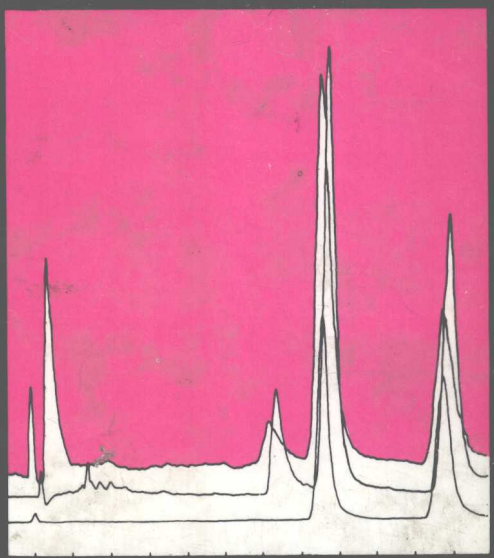
Analysis of Drugs and Metabolites by Gas Chromatography-Mass Spectrometry

Volume 4

Central Nervous System Stimulants



Benjamin J. Gudzinowicz
Michael J. Gudzinowicz

ANALYSIS OF DRUGS AND METABOLITES BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

VOLUME 4

Central Nervous System Stimulants

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ANALYSIS OF DRUGS AND METABOLITES
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

IN PREPARATION

VOLUME 5: Analgesics, Local Anesthetics, and Antibiotics

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

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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OTHER VOLUMES IN PREPARATION

CONTENTS

Chapter 1

AMPHETAMINES, XANTHINES, AND RELATED COMPOUNDS

I. INTRODUCTION

Although the mechanism of action of central nervous system (CNS) stimulants is largely unknown, a similar action may also be observed with other drugs that stimulate peripheral sensory receptors or indirectly block central inhibitory mechanisms. As noted by Bowman, Rand, and West [1], CNS stimulants may be classified into three major groups depending on the type of stimulation produced and the region of the CNS affected by the smallest effective doses. They categorized these groups as being (1) "psychomotor stimulants," which affect the higher levers of the cerebral cortex (producing increased mental and motor activity), (2) "analeptics," which act primarily on the nerve cells in the midbrain and medulla both to stimulate the respiratory and vasomotor centers in the brain and to antagonize the respiratory depressant action of other drugs, or (3) "spinal cord stimulants."

As pointed out by Goth [2] in his discussion of the general aspects of neuropharmacology, there are many neuropharmacologic agents that:

> ...can be categorized as mimicking or opposing the actions of acetylcholine or norepinephrine. While the terms cholinergic and adrenergic are reserved by tradition for nerves rather than drugs, they can also be used as synonyms for cholinomimetic and sympathomimetic,

> respectively. Thus, in dealing with autonomic nervous system drugs, one may speak of (1) cholinergic (cholinomimetic) drugs, (2) adrenergic (sympathomimetic) drugs, (3) anticholinergic drugs (various drugs) and (4) adrenergic blocking agents. . . . The sympathetic nervous system performs its homeostatic function by releasing norepinephrine at adrenergic nerve endings and epinephrine in the adrenal medulla. Drugs that act directly on adrenergic receptors, or release mediators which then act on the receptors, may be termed sympathomimetic or adrenergic drugs.

In recent years, the mode of sympathomimetic action has been described by two concepts: (1) There are at least two types of receptors, these being designated α-receptors and β-receptors [1]. As emphasized by Innes and Nickerson [3], "in general, the effect of drugs on alpha receptors is excitatory whereas that on beta receptors is inhibitory, although this is by no means an absolute rule. The concept of α- and β-receptor sites simplifies the classification of both sympathomimetic drugs and adrenergic blocking agents. So selective are these blocking agents that it has become customary to identify the type of epinephrine receptors in a tissue on the basis of the effect of the adrenergic blocking agents on the responses to sympathomimetic amines." (2) The actions of sympathomimetic agents are receptor-dependent as well as being influenced by the presence of norepinephrine (noradrenaline) storage sites in the adrenergic neurones.

In their general discussion of drugs acting on postganglionic adrenergic nerve endings and structures innervated by them (sympathomimetic drugs), Innes and Nickerson classified the adrenergic drugs by their actions:

> (1) a peripheral excitatory action on certain types of smooth muscle, such as those in blood vessels supplying skin and mucuous membranes, and also on salivary and certain sweat glands; (2) a peripheral inhibitory action on certain other types of smooth muscle, such as those in the wall of the gut, in the bronchial tree, and in blood vessels supplying skeletal muscle; (3) a cardiac excitatory action, responsible for an increase in heart rate and force of contraction; (4) metabolic actions, such as an increase in the rate of glycogenolysis in liver and muscle, and liberation of free fatty acids from adipose tissue; and (5) CNS excitatory actions, such as respiratory stimulation and, with some of the drugs, an increase in wakefulness and a reduction in appetite.

In this chapter, some of the more predominant CNS stimulants (psychoactivators and appetite-suppressant drugs such as amphetamine, methamphetamine, phentermine, diethylpropion, caffeine, nikethamide, pentylenetetrazol, etc.) will be discussed, whereas Chapter 2 will deal entirely

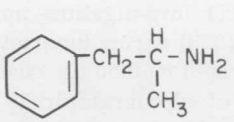
with several species of compounds, namely, those related to catecholamines, phenethylamine species, tryptamine-indole bases, and amines of biological significance, as well as β-adrenergic blocking agents (practolol, propranolol, etc.) and related compounds.

II. AMPHETAMINES AND RELATED COMPOUNDS

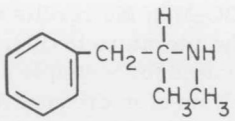
Unlike some hallucinogenics, amphetamines are synthetic drugs used to treat mild depression, obesity, narcolepsy (a tendency to fall asleep at any time), and certain behavioral disorders in children. The most commonly available amphetamines are amphetamine (racemic β-phenylisopropylamine), dextroamphetamine, and methamphetamine (dextro-desoxyephedrine).

As noted by Cohen [4] in 1970, three fairly distinct patterns of amphetamine abuse can be identified. Included in the first category, a relatively minor form of abuse

> the occasional ingestion of an average dose by people who need a temporary lift. Falling into this category are students cramming for an exam, overtired businessmen, or athletes who need a temporary boost to their endurance. Amphetamines are allegedly available at certain truck stops under names such as "Los Angeles turnarounds." These are said to allow a driver to go from New York to Los Angeles and back again without sleeping, as long as he continues to take his pills.
>
> A second form of abuse consists of swallowing 50 to 75 mg per day without medical supervision. A person in this second category might be an obesity patient who continues to take diet pills primarily for the lift they give, long after medication has been discontinued by a doctor.
>
> The third form of abuse has been on the upswing in recent years. Intravenous injection of hundreds of milligrams of amphetamines in a single dose-"shooting speed"-is probably the most widespread abuse of amphetamine in this country.



Amphetamine



Methamphetamine

In addition to the structures given for amphetamine and methamphetamine, many amphetamine analogs have been synthesized; some of these are shown in Table 1.1. As for structure-activity relationships as reviewed by Biel [6], Caldwell and Sever [7] summarize these findings as follows:

1. Excluding methamphetamine (MAMP), side-chain or amino group alkylation diminishes the activity of amphetamine (AMP).
2. Diminished anorectic activity and CNS stimulant effect are abolished and markedly diminished, respectively, by ring hydroxylation.
3. Both effects noted in (2) are decreased by side-chain hydroxylation.
4. Introduction of methoxy groups into the ring gives the molecule hallucinogenic properties.
5. CNS stimulant action is reduced by methylation of the side-chain carbon atom to which is attached the amino group (the basis of the phentermine series which will be discussed subsequently) and by N-alkylation with large groups.

On the other hand, the various metabolic pathways for amphetamine are shown in Figure 1.1; these include N-hydroxylation (N-hydroxy-amphetamine), side-chain hydroxylation (norephedrine), ring hydroxylation (p-hydroxy-amphetamine), both side-chain and ring hydroxylation (p-hydroxy-norephedrine, where the β-hydroxylation is carried out by dopamine-β-hydroxylase).

Up to 50% of the amphetamines may be eliminated via the urine route in unchanged form, this elimination route being highly dependent upon pH.

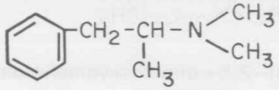
Acting upon postganglionic endings of adrenergic nerves or organs supplied by these nerves, amphetamines share some characteristic actions of biogenic amines--epinephrine, for instance--on the peripheral nervous systems, but they are much more powerful stimulants of the central nervous system [4].

In the 1960s, in keeping with the escalation of all types of drug abuse, amphetamines became a major problem which subsequently led to a greater in-depth study of their side effects in humans as well as the development of analytical methods (based on gas chromatography, mass spectrometry, or integrated GC-MS); the results of such investigations appearing more frequently in the literature [8-153, 237, 325] during the past decade.

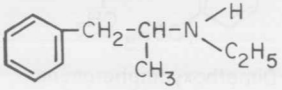
In 1962, the need for a simple and rapid method for quantitative and qualitative analysis of microquantities of adrenergic drugs in pharmacy, pharmacology, and toxicology led to the development of a gas chromatographic technique by Brochmann-Hanssen and Svendsen [8], who first reported the separation and identification of 11 sympathomimetics at temperatures ranging from 104°C for the nonphenolic to 135°C for the phenolic

TABLE 1.1

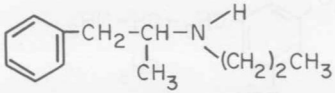
Structures of Amphetamine-type Compounds



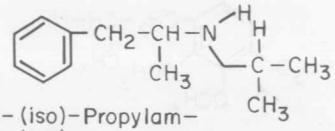
N-Dimethylamphetamine



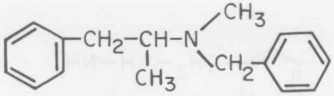
N-Ethylamphetamine



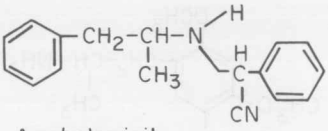
N-(n)-Propylamphetamine



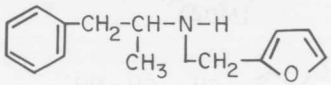
N-(iso)-Propylamphetamine



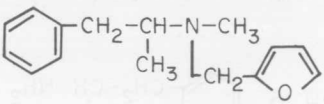
N-Benzylmethamphetamine
(Benzphetamine)



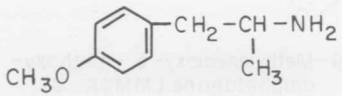
Amphetaminil



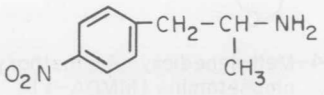
N-Furfurylamphetamine



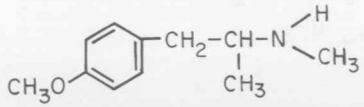
N-Furfurylmethamphetamine



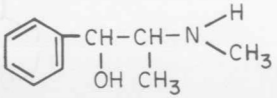
p-Methoxyamphetamine



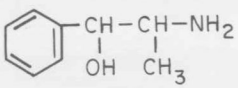
p-Nitroamphetamine



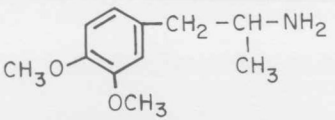
p-Methoxymethamphetamine



Ephedrine

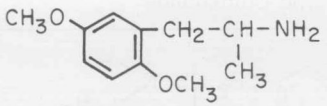


Norephedrine

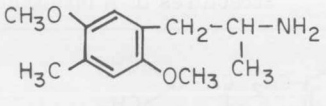


3,4-Dimethoxyamphetamine

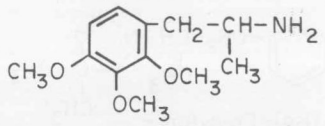
TABLE 1.1 (continued)



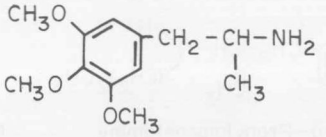
2,5-Dimethoxyamphetamine



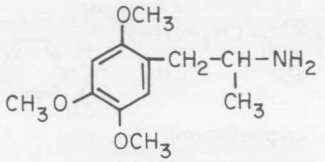
4-Methyl-2,5-dimethoxyamphetamine (STP)



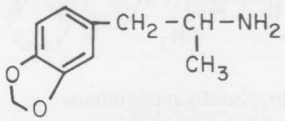
2,3,4,-Trimethoxyamphetamine



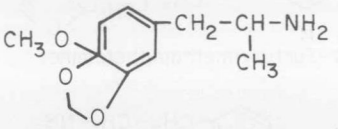
3,4,5-Trimethoxyamphetamine



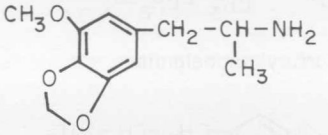
3,4,6-Trimethoxyamphetamine



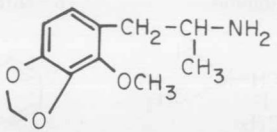
3,4-Methylenedioxyamphetamine (MDA)



3,4-Methylenedioxy-4-methoxy-amphetamine (MMDA-1)



3,4-Methylenedioxy-5-methoxy-amphetamine (MMDA-2)



3,4-Methylenedioxy-2-methoxy-amphetamine (MMDA-3)