GLC Analysis of Loxapine, Amoxapine, and Their Metabolites in Serum and Urine

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Abstract □ A GLC analysis is presented for loxapine, amoxapine, and their major metabolites in serum and urine. Electron-capture detection is employed for serum analysis, and flame ionization is used for urine analysis. The procedure includes trifluoroacetylation of secondary amine functions, followed by trimethylsilylation of phenolic groups after ethyl acetate extraction of the sample. Urine requires prior enzymatic hydrolysis of conjugates. Data indicating the utility of the procedure in hospitalized patients and normal volunteers are presented.

Keyphrases □ Loxapine—and metabolites, GLC analysis in serum and urine □ Amoxapine—and metabolites, GLC analysis in serum and urine □ GLC—analysis, loxapine, amoxapine, and their metabolites in serum and urine □ Antipsychotics—loxapine and metabolites, GLC analysis in serum and urine □ Antidepressants—amoxapine and metabolites, GLC analysis in serum and urine

Loxapine[1] succinate, 2-chloro-11-(4-methyl-1-piperazinyl)dibenz[*b,f*][1,4]oxazepine succinate, is a new antipsychotic agent effective in the treatment of acute and chronic schizophrenia (1–5). It is the only member of the dibenzoxazepine class currently available in the United States. Amoxapine (*N*-desmethylloxapine), a new drug with antidepressant properties, is currently in advanced clinical evaluation. The metabolic pathways (Scheme I) for these drugs were investigated[2]. This paper describes methods for the quantitation of loxapine, amoxapine, and their 7- and 8-hydroxylated metabolites using GLC following trifluoroacetylation of the secondary amine functions and trimethylsilylation of the phenolic groups.

EXPERIMENTAL

Reagents—Ethyl acetate, ACS reagent grade, was passed through an alumina column or glass distilled immediately prior to use. Carbonate buffer, pH 9.7, was prepared by mixing nine parts of 1 *M* Na_2CO_3 with one part of 1 *M* $NaHCO_3$. Trifluoroacetic anhydride[3], *N*-trimethylsilyldiethylamine[3], β-glucuronidase[4], and 8-methoxyloxapine[5] were used as received.

Apparatus—The gas-liquid chromatograph[6] was equipped with a ^{63}Ni-constant current electron-capture detector and a flame-ionization detector. Glass-stoppered 15- and 5-ml centrifuge tubes were soaked overnight in a concentrated detergent solution and then washed in tap water. The washed tubes were immersed for a minimum of 3 hr in dichromate cleaning fluid and washed again with a final rinse in approximately 1 *M* NH_4OH, followed by three distilled water rinses.

Chromatographic Conditions—Columns were 1.8-m × 2- or 4-mm glass tubes packed with 3% SP 2100 on 100–120-mesh Supelcoport[7]. The temperatures were: oven, 255°; injection port, 280°; and detector, 300°. Argon–methane (95:5) was used as both the carrier and electron-capture detection gas for that mode of detection. Helium was the carrier gas in flame-ionization detection.

Procedure—For the analysis of serum, a sample (1–3 ml) was added to a 15-ml glass-stoppered tube, followed by 0.2 ml of a 500-ng/ml solution of 8-methoxyloxapine (internal standard), 1 ml of 1 *M* pH 9.7 carbonate buffer, and 5 ml of ethyl acetate. The tube was shaken and centrifuged. The organic layer was transferred to a clean 5-ml tube, 1 ml of 0.1 *N* HCl was added, and the contents were shaken and centrifuged. The ethyl acetate was aspirated, and clean ethyl acetate (4 ml) was added to wash the acid extract.

The organic solvent was aspirated, and fresh ethyl acetate (4 ml) was added along with 1 ml of pH 9.7 carbonate buffer. The tube was shaken and centrifuged, the ethyl acetate phase was transferred to a clean 5-ml centrifuge tube, and the solvent was removed by a nitrogen stream. A 0.1-ml aliquot of a solution containing two parts of trifluoroacetic anhydride and five parts of ethyl acetate was added, and the tube was permitted to stand 10 min.

The reagent was removed by evaporation with a nitrogen stream, and 20 μl of *N*-trimethylsilyldiethylamine was added to the residue. The tube was stoppered and heated for 3 hr at 60°. Alternatively, the tube was permitted to stand overnight at room temperature. A 1–2-μl aliquot was injected into the gas chromatograph, and peaks were detected by electron capture.

For urine analysis, a sample (2–5 ml) was added to a tube containing

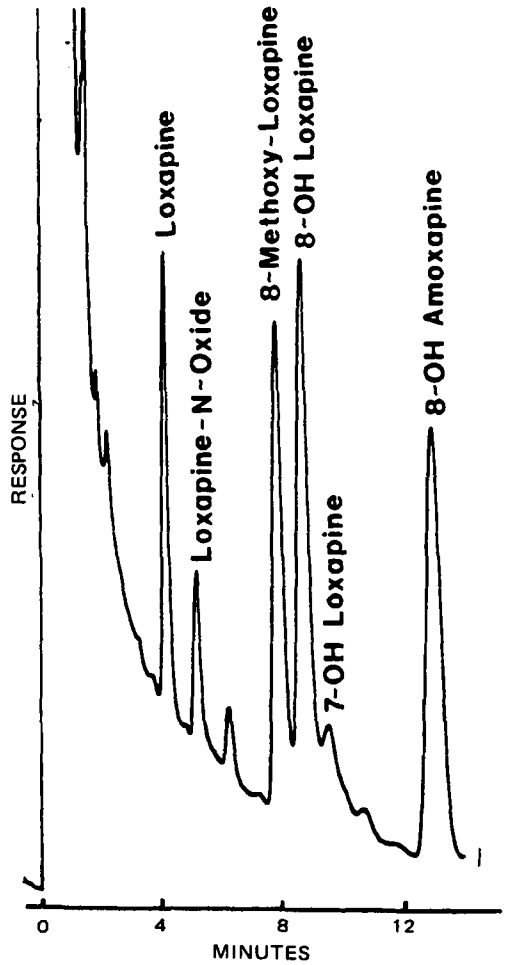

Figure 1—*Chromatogram of serum from a subject receiving loxapine intramuscularly.*

[1] Loxitane, Lederle Laboratories.
[2] Unpublished results.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Glusulase, Endo Laboratories, Garden City, N.Y.
[5] Supplied by Dr. R. A. Hardy, Jr., and Dr. C. F. Howell, Lederle Laboratories, Pearl River, N.Y.
[6] Either a model 900, Perkin Elmer, Norwalk, Conn., or a model 222, Tracor, Austin, Tex.
[7] Supelco, Bellefonte, Pa.

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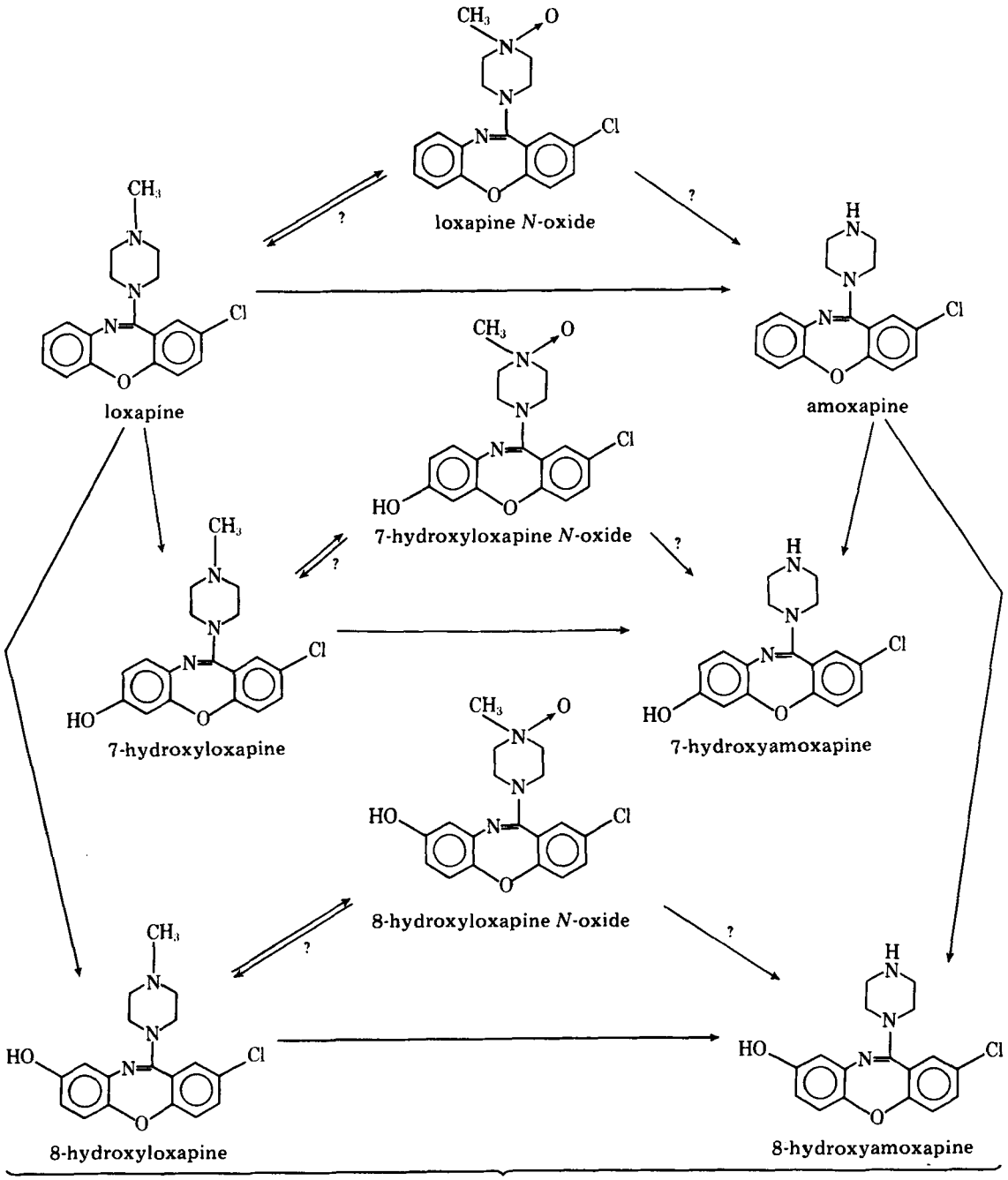
Table I—Precision Observed on Analysis of 10 Consecutive Standard Curves in Serum Containing Loxapine and Amoxapine Metabolites at the Concentration Indicated

Amount Added, ng/ml	Peak Height Ratio											
	Loxapine		Amoxapine		8-Hydroxyloxapine		7-Hydroxyloxapine		8-Hydroxyamoxapine		7-Hydroxy-amoxapine	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
5	0.128	0.0134	0.249	0.0444	0.132	0.0171	0.141	0.0154	0.275	0.0653	0.302	0.0482
10	0.268	0.0299	0.445	0.0870	0.277	0.0430	0.277	0.0377	0.524	0.0626	0.569	0.0521
15	0.422	0.0457	0.673	0.1298	0.446	0.0723	0.485	0.0415	0.790	0.1097	0.888	0.1083
20	0.585	0.0666	0.964	0.1377	0.604	0.0871	0.700	0.0511	1.056	0.1423	1.207	0.1370
25	0.750	0.1146	1.236	0.2230	0.779	0.1362	0.895	0.0808	1.320	0.3298	1.476	0.3057

10 μg of added 8-methoxyloxapine. (For analysis of urinary loxapine, 10 μg of amoxapine may be used as the internal standard.) The urine pH was adjusted to 5.0 ± 0.2 with 10% acetic acid. Glucuronidase solution, 0.5 ml, was added, and the sample was permitted to incubate for 48 hr at room temperature. A 1-ml aliquot of a 20-mg/ml solution of sodium hydrosulfite in 10% $NaHCO_3$ was added, and the pH was adjusted to 9.0–9.5 with 4 *N* NaOH.

The tube was heated in a boiling water bath for 10 min and cooled, and 6 ml of ethyl acetate was added. It was then stoppered, shaken, and centrifuged. The ethyl acetate phase was transferred to a clean tube, and



Scheme I

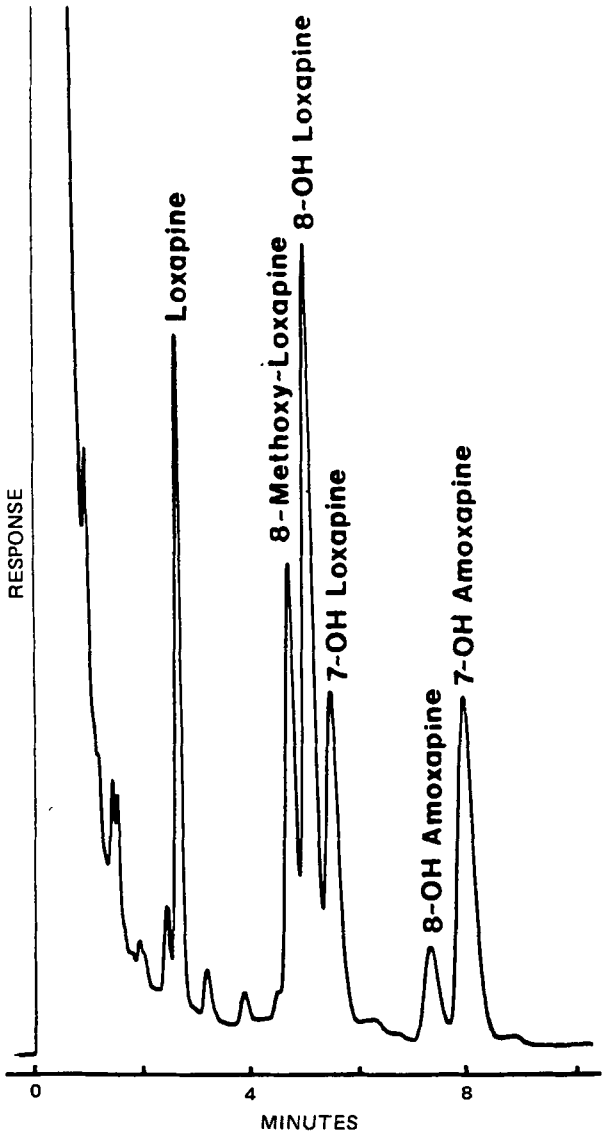


Figure 2—*Chromatogram of the 4–8-hr urine from a subject receiving a single 25-mg dose of loxapine.*

1 ml of 0.1 *N* HCl was added. The contents were mixed and centrifuged, and the ethyl acetate was aspirated. The tube was then covered with a 1.25-cm^2 piece of filter paper, held in place with a rubber band, and lyophilized.

The residue was reacted with a mixture of 0.5 ml of trifluoroacetic anhydride and 0.2 ml of dichloromethane for 30 min, after which the solvent was removed by evaporation. The acylated residue was dissolved in 50 μl of *N*-trimethylsilyldiethylamine and either heated for 3 hr at 60° or permitted to stand overnight at room temperature. A 2-μl aliquot was injected into the gas chromatograph, and peaks were detected by flame ionization.

Standards for analysis of loxapine and its metabolites in serum contained loxapine, 8-hydroxyloxapine, and 8-hydroxyamoxapine at concentrations ranging from 5 to 25 ng/ml. In urine, standards contained loxapine, 7-hydroxyloxapine, 8-hydroxyloxapine, 7-hydroxyamoxapine, and 8-hydroxyamoxapine at concentrations ranging from 5 to 25 μg/analytical sample. For amoxapine analysis, standards contained amoxapine, 7-hydroxyamoxapine, and 8-hydroxyamoxapine in both serum and urine. Serum standards ranged from 20 to 100 ng/ml, and urine standards ranged from 5 to 25 μg/sample.

RESULTS AND DISCUSSION

The chromatogram shown in Fig. 1 demonstrates the separation obtained on an extract of serum from a patient who had been receiving loxapine intramuscularly. A chromatogram from an amoxapine-dosed subject would have shown a peak at approximately 6 min corresponding to amoxapine and would not have peaks due to loxapine or 7- and 8-hydroxyloxapines. As is apparent, it is frequently difficult to measure 7-hydroxyloxapine in serum because of an overwhelmingly large amount of the 8-hydroxy analog. This problem did not occur with urine chromatograms (Fig. 2) where better separations were achieved with the helium carrier gas and where concentrations of these isomers were more nearly the same.

Table II—Plasma Loxapine Metabolites (Steady-State Levels) in 2-ml Aliquots of Serum Analyzed [a]

Patient	Dosage, mg/day	Loxapine, ng/ml	8-Hydroxy-loxapine, ng/ml	8-Hydroxy-amoxapine, ng/ml
1	20	7	18	20
2	25	0	11	11
3	30	1	16	6
4	30	8	12	11
5	40	2	24	14
6	50	1	22	16
7	50	11	36	18
8	60	9	29	23
9	70	4	34	23
10	70	4	37	28
11	80	17	88	29
12	80	7	33	16
13	100	31	72	64
14	100	6	41	31
15	120	2	16	7
16	120	12	40	33
17	150	2	6	4
18	200	12	42	28
19	200	8	47	94
20	250	73	97	114

[a] Samples were obtained just prior to next morning dose.

The precision of the reported procedure for serum analysis was estimated by preparing and running 10 consecutive standard curves containing both loxapine and amoxapine derivatives in serum (Table I). These data indicate a high reproducibility of the technique. An even higher reproducibility was achieved with urine standard curves.

The absolute recoveries of added loxapine, 8-hydroxyloxapine, and 8-hydroxyamoxapine from serum were estimated by comparing results from 10 serum samples to which these compounds had been added and that were carried through the analytical procedure without the internal standard with values from 10 samples prepared from the same stock solution in methanol that were simply derivatized after solvent removal. Extract transfers were measured accurately, and the same amount of the internal standard was added to all of the samples just prior to the trifluoroacetylation step. With an adjustment for extraction volumes, peak height ratio comparisons indicated the recovery of loxapine to be 70% (65–72%); that of 8-hydroxyloxapine was 90% (88–92%), and that of 8-hydroxyamoxapine was 86% (83–90%). These results indicate the desirability of preparing standards for serum analysis in serum.

Concentrations of loxapine, 8-hydroxyloxapine, and 8-hydroxyamoxapine found in the serum of normal volunteers after a single oral dose of 25 mg are illustrated by the curves shown in Fig. 3. Kinetic curves obtained after an amoxapine dosage of 50 mg are shown in Fig. 4. 7-Hydroxyamoxapine is not a metabolite of significance in serum following

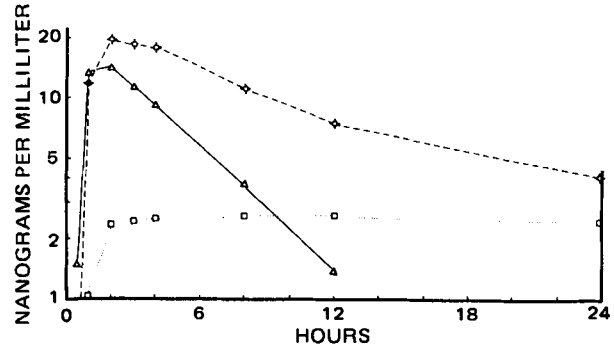


Figure 3—*Serum concentration-time curves of loxapine (△), 8-hydroxyloxapine (○), and 8-hydroxyamoxapine (□) after a single oral 25-mg dose of loxapine (mean values of 23 subjects).*

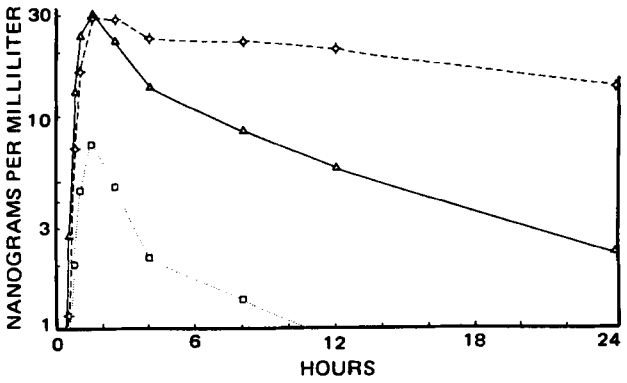


Figure 4—*Serum concentration–time curves of amoxapine (△), 7-hydroxyamoxapine (□), and 8-hydroxyamoxapine (○) after a single oral 50-mg dose of amoxapine (mean values of 26 subjects).*

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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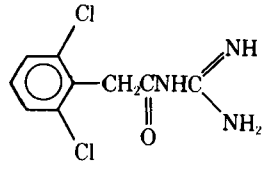
Abstract □ A quantitative electron-capture GLC method with good sensitivity is described for the determination of guanfacine in human plasma and urine. By condensing its amidino group with hexafluoroacetylacetone, guanfacine is converted to a pyrimidino derivative with better GLC properties than the 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.



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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

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[3] E. Merck A. G., Darmstadt, West Germany.
[4] Koch Light Laboratories, Colnbrook, England.
[5] J. T. Baker, Phillipsburg, N.J.
[6] Hewlett-Packard model 5713A.

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