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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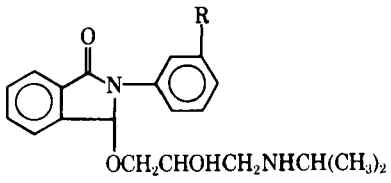
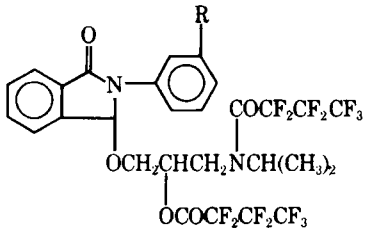


I: R = H

II: R = CF_3



III

N NaOH, and 1 ml of pH 9.5 buffer was added. The extraction with ethylene dichloride was repeated.

The organic phase was transferred to a small tube fitted with a glass stopper and evaporated to dryness under a nitrogen stream in a 50° water bath. The residue was dissolved in a mixture of 0.1 ml of ethyl acetate and 0.4 ml of toluene[13]. After shaking for 30 sec, 50 μl of heptafluorobutyric anhydride was added, and the solution was allowed to stand for 5 min at room temperature. The excess reagent was eliminated by adding 1 ml of a 5% aqueous solution of sodium bicarbonate[14]. After gas release, 0.1 ml of isopentyl alcohol was added. The lower aqueous phase was removed by pipet and discarded. The organic phase was washed by shaking with 0.5 ml of a 2% solution of isopentyl alcohol in distilled water. The mixture

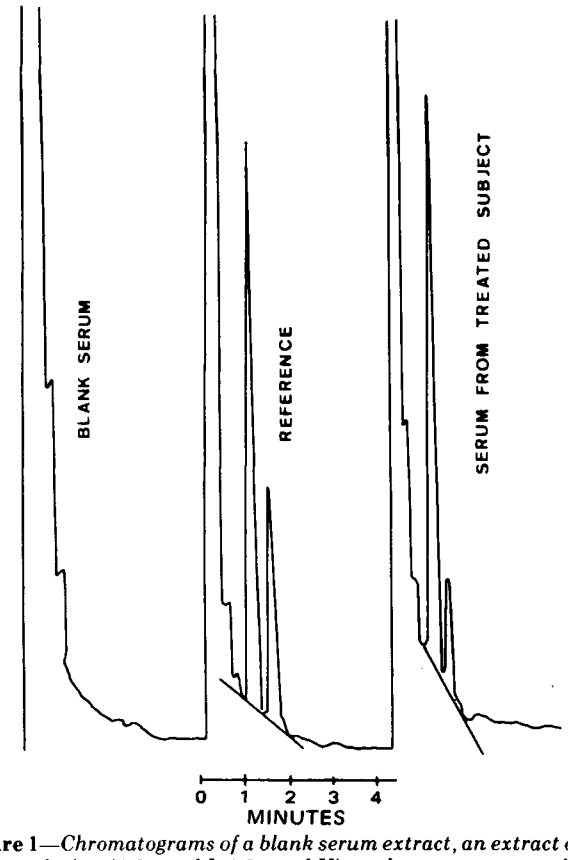


Figure 1—*Chromatograms of a blank serum extract, an extract of reference solution (1.6 ng of I–1.6 ng of II), and a serum extract from a treated subject.*

[13] Catalog No. 28 676, Prolabo, 75011 Paris, France.
[14] Catalog No. 27 778, Prolabo, 75011 Paris, France.

Table I—Apparent Recovery of Nofedone Added to Control Serum

Nofedone Added, ng/ml	Nofedone Found, ng/ml	Apparent Recovery[a], % (Mean ± *RSD*)
20	18.6	93 ± 16.4
50	49.7	99 ± 17.3
100	104	104 ± 10.7
250	270	108 ± 11.5
500	504	101 ± 3.2
1000	978	98 ± 5.0
2000	2070	103 ± 12.1
4000	4270	107 ± 9.72

[a] Average of seven determinations.

was allowed to stand, and 2 or 3 μl of the upper organic phase was injected into the gas chromatograph.

The ratio of the peak heights, H/H_1 (H is the derivative of I and H_1 is the derivative of II), was calculated and compared to a calibration curve. This calibration was obtained by performing derivations on a mixture of 0.025, 0.050, 0.1, or 0.2 ml of a 100-μg/ml stock solution of I in ethyl acetate with 0.1 ml of a 100-μg/ml stock solution of II in ethyl acetate. These reference samples were derivatized and diluted with a mixture of toluene–ethyl acetate–isopentyl alcohol (40:10:10) and were recorded in the best sensitivity range (1:25 dilution for an attenuation of $4 \times 2.5 \times 10^{-10}$). The highest reference sample was not linearly related to the lowest sample when the column was performing properly, but the samples were always derivatized to permit estimation of serum samples added with a too low an amount of internal standard.

To increase reliability, the calibration curve was checked for each series of samples. Each analysis on serum samples and each injection into the chromatograph were repeated at least twice. The injected volume was kept constant during a series of injections.

RESULTS AND DISCUSSION

The use of II as an internal standard was satisfactory because its physicochemical properties are closely related to those of I. The presence of the CF_3 radical in the molecule ensured a higher response for electron-capture detection (Fig. 1).

Several derivatization techniques were tested to form derivatives of I, and the method selected for use in this study was the most rapid. After neutralizing the excess heptafluorobutyric anhydride with sodium bicarbonate, no interfering peaks due to endogenous substances were noted in the upper organic phase. The bis-derivative of I (III) from the serum

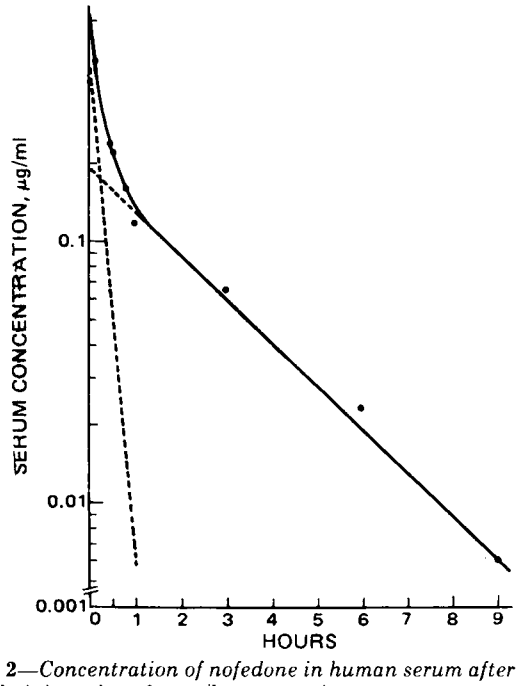


Figure 2—*Concentration of nofedone in human serum after intravenous administration of 1 mg/kg over 2 min.*

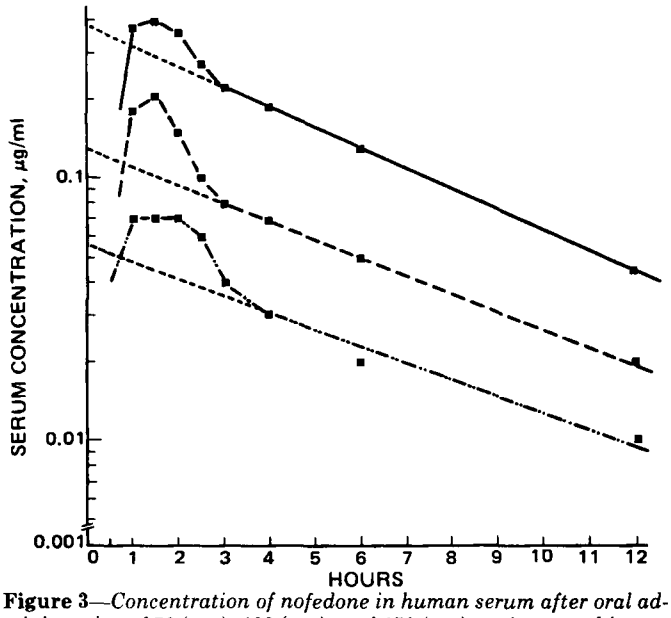


Figure 3—*Concentration of nofedone in human serum after oral administration of 50 (-·-·-), 100 (- - -), and 150 (——) mg in one subject.*

extract had the same chromatographic behavior as the authentic compound. The minimum detectable quantity was 100 pg, and the linearity of the ^{63}Ni-electron-capture detection response was observed from 0.5 to 2 ng in the organic phase injected (attenuation $4 \times 2.5 \times 10^{-10}$).

The theoretical sensitivity initially was 10 ng/ml, but it improved during use. The sensitivity limit for the quantitative determination of I in serum by electron-capture detection was 5 ng/ml of serum after a 2-ml sample extraction. We observed, as did Leitch *et al.* (3), that the sensitivity increased with the number of plasma extracts injected. At the beginning of the GLC analysis, plasma extracts were injected to obtain the plateau of best sensitivity. This plateau was maintained by alternating injections of serum extracts and reference samples. The apparent recovery of I compared with that of the internal standard and the accuracy of the results obtained are shown in Table I. The determinations were repeated seven times for each quantity. The last column in Table I shows the validity of the method. A plot of added drug *versus* found drug had a slope of unity. According to the data provided, a single determination is expected to be within about 12% of the true value, and multiple determinations should vary by ±12% (reproducibility).

The chromatographic analysis of the serum blanks showed that no compound extracted under the conditions described interfered with I or the internal standard (II) (Fig. 1). TLC of derivatized extracts from serum taken from patients treated with nofedone showed that there was no interfering substance with a close R_f value. In the urine of treated patients that contained metabolites, the concentrations of nofedone in the form of bis-derivatives were the same as those measured after elution of a silica plate and GLC analysis.

The sensitivity of this technique allowed determination of serum levels in humans for ~9 hr after an intravenous bolus injection (1 mg/kg in 2 min) and for 12 hr after a single oral dose (50–150 mg in a capsule or tablet). Figures 2 and 3 show the concentrations of nofedone in human serum after an intravenous bolus injection of 1 mg/kg and after an oral dose of 50, 100, and 150 mg in a capsule in another subject. Pharmacokinetic data can be derived from these distribution values. The selectivity of this method allows determination of the compound in saliva, urine, and feces.

Isopentyl alcohol was used because it prevents the molecule from adhering to the glass walls during extraction, derivatization, and GLC analysis. It also prevents tailing peaks and thus increases sensitivity. For serum concentrations below 50 ng/ml, optimal stability of the chromatograph is required to minimize background noise. For higher concentrations, the method provided good reproducibility and allowed calculation of pharmacokinetic parameters up to 9 hr following an intravenous bolus injection (1 mg/kg) and up to 12 and 24 hr after oral doses of 50, 100, and 150 mg.

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Fluorometric Determination of Thiazole-Containing Compounds

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Abstract □ Fluorescence spectroscopy was applied to the development of sensitive analytical methods for the determination of thiazole and several congeners that contain substituted thiazole rings. Treatment to yield thionine, previously used spectrophotometrically to measure thiazole and fluorometrically only for sulfur determinations in inorganic systems, is further characterized and illustrated with the determination of the antibiotic thiopeptin. This method is selective for submicrogram quantities of thiazole rings in the presence of fused-ring derivatives and reduced analogs. It has a precision of ±2% *RSD* (n = 11) at the 15-ng/ml thiazole concentration level with a signal-to-noise ratio of 3:1. For thiopeptin, this method has an accuracy of 5% mean relative error (n = 8) over the 5–20-ppm range in medicated feed.

Keyphrases □ Thiazole—thiazole-containing compounds, fluorometry □ Fluorometry—analysis, thiazole-containing compounds □ Spectroscopy, fluorescence—thiazole-containing compounds

The thiazole ring is a common structural element found in many compounds exhibiting biological and therapeutic activity and in several molecules used in agricultural products as well as in pharmaceutical formulations (1, 2). Because of these properties, sensitive analytical methods for thiazole-containing compounds are of interest for application in low concentration level formulations, bioavailability studies, and environmental samples.

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