

# Analysis of Anabolic Steroids Using GC/MS with Selected Ion Monitoring

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## Abstract

This study describes the use of gas chromatography/mass spectrometry with selected ion monitoring to screen 18 anabolic steroids banned by the International Olympic Committee. These anabolic steroids are analyzed in two fractions depending on their excretion pattern: nonconjugated (free) or conjugated fraction. The wet procedure of extracting steroids from urine consists of an initial isolation of lipophilic compounds on a column packed with Amberlite XAD-2 resin, followed by enzymatic hydrolysis with  $\beta$ -glucuronidase from *Escherichia coli*. After extraction, the hydrolyzed steroids are derivatized to the corresponding trimethylsilyl ethers. The derivatized steroids are analyzed by gas chromatography/mass spectrometry with selected ion monitoring of their characteristic ions. It takes 12 and 26 min to run GC/MS and edit the raw data for nonconjugated and conjugated fractions respectively.

## Introduction

In 1974, steroids were added to the list of doping agents banned by the International Olympic Committee on the grounds that drug abuse is against the Olympic spirit based on fair play, and also it is known that steroid abuse will harm the well-being of athletes. Nevertheless, it is suspected that the use of steroids has been steadily increasing among the players, who believe that the use of steroids improves their physical strength remarkably over a short period of time and who are willing to barter their health for fame and monetary gain. Precise and rapid determination of anabolic steroids and their metabolites continues to be of interest.

Some steroids can be analyzed with high-performance liquid chromatography (HPLC) (1-5), and it has been possible to detect 17 $\alpha$ -methyl, 17 $\alpha$ -ethyl, and 19-nortestosterone steroids by radioimmunoassay (RIA) despite the method's low specificity (6-9). However, both of these methods cannot provide the data necessary to confirm a presumptive positive sample. Therefore, gas chromatography/mass spectrometry (GC/MS) with high sensitivity and specificity has been regarded as the most reliable technique so far. In many cases, GC/MS has been used to

analyze the steroids either qualitatively or quantitatively (10-23).

Anabolic steroids are extensively metabolized: hydroxylation, reduction, oxidation, and conjugation are common metabolic pathways of anabolic steroids in humans. As far as conjugation patterns are concerned, steroids can be divided into two groups, nonconjugated (free) steroid and conjugated steroid. Dosić reported that methandienone, oxandrolone, fluoxymesterone, stanozolol, dehydrochloromethyltestosterone, and formyldecaalone can be detected somewhat easily in the nonconjugated fraction. These anabolic steroids have a 17 $\beta$ -hydroxy-17 $\alpha$ -methyl group in their structure (Figure 1). The other anabolic steroids whose structures are shown in Figure 2 are excreted mainly as glucuronides.

## Materials

**Reagents.** Amberlite XAD-2 resin, 150-200  $\mu$ m, from Serva was used. Methanol (500 mL) was distilled at 64°C; the first 50 mL was discarded and the next 300 mL was collected. The phosphate buffer, pH 7.0, was 250 mL of 0.2M  $K_2HPO_4$  mixed with 50 mL of 0.2M  $KH_2PO_4$ . Diethylether was distilled with calcium hydride and stored in a refrigerator at ca. 1°C.  $\beta$ -Glucuronidase from *Escherichia coli* was from Boehringer. *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA), trimethylchlorosilane (TMSi), and trimethylchlorosilane (TMSCl) were from Sigma, and *N*-methyl-*N*-trimethylsilyl heptafluorobutyramide (MSHFB) and *N*-methyl-bisheptafluorobutyramide were from Macherey-Nagel.

**Instrumentation.** Analyses were performed with a Hewlett-Packard Model 5890A gas chromatograph and Model 5970B mass selective detector.

**GC/MS operating conditions for nonconjugated fraction.** A cross-linked 5% phenylmethylsilicone capillary column (length 17 m, i.d. 0.2 mm, film thickness 0.33  $\mu$ m) was connected into the ion source. Samples were injected in the splitless mode. Temperatures of injector and transfer line were set at 300°C. Oven temperature was initially 180°C, ramped by 25°C/min to 300°C, and held for 3 min. The carrier gas was helium at a flow rate of 0.7 mL/min (at 180°C).

**GC/MS operating conditions for conjugated fraction.** The GC was equipped with a 17-m HP-1 fused-silica capillary column, 0.2 mm i.d., 0.11- $\mu$ m film thickness. The oven temperature was

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# Research On Steroids Volume 8

**E. Ronald de Kloet**



## **Research On Steroids Volume 8:**

**Report of the Hypertension Task Force: Current research and recommendations from the task force subgroups on pediatrics, genetics** National Heart, Lung, and Blood Institute. Hypertension Task Force, 1979 Abstract The current state of hypertension research is reviewed focusing on 12 special hypertension concerns and covering each concern with the following an evaluation of the current literature recommendations for future research and a comprehensive list of references Volume 1 written for the public examines hypertension as both a public health and a research problem and Volume 2 written for the biomedical community explores general recommendations and summaries of scientific reports are covered in volumes 3 through 9 *Quantitative Analysis of Steroids* S. Görög, 2012-12-02 Studies in Analytical Chemistry Volume 5 *Quantitative Analysis of Steroids* covers the pharmaceutical aspects of the analysis of steroid hormones This book is divided into nine chapters that examine the biological clinical analysis of other important groups of steroids including sterols vitamin D bile acids cardiac glycosides and sapogenins The material in the book is classified according to the main groups of steroids individual chapters being devoted to sex hormones corticosteroids sterols vitamins D bile acids cardiac glycosides sapogenins and miscellaneous steroids The structure of each chapter is the same In section 1 the fundamentals of the chemistry of the group of steroids in question are outlined Section 2 provides a detailed description of the use of various spectroscopic chromatographic protein binding and other methods while Section 3 deals with the main problems encountered in the analysis of the group of steroids in question and with their solution using the methods described in Section 2 This book will prove useful to steroid and analytical chemists **Terpenoids and Steroids, Volume 4, A Review of the Literature Published Between September 1972 and August 1973** K. H. Overton, 1974 This product is not available separately it is only sold as part of a set There are 750 products in the set and these are all sold as one entity Specialist Periodical Reports provide systematic and detailed review coverage of progress in the major areas of chemical research Written by experts in their specialist fields the series creates a unique service for the active research chemist supplying regular critical in depth accounts of progress in particular areas of chemistry For over 90 years The Royal Society of Chemistry and its predecessor the Chemical Society have been publishing reports charting developments in chemistry which originally took the form of Annual Reports However by 1967 the whole spectrum of chemistry could no longer be contained within one volume and the series Specialist Periodical Reports was born The Annual Reports themselves still existed but were divided into two and subsequently three volumes covering Inorganic Organic and Physical Chemistry For more general coverage of the highlights in chemistry they remain a must Since that time the SPR series has altered according to the fluctuating degree of activity in various fields of chemistry Some titles have remained unchanged while others have altered their emphasis along with their titles some have been combined under a new name whereas others have had to be discontinued The current list of Specialist Periodical Reports can be seen on the inside flap of this volume

**Neurobiology of Steroids** E. Ronald de Kloet, 2013-10-22 Steroid hormones are unique compounds in that they are active at the interface of peripheral endocrine events and neural mechanisms. Thus their effects present an important peripheral signaling system to alter brain function. This volume presents state of the art and classical techniques for the study of steroid hormones and their receptors and their effects and actions. Comprehensive protocols included for the study of Steroid kinetics and metabolism. Steroid receptors. Molecular and cellular effects of steroids. Steroid effects on integrated systems.

**Steroids** Hassan Abduljabbar, 2012-01-11 This book explains the basic science of steroids and is targeted towards professionals engaged in health services. It should be noted that medical science evolves rapidly and some information like the understanding of steroids and their therapeutic use may change with new concepts quickly. Steroids are either naturally occurring or synthetic fat soluble organic compounds. They are found in plants, animals and fungi. They mediate a very diverse set of biological responses. The most widespread steroid in the body is cholesterol, an essential component of cell membranes and the starting point for the synthesis of other steroids. Since the science of steroids has an enormous scope, we decided to put the clinical aspects of steroids in a different book titled *Steroids: Clinical Aspects*. The two books complete each other. We hope that the reader will gain valuable information from both books and enrich their knowledge about this fascinating topic.

**Molecular Structure and Biological Activity of Steroids** Martin Bohl, 2018-01-18 *Molecular Structure and Biological Activity of Steroids* focuses on the identification of steroid structural features that control particular biological effects. Steroid geometry plays a critical role in steroid activity; therefore the results of X-ray crystallographic, NMR spectroscopic and theoretical studies on structure-activity relationships form an integral part of the book. Well-established observations and proposed models are presented, summarizing knowledge of molecular features, e.g. steroid backbone structures, side chain constitutions, substitution sites, configurations, hydrogen bonding patterns that can affect steroid action. The book also provides overviews about recent progress in various frontier fields of steroid research, including such areas as sex steroids, glucocorticoids, aromatase inhibitors, vitamin D metabolites, brassinosteroids, neuromuscular blocking agents and cardiotonic steroids. *Molecular Structure and Biological Activity of Steroids* is a must-have resource for biochemists, pharmacologists, endocrinologists, biophysicists and others actively involved in steroid research.

**Testicular Tangrams** Focko Rommerts, K.J. Teerds, 2002-04-03 During the past two decades, research on the testis has created a vast number of publications. New techniques such as cell and gene cloning, germ cell manipulation and transplantation as well as gene expression have surpassed the classical approaches that involved studies on receptor activation and endocrine, paracrine and autocrine regulatory mechanisms. Due to the expansion of this field of research, it has become increasingly difficult to integrate the large number of observations and relate them to the physiology and pathophysiology of the testis. More emphasis must therefore be put on the integration of isolated findings and the construction of the total picture. In order to stimulate this specific mental activity, we have named this book *Testicular Tan*

gram We hope that the chapters in this book will be used as pieces of the testicular puzzle and that the reader enjoys composing new shapes The chapters of this book are representative of the plenary lectures presented at the 1<sup>st</sup> European Workshop on Molecular and Cellular Endocrinology of the Testis held in Doorwerth The Netherlands from April 6-10 2002 Publication of this book has been made possible by the generous support of the Ernst Schering Research Foundation We would also like to thank the distinguished scientists who have provided us with their excellent manuscripts well before the start of the Workshop We acknowledge the help of Dr U F Habenicht Dr M *Research Grants Index* National Institutes of Health (U.S.). Division of Research Grants,1972 **National Library of Medicine Current Catalog** National Library of Medicine (U.S.),1985 Current Catalog National Library of Medicine (U.S.),1985 First multi year cumulation covers six years 1965-70 **Small Molecule – Protein Interactions** Herbert Waldmann,Marcus Koppitz,2003-03-07 Based on the international workshop on Small Molecule Protein Interactions held in Berlin April 24-26 2002 researchers from industry and academic laboratories describe novel and efficient ways selecting promising new drug targets and developing small molecule inhibitors against them The structure of the book corresponds to the different aspects of the drug discovery process All chapters are written by leading experts in the field who present and discuss the most recent state of the art tools and techniques for the development of novel drugs The value of the book lies in surveying and summarizing the approaches taken by different companies and institutions giving the reader a balanced view on the use of the latest techniques on the one hand and experience based assistance in selecting appropriate tools for their own work on the other hand **The Role of Natural Products in Drug Discovery** J. Mulzer,R. Bohlmann,2013-04-17 Natural Products have been important sources of useful drugs from prehistoric times to the present This book gives an overview about this field and provides important recent contributions to the discovery of new drugs generated by research on natural products Total synthesis of natural products with interesting biological activities is paving the way for the preparation of new and improved analogs The methods of combinatorial chemistry permit the selection of the best drug from a large number of candidates Beyond synthesis and evaluation of organic molecules a number of new bioorganic methods are coming to the fore and will be discussed in this issue of the Ernst Schering Research Foundation workshop proceedings Gene Therapy Robert E. Sobol,Kevin J. Scanlon,Eirik Nestaas,2013-11-11 This book is a collection of preclinical and clinical reports on the application of gene therapy to human disease The two methods available for delivering therapeutic genes to diseased cells viral and non viral vector systems are detailed and characterized Several reports describe both approaches currently used in gene therapy the introduction of a wild type gene to restore normal gene function in diseased cells and the use of antisense molecules to hinder abnormal gene expression Clinical studies detail several different strategies for the treatment of cancer and cardiovascular diseases using genetic material as therapeutic agents The regulatory issues governing the use of gene therapy in Europe and the United States are also presented This book highlights the range of applications and demonstrates the rapid progress being made in

the field of gene therapy      **Biocombinatorial Approaches for Drug Finding** W. Wohlleben, T. Spellig, B. Müller-Tiemann, 2007-01-19 Genome and proteome based research is generating a significant increase in the number of available drug targets Correspondingly there is an increasing need for novel diverse compounds particularly based on natural compounds as screening resource The purpose of the Ernst Schering Research Foundation Workshop 51 was to provide a forum for an open exchange on perspectives and limitations of biocombinatorial synthesis and the significance of this technology for future drug discovery in light of this challenge Experts from academia and industry provided contributions covering the significance of natural compounds for state of the art drug discovery the underlying basic principle for the biosynthesis of highly complex compounds and the scope and limitations of combinatorial biosynthesis regarding formation identification optimisation isolation and manufacturing of novel biologically active entities      Neuroinflammation in Stroke Ulrich Dirnagl, Bernd Elger, 2004-01-14 The successful treatment of acute stroke remains one of the major challenges in clinical medicine Over the last decades the understanding of stroke pathophysiology has greatly improved while the therapeutic options in stroke therapy remain very limited Today hyperacute mechanisms of damage such as excitotoxicity can be discriminated from delayed ones such as inflammation and apoptosis Targeting of inflammation has already been successfully applied in various stroke models but translation into a clinically efficacious strategy has not been achieved so far In this book leading experts in basic cerebrovascular research as well as stroke treatment review the current evidence for and against an important role for inflammation in stroke and explore the potential of treating or modulating inflammation in stroke therapy      Opportunities and Challenges of the Therapies Targeting CNS Regeneration H.D. Perez, B. Mitrovic, A. Baron Van Evercooren, 2007-01-19 The therapeutic options for the treatment of Multiple Sclerosis MS and other neurodegenerative and traumatic diseases such as spinal cord injury Alzheimer s Parkinson s disease etc have experienced enormous progress over recent years Despite these encouraging developments available therapies are only partially effective and the ultimate goal is still far from being attained Improved understanding of the cellular and molecular mechanisms of the pathogenesis of neurodegeneration and demyelination has led to a variety of new therapeutic targets and approaches In addition to modulation of the inflammatory process MS and classical neuroprotection stroke AD therapeutic approaches focusing on active remyelination and neuronal regeneration have become increasingly important Based on current concepts this book summarizes new therapeutic approaches      **Chemokine Roles in Immunoregulation and Disease** Philip M. Murphy, Richard Horuk, 2003-10-23 The Ernst Schering Workshop on Chemokine Roles in Immunoregulation and Disease with contributions by leading experts in the field succinctly summarizes cutting edge research in immunology protein chemistry and disease This book gives an up to date overview of important developments in immunology with a concise state of the art review of chemokines It is a time saving reference for both researchers and students providing fast and easy access to the latest information in this important area of research      **The Promises and Challenges of Regenerative Medicine**

John Morser, S. -I. Nishikawa, 2005-07-06 Melanocyte system for studying stem cell niche Establishment and manipulation of monkey and human embryonic stem cell lines for biomedical research Human embryonic stem cell derivation In search of the elusive epidermal stem cell Corneal cells for regeneration Polycomb gene product Bmi 1 Directed differentiation of neural and sensory tissues from embryonic stem cells in vitro Stem cell biology for vascular regeneration      **New Pharmacological Approaches to Reproductive Health and Healthy Ageing** Egon Diczfalusy, 2001-10-26 Professor Egon Diczfalusy a pioneer in gynaecological endocrinology and in particular female contraception celebrated his 80th birthday with a scientific symposium The participants were scientists from all over the world who had been scholars and close collaborators at different points in time The interdisciplinary meeting consisted of lectures on the epidemiological impacts of ageing its challenge for the pharmaceutical industry philosophical aspects of the future of mankind and the history and future of contraception Three round table discussions addressed the hormonal control of reproduction and ageing new approaches and future perspectives of contraception for women and men and hormone replacement in the elderly      **Human Gene Therapy: Current Opportunities and Future Trends** G. M. Rubanyi, S. Ylä-Herttuala, 2003-07-14 Advances in genetics molecular biology and gene delivery technologies in recent years have led to new gene therapy strategies for treatment of a variety of diseases This book gives a comprehensive overview of the present status and future directions of gene delivery systems and therapeutic strategies for the clinical application of gene therapy in cancer cardiovascular and central nervous system diseases Stem cell based therapies and gene expression regulatory systems as novel platform technologies for various gene therapy applications are also discussed Leading experts give excellent overviews of basic molecular aspects and clinical applications in this new emerging biomedical field

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