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Solvent Effects on IR Spectra of Flurazepam

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Abstract □ Differences among the IR spectra of flurazepam samples obtained by recrystallizing flurazepam dihydrochloride from different solvents were studied and found to be caused by different mixtures of flurazepam mono- and dihydrochlorides and the free base; these spectral differences are not caused by polymorphism or decomposition of flurazepam.

Keyphrases □ Flurazepam—IR spectral analysis, effects of various extraction solvents, transformation from dihydrochloride to monohydrochloride and free base, comparison of spectra of extracted, untreated, and solution samples □ Spectroscopy, IR—flurazepam analysis, effect of various extraction solvents □ Sedatives—flurazepam, IR spectroscopy

To identify the active ingredient in flurazepam dihydrochloride capsules, flurazepam dihydrochloride was extracted from the capsules and its IR spectrum was matched with a reference spectrum. The IR spectrum of the extracted substance in potassium bromide was significantly different from the spectrum of untreated flurazepam dihydrochloride in potassium bromide, as well as from that published previously (1). Different spectra also were obtained in potassium bromide when different extraction solvents were used. The observed differences in the IR spectra were investigated to determine their origin.

EXPERIMENTAL

The spectra were recorded by a grating IR spectrophotometer[1]. The samples were measured as potassium bromide disks, as solutions in cells with sodium chloride windows, and as a capillary film between two sodium chloride disks.

The samples were prepared using the following extraction methods.

1. The contents of three 15-mg flurazepam dihydrochloride capsules[2] were extracted with 10 ml of methanol or acetone and filtered. The filtrate was evaporated to dryness under a nitrogen stream, and the residue was collected.
2. Flurazepam dihydrochloride (0.5 g) was dissolved in 3 ml of methanol and evaporated to dryness under nitrogen.
3. Flurazepam dihydrochloride (0.5 g) was dissolved in 100 ml of chloroform with stirring, and the clear solution was evaporated to dryness under nitrogen.
4. Flurazepam dihydrochloride (0.5 g) was dissolved in 2 ml of distilled water and evaporated to dryness under nitrogen. The residue was dried in a vacuum oven at ~50°, if necessary.
5. The contents of two to three flurazepam dihydrochloride capsules (or 100 mg of flurazepam dihydrochloride) were mixed well with 10 ml of distilled water. The mixture was neutralized with 1.0 *N* NaOH, and the milky solution was extracted with 10 ml of chloroform. The chloroform layer (flurazepam free base) was passed through anhydrous sodium sulfate and evaporated to dryness under nitrogen.

[1] Model 283, Perkin-Elmer.
[2] Dalmane, Hoffmann-La Roche.

RESULTS AND DISCUSSION

The IR spectra of materials (in potassium bromide) extracted from three 15-mg flurazepam dihydrochloride capsules with methanol and acetone are presented in Fig. 1 (curves A and B, respectively). Figure 1 also includes the spectra of untreated flurazepam dihydrochloride, flurazepam monohydrochloride, and flurazepam free base as potassium bromide pellets (curves C, D, and E, respectively). The spectra of the materials extracted with methanol and acetone disagree with each other as well as with the spectra of untreated flurazepam dihydrochloride. At this point, the suggestion of polymorphism arose. The IR spectra (as potassium bromide pellets) of the substances recrystallized by dissolving flurazepam dihydrochloride in methanol, chloroform, and water were obtained (curves A, B, and C, respectively, Fig. 2). Again, these spectra are not identical to one another or to the spectrum of untreated flurazepam dihydrochloride. To investigate whether polymorphism was causing these differences, solution spectra of the same substances were obtained (Fig. 3). Figure 3 also contains the solution spectrum of untreated flurazepam dihydrochloride. Since significant differences were found in the solution spectra as well, these differences could not be attributed to polymorphism.

The differences among curves C–E in Fig. 1 are most pronounced between 1800 and 2500 cm^{-1} and at 1635 cm^{-1}. The 1800–2500-cm^{-1} region contains the absorption bands due to the R_3N^+H and $R_2C{=}N^+H$ ions. Saturated tertiary amine salts (R_3N^+H) exhibit a strong and broad ammonium band (2) centered between 2300 and 2500 cm^{-1}, particularly when a large asymmetric tertiary ammonium ion combines with a relatively small negative ion such as chloride. This band has been observed in many tertiary amine salts of hydrochloric acid and hydrobromic acid.

Unsaturated amine salts ($C{=}N^+H$) exhibit a characteristic immonium band between 1800 and 2000 cm^{-1} in addition to the ammonium band between 2300 and 2500 cm^{-1}, which overlaps with the ammonium band caused by the R_3N^+H ion. The IR spectrum of flurazepam dihydrochloride is characterized by the immonium band between 1800 and 2000 cm^{-1} as well as the ammonium band between 2300 and 2500 cm^{-1} (curve C, Fig. 1). The IR spectrum of flurazepam monohydrochloride is characterized only by the ammonium band between 2300 and 2500 cm^{-1} and the absence of the immonium band between 1800 and 2000 cm^{-1} (curve D, Fig. 1). In flurazepam monohydrochloride, where the hydrochloride

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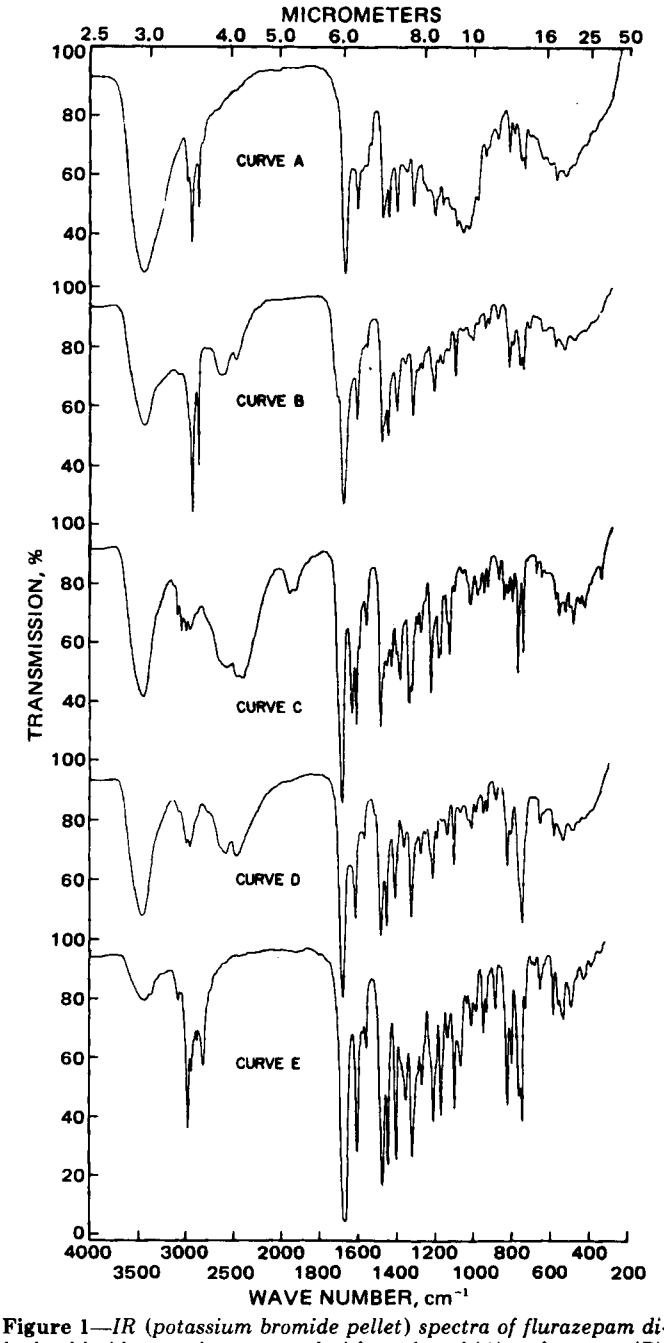
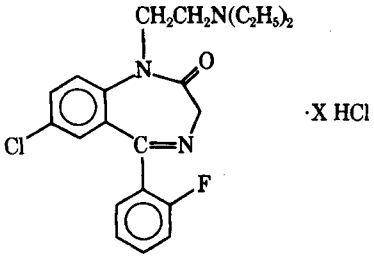


Figure 1—*IR (potassium bromide pellet) spectra of flurazepam dihydrochloride capsules extracted with methanol (A) and acetone (B). Curve C is the spectrum of untreated flurazepam dihydrochloride, curve D is that of flurazepam monohydrochloride, and curve E is that of flurazepam free base.*



X = 2 (dihydrochloride)
X = 1 (monohydrochloride)
X = 0 (free base)

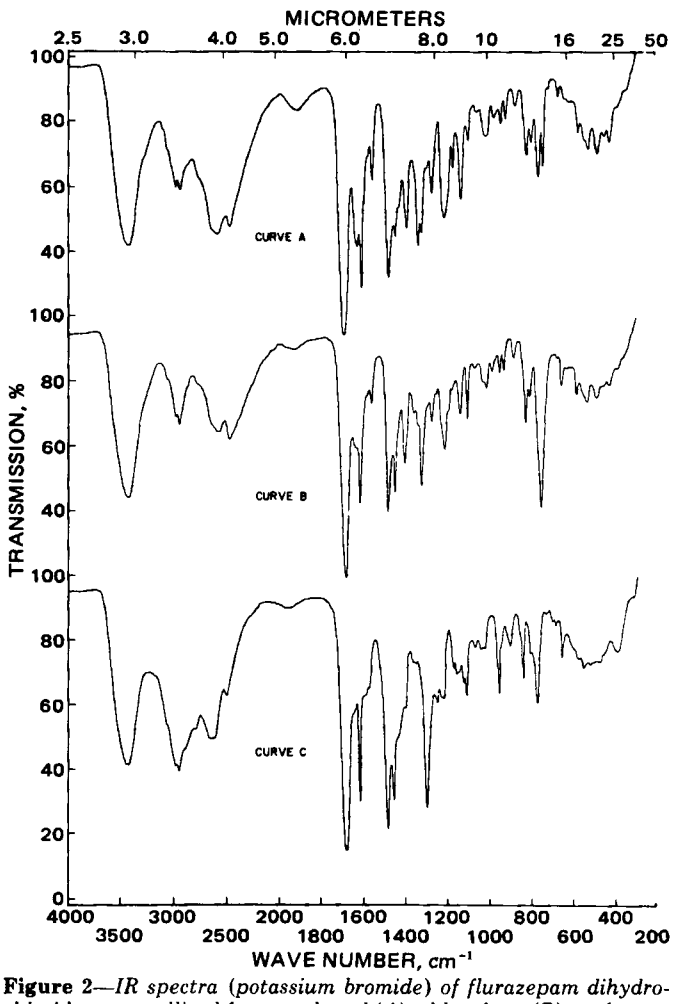


Figure 2—*IR spectra (potassium bromide) of flurazepam dihydrochloride recrystallized from methanol (A), chloroform (B), and water (C).*

concentration is less than that in flurazepam dihydrochloride, the acidic protons in flurazepam monohydrochloride participate in the formation of saturated tertiary ammonium ions (R_3N^+H) only because of the higher basicity of the tertiary amine (R_3N) than the imine (C=N—). The free base is recognized by the absence of such bands in the 1800–2500-cm^{-1} region (curve E, Fig. 1). The next most distinguishing feature of flurazepam dihydrochloride is the sharp band at 1635 cm^{-1} assigned to the $C{=}N^+$ stretching vibration; this band is absent in the spectra of the monohydrochloride and the free base. Furthermore, the carbonyl stretching band shifts from ~1680 cm^{-1} in the monohydrochloride and in the free base, to ~1700 cm^{-1} in the dihydrochloride.

Interpretation of Spectra from Capsules—The spectrum of flurazepam dihydrochloride capsules extracted with methanol lacks the ammonium and immonium absorption bands from 1800 to 2500 cm^{-1} (curve A, Fig. 1); this material is mostly flurazepam free base contaminated by other ingredients extracted from the capsule. The spectrum of flurazepam dihydrochloride capsules extracted with acetone displays only the ammonium band between 2300 and 2500 cm^{-1} (curve B, Fig. 1). Since the intensity of this band is weaker than that observed with the monohydrochloride, this material is a mixture of flurazepam monohydrochloride and flurazepam free base.

The intensities of the observed bands between 1800 and 2500 cm^{-1} and at 1635 cm^{-1} in Fig. 2 show that each spectrum is a linear superposition of the spectra of flurazepam dihydrochloride and flurazepam monohydrochloride, each having a different ratio of dihydrochloride to monohydrochloride. The material recrystallized by dissolving flurazepam dihydrochloride in methanol seemed to be approximately an equal mixture of mono- and dihydrochlorides (curve A, Fig. 2). The substances obtained from chloroform and water contain much more mono- than dihydrochlorides, as evidenced by the reduced intensities of the $C{=}N^+$ band at 1635 cm^{-1} and the immonium band at 1800–2000 cm^{-1} (curves B and C, Fig. 2).

Interpretation of Spectra from Solutions—These spectra (curves

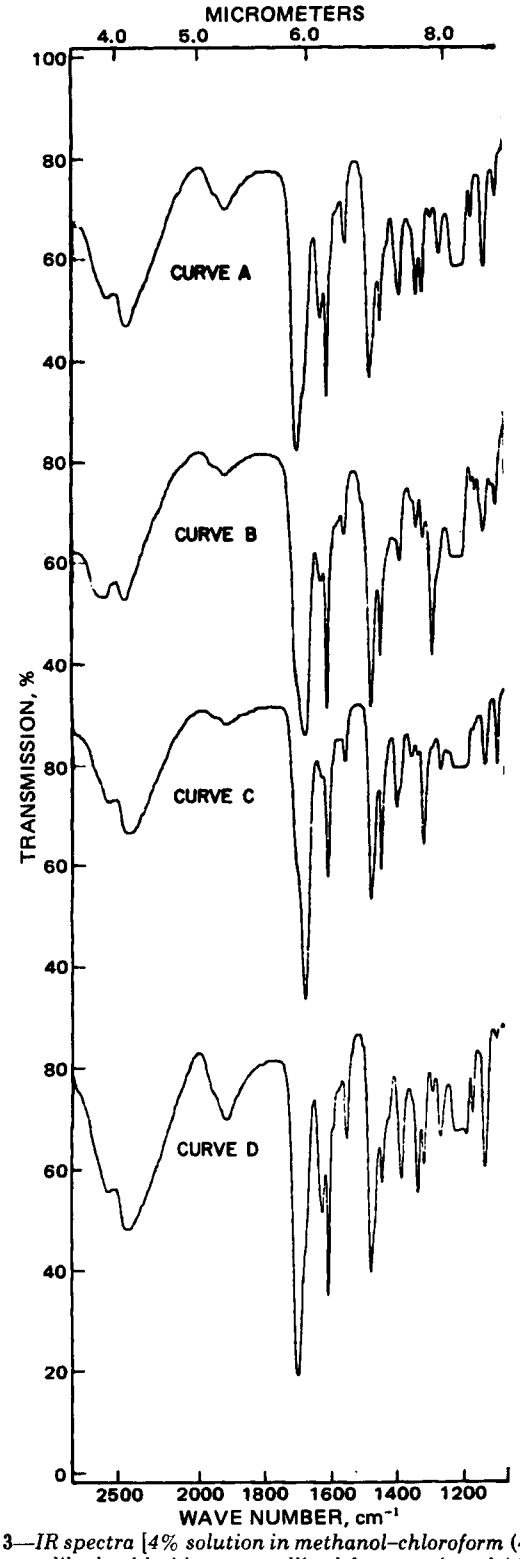


Figure 3—*IR spectra [4% solution in methanol–chloroform (4:96)] of flurazepam dihydrochloride recrystallized from methanol (A), chloroform (B), and water (C). Curve D is the solution spectrum of untreated flurazepam dihydrochloride.*

A–C, Fig. 3) display varying intensities of the ammonium, immonium, and C=N$^+$ bands, which can be attributed to different equilibrium mixtures of the mono- and dihydrochlorides in the materials recrystallized from methanol, chloroform, and water. The material recrystallized from water contained the lowest amount of dihydrochloride. The solution spectra were sharp enough to show the presence of two carbonyl stretching resonances due to the mono- and dihydrochlorides. The shift of the carbonyl band from 1700 to 1680 cm^{-1} provides further evidence of the transformation from the di- to the monohydrochloride.

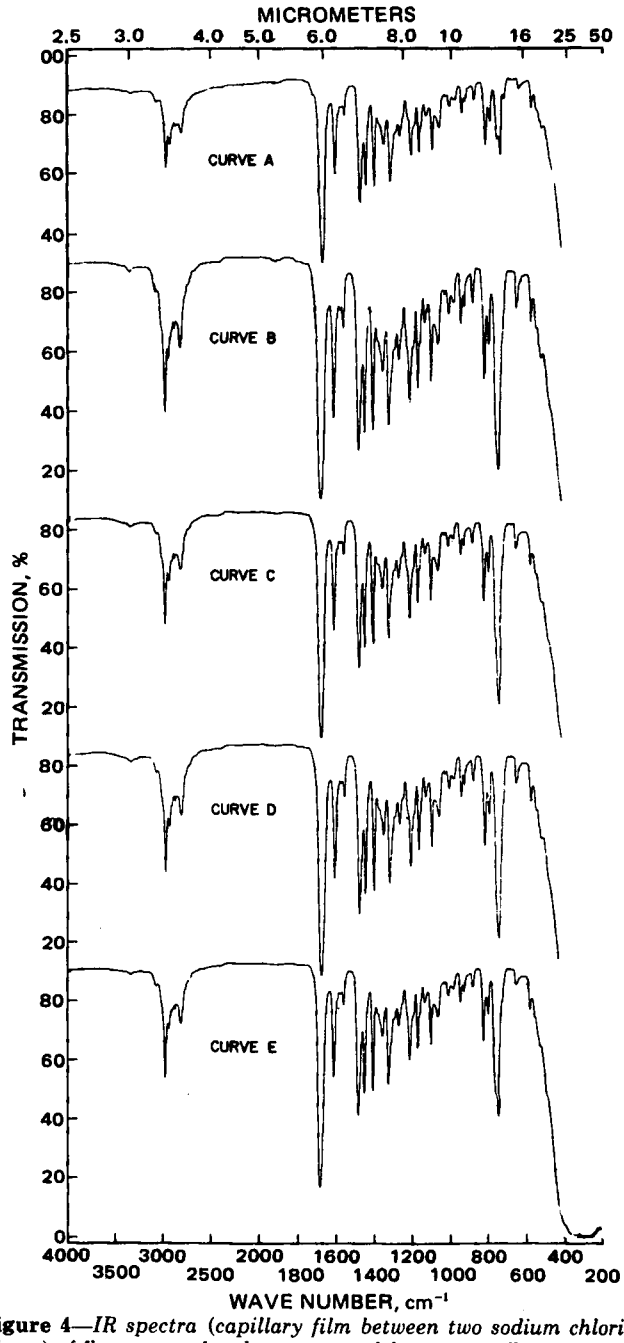


Figure 4—*IR spectra (capillary film between two sodium chloride plates) of flurazepam free base prepared from 15-mg flurazepam dihydrochloride capsules (A) and of flurazepam dihydrochloride recrystallized from methanol (B), chloroform (C), and water (D). Curve E is the spectrum of the free base prepared from untreated flurazepam dihydrochloride.*

In the spectrum (curve A) of the substance recrystallized from methanol, a small shoulder is observable at 1680 cm^{-1} on the right side of the carbonyl band at 1700 cm^{-1}. In the spectra (curves B and C) of the samples recrystallized from chloroform and water, the shoulder becomes the main carbonyl band at 1680 cm^{-1}, while the intensity of the band at 1700 cm^{-1} decreases such that it becomes a shoulder at 1680 cm^{-1} on the left side of the main band. The compatibility of the spectra (Fig. 4) of the flurazepam free base prepared from untreated flurazepam dihydrochloride, flurazepam dihydrochloride capsules, and flurazepam dihydrochloride recrystallized from methanol, chloroform, and water ruled out decomposition of flurazepam in the various studies.

CONCLUSIONS

The immonium and ammonium bands between 1800 and 2500 cm^{-1} and the C=N$^+$ stretching band at 1635 cm^{-1}, as well as the carbonyl band

position (1700 cm^{-1} for dihydrochloride and 1680 cm^{-1} for monohydrochloride and for free base), were the distinguishing diagnostic features used in this investigation to establish that the different IR spectra of flurazepam dihydrochloride recrystallized from different solvents result from different equilibrium mixtures of flurazepam mono- and dihydrochlorides and/or free base in these solvents. The solvent effect on the transformation of flurazepam di- to monohydrochloride was methanol < chloroform < water. The solution spectra also ruled out polymorphism, and the spectra in Fig. 4 show no compound decomposition.

In view of these findings, the IR identity test for the active ingredient in bulk flurazepam dihydrochloride or flurazepam dihydrochloride capsules should be based on extraction of flurazepam free base using the procedure described in the *Experimental* section and then on comparison of its IR spectrum with a spectrum of the free base similarly extracted from a standard flurazepam dihydrochloride sample, which can serve as a reference spectrum for future work.

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Determination of Nofedone in Human Serum by Electron-Capture GLC

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Abstract □ An electron-capture GLC method to measure nofedone in human serum was developed. A homolog of nofedone was added to the serum as an internal standard before the sample was alkalinized with pH 9.5 phosphate buffer and extracted with ethylene dichloride containing 0.5% isopentyl alcohol. This organic phase was extracted with 0.2 *N* HCl, the acidic aqueous phase was neutralized immediately, and the extraction with ethylene dichloride was repeated. The ethylene dichloride phase was evaporated to dryness, and the residue was reacted with heptafluorobutyric anhydride. The derivatives were chromatographed at 290° on a 1% Dexsil 300 column. Data on apparent recovery, accuracy, and specificity are given. The detection limit was 5 ng/ml of serum. Serum levels over time in one patient after intravenous administration of 1 mg/kg and after oral administration of 50, 100, and 150 mg of nofedone are presented.

Keyphrases □ Nofedone—analysis, electron-capture GLC, human serum □ GLC, electron capture—nofedone, human serum □ Antiarrhythmic agents—nofedone, analysis, electron-capture GLC, human serum

Nofedone[1] (I) fumarate, 3-[2-hydroxy-3-(isopropylamino)propoxy]-2-phenyl-1-isoindolinone B form, shows marked experimental and clinical antiarrhythmic activity after intravenous and oral administration (1). Compound I is under clinical study. To investigate its pharmacokinetics and to establish the optimal dose range, a procedure was developed for the determination of the drug in human serum. The described method involves GLC of a heptafluorobutyrate derivative with ^{63}Ni-electron-capture detection. A similar method was used for the determination of a β-adrenoreceptor antagonist with the same aminohydroxylated chain (2).

EXPERIMENTAL

Reagents—All solvents and reagents (0.2 *N* HCl[2], 1 *N* NaOH[3], and pH 9.5 phosphate buffer prepared from a 0.5 *M* aqueous solution of dibasic sodium phosphate[4]) were analytical grade. Ethylene dichloride[5], UV spectrophotometric grade, was freshly distilled before use. The heptafluorobutyric anhydride[6] was the derivatizing agent for the electron-capture analysis. It was kept at 4° in sealed vials to prevent the ingress of water from the atmosphere. Nofedone and the internal standard, 3-[2-hydroxy-3-(isopropylamino)propoxy]-2-(3-trifluoromethylphenyl)-1-isoindolinone[7] (II), were used as the free bases.

Apparatus and Operating Conditions—The gas chromatograph[8] was equipped with a ^{63}Ni-electron-capture detector[9] and a glass column (2 m long × 6.25 mm i.d.) packed with 1% Dexsil 300 on a 100–120-mesh support[10]. The column temperature was 290°, the injection port temperature was 300°, the detector temperature was 300°, the carrier gas (nitrogen) flow rate was 20 ml/min, and the scavenger gas (nitrogen) flow rate was 80 ml/min.

The column was conditioned for 24 hr by temperature programming from 250 to 350° at 1°/min and was regulated at 350° for 10 min. It was treated before analysis by several injections of the derivatized extract of serum blank with or without I and the internal standard to ensure maximal performance. Under the conditions described, the retention times of the derivatives of I and II were 2 and 1.5 min, respectively.

Glassware—All glassware was cleaned well and tested with an aqueous solution of I·HCl and II of known content.

Procedure—Compound II was used as a 100-μg/ml solution in ethyl acetate[11] and kept from bright light. This solution was diluted (1:100) with ethyl acetate and immediately inserted in a centrifuge tube. Samples of the internal standard, 50, 200, and 500 ng, were commonly used for such analyses. The quantity of the internal standard was selected to give a peak height greater than or equal to that of I. The solvent was evaporated under a nitrogen stream, and 0.25, 0.50, or 1 ml of serum was added. After vibrating for 30 sec, 2 ml of pH 9.5 phosphate buffer was added.

This aqueous solution was extracted with 6 ml of ethylene dichloride containing 0.5% isopentyl alcohol[12], mechanically shaken for 20 min, and centrifuged. The lower organic phase was removed by pipet and washed by shaking for 5 min with 2 ml of distilled water. The organic phase then was extracted with 2 ml of 0.2 *N* HCl and mechanically shaken for 20 min. The acidic aqueous phase was neutralized immediately with 0.5 ml of 1

[1] 30,356 RP.
[2] Catalog No. 20 252, Prolabo, 75011 Paris, France.
[3] Catalog No. 31 627, Prolabo, 75011 Paris, France.
[4] Catalog No. 28 028, Prolabo, 75011 Paris, France.
[5] Catalog No. 23 341, Prolabo, 75011 Paris, France.
[6] Puriss p.a., Fluka A.G., Buchs S.G., Switzerland.
[7] 28,731 RP.
[8] Model 419, Packard Instrument Co., Downers Grove, Ill.
[9] Model 714, Packard Instrument Co., Downers Grove, Ill.
[10] Supelcoport, Supelco, Bellefonte, Pa.
[11] Catalog No. 23 882, Prolabo, 75011 Paris, France.
[12] Catalog No. 20 799, Prolabo, 75011 Paris, France.

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