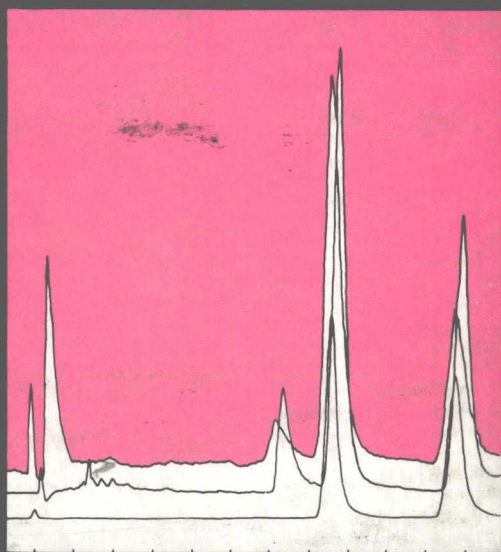


# Analysis of Drugs and Metabolites by Gas Chromatography- Mass Spectrometry

Volume 6

Cardiovascular, Antihypertensive,  
Hypoglycemic, and Thyroid-Related Agents

Benjamin J. Gudzinowicz  
Michael J. Gudzinowicz



# **ANALYSIS OF DRUGS AND METABOLITES BY GAS CHROMATOGRAPHY- MASS SPECTROMETRY**

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## **VOLUME 6**

**Cardiovascular, Antihypertensive, Hypoglycemic, and  
Thyroid-Related Agents**

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BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

VOLUME 1: Respiratory Gases, Volatile Anesthetics, Ethyl Alcohol,  
and Related Toxicological Materials

VOLUME 2: Hypnotics, Anticonvulsants, and Sedatives

VOLUME 3: Antipsychotic, Antiemetic, and Antidepressant Drugs

VOLUME 4: Central Nervous System Stimulants

VOLUME 5: Analgesics, Local Anesthetics, and Antibiotics

VOLUME 6: Cardiovascular, Antihypertensive, Hypoglycemic, and  
Thyroid-Related Agents

IN PREPARATION

VOLUME 7: Natural, Pyrolytic, and Metabolic Products of Tobacco and  
Marijuana

OTHER VOLUMES IN PREPARATION

Dedicated to

HELEN L. GUDZINOWICZ  
a devoted and understanding wife and mother

## PREFACE

In the past two decades, remarkable progress has been made in the analysis of drugs, pharmaceuticals, and related toxicological materials. In great measure, these notable advances can be attributed to technological advancements in two specific types or areas of analytical instrumentation; namely, gas chromatography and integrated gas chromatography-mass spectrometry.

Since James and Martin revealed to the scientific community their gas chromatographic technique which permitted the separation of fatty acid mixtures into their individual components, the rapid growth of gas chromatography has been very evident. This remarkable progress can be directly correlated with the improvements that we have witnessed over the years in gas chromatographic stationary phase, carrier gas, column, and temperature- and pressure-controlling technology. Furthermore, it has assumed a position of even greater analytical significance since the advent of highly specific, rapid, sensitive detection systems.

On the other hand, the integrated GC-MS analytical system is rather unique and exceptional in that it combines the mass spectrometer's unexcelled identification potential with the gas chromatograph's separation capabilities. Although the integration of GC and MS was first reported in 1957 by Holmes and Morrell, it nevertheless remained a dormant, costly, and seemingly unappreciated technique until 1970. Since then, with improved instrumentation at a more reasonable price and newly developed operating techniques, numerous publications have appeared in the literature showing its applicability to a wide variety of difficult analytical problems, thus opening up new horizons for analytical research in toxicology, biochemistry, pharmacology, forensics, medicine, etc. To be able to monitor a drug, its persistence and metabolic fate in biological fluids of man via mass fragmentography at picogram concentration levels provides the researcher with a tool of immeasurable significance.

Because much has been written over the years about the analysis of drugs and their metabolites by either or both techniques, the objectives of these volumes are several-fold: (1) to compile from existing literature in a chronological manner the various GC and/or GC-MS procedures available for the analysis of specific drugs and their metabolites, (2) to describe with as much detail as possible all procedures (qualitative and quantitative) in order that they might be reproduced faithfully in one's laboratory, and (3) to indicate, wherever possible, not only the results, precision, accuracy, and limits of detection achieved by a given procedure, but also its applicability to pharmacokinetic studies. For this reason, in addition to the text, which is well referenced in each section, many illustrations of actual applications and tables of data for each instrumental technique are included as aids to the analyst for his greater appreciation and understanding of the limitations as well as potentials ascribed to each method. As stated in the past, from an analytical chemist's point of view, it is hoped that this deliberately combined visual and factual approach will find acceptance by the reader who would otherwise rely only on his interpretation of the written word relative to some published procedure.

Without wishing to be repetitious, in retrospect it must be again stated that this volume really represents the end result of many tedious and arduous investigations by numerous eminent scientists whose research efforts have appeared in the literature throughout the world. We are indeed humbly indebted to them, and to those journals, publishers, and organizations that granted special copyright permission to the authors.

Benjamin J. Gudzinowicz

Michael J. Gudzinowicz



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## Chapter 1

### CARDIOVASCULAR DRUGS

In this chapter, drugs with the ability to alter cardiovascular function will be considered: digitalis-type glycosides, antiarrhythmic, antianginal, anticoagulant, and diuretic drugs, as well as pharmacological approaches to atherosclerosis using agents aimed at altering hyperlipidemia.

#### I. DIGITALIS-TYPE GLYCOSIDES

As noted by Goth [1]:

Certain steroids and their glycosides have characteristic effects on the contractility and electrophysiology of the heart. Most of these glycosides are obtained from the leaves of the foxglove, Digitalis purpurea or Digitalis lanata, or from the seeds of Strophanthus gratus. These cardioactive steroids [Fig. 1.1] are widely used in the treatment of heart failure and in the management of certain arrhythmias. They are collectively referred to as digitalis.

Although catecholamines, methylxanthines, and glucagon also increase the contractility of the myocardium, digitalis must accomplish its effect by a unique mechanism and is by far the most important drug in the treatment of heart failure.

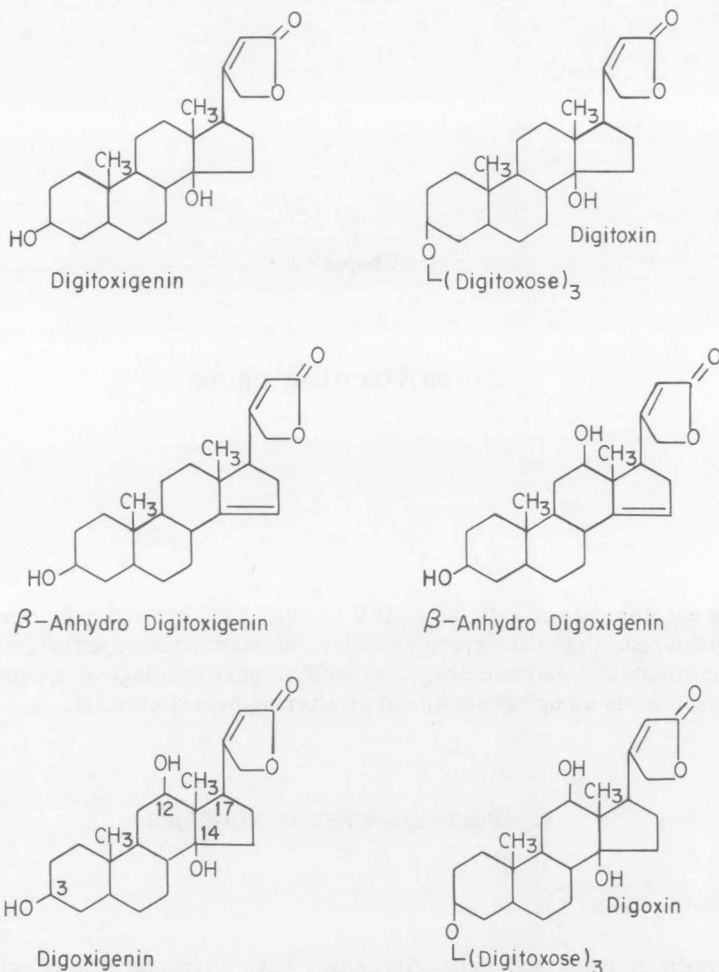


Figure 1.1. Structure of cardiac glycosides and related compounds.

In recent years various aspects of the pharmacokinetics of drugs have appeared in the literature and, in this regard, Chow and Ronfeld [2] compiled data for antibiotics and antiarrhythmics which included percent of drug absorbed intact, time of peak concentration, volume of distribution, elimination half-life ( $\beta$  phase), percent of drug excreted unchanged, renal clearance, and plasma binding. Such information for digitoxin and digoxin are as shown in Table 1.1.

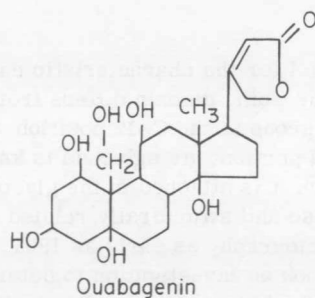
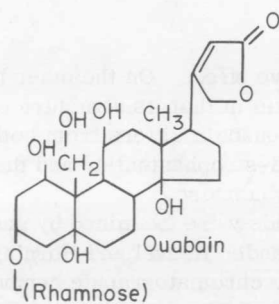
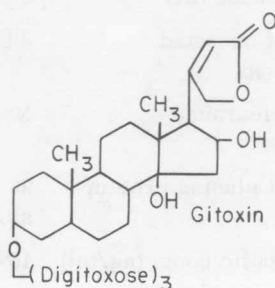
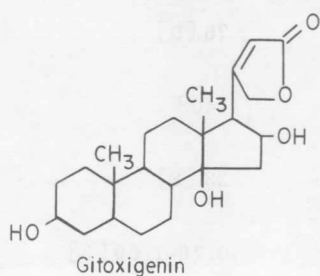
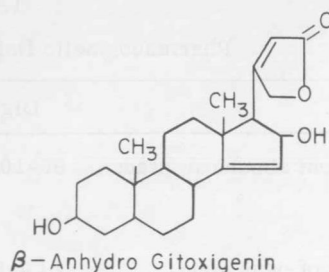
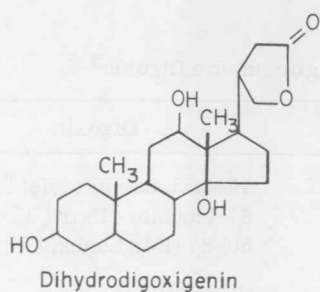


Figure 1.1. (continued)

Of the most commonly used glycosides, the structure of digitoxin is characterized by a steroid nucleus with an unsaturated lactone attached in the C-17 position. The three sugars attached to the C-3 position are unusual 20-deoxyhexoses. The molecule without the sugars is called an aglycone or genin. The steroidal structure and the unsaturated lactone are

TABLE 1. 1  
Pharmacokinetic Data for Digitoxin and Digoxin<sup>a</sup>

	Digitoxin	Digoxin
Percent absorbed intact	90-100 [3]	45-56 Lanoxin tablet [9, 10] 67 Lanoxin elixir [9] 80-83 (IM) Lanoxin
Time of peak conc. (hr)	1 [4, 5]	0.5-0.75 [9] 1-2 (IM) [9, 11]
Volume of distribution $V_d$	35 liters <sup>b</sup> [6]	580 liters [12]
$t_{1/2}$ $\beta$ phase (hr)	6 days [3, 7]	36 [13]
Percent excreted unchanged	8 [6]	76 [9]
Renal clearance (ml/min)	Negligible	140 <sup>b</sup>
Percent plasma protein binding	96 (bound to albumin) [8]	23 [8]
Therapeutic conc. (ng/ml)	15-20 [7]	0.76-1.60 [3]

<sup>a</sup>Adapted from Chow and Ronfeld [2].

<sup>b</sup>Calculated value.

essential for the characteristic cardioactive effect. On the other hand, as noted by Goth, digoxin differs from digitoxin in that its structure contains an OH group at the C-12 position whereas ouabain differs from both in its steroid portion; its aglycone is known as G-strophanthidin, and the sugar to which it is attached in the glycoside is rhamnose.

These and structurally related compounds were examined by gas chromatography as early as 1961, when Vanden Heuvel and Horning [14] undertook an investigation to determine the chromatographic behavior of the sapogenins, which are naturally occurring steroids that, in addition to a spiroketal system, have a varying degree of substitution with keto and hydroxyl groups.

With an argon ionization detector and a 6-ft by 5-mm column packed with 0.75% SE-30 on 100-140 mesh Gas Chrom P and operated isothermally at 225°C, the relative retention times of the compounds investigated all yielded single, well-defined peaks. The times are listed in Table 1.2.

Several correlations between structure and relative retention times

TABLE 1.2  
Relative Retention Times of Various Sapogenins<sup>a, b</sup>

Compound	C <sub>25</sub> <sup>c</sup>	C <sub>5</sub> <sup>d</sup>	Substituents	Time <sup>e</sup>
Sarsasapogenin	Neo	$\beta$	3 $\beta$ -OH	2.57
Smilagenin	Iso	$\beta$	3 $\beta$ -OH	2.47
Yamogenin	Neo	$\Delta^5$	3 $\beta$ -OH	2.66
Diosgenin	Iso	$\Delta^5$	3 $\beta$ -OH	2.64
Tigogenin	Iso	$\alpha$	3 $\beta$ -OH	2.71
Yuccagenin	Iso	$\Delta^5$	2 $\alpha$ , 3 $\beta$ -(OH) <sub>2</sub>	4.68
Gitogenin	Iso	$\alpha$	2 $\alpha$ , 3 $\beta$ -(OH) <sub>2</sub>	4.81
Chlorogenin	Iso	$\alpha$	3 $\beta$ , 6 $\alpha$ -(OH) <sub>2</sub>	5.40
Hecogenin	Iso	$\alpha$	3 $\beta$ -OH 12-Keto	4.96
Mexogenin	Iso	$\beta$	2 $\beta$ , 3 $\beta$ -(OH) <sub>2</sub> 12-Keto	7.82
Manogenin	Iso	$\alpha$	2 $\alpha$ , 3 $\beta$ -(OH) <sub>2</sub> 12-Keto	8.76
Kammogenin	Iso	$\Delta^5$	2 $\alpha$ , 3 $\beta$ -(OH) <sub>2</sub> 12-Keto	8.33
Cholestane				1.00 <sup>f</sup>

<sup>a</sup>From Vanden Heuvel and Horning [14], courtesy of the Journal of Organic Chemistry.

<sup>b</sup>Conditions: Column, 6 ft x 5 mm; 0.75% SE-30 polymer on 100/140 mesh Gas Chrom P; 225°C; 14 psi; argon ionization detector.

<sup>c</sup>The configurations are 25L or neo and 25D or iso.

<sup>d</sup>The notation refers to 5-H.

<sup>e</sup>Relative to cholestane.

<sup>f</sup>Time, 7.0 min.

were reported by Vanden Heuvel and Horning, based on the chromatographic retention time data. For example, they noted that:

1. An additional hydroxyl or carbonyl incorporated into the structure led to a very large increase in retention time.



2. Compounds with differing ring A/B relationship could be resolved (tigogenin and smilagenin).
3. Compounds with a  $\Delta^5$  structure gave retention times which differed from those noted for their corresponding saturated compounds.
4. A relatively small effect due to a change in configuration of the methyl group at C-25 was noted; this effect paralleled their observations on the behavior of C-25 epimers in the steroidal amine series.

Since a number of steroid hormones are synthesized from sapogenins, the investigators postulated that these steroid transformation reactions could be monitored by gas chromatography.

Since the determination of the urinary excretion of digitalis compounds can improve dose control of digitalis in difficult clinical situations [15] and the aglycone moieties of digitalis compounds are steroids as evidenced by the structures of digitoxigenin and digoxigenin, Jelliffe and Blankenhorn [16] investigated their gas chromatographic behavior with a Barber Colman model 10 gas chromatograph equipped with an argon ionization detector and a 12-ft by 4-mm-i.d. glass column packed with 0.75% SE-30 coated onto 100-140 mesh, silanized Gas Chrom P. Whereas the free hydroxy compounds could not be chromatographed directly, trimethylsilyl (TMSi) ether derivatives of digitoxin, digoxin, digitoxigenin, and digoxigenin were prepared with hexamethyldisilazane and trimethylchlorosilane added to catalyze the reaction



The digitoxigenin-TMSi and digoxigenin-TMSi derivatives (shown below) yielded single, reproducible, symmetrical peaks with the following operating parameters: column temperature, 228°C; detector temperature, 220°C; flash heater temperature, 348°C; detector voltage, 1000 V; argon carrier-gas flow rate, 104 ml/min; electrometer gain,  $10^{-9}$  A. The retention times for cholestane (reference marker), digitoxigenin-TMSi, and digoxigenin-TMSi were 6.2, 37.5, and 47.5 min, respectively. However, no useful peaks were obtained for digitoxin, digoxin, and digoxigenin.

In 1967, Wilson, Johnson, Perkins, and Ripley [17] chromatographed on a single column the TMSi ether derivatives of digoxigenin, digoxigenin monodigitoxoside, digoxigenin bisdigitoxoside, and digoxin as well as the free sterols digoxigenin, digitoxigenin, and gitoxigenin.

The aglycones of digoxin and digitoxin were determined quantitatively by hydrolyzing the glycosides in a dilute hydrochloric acid-dioxane solution followed by GC analysis of the TMSi derivatives of the resulting sterols. In their procedure, the TMSi derivatives were prepared by adding 1 ml of silanizing mixture (hexamethyldisilazane-trimethylchlorosilane-pyridine,