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 #-glucuronidese from Escherichie coll. After extraction, the hydrolyzed steroids are derivatized to the corresponding trimethylisilyl 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 barrer 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 17α-methyl. 17α-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. Donike reported that methandienone, exandrolone, fluoxymesterone, stanozolol, dehydrochlormethyliestosterone, and formyldienolone can be detected somewhat easily in the nonconjugated fraction. These anabolic steroids have a 17β -hydroxy- 17α -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 μm, 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,HPO, mixed with 50 mL of 0.2M KH,PO. Diethylether was distilled with calcium hydride and stored in a refrigerator at ca. 1°C. β-Glucuronidase from Escherichia coli was from Boeringer. N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA), trimethyliodosil-ane (TMSI), and trimethylchlorosilane (TMSCI) 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 µm) 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 belium 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-µm film thickness. The oven temperature was

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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 Steroids Hassan Abduljabbar, 2012-01-11 This book explains the basic science of steroids covered in volumes 3 through 9 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 guickly 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 Terpenoids and Steroids, Volume 4, A Review of the Literature Published Between knowledge about this fascinating topic 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 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 Advances in Lipid Research Rodolfo Paoletti, David Kritchevsky, 2014-06-28 Advances in Lipid Research Volume 16 is a seven chapter text that contains research studies on the chemistry and physiology of lipids The introductory chapter reviews the metabolism of distinct molecular species of phospholipids The succeeding chapters describe the relationship between fatty acid and immunity as well as the influence of vitamin C on cholesterol metabolism gallstone formation and atheroschlerosis These chapters also examine the possible link between vitamin C deficiency and lipid disorders A chapter discusses the arterial enzymes of cholesteryl ester metabolism specifically the synthesis and hydrolysis of cholesteryl esters under a variety of conditions The remaining chapters consider the occurrence characteristics and factors affecting reaction of phospholipase D Surveys of the certain aspects of prostaglandin metabolism and the effects of thyroid hormone on atherosclerosis are also provided in these chapters This book will prove useful to lipid and enzyme chemists biochemists and researchers Theriogenology, 1980 An international journal of animal reproduction **Practical Management of Pain E-Book** Honorio Benzon, James P. Rathmell, Christopher L. Wu, Dennis Turk, Charles E. Argoff, Robert W Hurley, 2022-02-10 For more than 30 years Practical Management of Pain has offered expert guidance to both clinicians and trainees covering every aspect of acute and chronic pain medicine for adult and pediatric patients. The fully revised 6th Edition brings you fully up to date with new developments in patient evaluation diagnosis of pain syndromes rationales for management treatment modalities and much more Edited by a team of renowned pain clinicians led by Dr Honorio Benzon this authoritative reference is a comprehensive practical resource for pain diagnosis and treatment using a variety of pharmacologic and physical modalities Presents a wealth of information in a clearly written easily accessible manner enabling you to effectively assess and draw up an optimal treatment plan for patients with acute or chronic pain Takes a practical multidisciplinary approach making key concepts and techniques easier to apply to everyday practice Shares the knowledge and expertise of global contributors on all facets of pain management from general principles to specific management techniques Discusses the latest best management techniques including joint injections ultrasound guided therapies and new pharmacologic agents such as topical analgesics Covers recent global developments regarding opioid induced hyperalgesia neuromodulation and pain management and identification

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of EAE in research all over the world The book will provide established researchers and students with novel insights and guidance for their research and will help to push the field forward **Spinal Injections & Peripheral Nerve Blocks** E-Book Marc Huntoon, Honorio Benzon, Samer Nauroze, Timothy Deer, 2011-08-02 Spinal Injections Marc Huntoon MD and Samer Nauroze MD offer expert advice and scientific evidence supporting the use of spinal injections and sympathetic nerve blocks Comprehensive evidence based coverage on selecting and performing these techniques as well as weighing relative risks and complications helps you ensure optimum outcomes With access to the fully searchable text at www expertconsult com and procedural videos on Expert Consult you ll have the detailed visual assistance you need right at your fingertips Understand the rationale and scientific evidence behind spinal injections and sympathetic nerve blocks when and why they should be performed the mechanisms of action on pain and current guidelines for practice and master their execution Optimize outcomes reduce complications and minimize risks by adhering to current evidence based practice guidelines Apply the newest techniques in employing ultrasound fluoroscopy and computed tomography CT to guide needle placement Quickly find the information you need in a user friendly format with strictly templated chapters supplemented with illustrative line drawings images and treatment algorithms See how it s done through step by step procedural videos on Expert Consult Access the fully searchable contents at expertconsult com **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 clon ing germ cell manipulation and transplantation as well as gene ex pression have surpassed the classical approaches that involved studies on receptor activation and endocrine paracrine and autocrine regula tory 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 test is 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 I in European Workshop on Molecular and Cellular Endocrinology of the Testis held in Doorwerth The Netherlands from April 6 I 0 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 pro vided us with their excellent manuscripts well before the start of the Workshop We acknowledge the help of Dr U F Habenicht Dr M National Library of Medicine Current Catalog National Library of Medicine (U.S.), 1985 **Current Catalog**

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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

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