) by GLC-electron-capture detection. It involves the selective extraction of the compound into ether from the blood or plasma made alkaline with sodium hydroxide, followed by reextraction into 0.1 *N* HCl. After washing the acid with ether, the aqueous solution is alkalinized and the compound is reextracted into ether. The ether is evaporated, and the residue is dissolved in a 1% solution of diethylamine in *n*-hexane which contains 5-chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone as a reference standard; this solution is analyzed by GLC. The overall recovery of I-hydrochloride added to blood or plasma is of the order of 85%, and the sensitivity limit is about 0.015 mcg. I/ml. blood or plasma using a 4-ml. sample per assay. The method was applied to the determination of plasma levels of the drug in man following single 100- and 200-mg. oral doses of the drug administered 7 days apart.

Keyphrases □ 8-Chloro-1-(3-dimethylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one—analysis in blood, GLC-electron-capture detection □ Benzothiazepin-2-ones—analysis of 8-chloro-1-(3-dimethylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one in blood, GLC-electron-capture detection □ GLC-electron-capture detection—analysis, 8-chloro-1-(3-dimethylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one in blood

The tranquilizing properties of the phenothiazines (1) has led to the synthesis of a variety of heterocyclic compounds containing the nitrogen and sulfur moieties in the heterocyclic ring. Many of these compounds have antidepressant activity (2). Thiazesim[1] (3) is a member of the benzothiazepine class of compounds (4) that has shown antidepressant activity in animals (5) and man (6).

8-Chloro-1-(3-dimethylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one hydrochloride (I-hydrochloride) (Table I), another member of this class of compounds, was synthesized by Wenner and Uskokovic (7) and is under clinical investigation as a psychostimulant (8, 9).

A sensitive and specific blood level method was developed for the quantitation of I by GLC–electron-capture detection with a linear range of detection of 10–250 ng. (Fig. 1). The method was applied to the determination of plasma levels of the intact drug (I) in man following administration of single 100- and 200-mg. oral doses.

EXPERIMENTAL

Reagents—All reagents were of analytical grade purity (>98%) and were used without further purification. All aqueous solutions were made up in double-distilled water. The following were used: 6 *N* NaOH, aqueous; 2 *N* NaOH, aqueous; 0.1 *N* HCl, aqueous; 1% diethylamine in spectrograde *n*-hexane[2], 1:99 (v/v), prepared as described under *Standard Solutions;* ether[3], analytical grade, anhydrous; sodium sulfate, anhydrous; and ethanol, absolute.

GLC Conditions—A gas chromatograph[4] with a cylindrical foil electron-capture detector (foil strength 225 mc. of titanium tritide) was used.

Column—A 1.22-m., 0.63-cm. (4-ft., 0.25-in.) o.d. and 0.45-cm. (0.18-in.) i.d., borosilicate glass column containing 5% silicone grease[5] on 60–80-mesh silanized Chromosorb W was conditioned for 72 hr. at 260° prior to use.

Carrier Gas—Nitrogen (oil pumped and dry) was used at a column head pressure of 100 psig. and a flow rate of 100–120 ml./min.

Temperature Settings—The injection port temperature was 250°, the detector temperature was 200°, and the column oven temperature was 230°.

Amplifier Range—The range used was 1.0 × attenuation 8.0 for a full-scale deflection for 100 ng. of I.

Detector—It was operated at plateau voltage (−) 70 v. d.c.

Recorder—A 1.0-mv. dual-pen recorder[6] was operated full scale at a chart speed of 101.6 cm. (40 in.)/hr.

Standard Solutions—Stock solutions of I and 5-chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone (III)[7] (the reference standard) have to be prepared, from which the working solutions containing varying amounts of I to a constant amount of III are prepared for making the GLC calibration curve.

Preparation of Stock Solution of I—Weigh out 11.20 mg. of I-hydrochloride, equivalent to 10.00 mg. of the free base, into a 15-ml. centrifuge tube. Dissolve in 1 ml. of water, add 1 ml. of 2 *N* sodium hydroxide to liberate the free base, and extract three times successively with 6 ml. of ether by shaking for 10 min. on a reciprocating shaker. Centrifuge and transfer the first ether extract quantitatively into a tared 10-ml. volumetric flask, evaporate to dryness, add the second and third ether extracts successively to the residue in the tared flask, and evaporate again to dryness. Vacuum dry the sample for 20-min. intervals until constant weight is achieved. Recovery of the free base should be better than 95%. Dissolve the residue in 1 ml. of ethanol and dilute with sufficient *n*-hexane to yield a stock solution containing 1 mg. (free base)/ml.

Preparation of Stock Solution of III (Reference Standard for GLC)—Weigh out 11.10 mg. of III-hydrochloride (Table I), equivalent to 10.00 mg. of free base (oily residue), and make a stock solution containing 1 mg./ml. exactly as described for I.

Preparation of External Standard Solutions of I (Free Base) Containing the Reference Standard (III) for GLC Calibration—Transfer 1 ml. of each of the above solutions into separate 10-ml. volumetric flasks and make up to volume with *n*-hexane. These solutions contain 100 mcg./ml. of I and III, respectively. Make standard solutions of I containing a constant amount of III in 10-ml. volumetric flasks, making to volume with a solution of 1% diethylamine in *n*-hexane such that each solution contains 2.0 mcg./ml. of the reference standard (III) and 2.0, 4.0, 6.0, 8.0, or 10 mcg./ml. of I. Add approximately 300 mg. of sodium sulfate (anhydrous) as a desiccant to each solution to maintain the anhydrous state of these solutions, which are used to make the analytical calibration curve.

A new calibration curve is prepared on each day of analysis from duplicate 10-μl. injections of each standard solution. The peaks due to I and III have retention times (R_t) of 4.2 and 7.5 min., respectively (Fig. 2), and the peak area (cm.²) of each component

[1] 5-[2-(Dimethylamino)ethyl]-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one hydrochloride.

[2] Fisher.

[3] Mallinckrodt; may be used for up to 7 days after the can is opened.

[4] Aerograph model 205-1-B.

[5] Dow Corning DC-11.

[6] Westronics.

[7] Synthesized by R. I. Fryer and J. Earley, Department of Chemical Research, Hoffmann-La Roche Inc., Nutley, N. J., 1968.

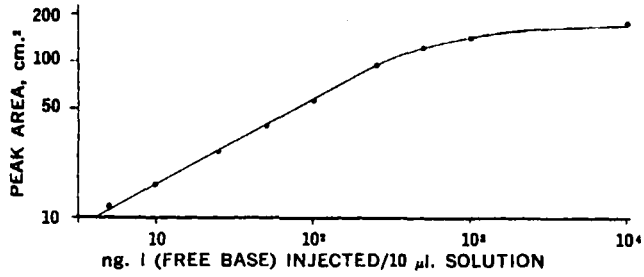


Figure 1—*Linear dynamic range of electron-capture detector response to I.*

is determined by the product of peak height (centimeters) times the width (centimeters) at half-height using the slope–baseline technique. A standard curve is prepared by plotting the peak area ratio of I:III *versus* nanograms of I (Fig. 3).

Preparation of Internal Standards for Determination of Percent Recovery from Blood—Weigh out 11.20 mg. of I-hydrochloride, which is equivalent to 10.00 mg. of free base, and dissolve in 10 ml. of absolute ethanol to give a stock solution containing 1 mcg./ml. Then dilute this solution to yield an internal standard solution containing 1 mcg./100 µl. of ethanol, suitable aliquots of which are added to blood or plasma as internal standards for determining the percent recovery.

Preparation of Stock Solution of 1% Diethylamine in n-Hexane Containing III for GLC Analysis of Biological Samples—Into a 100-ml. volumetric flask, transfer 1 ml. of diethylamine and 0.2 ml. of the 1.0-mg./ml. stock solution of III and make up to volume in spectrograde *n*-hexane. Add about 1 g. of anhydrous sodium sulfate and shake by inversion. This solution contains 200 ng. III/100 µl. and is used for dissolving the residue of the samples prior to GLC analysis.

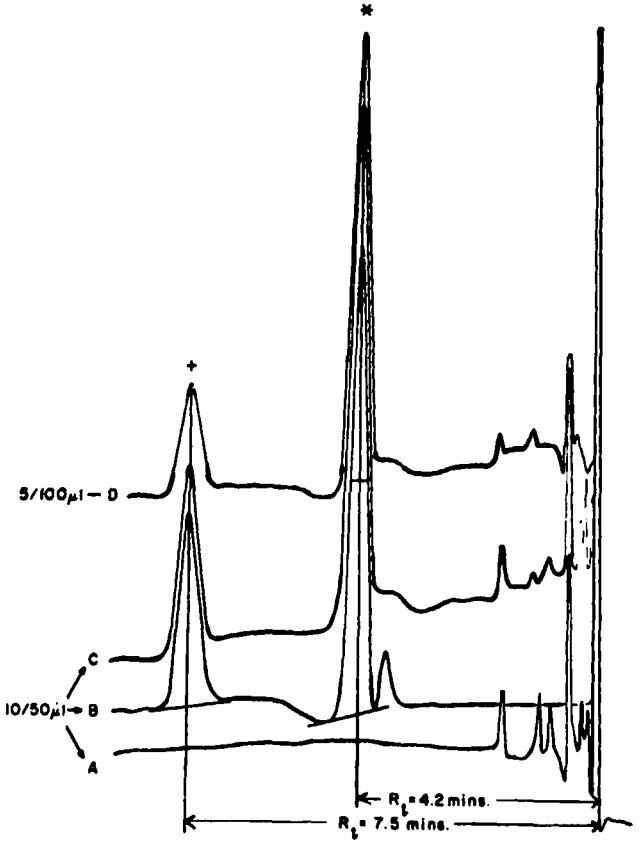


Figure 2—*Gas chromatograms of I extracted from blood. Key: *, I, analytical peak; +, III, reference standard; A, control plasma extract; B, authentic standards of I and III in 1% diethylamine–n-hexane; C, I, recovered from control plasma; and D, plasma extract from a patient dosed with I.*

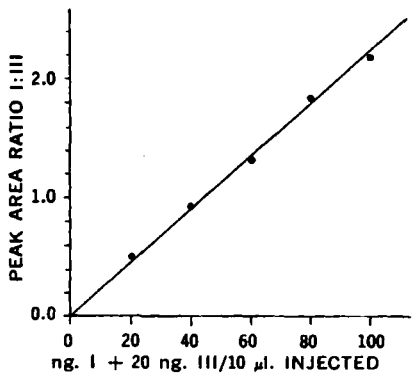


Figure 3—*Calibration curve of electron-capture detector response to I using the benzophenone (III) as a reference standard.*

Experimental Procedure for Extraction from Blood or Plasma—Into a 50-ml. centrifuge tube, add 4 ml. of blood or plasma, 4 ml. of 2 *N* NaOH, and 15 ml. of ether for the first extraction. Along with the samples, run a 4-ml. specimen of control blood or plasma (taken preferably from the patient prior to medication) and four separate 4-ml. specimens of control blood or plasma to which 200, 400, 600, and 800 ng. of I-hydrochloride (20-, 40-, 60-, and 80-µl. aliquots of the internal standard solution evaporated to dryness under nitrogen) are added as internal standards. Seal the stoppers with a drop of distilled water. Shake for 15 min. on a reciprocating shaker, centrifuge for 10 min. at 2000 r.p.m., and transfer the ether into another 50-ml. tube. Repeat the extraction with another 10-ml. portion of ether and combine the ether extracts. Back-extract the ether with 5 ml. of 0.1 *N* HCl, centrifuge, aspirate off the ether, and wash the hydrochloric acid twice with 10-ml. portions of ether; then centrifuge and remove the ether by aspiration as before. Alkalinize the hydrochloric acid to a bromthymol blue end-point with 0.1 ml. of 6 *N* NaOH. Extract with 2 × 10-ml. portions of ether by shaking for 10 min. Then centrifuge and combine the extracts sequentially in a 15-ml. centrifuge tube and evaporate to dryness. Transfer the sample residues to a vacuum desiccator and vacuum dry for 15–20 min. to remove all traces of moisture. Add 50 µl. of a 1% solution of diethylamine in *n*-hexane containing the reference standard (III) (reference standard stock solution) to each tube, and dissolve the residue by mixing for 60 sec. on a mixer[8] at high speed. Prepare two samples at a time for GLC to prevent solvent losses in the tubes. Inject a 10-µl. aliquot for GLC analysis. The peaks due to I and III in the unknowns are identified by their respective retention times of 4.2 and 7.5 min. compared to the retention times of the peaks of the authentic external standards (Fig. 2).

Calculations—The peak areas of I and III are determined as described. The peak area ratios of I:III are calculated, and the

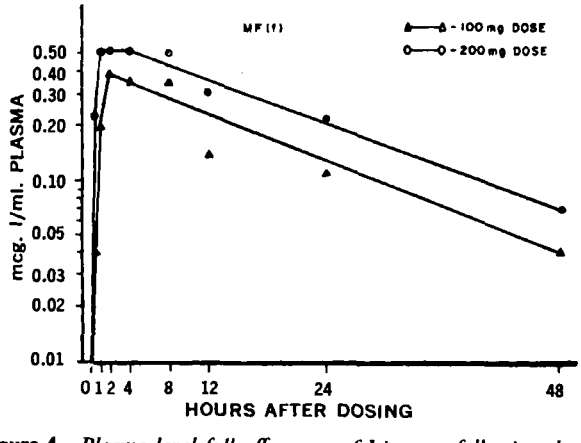
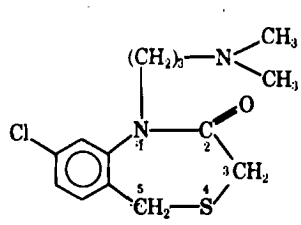


Figure 4—*Plasma level fall-off curves of I in man following the oral administration of 100- and 200-mg. doses given 7 days apart.*

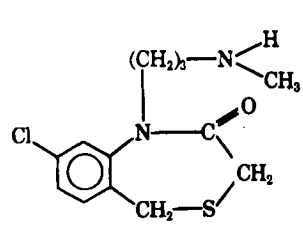
[8] Vortex Super Mixer (Lab-Line).

Table I—Chemical Names, Structural Formulas, and Physical Properties of the Compounds Mentioned

Compound	Chemical Name	Molecular Weight	Melting Point	Reference to Synthesis
I-hydrochloride	8-Chloro-1-(3-dimethylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one hydrochloride	335.31	201–202°	7
II-hydrochloride	8-Chloro-1-(3-methylaminopropyl)-3,5-dihydro-4,1-benzothiazepin-2(1*H*)-one hydrochloride	321.28	230–232°	7
III-hydrochloride	5-Chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone hydrochloride (reference standard for GLC analysis)	385.30	134–143°	



I



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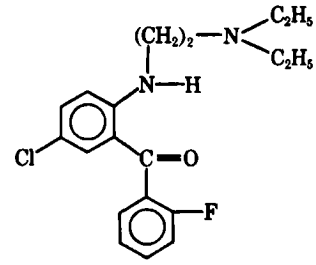

III

nanogram amounts of I in the unknowns are interpolated from the standard curve of the recovered internal standards or by direct comparison to any given internal standard using the formula:

$$\frac{\text{[peak area ratio] unknown}}{\text{[peak area ratio]internal standard}} \times \frac{\text{mcg. internal standard}}{\text{ml. of sample assayed}} = \text{mcg. I/ml. blood or plasma} \quad \text{(Eq. 1)}$$

The percent recovery of internal standards of I-hydrochloride added to blood or plasma is determined as follows:

$$\frac{\text{ng. I (recovered)}}{\text{ng. I-hydrochloride (added)}} \times \text{aliquot factor} \times 1.122 \times 100 = \%\ \text{recovery of I} \quad \text{(Eq. 2)}$$

where aliquot factor = aliquot of sample injected for GLC (10/50 μl.), and 1.122 = conversion factor for molar weight. The ratio of:

$$\frac{\text{I-hydrochloride}}{\text{I free base}} = \frac{335.31}{298.81} \quad \text{(Eq. 3)}$$

The percent recovery of internal standards should be determined routinely as a check on analytical precision and reproducibility.

DISCUSSION

During the investigation of the intrinsic analytical properties of I, it was noted that the compound had no useful absorbance in either the visible or UV region and, consequently, had no useful intrinsic fluorescence in basic, neutral, or acidic media. However, the free base of the compound was amenable to GLC analysis employing the sensitivity and selectivity of the electron-capture detector response to the halogen, sulfur, and carbonyl groups in the molecule for its detection. The limit of detection of the pure compound was 10 ng. when chromatographed on a 1.23-m. (4-ft.) column of 5% silicone grease[5] at 230°, giving a potential sensitivity limit of 0.015 mcg./ml. of blood or plasma. However, greater sensitivity may be obtained by using the phenyl silicone liquid phases such as OV-1 and OV-17 and the ^{63}Ni (15 mc.) electron-capture detector, especially in the pulsed d.c. operational mode.

The selective extraction of I into ether from the blood or plasma made alkaline with sodium hydroxide was based on the basic nature of the compound (pKa 8.7). Since the compound readily forms acid salts, the free base in ether was back-extracted into 0.1 *N* HCl, which was then subjected to suitable cleanup. The hydrochloric acid solution containing I is made strongly alkaline, extracted into ether, and concentrated by evaporation.

It was noted that I in the final ether residue was not quantitatively dissolved in *n*-hexane, apparently due to strong adsorption on the glass. However, it was determined by GLC that I was solubilized and quantitatively desorbed off the glass with a solution of 1% diethylamine in *n*-hexane. Since diethylamine is very hygroscopic anhydrous sodium sulfate was used to remove traces of moisture in the sample residues which would otherwise severely quench the response of the ^{3}H-electron-capture detector, giving nonlinear and irreproducible results. The reference standard (III) serves as an index of the performance of the electron-capture detector during the assay and monitors the stability of the GLC system.

The electron-capture detector response characteristics of III, its sensitivity limits, and its linear range of detection were compatible with those of I. Its retention time of 7.5 min. ensures complete resolution from I and from extracted impurities, making it a suitable reference standard for GLC analysis.

Application of Method to Biological Specimens—Plasma levels were determined in a female patient (M.F.; age 59 and 68.4 kg. in weight) following single oral doses of 100 and 200 mg. administered 7 days apart. Blood specimens were collected prior to medication (control) and at 0.5, 1, 2, 4, 8, 12, 24, and 48 hr. after dosing; the plasma was separated immediately. Semilogarithmic plots of the plasma levels are shown in Fig. 4 and indicate a slow and prolonged absorption of the drug, with a maximum level being reached 1–2 hr. after administration. These levels were maintained for up to 8 hr. following both the 100- and 200-mg. doses, after which time a progressive decline in the plasma levels was seen up to 48 hr. The plasma level half-life determined by the method of least squares was approximately 15 hr. for both the 100- and 200-mg. doses.

The sensitivity limit of the method was established at 0.015 mcg. I/ml. plasma using a 4-ml. sample per assay in additional studies where blood levels were determined in man following the oral administration of 20 mg. of I-hydrochloride/day in 5-mg. doses (q.i.d.).

Specificity of Assay—Following GLC analysis, the remainder of the 1% diethylamine in *n*-hexane solutions were pooled and analyzed by one-dimensional TLC in acetone–ammonium hydroxide (100:2). The chromatoplates showed the presence of a single spot corresponding to that of authentic I (R_f 0.90). The *N*-monodesmethyl analog (II, R_f 0.17) (Table I), a metabolite seen in dog blood, is absent in human blood extracts after single oral doses. It may, however, appear after chronic administration of the drug. On GLC analysis, Compound II had a retention time of 5.4 min. and was resolved from I whose retention time was 4.2 min. The GLC assay in blood is apparently specific for the intact drug.

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Determination of Coating Thickness of Microcapsules and Influence upon Diffusion

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Abstract □ Experiments in this study appear to show that the diffusion rate of the encapsulated drug is a function of microcapsule size. The object of this paper is to report the influence of the coating upon diffusion and the determination of the thickness of the coating. An equation was established, which was verified by microscopic measurement of microspheres previously sliced with a microtome.

Keyphrases □ Microcapsules—determination of coating thickness, effect on diffusion rate □ Coating thickness, microcapsules—determination, effect on diffusion rate □ Diffusion rate, encapsulated drugs—influence of nature and thickness of coating

The microencapsulation process is comparatively new (1–7). It consists of coating crystals or microdroplets of liquid with a polymer film. It has been applied successfully to a number of products, from gasoline to tetracycline. Its pharmaceutical use enables one to mix substances incompatible with each other, to mask an unpalatable taste, or to induce a prolonged action. Of course, the nature and thickness of the coating determine the diffusion rate.

THEORETICAL

Influence of Coating upon Diffusion—Studying the dissolution in water of cylinders of benzoic acid and lead chloride, Noyes and Whitney (8) gave the following dissolution equation:

$$\frac{dc}{dt} = K(C_\infty - c) \qquad \text{(Eq. 1)}$$

where K is a constant, C_∞ is the solubility of the substance, and c is the concentration at the expiration of the time t.

According to Brunner (9):

$$K = \frac{DS}{V\delta} \qquad \text{(Eq. 2)}$$

where K is the same constant as in the Noyes–Whitney equation, D is the solute molecule diffusion coefficient, V is the volume of solution, δ is the effective diffusion layer thickness, and S is the surface of the solid–solution interface.

The equation:

$$\frac{dc}{dt} = \frac{DS}{V\delta}(C_\infty - c) \qquad \text{(Eq. 3)}$$

allowed the computation of δ.

As reported by Wurster and Taylor (10): "the idealized film layer is not well defined, but it allows the correlation of experimental data with the physical properties of both the solute and solvent."

Dissolution of Microencapsulated Solute—In this experiment, the material to be encapsulated was viscous and sticky; however, the dry microcapsules did not aggregate. Thus one may disregard the possibility of a spontaneous diffusion of this material through the coating wall, and the phenomenon may be considered as follows: when the microcapsules were suspended in a liquid, the penetration of the solvent into the microspheres occurred first, followed by the dissolution of the encapsulated solute and the diffusion of the solution. The phenomena taking place during this exchange were dependent on osmotic pressure and diffusion.

In the event of the encapsulated substance being only slightly soluble in the liquid, one may disregard the effect of osmotic pressure and consider only diffusion. Thus, Eq. 3 may be expressed as:

$$\left[\frac{dc}{dt}\right]_{\text{coating}} = \frac{DS'\epsilon}{Vh}(C_\infty - c) \qquad \text{(Eq. 4)}$$

where S' is the external surface of a microcapsule, ϵ is a coefficient expressing the porosity and tortuosity of the coating, and h is the thickness of the coating. Other symbols are the same as already defined.

If it is assumed that microcapsules with the same radius have the same coating thickness, and that this thickness as well as that of Brunner's (9) layer are small and negligible with respect to the radius of microcapsules, one may write:

$$S = S' = n4\pi\bar{r}^2 \qquad \text{(Eq. 5)}$$

where $\bar{r}$ is the mean radius of the microcapsules and n is the number of microcapsules. The volume V_1 of the microcapsules is expressed as:

$$V_1 = \frac{4}{3}n\pi\bar{r}^3 = \frac{m}{d} \qquad \text{(Eq. 6)}$$