

# Determination of 7-Bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one (Ro 5-3350) in Blood by Gas-Liquid Chromatography

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A method for the determination of Ro 5-3350 [7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one] in whole blood of humans is described involving selective extraction into ether, acid hydrolysis, and analysis by gas-liquid chromatography. The method has good sensitivity (0.07 to 0.10 mcg./ml., blood), is specific for Ro 5-3350 after it is hydrolyzed to 2-amino-5-bromo-benzoylpyridine, and has an acceptable recovery of the order of  $61.0 \pm 3.0$  per cent. The method has been successfully applied to the determination of Ro 5-3350 in samples obtained from a blood level fall-off study.

Ro 5-3350 [7-BROMO-1,3-DIHYDRO-5-(2-PYRIDYL)-2H-1,4-BENZODIAZEPIN-2-ONE] (1) is a new psychotherapeutic drug of the 1,4-benzodiazepine class of compounds (2, 3). It is undergoing clinical evaluation as a psychotropic drug in the control of ambulatory schizophrenics.

The compound is chemically related to chlordiazepoxide<sup>1</sup> and diazepam.<sup>2</sup> Ro 5-3350 undergoes strong acid hydrolysis to an aromatic primary amine which can be measured by a Bratton-Marshall procedure as is chlordiazepoxide (4) (Scheme I).

Studies of Schwartz and Baukema (5) with <sup>14</sup>C-labeled Ro 5-3350 (labeled in the benzodiazepine ring at  $\text{C}=\text{O}$ , Scheme I) conducted in dogs revealed that the maximum concentration of the compound in blood after a 3.5-mg./Kg. oral dose totaling 36 mg. was 0.90 mcg./ml. 4 hr. after administration of the drug. This indicated the need for an assay capable of detection in the submicrogram range and suggested the use of gas-liquid chromatography (GLC).

Preliminary work showed that intact Ro 5-3350 could not be determined by GLC because of thermal decomposition at the high flash temperatures required for volatilization. However, strong acid hydrolysis of the compound to 2-amino-5-bromo-benzoylpyridine (ABBP), Scheme I, reaction 3, gave a good response to electron-capture detection when chromatographed on a 2-ft. column of 2% Carbowax 20M-TPA (6). The method finally adopted

utilized the electron-capture detector response to ABBP for the determination of the parent compound. Ro 5-3350 in blood or plasma was extracted at pH 9.0 into diethyl ether and then re-extracted from ether into 6 N H<sub>2</sub>SO<sub>4</sub>. The Ro 5-3350 in this acid medium was hydrolyzed to 2-amino-5-bromo-benzoylpyridine (ABBP) and glycine, after which the acid phase was neutralized, and the ABBP was extracted into diethyl ether. The residue after the evaporation of ether was taken up in a known volume of *n*-hexane, an aliquot of which was analyzed by GLC. The area of the ABBP peak was used to determine the concentration of Ro 5-3350 in the sample.

## EXPERIMENTAL

**Operational Parameters.**—Instrument: Jarrell-Ash chromatograph, Universal model 28-700 with a 100 mc. titanium tritide electron-capture detector (No. 28-750). Column: a 2-ft. column of 2% Carbowax 20M-TPA on silanized Gas Chrom P 100-120 mesh contained in stainless steel 1/4-in. tubing. Carrier gas: nitrogen passed through a molecular sieve before entering the column and adjusted to a flow rate of 250-300 ml./min. Column head pressure: 25-27 psig on a second stage of gas regulator. Conditions of column head pressure and flow rate may be varied so as to obtain a retention time for ABBP between 6 and 7 min. for effective separation from adjacent peaks. Temperature of oven:  $220 \pm 2^\circ$  (isothermal). Temperature of injection port:  $250 \pm 2^\circ$ . Temperature of detector:  $210 \pm 2^\circ$ . Amplifier range:  $1.0 \times 10^{-9}$  amp. full-scale deflection. Optimum detector voltage for ABBP: 20-30 v. d.c. Output on recorder: 1.0 mv. (Brown-Honeywell.) Chart speed: 1.25 cm./min. = 30 in./hr. Time constant: 1 sec. Minimum detectable amount of ABBP =  $5.0 \times 10^{-9}$  Gm. (5.0 ng.). Retention time (*R*<sub>t</sub>) of ABBP = 6-7 min.

**Preparation of Column Substrate.**—The inert support Gas Chrom P 100/120 mesh (Applied Science Labs, State College, Pa.) was silanized, and coated with Carbowax 20M-TPA (Wilkins Instrument and Research, Inc.) to give a 2% loading on a weight-weight basis (7).

**Packing and Conditioning the Chromatographic Column.**—A 2-ft. piece of stainless steel tubing was

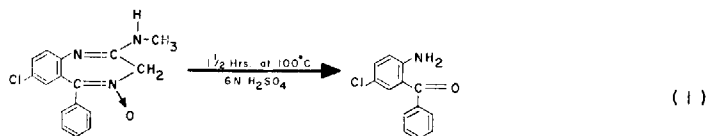
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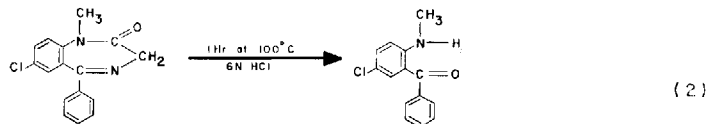
<sup>1</sup> Marketed as Librium by Hoffmann-La Roche, Inc., Nutley, N. J.

<sup>2</sup> Marketed as Valium by Hoffmann-La Roche, Inc., Nutley, N. J.



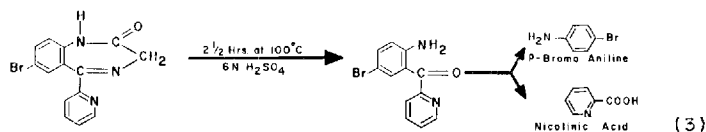
CHLORDIAZEPOXIDE

2-AMINO-5-CHLORO-BENZOPHENONE (ACB)



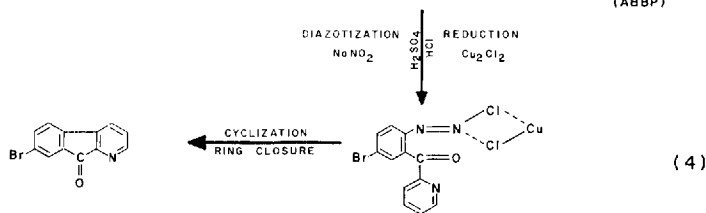
DIAZEPAM

2-METHYL-AMINO-5-CHLORO-BENZOPHENONE (MACB)



Ro 5-3350

2-AMINO-5-BROMO-BENZOYLPIRIDINE (ABBP)



"FLUORENONE" DERIVATIVE

Scheme I  
Chemical Reactions of  
Chloridiazepoxide, Diazepam,  
and Ro 5-3350.

packed with the prepared substrate and conditioned for 24–48 hr. at 240° (7). A properly conditioned and sensitized column had a useful life-span of about 6 months of continuous use.

#### Determination of Standard Curve of ABBP

2-Amino-5-bromo-benzoylpyridine synthesized by Fryer *et al.* (1) of >98% purity was dissolved in hexane to yield a stock standard solution of 1 mg./ml. From this solution suitable dilutions were made with *n*-hexane to yield final standard solutions covering the range of 5 ng./10  $\mu$ l. to 30 ng./10  $\mu$ l. Three 10- $\mu$ l. aliquots of each of the final solutions were injected and from their average peak areas a standard curve of peak area (cm.<sup>2</sup>) versus nanograms of ABBP was drawn as shown in Fig. 1. A standard curve should be determined for each day of analysis because column performance and detector response to ABBP changes with time.

#### Procedure

**Reagents.**—All reagents must be of reagent grade purity (>98%) and all inorganic reagents were made up in triple-distilled water. 6 *N* H<sub>2</sub>SO<sub>4</sub> in triple-distilled water. 6 *N* NaOH in triple-distilled water.

**1 *M* H<sub>3</sub>BO<sub>3</sub>-Na<sub>2</sub>CO<sub>3</sub>-KCl Buffer.**—Dissolve 62.8 Gm. of boric acid (H<sub>3</sub>BO<sub>3</sub>) and 74.6 Gm. of KCl/L. of triple-distilled water. Dissolve 106.0 Gm. Na<sub>2</sub>CO<sub>3</sub>/L. of triple-distilled water. To 630 ml. of the boric acid-KCl solution add 370 ml. of the Na<sub>2</sub>CO<sub>3</sub> solution to make a liter of buffer solution.

Shake well and check pH, buffer it up to pH 9.0 if necessary with the Na<sub>2</sub>CO<sub>3</sub> solution. This solution is 1 *M* with respect to H<sub>3</sub>BO<sub>3</sub>-Na<sub>2</sub>CO<sub>3</sub>-KCl. The solution should be stored at about 35–37° to prevent crystallization of the salts out of the solution.

**Diethyl Ether.**—Analytical reagent grade ether (absolute) containing not more than 0.0005% residue after evaporation and peroxide content not more than 0.00005% (Mallinckrodt) must be used from a freshly opened can on the day of use or up to 3 days after opening.

***n*-Hexane.**—Reagent grade "spectranalyzed" hexane (Fisher) containing not more than 0.0003% residue after evaporation must be used.

Into a 40-ml. glass-stoppered centrifuge tube, add 1 ml. of sample of blood containing Ro 5-3350, 2 ml. of triple-distilled H<sub>2</sub>O, and 5 ml. of pH 9.0 1 *M* borate buffer, and mix well by tapping.

Add 15 ml. of ether, seal stopper with triple-distilled water, and shake on a reciprocating shaker for 10 min. Centrifuge at 0–5° (refrigerated centrifuge) for 5 min. at 2100 r.p.m. to separate the layers and transfer ether phase into another 40-ml. glass-stoppered tube.

Re-extract the blood with another 10 ml. of ether and combine the ether extracts.

Add 5 ml. of 6 *N* H<sub>2</sub>SO<sub>4</sub> to the combined ether extract, shake for 10 min., and centrifuge for 5 min. to effect a quantitative separation of the ether and acid phases. Remove the ether layer by aspiration with a capillary pipet.

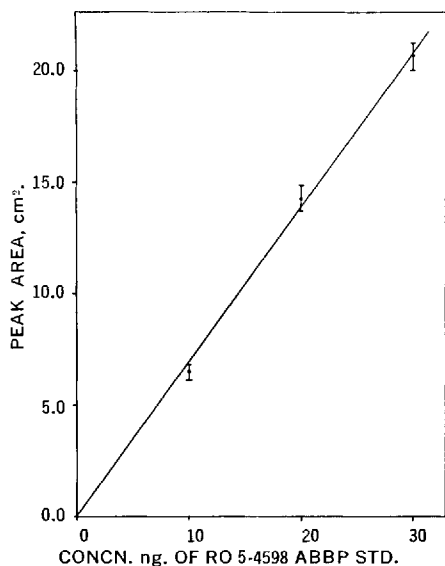


Fig. 1.—Linear response of ABBP standards on a 2-ft. column of 2% Carbowax 20M-TPA on silanized Gas Chrom P.

Extract the acid phase once by shaking with 10 ml. of ether for 10 min., followed by a second extraction with 10 ml. of ether for 5 min. After each extraction centrifuge for 5 min. to separate the phases and remove the respective ether layers by aspiration with a capillary pipet.

Place the tube containing the ether-washed  $H_2SO_4$  extract (unstoppered) in a hot water bath ( $80-90^\circ$ ) for 10 min. to drive off all the remaining ether. Then stopper the tube (seal with triple-distilled water) and place it in a boiling water bath for 2.5 hr. to hydrolyze the Ro 5-3350 to ABBP.

After hydrolysis, cool the sample in ice, and add 1 drop of bromthymol blue indicator (0.1% in 50% alcohol). Neutralize the cooled  $H_2SO_4$  extract in ice with 6 *N* NaOH to a blue color.

Add 10 ml. of ether to the neutralized sample, seal the stopper with triple-distilled water, and shake for 10 min. on a reciprocating shaker. Centrifuge for 5 min. to separate the phases and transfer the ether layer into a 15-ml. glass-stoppered conical centrifuge tube, and evaporate the ether down to a volume of about 1 ml.

Re-extract the sample with another 10 ml. of ether and combine the ether extracts in the 15-ml. centrifuge tube.

Centrifuge the combined ether extracts for 5 min. and carefully remove any aqueous layer present using a hypodermic syringe fitted with a cannula (BD-20, 15 cm. in length).

Evaporate the combined ether extracts to dryness in a hot water bath and dry the residue in a vacuum desiccator for 30 min. The samples must not be maintained under vacuum for more than 1 hr. or loss of ABBP is incurred.

Dissolve the residue in 100  $\mu$ l. of *n*-hexane and ensure uniform distribution and solution of the material by tapping the tube for 60 sec.

A suitable-sized aliquot, 1–10  $\mu$ l., of the final 100  $\mu$ l. of *n*-hexane extract of the sample residue is chro-

matographed and the ABBP peak is identified by its retention time.

The area of this peak is determined and the nanogram amount of ABBP present is obtained from a standard curve. The nanogram amount of Ro 5-3350/ml. of blood or plasma is determined from the total nanograms of ABBP  $\times$  1.142 [conversion factor, based on the molecular weights of Ro 5-3350 (316.16) and ABBP (276.9), respectively]. The amount of compound present in the samples should be corrected for the per cent recovery of internal standards run concurrently.

## RESULTS AND DISCUSSION

**Preparation of the Sample for GLC.**—Two problems were encountered in this phase of the study. The first one was to effect a quantitative extraction of Ro 5-3350 from blood and back into 6 *N*  $H_2SO_4$ , the second was the complete hydrolysis to ABBP and the extraction of this compound into ether. Because of the basic nature of the compound it was found that Ro 5-3350 was quantitatively extracted (97–99%) into diethyl ether from blood buffered to pH 9.0 and back into 2 *N* HCl as determined by its U.V. absorbance values in 2 *N* HCl,  $A_{240}^{max} = 0.057/\text{mcg}$ . The recovery from blood into ether was also determined to be 98–99% of added Ro 5-3350 using  $^{14}\text{C}$ -labeled material determined by radioisotope scintillometry (Table I). When the ether phase was extracted with 6 *N*  $H_2SO_4$ , all the radioactivity (dpm) was quantitatively removed into the acid.

The next step, conversion of Ro 5-3350 to ABBP by acid hydrolysis in 6 *N*  $H_2SO_4$  and the extraction of ABBP into ether was studied, and was followed by thin-layer chromatography (TLC) on Silica

TABLE I.—RECOVERY OF  $^{14}\text{C}$ -RO 5-3350 ADDED TO 1 ml. OF BLOOD

Concn., mcg.	Determined by Radioisotope Scintillometry		Recovery, %
	Activity Added, dpm	Activity Recovered, dpm	
50	18,200	18,042	99.1
		18,307	100.6
		18,371	101.0
100	36,000	36,204	100.5
		35,211	97.8
		34,218	95.1
		35,679	99.1
		Over-all av.	99.0 $\pm$ 2.1

TABLE II.—RECOVERY OF RO 5-3350 AFTER HYDROLYSIS TO ABBP IN 6 *N*  $H_2SO_4$

Ro 5-3350 Added, ng.	Determined by Gas Liquid Chromatography	
	Total Ro 5-3350 <sup>a</sup> Recovered, ng.	% Hydrolysis
300	224	75.0
	226	75.0
	391	78.0
	389	77.0
	393	79.0
500	407	82.0
	365	73.0
	407	82.0
	Over-all av.	77.6 $\pm$ 3.3

<sup>a</sup> Total ng. ABBP  $\times$  1.142 = Ro 5-3350 equivalent.

Gel G (Stahl) with a fluorescent indicator in two solvent systems: *n*-heptane-chloroform-ethanol (10:10:1) and in ethyl acetate. By running separate standards of Ro 5-3350 and ABBP alongside the hydrolysate extract and by viewing the developed plate under shortwave U.V. light, the position of each compound was made visible so that the segments of Silica Gel G containing them could be scraped off the plate and either counted by radioisotope scintillometry or analyzed by GLC. Using  $^{14}\text{C}$ -labeled Ro 5-3350 extracted from blood into ether and back in 6 *N*  $\text{H}_2\text{SO}_4$ , and also added directly to 6 *N*  $\text{H}_2\text{SO}_4$  and hydrolyzed for 2.5 hr. at  $100^\circ$ , no significant radioactivity was measurable in the Ro 5-3350 area on TLC. This indicated that the compound was quantitatively converted. The area on the TLC plate having the same  $R_f$  as the ABBP standard showed an intense yellow band which was scraped off, extracted into ether, and analyzed by GLC. This compound had the same retention time as the ABBP standards and was quantized from a standard curve of ABBP.

Studies on the kinetics of the hydrolysis of Ro 5-3350 to ABBP showed that 6 *N*  $\text{H}_2\text{SO}_4$  produced the more reproducible and optimal yield of the compound when compared with 6 *N* HCl. The average recovery of Ro 5-3350 after hydrolysis for 2.5 hr. at  $100^\circ$  (boiling water bath) was determined by GLC and was found to be of the order of  $77\% \pm 3\%$  of initially added compound (Table II). This yield could not be improved, and it is suspected that a loss of ABBP under the hydrolysis conditions to *p*-bromoaniline and nicotinic acids (Scheme I, reaction 3) occurs with the resultant loss in yields of ABBP. The conversion of Ro 5-3350 to ABBP is quantitative as there was no measurable amount of residual Ro 5-3350- $^{14}\text{C}$  left after hydrolysis, as determined by TLC and radioisotope scintillometry. The amount of ABBP recovered after hydrolysis was determined by GLC. The possible interference of *p*-bromoaniline with the GLC determination of ABBP was investigated. The compound does not interfere with the quantization of ABBP. The use of 6 *N*  $\text{H}_2\text{SO}_4$  over 6 *N* HCl also gave cleaner chromatograms for control blood after a 2.5-hr. hydrolysis (Fig. 2, curve A).

During the investigation of suitable clean-up methods for the purpose of eliminating interfering peaks, it was found that centrifuging at each extraction gave clean separation of ether and aqueous phases resulting in a minimal contamination of the ether extracts with water-soluble material. Washing the  $\text{H}_2\text{SO}_4$  with ether, centrifuging the final ether extract, and careful removal of any droplets of water and alkali carried over in the transfer operation very effectively cleaned up the extract and gave a very clean chromatogram in the area of the ABBP peak.

#### Parameters for Gas Chromatography

The development of GLC parameters for the determination of ABBP involved the investigation of several phases of which Carbowax 20M (CBW 20M) and ethylene glycol adipate (EGA) proved to be the most satisfactory. Using 2-ft. columns of 2% CBW 20M or 2% EGA on silanized Gas Chrom P 100/120 mesh, the minimum detectable amounts of ABBP were in the order of 50 to 100 ng. by electron capture. This level was not sensitive

enough since the blood levels from the dog experiments (5) indicated the need for an assay in the 10–20 ng. order of sensitivity for ABBP.

The compound ABBP has an aromatic amine group and the pyridyl-*N* moiety, both of which are electrophilic groups, and thus would tend to reduce the electronegativity of the Br and carbonyl groups. This would tend to reduce the response of ABBP to electron-capture detection (ECD) by GLC. The formation of a derivative (8) which would reduce or eliminate such effects was attempted (Scheme I, reaction 4) but was unsuccessful in the submicrogram range. A few grams of the "fluorenone" derivative was synthesized (9) for use as an analytical standard. It showed excellent response to ECD with a minimum detectable range of 5–10 ng.

The use of lightly loaded columns of 2-ft. 0.20–0.25% Carbowax 20M on micro glass beads 100–120 mesh gave better results on GLC and enabled the determination of 10–20 ng. of ABBP as minimum detectable amounts. The major disadvantage with this preparation was a marked tailing of the ABBP peak and short column life of 4–6 weeks after which the entire column had to be discarded. Finally the use of 2% Carbowax 20M-TPA as the

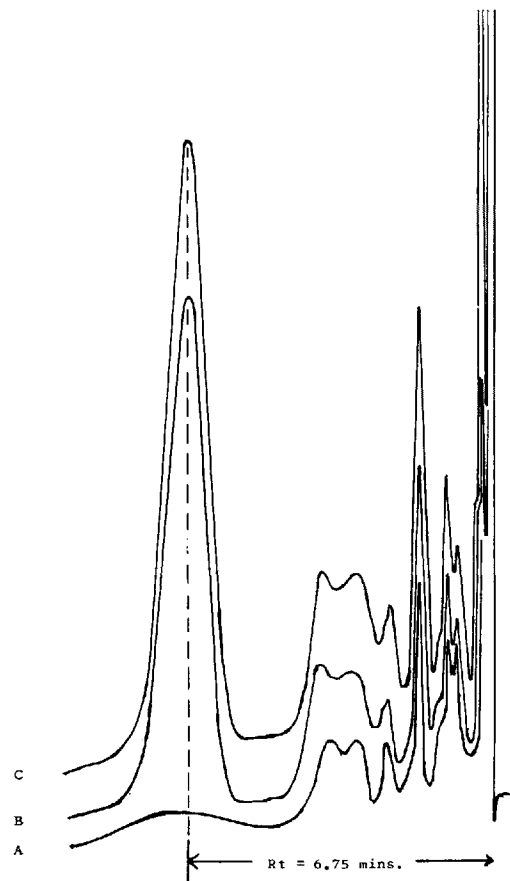


Fig. 2.—Chromatogram of control blood (A) and control blood containing 400 ng. (B) and 500 ng. (C) of added Ro 5-3350 recovered as the ABBP compound (10/100  $\mu\text{l}$ ).

TABLE III.—RECOVERY OF Ro 5-3350 ADDED TO 1 ml. OF BLOOD DETERMINED BY GLC

Ro 5-3350 Added, ng.	Total Ro 5-3350 <sup>a</sup> Recovered, ng.	% Recovered
200	116	58.0
	116	58.0
	183	61.0
	185	62.0
	179	59.5
300	174	58.0
	201	67.0
	199	66.0
	249	62.0
	219	55.0
400	242	57.0
	307	61.0
	299	60.0
	297	59.0
500	313	63.0
	290	58.0
	295	59.0
	301	60.0
	320	64.0
	322	64.0
	315	63.0
	Over-all av. 61.0 ± 3.1	

<sup>a</sup> Total ng. ABPP × 1.142 = Ro 5-3350 equivalent.

liquid phase gave the best results, with well-resolved symmetrical peaks, and a minimum detectable limit of 5–10 ng. of ABPP. It is believed that the terminal TPA moiety of this polyester phase inactivates adsorbent sites still remaining exposed after silanizing and produces a very thin uniform coating which enhances resolution and sensitivity by minimizing losses on the column due to adsorption.

**Gas Liquid Chromatography of Sample Extracts.**—The response of the electron capture detector to ABPP standards of concentrations ranging from 10.0–30.0 ng. was found to be linear when chromatographed on a properly conditioned column of 2% Carbowax 20M-TPA, as is demonstrated by the standard curve (Fig. 1). Using a constant-injection volume of 10  $\mu$ l. it was possible to reproduce repeated injections of the same standard within very close limits. Since the response of the detector changes with time possibly due to contamination of the foil with a resultant change in standing current, it was necessary to plot a standard curve for each day of use. A properly conditioned and sensitized column has an average useful life span of about 6 months of continuous use, after which it begins to show signs of deterioration. When the peak response of the column to any given ABPP standard has dropped by 10% or more of its previously determined peak value it is advisable to discharge the column and repack it with fresh substrate and repeat the conditioning operation.

#### Recovery Experiments

The over-all recovery of known amounts of Ro 5-3350 added to blood and taken through the entire procedure was determined by gas chromatography as the ABPP compound, Fig. 2, curves B and C. The average over-all recovery of 200–500 ng. of Ro 5-3350 added to 1 ml. of blood was in the order of 61% ± 3.0% (Table III). This represents the product of a 98–99% recovery of Ro

5-3350 from blood into 6 *N* H<sub>2</sub>SO<sub>4</sub>, and an average hydrolysis and recovery of the compound as ABPP of 77%. This represents a theoretical over-all recovery of 75–76%, but in actuality only a 61% recovery is obtained. The discrepancy of 15% recovery has not been satisfactorily accounted for as yet. However, the practical recovery obtained is very reproducible. The minimum detectable amount of ABPP is 5–10 ng./10  $\mu$ l., and the sensitivity limit of the method is of the order of 0.07–0.10 mcg./ml. of blood or plasma. Blood specimens from a patient who had received a single 15-mg. oral dose of the compound were pooled and extracted into ether using 1 *M* pH 9.0 buffer. The extract was analyzed by thin-layer chromatography (TLC) in two solvent systems: ethyl acetate–NH<sub>4</sub>OH (97:3, v/v) and chloroform–heptane–ethanol (10:10:5, v/v) and showed the presence of Ro 5-3350 which yielded ABPP on strong acid hydrolysis and GLC analysis. This demonstrates the specificity of the extraction procedure and GLC assay for Ro 5-3350 (Fig. 3).

#### Application of the GLC Method to Biological Samples

**Blood Level Fall-Off Curves of Ro 5-3350 Following the Administration of a Single 15-mg. Oral Dose in Man.**—Three patients were each given a

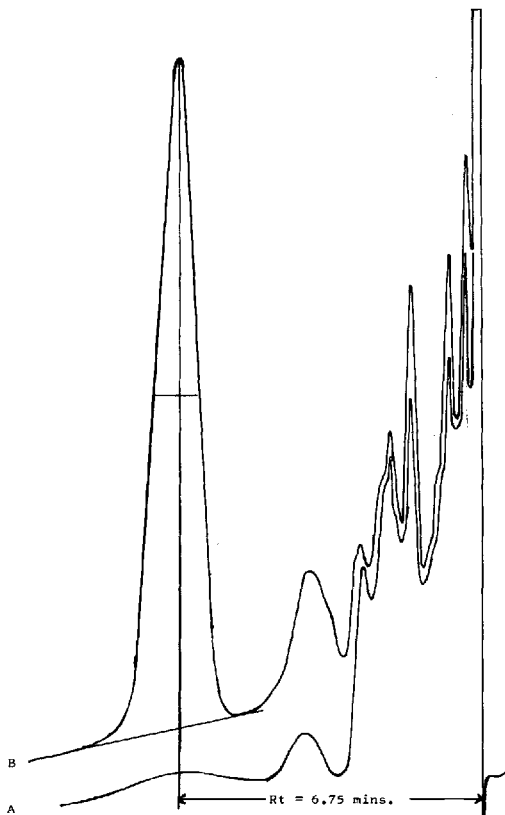


Fig. 3.—Chromatogram of blood extracts from a patient (E.M.) prior to medication, control blood (A), and 4 hr. after a single 15-mg. oral dose of Ro 5-3350; B, recovered as the ABPP compound (10/100  $\mu$ l.).

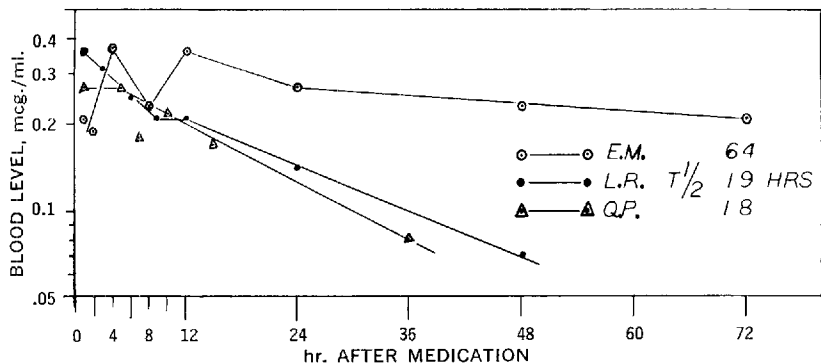


Fig. 4. — Blood level fall-off curves in three humans following the administration of a single 15-mg. oral dose of Ro 5-3350.

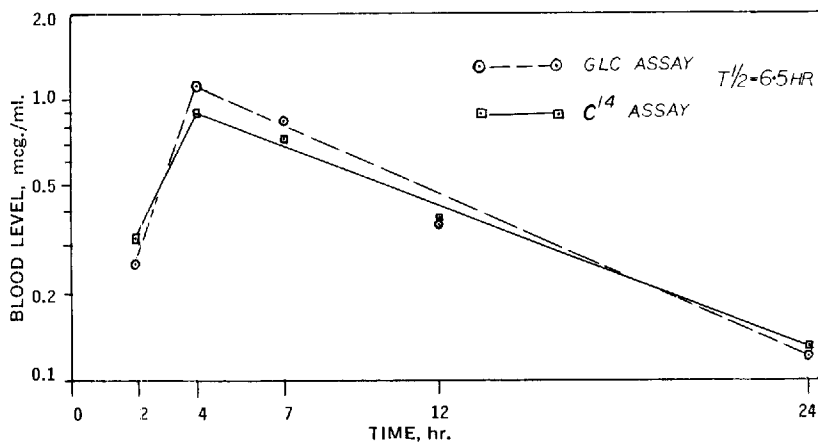


Fig. 5. — Blood level fall-off curve in a dog given a single oral dose (3.5 mg./Kg.) 36 mg. of Ro 5-3350-<sup>14</sup>C determined by liquid scintillation counting and by GLC.

single oral 15-mg. dose of Ro 5-3350 and specimens of whole blood collected prior to medication (control), and thereafter at suitable intervals of time covering a period of 72 hr. (3 days) from which blood level fall-off curves could be constructed (Fig. 4). In two out of three patients blood level maxima were obtained 1 hr. after dosage of 0.27 (Q. P.) and 0.36 mcg./ml. (L.R.) after which a progressive decline with time was seen with half-lives of 18 hr. and 19 hr., respectively. One patient (E.M.), showed a level of 0.21 mcg./ml. at 1 hr. which rose to 0.37 mcg./ml. at 4 hr., which declined to 0.23 mcg./ml. at 8 hr., rose to 0.36 mcg./ml. at 12 hr., after which a very gradual decline was seen with a half-life of about 64 hr. This erratic behavior may be due to a metabolic factor inherent in this patient.

#### Blood Level Fall-Off of Ro 5-3350 in the Dog.—

The blood level fall-off pattern of Ro 5-3350 was determined in a dog given a single oral dose of 3.5 mg./Kg. totaling 36 mg. of Ro 5-3350-<sup>14</sup>C. Specimens of whole blood were taken prior to medication and thereafter at 1, 2, 4, 7, 12, and 24 hr. after medication (5) and analyzed for intact Ro 5-3350 by (a) liquid scintillation counting and (b) GLC. The fall-off curves are shown in Fig. 5 and demonstrate the close reproducibility of the two methods. A blood level maximum of 1.11 mcg./ml. (GLC) and 0.90 mcg./ml. (scintillometry) was obtained 4 hr. after dosage, which declined with a half-life of 6 hr. (GLC) and 7 hr. (scintillometry), indicating an average half-life of about 6.5 hr. in blood.

## SUMMARY

The GLC method for the determination of Ro 5-3350 in blood was developed and involves the selective extraction of the compound from blood with pH 9.0 1 M borate-Na<sub>2</sub>CO<sub>3</sub>-KCl buffer using ether as the solvent, followed by extraction of the ether with 6 N H<sub>2</sub>SO<sub>4</sub>. The compound was hydrolyzed in 6 N H<sub>2</sub>SO<sub>4</sub> for 2.5 hr. at 100° (boiling water bath) to convert it into 2-amino-5-bromobenzoylpyridine (ABBP) which was quantized by GLC.

The average over-all recovery of 200 to 500 ng. of Ro 5-3350 added per milliliter of blood was in the order of 61 ± 3.0%. The minimal detectable amount of ABBP by electron capture was 5-10 ng.

The method was applied to the determination of blood levels in a dog and in man following single oral doses of Ro 5-3350.

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