ULTRATRACE
ANALYSIS OF
PHARMACEUTICALS
AND OTHER
COMPOUNDS
OF INTEREST

Edited by Satinder Ahuja

> Volume 85 in Chemical Analysis. A Series of Monographs on Analytical Chemistry and Its Applications Edited by P.J. Elving and J.D. Winefordner, Editor Emeritus. I.M. Kolthoff

Ultratrace Analysis of Pharmaceuticals and Other Compounds of Interest

Edited by

Satinder Ahuja

Development Department Pharmaceuticals Division CIBA-GEIGY Corporation Suffern, New York

A WILEY-INTERSCIENCE PUBLICATION

JOHN WILEY & SONS
New York / Chichester / Brisbane / Toronto / Singapore

Copyright © 1986 by John Wiley & Sons, Inc.

All rights reserved. Published simultaneously in Canada.

Reproduction or translation of any part of this work beyond that permitted by Section 107 or 108 of the 1976 United States Copyright Act without the permission of the copyright owner is unlawful. Requests for permission or further information should be addressed to the Permissions Department, John Wiley & Sons, Inc.

Library of Congress Cataloging-in-Publication Data:

Main entry under title:

Ultratrace analysis of pharmaceuticals and other compounds of interest.

(Chemical analysis, ISSN 0069-2883; v. 85)

"A Wiley-Interscience publication." Includes index.

1. Ultratrace analysis. 2. Chemistry, Analytic.

3. Drugs-Analysis. 4. Chromatographic analysis.

I. Ahuja, Satinder, 1933-. II. Series.

QP519.9.U48U48 1986

ISBN 0-471-82673-1

85-29407 543

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

CHEMICAL ANALYSIS

A SERIES OF MONOGRAPHS ON ANALYTICAL CHEMISTRY AND ITS APPLICATIONS

Editors

P. J. ELVING, J. D. WINEFORDNER

Editor Emeritus: I. M. KOLTHOFF

Advisory Board

Fred W. Billmeyer, Jr.
Eli Grushka
Barry L. Karger
Viliam Krivan

Victor G. Mossotti
A. Lee Smith
Bernard Tremillon
T. S. West

VOLUME 85

A WILEY-INTERSCIENCE PUBLICATION

JOHN WILEY & SONS
New York / Chichester / Brisbane / Toronto / Singapore

Ultratrace Analysis of Pharmaceuticals and Other Compounds of Interest

CONTRIBUTORS

Satinder Ahuja

Development Department Pharmaceuticals Division CIBA-GEIGY Corporation Suffern, New York

C. J. Barnes

Department of Health & Human Services Food and Drug Administration Washington, D.C.

Donald A. Cooper

Special Testing and Research Laboratory Drug Enforcement Administration McLean, Virginia

J. C. Garriot

Office of the Medical Examiner County of Bexar San Antonio, Texas

John W. Howard

Department of Health & Human Services Food and Drug Administration Washington, D.C.

J. M. Liesch

Merck Sharp & Dohme Research Laboratories P.O. Box 2000 Rahway, New Jersey

Robert C. Livingston

Department of Health & Human Services Food and Drug Administration Washington, D.C.

James Luch

Development Department Pharmaceuticals Division CIBA-GEIGY Corporation Suffern, New York

Ira S. Lurie

Special Testing and Research Laboratory Drug Enforcement Administration McLean, Virginia

James M. Moore

Special Testing and Research Laboratory Drug Enforcement Administration McLean, Virginia

Terence H. Risby

Division of Environmental Chemistry Department of Environmental Health Science The Johns Hopkins University Baltimore, Maryland

J. B. Smith

Development Department Pharmaceuticals Division CIBA-GEIGY Corporation Suffern, New York

L. R. Snyder 26 Silverwood Ct. Orinda, California

Joseph C. Touchstone
University of Pennsylvania
School of Medicine
Department of Obstetrics and
Gynecology
Philadelphia, Pennsylvania

W. J. A. Vanden HeuvelMerck Sharp & Dohme ResearchLaboratoriesP.O. Box 2000Rahway, New Jersey

T. Walle

Department of Pharmacology Medical University of South Carolina Charleston, South Carolina

U. K. Walle

Department of Pharmacology Medical University of South Carolina Charleston, South Carolina

PREFACE

Ultratrace analysis, simply defined, entails any analysis performed below trace levels. Analysis carried out at parts per million or microgram amounts is frequently considered trace analysis. For the purpose of this book, analyses performed at submicrogram levels are considered ultratrace analyses. Therefore, coverage is provided primarily for such methodologies and their applications. Some analyses carried out at microgram levels in complex matrices in forensic or animal feed samples are also included because they are beyond what is ordinarily considered trace analysis in those fields.

Ultratrace analyses are highly specialized and complicated. Therefore, experts from various fields were invited to write about the analyses performed at the lowest level in their respective fields. This book not only covers analyses performed in a variety of areas, but also highlights how these analyses are instrumental in solving some of the complex scientific problems pertaining to mode of action of drugs and safety of our food, water, and environment.

The object of this book is to provide a ready reference to methodologies that are used in different related fields to allow optimal method selection. This may not be ordinarily possible since researchers tend to review primarily literature in their own fields. Yet the information a pharmaceutical analyst seeks might be available from a clinical chemist or environmental chemist. As a matter of fact, ultratrace analyses are performed by clinical, forensic, environmental, and food chemists, and other researchers involved in determining mechanism of action, disposition, and toxic properties of chemicals. This book was planned to provide valuable sources of information from a variety of fields with sufficient methodological details to allow methodology evaluation.

The first portion of this book includes six chapters detailing methodologies commonly used for ultratrace analyses: derivatization chromatography, selected ion-monitoring including MS/MS, liquid chromatography including LC/MS, modern thin layer chromatography, and atomic spectrometry. Since immunoassays provide selective applications, they are appropriately covered in Chapter 9 on analysis of drugs and their metabolites.

Applications of ultratrace analyses are covered in the second part of the book. The concern for purity of pharmaceutical compounds and excipients is addressed in two separate chapters. The next two chapters highlight how viii PREFACE

ultratrace analyses help in the performance of metabolic and toxicological studies. The last three chapters deal with the importance and applications of these analyses in the use and abuse of drugs in animals and humans.

I wish to express my sincere appreciation to all the authors for their valuable contributions to this volume. Special thanks are due to my father, Jawahar Lal, and my mother, Sushil Vati, for providing inspiration, and my wife, Fay, for helping in many ways.

SATINDER AHUJA

Monsey, New York May 1986

CONTENTS

1.	THE SCOPE OF ULTRATRACE ANALYSIS OF PHARMACEUTICALS AND OTHER COMPOUNDS OF				
	INTEREST	UTICALS AND OTHER COMPOUNDS OF	1		
	Satinder Ahuja				
		nd Sample Preparation	2		
		lidation and Interlaboratory Variations	3		
		Methodologies and Applications	4		
		c Spectroscopy	5		
	3.2. Liquid	Chromatography	7		
	3.3. Gas C	hromatography	8		
	3.4. Combi	nation Methods	11		
	3.4.1.	Liquid Chromatography-Mass Spectroscopy (LC/MS)	12		
		Gas Chromatography-Mass Spectroscopy (GC/MS)	13		
2.	DERIVATIZA CHROMATO	ATION FOR GAS AND LIQUID GRAPHY	19		
	Satinder Ahuj	a			
	1. Derivatization in Gas Chromatography				
	1.1. Deriva	tization Methods	21		
	1.1.1.	On-Column Reactions	23		
	1.1.2.	Reactions with Diakyl Acetals	24		
	1.1.3.	Silylation	25		
	1.1.4.	Esterification	26		
	1.1.5.	Acetylation	28		
	1.1.6.	Hydrazone Formation	28		
	1.1.7.	Ion-Pair Formation	28		
	1.1.8.	Derivatization for Electron Capture Detection	29		

	1.2. Classes of Compounds	32
	1.2.1. Acids	33
	1.2.2. Alkaloids	39
	1.2.3. Amides, Imides, and Related Compounds	42
	1.2.4. Amines	44
	1.2.5. Antibiotics	48
	1.2.6. Azepines	50
	1.2.7. Barbiturates and Other Urea Derivatives	52
	1.2.8. Esters	53
	1.2.9. Hydroxy Compounds and Mercaptans	54
	1.2.10. Steroids	61
	1.2.11. Vitamins	64
	1.2.12. Miscellaneous Compounds	66
	2. Derivatization in Liquid Chromatography	68
	2.1. Derivatization Methods	68
	2.1.1. UV-Absorbing Derivatives	68
	2.1.2. Fluorescent Derivatives	69
	2.2. Classes of Compounds	69
	2.2.1. Acids	71
	2.2.2. Alkaloids	73
	2.2.3. Amines	74
	2.2.4. Antibiotics	75
	2.2.5. Barbiturates and Related Compounds	75
	2.2.6. Carbonyl Compounds	75
	2.2.7. Hydroxy Compounds	76
	2.2.8. Steroids and Other Hormones	77
	2.2.9. Miscellaneous	77
3.	SELECTED ION-MONITORING METHODS	91
••	W. J. A. Vanden Heuvel and J. M. Liesch	71
	Packed-Column GLC Low-Resolution SIM	94
	Capillary Column GLC Low-Resolution SIM	98
	3. Techniques for Enhancement of Detection Selectivity	101
	4. Ionization Methods for Enhancement of Sensitivity and	101
	Specificity	104

CONTENTS	X

4.	LIQUID CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY	109
	L. R. Snyder and Satinder Ahuja	112
	1. Equipment Requirements	113
	1.1. Pumps	113
	1.2. Injection Systems 1.3. Detectors	114 115
	2. Separation Conditions	117
	2.1. Resolution	119
	2.2. Detector Response	120
	2.3. Gradient Elution	123
	3. Sample Derivatization Procedures	125
	3.1. Reaction Detectors	126
	4. Sample Cleanup and Column-Switching Procedures	130
	4.1. Sample Extraction	130
	4.2. Column Switching	131
	4.3. Automation	136
	5. Liquid Chromatography-Mass Spectrometry	138
	5.1. Techniques	139
	5.1.1. Direct Liquid Introduction (DLI)	139
	5.1.2. Jet Expansion	141
	5.1.3. Mechanical Transfer	142
	5.1.4. Miscellaneous Techniques	142
	5.2. Classes of Compounds	143
5.	MODERN THIN LAYER CHROMATOGRAPHY	149
	Joseph C. Touchstone	
	1. Sample Preparation for Ultratrace Analysis	151
	2. Conventional TLC	154
	2.1. Materials	155
	2.2. Extraction Procedure for Plasma	155
	2.3. Extraction Procedure for Urine	155
	2.4. Chromatography	155
	2.5. Fluorescence Measurement	156

xii CONTENTS

3. Hig	h-Performance Thin Layer Chromatography	156
3.1.	Sample Application	157
3.2.	Development Techniques for HPTLC	159
	3.2.1. Linear Development	159
	3.2.2. Circular Development	160
	3.2.3. Anticircular Development	161
	3.2.4. Continuous Development	161
	3.2.5. Considerations for Detection and Densitometry	162
3.3.	Classes of Compounds	163
	3.3.1. Phenothiazines	163
	3.3.2. Other Compounds	164
4. Rev	ersed-Phase Thin Layer Chromatography	166
4.1.	Types of Plates	167
4.2.	Theories of Separation	169
4.3.	Sources of RPTLC Layers	170
		171
4.5.	Mobile-Phase Selection	172
4.6.	Detection	174
4.7.	Classes of Compounds	176
	4.7.1. Ubiquinones	176
	4.7.2. Other Compounds	177
ATOM	IIC SPECTROMETRIC ANALYSIS	179
		1//
1. Ato	om Reservoirs	179
1.1.	Flame Atomizers	179
1.2.	Nonflame Atomizers	180
	1.2.1. Electrothermal Atomizers	181
	1.2.2. Inductively Coupled Plasmas	181
2. Ato	mic Emission Spectrometry	182
2.1.	Theory	182
2.2.	Instrumentation	183
2.3.	General Considerations	184
	3.1. 3.2. 3.3. 4. Rev 4.1. 4.2. 4.3. 4.4. 4.5. 4.6. 4.7. ATOM Terenc 1. Ato 1.1. 1.2.	3.2.2. Circular Development 3.2.3. Anticircular Development 3.2.4. Continuous Development 3.2.5. Considerations for Detection and Densitometry 3.3. Classes of Compounds 3.3.1. Phenothiazines 3.3.2. Other Compounds 4. Reversed-Phase Thin Layer Chromatography 4.1. Types of Plates 4.2. Theories of Separation 4.3. Sources of RPTLC Layers 4.4. Application of Samples 4.5. Mobile-Phase Selection 4.6. Detection 4.7. Classes of Compounds 4.7.1. Ubiquinones 4.7.2. Other Compounds ATOMIC SPECTROMETRIC ANALYSIS Terence H. Risby 1. Atom Reservoirs 1.1. Flame Atomizers 1.2. Nonflame Atomizers 1.2.1. Electrothermal Atomizers

			CONTENTS	xiii
	3.	Aton	nic Absorption Spectrometry	184
		3.1.	Theory	184
		3.2.	Instrumentation	185
		3.3.	General Considerations	186
	4.	Aton	nic Fluorescence Spectrometry	186
			Theory	186
		4.2.	Instrumentation	187
		4.3.	General Considerations	187
	5. Methodological Considerations		odological Considerations	188
		5.1.	Sample Preparation	188
		5.2.	Selection of an Analytical Procedure	189
		5.3.	Quantification	193
		5.4.	Speciation	193
7.			ITY ANALYSIS OF PHARMACEUTICAL	
	COMPOUNDS Satinder Ahuja 1. Method Development Strategies			195
			-	106
				196
		1.1.	Extractable Impurities	196
	•	1.2.	Chromatographable Impurities	196
	2.		es of Compounds	197
		2.1.	Alkaloids	199
			Amines	200
			Amino Acids Analgesics	201 202
			Antibiotics	202
			Antidonics Antidepressants and Tranquilizers	206
			Antineoplastic Agents	208
			Local Anesthetics	208
			Prostaglandins	209
			Steroids	209
			Sulfonamides	210
			Vitamins	212
			Miscellaneous	212

xiv CONTENTS

8.	IMPURITY ANALYSIS OF EXCIPIENTS	217	
	J. B. Smith and Satinder Ahuja		
	1. Ultratrace Contaminants		
	1.1. Cationic Contaminants or Metals	219	
	1.1.1. Arsenic	219	
	1.1.2. Heavy Metals	225	
	1.1.3. Iron	228	
	1.2. Anionic Contaminants	228	
	1.2.1. Chloride	229	
	1.2.2. Sulfide	230	
	1.2.3. Sulfite	230	
	1.3. Gaseous Contaminants	230	
	1.3.1. Ammonia	230	
	1.3.2. Hydrogen Sulfide	231	
	1.3.3. Nitric Oxide and Nitrous Dioxide	231	
	1.4. Organic Contaminants	231	
	1.4.1. Contaminants in Alcohol	231	
	1.4.2. Contaminants in Chloroform	231	
	1.4.3. Contaminants in Saccharin	232	
	1.5. Miscellaneous Contaminants	232	
9.	ANALYSIS OF DRUGS AND THEIR METABOLITES	235	
	T. Walle and U. K. Walle		
	1. Fluorometry	236	
	2. Thin Layer Chromatography	238	
	3. High-Performance Liquid Chromatography	239	
	4. Gas Chromatography	247	
	5. Gas Chromatography-Mass Spectrometry	254	
	6. Radioimmuno- and Radioreceptor Assays	260	
	6.1. RIA	260	
	6.2. RRA	264	
10.	DRUG ANALYSIS IN ANIMAL FEEDS	273	
	James Luch and Satinder Ahuja		
	1. Analysis of Drugs in Feed to Support Toxicological	y spanne	
	Testing in Animals	277	
	1.1. Liquid-Liquid Partition and Column Cleanup	278	

CONTENTS	XV

	1.2. Liquid-Liquid Partition and TLC Cleanup	282
	1.3. Column Chromatography Cleanup	284
	1.4. Liquid-Liquid Partition Cleanup	288
	2. Medicated Feeds	289
	2.1. Classes of Compounds	289
	2.1.1. Antibacterials	289
	2.1.2. Coccidiostats	290
	2.1.3. Antidysentery Compounds	291
	2.1.4. Anabolic Agents	291
11.	REGULATORY ANALYSIS OF DRUG RESIDUES IN	
	ANIMAL-DERIVED FOODS	295
	Robert C. Livingston, C. J. Barnes, and John W. Howard	
	1. Regulatory Background	296
	1.1. Federal Food, Drug, and Cosmetic Act	296
	1.2. Safety Assessment of Animal Drug Residues	296
	1.3. Criteria for Methods of Analysis	300
	2. Regulatory Methods	301
	2.1. Melengestrol Acetate	302
	2.2. Lasalocid	304
	2.3. Fenbendazole	305
	2.4. Ivermectin	306
	2.5. Carbadox	307
	2.6. Pyrantel and Morantel Tartrate2.7. Arsenicals	309
	2.7. Arsenicais 2.8. Tiamulin	310
	2.9. Sulfonamides	. 312
	2.9. Suitonamides	314
12.	ULTRATRACE DRUG ANALYSIS IN FORENSIC	
	CHEMISTRY	319
	Ira S. Lurie, James M. Moore, and Donald A. Cooper	
	1. MS and GC/MS Applications	320
	1.1. Modified Procedures for Forensic Drug Analysis	321
	1.2. Instrumental Methods	322
	2. HPLC Applications	323
	2.1. LSD	323
	2.2. Marijuana	325

XV1		

CONTENTS

		2.3. Psilocybin and Psilocin	328
		2.4. Manufacturing By-products	331
		2.5. General Analysis	333
	3.	GC Applications	338
		3.1. GC-FID	338
		3.2. GC-ECD	338
13.	DR	UG ANALYSIS IN POSTMORTEM TOXICOLOGY	353
	J. (C. Garriott	
	1.	Circumstances Affecting Postmortem Drug Analyses	353
	2.	Choice of Specimens	354
	3.	Analytical Instrumentation	355
	4.	Analytical Procedures	356
		4.1. Extraction and Separation	356
		4.2. Analysis for Drugs with Basic Properties	357
	,	4.3. Analysis for Drugs with Acidic and Neutral	
		Properties	358
		4.4. Alcohols and Other Volatile Substances	359
		Detection of Ultratrace Concentrations of Drug Agents in Biological Fluids	360
		5.1. Opiate Narcotics	360
		5.2. Synthetic Narcotics	361
		5.3. Basic Drugs	362
		5.4. Cannabinoids	363
	6.	Other Postmortem Analyses Requiring Specialized	
		Techniques	364
	7.	Special Problems in Postmortem Specimen Analysis	365
	•	7.1. The Exhumed or Decomposed Body	365
		7.2. Stability of Drugs	366
		7.3. Findings in Postmortem Drug Analysis	368
		7.4. Interpretation of Postmortem Drug Findings	370
		INDEX	377