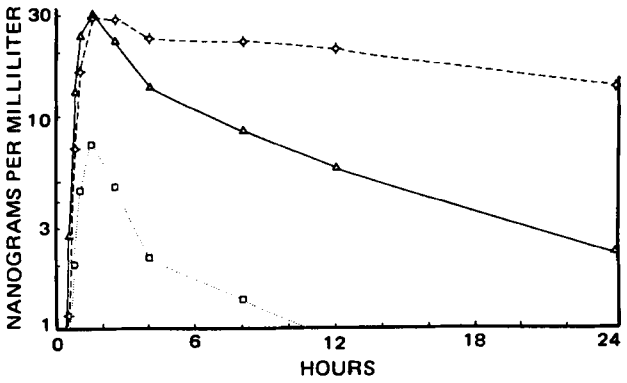

Figure 4—*Serum concentration–time curves of amoxapine (△), 7-hydroxyamoxapine (□), and 8-hydroxyamoxapine (○) after a single oral 50-mg dose of amoxapine (mean values of 26 subjects).*

loxapine dosage; 7-hydroxyloxapine, although present in appreciable quantity, is overwhelmed by the corresponding 8-hydroxy isomer, as previously described.

Observed concentrations of loxapine and its 8-hydroxylated metabolites at steady state following multiple dosage are shown in Table II. The patients under treatment received a twice a day dosage at the level indicated, and serum was obtained for analysis immediately prior to a morning dose. A similar study involving amoxapine dosage was not performed.

The development of the described procedure led to an interesting observation concerning *N*-oxides of loxapine and its *N*-methylated metabolites (7-hydroxyloxapine and 8-hydroxyloxapine). These compounds form stable derivatives with trifluoroacetic anhydride, which survive the described GLC conditions and yield peaks differing in retention time from any of the compounds described. Loxapine *N*-oxide is found in human serum and is a major metabolite in dog serum.

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Determination of Guanfacine in Biological Fluids by Electron-Capture GLC

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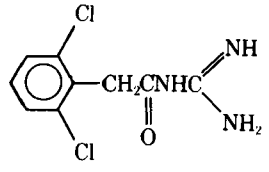
Received February 6, 1978, from the *Centre de Recherches Pharmacocinétiques, Laboratoires Sandoz, 14, Boulevard Richelieu, 92505 Rueil Malmaison, France.* Accepted for publication July 18, 1978.

Abstract □ A quantitative electron-capture GLC method with good sensitivity is described for the determination of guanfacine in human plasma and urine. By condensing its amidino group with hexafluoroacetylacetone, guanfacine is converted to a pyrimidino derivative with better GLC properties than the parent drug. This method allows the determination of guanfacine in plasma and urine at concentrations as low as 0.5 ng/ml and was applied successfully to measurement of plasma levels in humans after therapeutic dosing.

Keyphrases □ Guanfacine—GLC analysis in plasma and urine □ GLC—analysis, guanfacine in plasma and urine □ Antihypertensive agents, potential—guanfacine, GLC analysis in plasma and urine

Guanfacine[1], *N*-amidino-2-(2,6-dichlorophenyl)acetamide hydrochloride (I), is a guanidine derivative showing antihypertensive activity in animals (1) and humans (2, 3). Like clonidine, guanfacine possesses peripheral and central α-adrenoceptor stimulant activity resulting in a reduction in sympathetic nerve activity.



I

This report describes an electron-capture GLC method adapted to the determination of guanfacine in biological fluids. After extraction, the compound is derivatized by condensing the amidino group with hexafluoroacetylacetone to form a pyrimidino compound, as reported[2] for guanido compounds (4, 5).

EXPERIMENTAL

Reagents—Methanol[3] RP, hexafluoroacetylacetone[4], and ethylanthraquinone[4], all analytical grade, were used without further purification. Dichloromethane[5], benzene[3], and methyl isobutyl ketone[5] were freshly distilled under nitrogen.

A standard solution of ethylanthraquinone (internal standard) was prepared by dissolving 100 mg of the product in 50 ml of methanol.

Apparatus—A chromatograph[6] equipped with a ^{63}Ni-electron-capture detector was fitted with a glass column (1.6 m × 3 mm i.d.) packed with 3% OV-225 on 100–120-mesh Gas Chrom Q. The flow rate of the carrier gas, 5% methane in argon, was 60 ml/min. The temperatures of the injection port, column, and detector block were 250, 210, and 300°, respectively.

[1] Clinical code BS 100-141

[2] R. Laplanche, Sandoz-Bale Internal Report, 1976.
[3] E. Merck A. G., Darmstadt, West Germany.
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[5] J. T. Baker, Phillipsburg, N.J.
[6] Hewlett-Packard model 5713A.

0022-3549/79/0200-0219$01.00/0

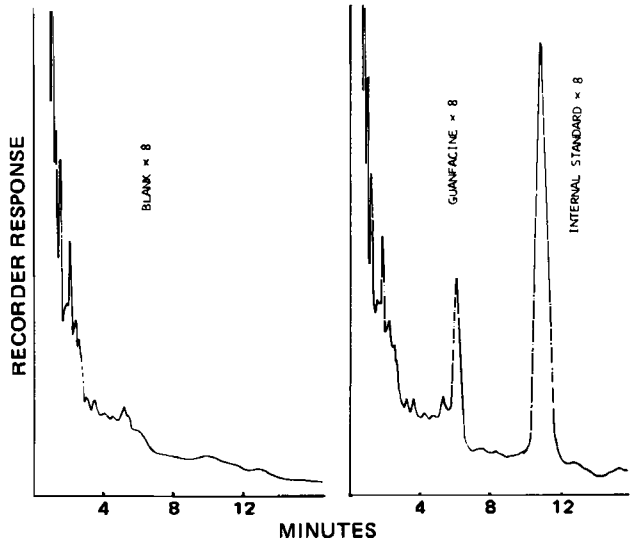


Figure 1—*Gas chromatograms of human plasma assayed as described. Key: left, chromatogram from normal human plasma; and right, chromatogram from normal human plasma containing 2 ng of guanfacine/ml.*

The mass spectrum of the derivative of the parent drug was determined on a mass spectrometer[7] with a multiple ion detector[8] coupled to a gas chromatograph *via* a membrane separator. The source was an electron impact operating at 70 ev and 20 mamp. The source temperature was 220°, and the analyzer temperature was 180°.

Assay—To 4 ml of plasma (or diluted urine) was added 0.5 ml of 1 *N* NaOH. Preliminary extraction was performed by adding 10 ml of methyl isobutyl ketone. After shaking for 20 min and then centrifugation at 2000 rpm for 5 min, the organic phase was transferred to a glass-stoppered tube and shaken for 20 min with 4 ml of 0.1 *N* HCl. After centrifugation (5 min at 2000 rpm), the organic phase was discarded. The acidic phase was alkalinized with 1 ml of 1 *N* NaOH and again extracted with 10 ml of dichloromethane–methyl isobutyl ketone (90:10 v/v).

After centrifugation, an aliquot of the organic phase was evaporated at 40° under nitrogen. The residue was taken up in 1 ml of 10% acetic acid in methanol and dried again. The new residue was dissolved in 200 μl of 10% hexafluoroacetylacetone in methanol. The tubes, stoppered with polytef capsules, were heated in an aluminum heating block at 100° for 1 hr and then evaporated to dryness under nitrogen.

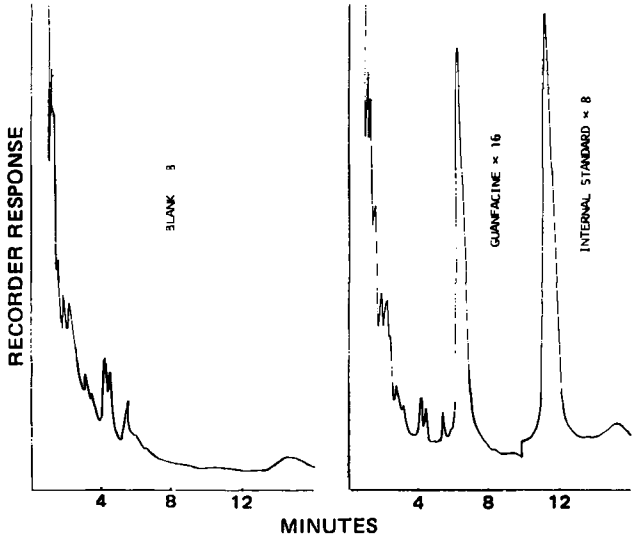
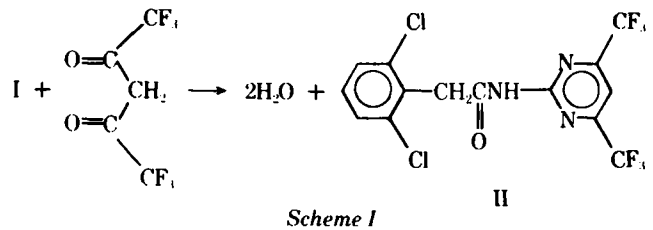


Figure 2—*Gas chromatograms of human urine assayed as described. Key: left, chromatogram from normal human urine; and right, chromatogram from normal human urine containing 50 ng of guanfacine/ml before dilution.*



Scheme I

The dry extract was taken up in 400 μl of benzene containing 100 ng of the internal standard (ethylanthraquinone); then 3 ml of 3 *N* NaOH was added to hydrolyze the excess hexafluoroacetylacetone. The samples were vortexed and centrifuged, and 4 μl of the benzene phase was injected into the chromatograph.

RESULTS AND DISCUSSION

To determine the optimal chromatographic extraction and derivatization conditions for the analysis of guanfacine at very low concentrations, various experiments were performed. Initially, the substance was analyzed without derivatization by using a glass column packed with very polar phases[9]. However, due probably to the high polarity of the compound, retention on the column wall led to poor reproducibility.

To reduce this phenomenon and to increase the sensitivity, a pyrimidino derivative was formed by condensing the amidino group with hexafluoroacetylacetone as reported previously (4, 5). The detector response then was stable, and the sensitivity was increased slightly.

Identification of Derivative—A stable derivative, II (Scheme I), was obtained and its structure was confirmed by its mass spectrum[10] (Table I).

Sample Extraction—Various parameters of the procedure were analyzed. Among the different solvents used for extraction, only methyl isobutyl ketone and 10% methyl isobutyl ketone in dichloromethane gave a good yield. Heptane–1.5% isoamyl alcohol, ethyl acetate, ether, benzene, chloroform, hexane, and dichloromethane resulted in less than a 20% yield.

For extraction of the alkalinized acid phase, 10% methyl isobutyl ketone in dichloromethane was substituted for pure methyl isobutyl ketone to minimize the water retention by the residue. Back-extraction into diluted acid removed most of the neutral compounds from the initial methyl isobutyl ketone extract, which may have interfered in the chromatographic analysis. A very clean plasma extract (blank) was thus obtained (Fig. 1). Figure 1 also shows a chromatogram of assayed human plasma with ethylanthraquinone as the internal standard.

The present GLC method also was applied to the determination of guanfacine in urine (Fig. 2).

The data in Table II show that guanfacine transfer from an aqueous into an organic phase was almost complete at pH 11 and that its reextraction into the aqueous phase should occur at pH <3.

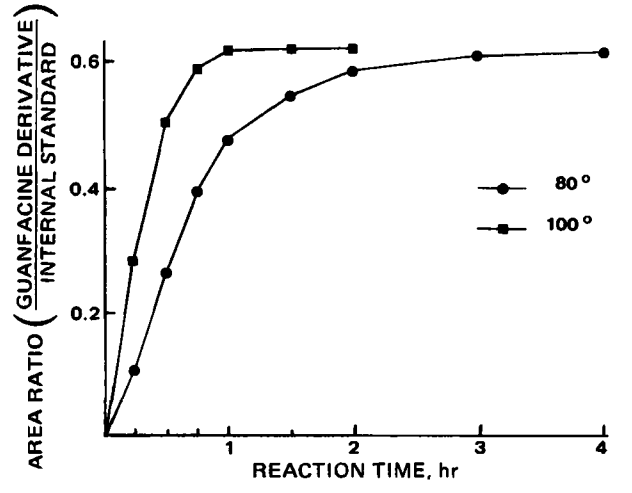


Figure 3—*Influence of temperature and heating time on derivatization kinetics.*

[7] Hewlett-Packard model 5980A.
[8] Hewlett-Packard model 5947A.
[9] Carbowax 20M or neopentylglycolsuccinate from Carlo Erba.
[10] Performed by R. Laplanche.

Table I—Principal Mass Peaks in Mass Spectra of the Compounds

			Guanfacine (Direct Inlet in Ion Source), mol. wt. = 245							
m/e	86	69	123	159	89	100	161	125	87	149
I[a], %	100	(46)[b]	38	35	34	(30)	26	20	15	(15)
			Guanfacine Derivative (GLC–Mass Spectral Coupling), mol. wt. = 417							
m/e	186	159	188	161	101	123	382	238	89	69
I, %	100	88	75	56	(48)	41	27	21	(16)	(16)

[a] Intensity of the mass peak relative to the parent peak = 100. [b] The intensities in parenthesis are possibly too high because of the interference of an impurity or pyrolysis product.

Table II—Influence of pH on Extraction of 8 ng of Guanfacine from 4 ml of Aqueous Phases (*i.e.*, 2 ng/ml)

pH	Recovery[a] ± *SEM*, %
1 (0.1 *N* HCl)	—
3[b] (buffer)	17 ± 1.2
5[b] (buffer)	32 ± 0.9
7[b] (buffer)	55 ± 3.0
9[b] (buffer)	70 ± 1.2
11[b] (buffer)	90 ± 1.5
13 (1 *N* NaOH)	93 ± 1.5

[a] Mean of four determinations. [b] All buffers (Titrisol) were purchased from Merck, Darmstadt, West Germany.

Derivatization—Excess hexafluoroacetylacetone was used for derivatization. A 200-μl aliquot of 10% hexafluoroacetylacetone in methanol afforded complete derivatization over the 1–20-ng/ml range. Urine samples containing more than 20 ng of guanfacine/ml were diluted before analysis.

Figure 3 shows the influence of temperature and heating time on the kinetics of the derivative reaction. At 100°, the reaction was virtually complete after 1 hr; 3 hr was required at 80°.

Initially, toluene was used as the solvent for derivatization[2]. However, the reaction between guanfacine and hexafluoroacetylacetone was more complete with methanol, and the peak area corresponding to II was approximately four times higher than with toluene.

Recovery and Linearity—Guanfacine recovery was determined by adding various known amounts of product to human plasma (Table III) or human urine (Table IV) and analyzing each sample in quadruplicate according to the described procedure. As compared to a similar series of unextracted reference standards of guanfacine, the mean recoveries varied from 73.5 to 84.6% with an average of 79% and were independent of the concentration within the range used for plasma.

The standard curve obtained from plasma spiked with guanfacine was linear in the 0.5–10-ng/ml range as reflected by the values of the correlation coefficient ($y = 0.087x + 0.051$, $r = 0.994$). For urine, a good correlation also was obtained for the 20–400-ng/ml range ($y = 5.46 \times 10^{-3}x + 0.216$, $r = 0.998$). Triplicate assays on different days gave a standard deviation of 3.4% at 10 ng/ml and of 4.2% at 1 ng/ml.

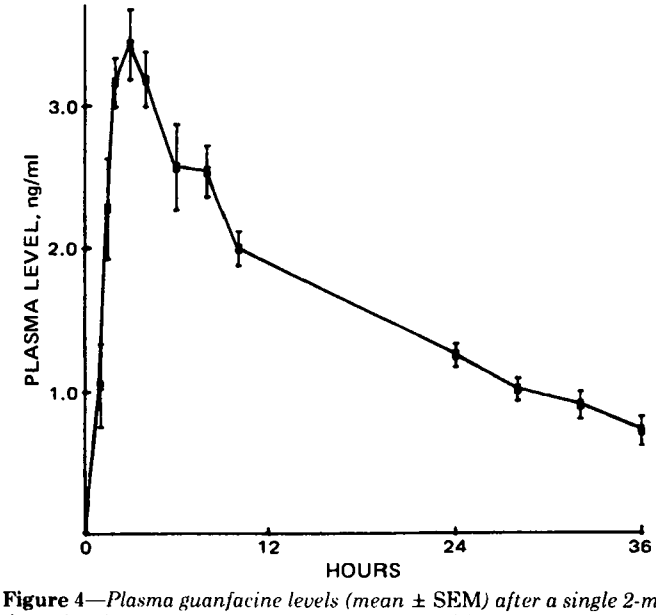


Figure 4—*Plasma guanfacine levels (mean ± SEM) after a single 2-mg dose of active compound in six hypertensive subjects.*

Table III—Recovery of Guanfacine Added to Human Plasma[a] Samples

Guanfacine Added, ng/ml	*n*	Amount Recovered[b], ng/ml	Recovery, mean ± *SEM*, %
0.5	4	0.38	77.0 ± 1.3
1	4	0.85	84.6 ± 2.1
2	4	1.66	83.2 ± 4.0
4	3	3.04	76.1 ± 1.8
8	4	5.88	73.5 ± 1.0

[a] Assays were performed on 4 ml of plasma. [b] Average of *n* determinations.

Table IV—Recovery of Guanfacine Added to Human Urine[a] Samples

Guanfacine Added, ng/ml[b]	*n*	Amount Recovered[c], ng/ml	Recovery, mean ± *SEM*, %
50	2	36.7	73.3 ± 1.4
100	2	76.4	76.4 ± 4.1
200	4	163.4	81.7 ± 1.9
400	4	341.6	85.4 ± 2.3

[a] Assays were performed on 4 ml of diluted urine (1:10). [b] Levels before dilution. [c] Average of *n* determinations.

Sensitivity and Specificity—The detection limit, defined as five times the background level, for guanfacine was 4 pg, corresponding to 0.125 ng/ml of plasma under the assay conditions. Four times this concentration (*i.e.*, 0.5 ng/ml of plasma) was the lowest measurable concentration with an accuracy of ±5%.

The specificity of the method in the presence of other drugs such as diuretics and β-blocking agents, which may be administered concurrently with an antihypertensive drug, was checked. No interfering peak was observed on the chromatogram under the same conditions.

Application to Pharmacokinetic Studies—The suitability of the proposed method for the determination of plasma guanfacine was tested in six hypertensive patients who received 2 mg of guanfacine as a single oral dose. Blood samples (10 ml) were drawn by venipuncture at 0, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 28, 32, and 36 hr. Plasma was separated, and the samples were frozen until analyzed.

Guanfacine concentrations then were determined by the described method. The mean plasma concentration–time curve (±*SEM*) is shown in Fig. 4. No guanfacine metabolites interfered with the determination of the parent compound.

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