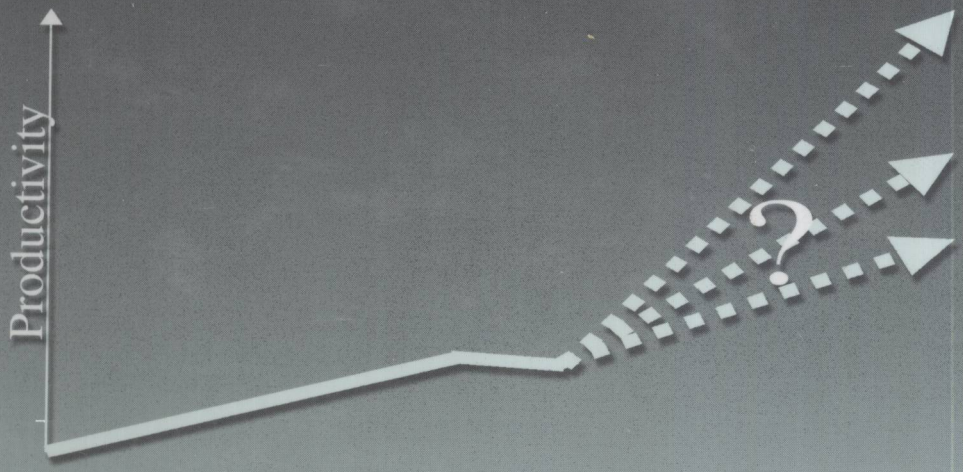
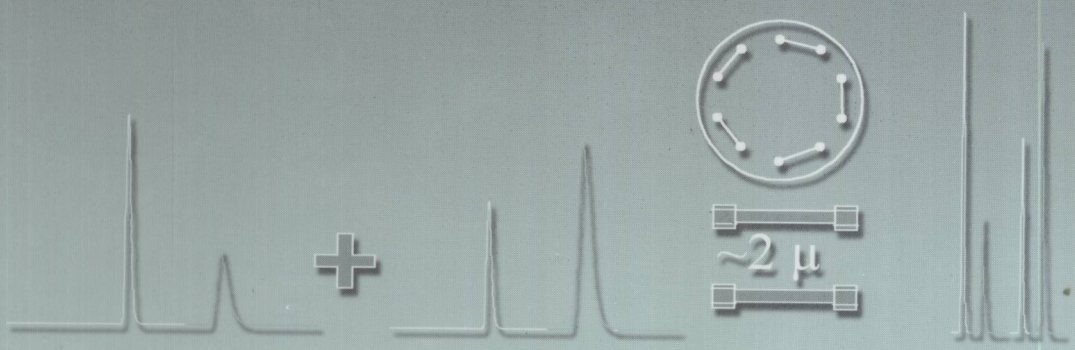
High-Throughput Analysis in the Pharmaceutical Industry





CRITICAL REVIEWS IN COMBINATORIAL CHEMISTRY

Edited by

Perry G. Wang

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High-Throughput Analysis in the Pharmaceutical Industry

CRITICAL REVIEWS IN COMBINATORIAL CHEMISTRY

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High-Throughput Analysis in the Pharmaceutical Industry
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Preface

I had the pleasure of developing and exploiting the high-throughput techniques used for drug analysis in the pharmaceutical industry at Abbott Laboratories. My major duties as project leader involved bioanalytical method development and validation by liquid chromatography with tandem mass spectrometry (LC/MS/MS). While organizing a symposium titled "High-Throughput Analyses of Drugs and Metabolites in Biological Matrices Using Mass Spectrometry" for the 2006 Pittsburgh Conference, it became my dream to edit a book called *High-Throughput Analysis in the Pharmaceutical Industry.*

It is well known that high-throughput, selective and sensitive analytical methods are essential for reducing timelines in the course of drug discovery and development in the pharmaceutical industry. Traditionally, an experienced organic chemist could synthesize and finalize approximately 50 compounds each year. However, since the introduction of combinatorial chemistry technology to the pharmaceutical industry, more than 2000 compounds can be easily generated yearly with certain automation. Conventional analytical approaches can no longer keep pace with the new breakthroughs and they now constitute bottlenecks to drug discovery. In order to break the bottlenecks, a revolutionary improvement of conventional methodology is needed. Therefore, new tools and approaches for analysis combined with the technologies such as combinatorial chemistry, genomics, and biomolecular screening must be developed. Fortunately, liquid chromatography/mass spectrometry (LC/MS)-based techniques provide unique capabilities for the pharmaceutical industry. These techniques have become very widely accepted at every stage from drug discovery to development.

This book discusses the most recent and significant advances of high-throughput analysis in the pharmaceutical industry. It mainly focuses on automated sample preparation and high-throughput analysis by high-performance liquid chromatography (HPLC) and mass spectrometry (MS). The application of high-performance liquid chromatography combined with mass spectrometry (HPLC-MS) and the use of tandem mass spectrometry (HPLC/MS-MS) have proven to be the most important analytical techniques for both drug discovery and development. The strategies for optimizing the application of these techniques for high-throughput analysis are also discussed. Microparallel liquid chromatography, ADME/PK high-throughput assays, MS-based proteomics, and advances in capillary and nano-HPLC technology are also introduced in this book.

I sincerely hope that readers—ranging from college students to professionals and academics in the fields of pharmaceutics and biotechnology—will find the chapters in this book to be helpful and valuable resources for their current projects and recommend this volume to their colleagues.

I would like to note my appreciation to all the contributors who found time in their busy schedules to provide the chapters herein. Many thanks to my previous colleagues, Shimin Wei, Min S. Chang, and Tawakol El-Shourbagy for their friendship and support. I would like to take this opportunity to acknowledge and thank the late Dr. Raymond Wieboldt for his priceless mentoring, without which I could not have been so successful in establishing my career in the pharmaceutical industry. I would also like to thank Bing Yan, Lindsey Hofmeister, Pat Roberson, Marsha Hecht, and Hilary Rowe for their much valued assistance throughout the preparation of this book. My thanks and gratitude go also to my family, whose support and encouragement greatly assisted me in editing this book.

Perry G. Wang
Wyomissing, Pennsylvania

Editor

Dr. Perry G. Wang is currently a principal scientist at Teleflex Medical. His interests include analytical method development and validation, medicated device products, and environmental engineering. His expertise focuses on high-throughput analysis of drugs and their metabolites in biological matrices with LC/MS/MS.

Dr. Wang received a President's Award for Extraordinary Performance and Commitment in 2005 for his dedication in leading the Kaletra® reformulation project at Abbott Laboratories. He was presented with a President's Award for Excellence while he worked in the U.S. Environmental Protection Agency's research laboratories.

Dr. Wang is an author of more than 20 scientific papers and presentations. He organized and presided over symposia for the Pittsburgh Conference in 2006 and 2008, respectively. He has been an invited speaker and presided over several international meetings including the Pittsburgh Conference and the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS). His current research focuses on developing new medicated-device products applied to critical care medicine and testing drug release kinetics and impurities released from drug-device combination products. He earned a B.S. in chemistry from Shandong University and an M.S. and Ph.D. in environmental engineering from Oregon State University.

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1 High-Throughput Sample Preparation Techniques and Their Application to Bioanalytical Protocols and Purification of Combinatorial Libraries

Krishna Kallury

CONTENTS

1.1 NEED FOR HIGH-THROUGHPUT SAMPLE PURIFICATION AND CLEAN-UP IN DRUG DISCOVERY

The drug discovery process took a revolutionary turn in the early 1990s through the adaptation of combinatorial chemistry for generating large volumes of small organic molecules (generally having molecular weights below 750 Daltons) so that the products of all possible combinations of a given set of starting materials (building blocks) can be obtained at once. The collection of these end products is called a combinatorial library.

Production of such libraries can be achieved through either solid phase synthesis or solution chemistry. This newly acquired capability of synthetic chemists to produce a large number of compounds with a wide range of structural diversity in a short time, when combined with high-throughput screening, computational chemistry, and automation of laboratory procedures, led to a significantly accelerated drug discovery process compared to the traditional one-compound-at-a-time approach. During the high-throughput biological screening of combinatorial compounds, initial sample purification to remove assay-interfering components is required to ensure "true hits" and prevent false positive responses. This created needs for rapid purification of combinatorial synthesis products along with rapid evaluation of the purities of these large numbers of synthetic products. In addition, screening biological activities of combinatorial libraries at the preclinical and clinical (phases I through III) trial stages generates drug and metabolite samples in blood, plasma, and tissue matrices. Because these biological matrices carry many other constituents (proteins, peptides, charged inorganic and organic species) that can interfere with the quantitation of the analytes and also damage the analytical instrumentation (especially mass spectrometers and liquid chromatographic columns), rapid clean-up methods are required to render the samples amenable for analysis by fast instrumental techniques. This chapter addresses the progress made during the past decade in the areas of rapid purification of combinatorial libraries and sample preparation and clean-up for high-throughput HPLC and/or LC/MS/MS analysis.

In addition to the large volume synthesis of small molecules, combinatorial approaches are also used to generate catalysts, oligonucleotides, peptides, and oligosaccharides. High-throughput purification has also found applicability for the isolation and clean-up of natural products investigated for biological activity. Several reviews and monographs are available on various topics related to the synthetic and biological screening aspects of the drug discovery process. Since the focus of this chapter is on the purification of combinatorial libraries and clean-up of drugs and their metabolites in biological matrices, it is suggested that the readers refer to the latest literature available on solid phase[1–10] and solution phase[11–17] combinatorial synthesis, ADME studies,[18–26] rapid instrumental analysis techniques,[27–33] and high-throughput methods in natural products chemistry[34–40] for more detailed insights into these areas of relevance to combinatorial synthesis and high-throughput screening.

1.2 RAPID PURIFICATION TECHNIQUES FOR DRUGS AND METABOLITES IN BIOLOGICAL MATRICES

1.2.1 State-of-Art Sample Preparation Protocols

A number of advances have been made during the past decade to convert sample preparation techniques used for about 30 years for the clean-up of drugs in biological matrices into formats that are amenable for high volume processing with or without automation. Detailed accounts about the fundamentals of these techniques can be found in the literature.[41–50] Therefore, only brief descriptions of the principles of these methods are presented. For isolating drugs and metabolites from biological matrices, several approaches have been reported, which consist of:

- Solid phase extraction (SPE)
- Liquid—liquid extraction (LLE)
- Protein precipitation (PPT)
- Affinity separations (MIP)
- Membrane separations
- Preparative high performance liquid chromatography (HPLC)
- Solid phase microextraction (SPME)
- Ultrafiltration and microdialysis

SPE, LLE, and PPT are the most commonly used sample preparation techniques and hence most of the discussion will be devoted to them. The others will be dealt with briefly. All of these methods have certain ultimate goals:

- Concentrate analyte(s) to improve limits of detection and/or quantitation
- Exchange analyte from a non-compatible environment into one that is compatible with chromatography and mass spectrometric detection
- Remove unwanted matrix components that may interfere with the analysis of the desired compound
- Perform selective separation of individual components from complex mixtures, if desired
- Detect toxins in human system or in environment (air, drinking water, soil)
- Identify stereochemical effects in drug activity and/or potency
- Follow drug binding to proteins
- Determine stability and/or absorption of drugs and follow their metabolism in human body

1.2.2 Matrix Components and Endogenous Materials in Biological Matrices

Biological matrices include plasma, serum, cerebrospinal fluid, bile, urine, tissue homogenates, saliva, seminal fluid, and frequently whole blood. Quantitative analysis of drugs and metabolites containing abundant amounts of proteins and large numbers of endogenous compounds within these matrices is very complicated. Direct injection of a drug sample in a biological matrix into a chromatographic column would result in the precipitation or absorption of proteins on the column packing material, resulting in an immediate loss of column performance (changes in retention times, losses of efficiency and capacity). Similar damage can occur to different components of the ESI/MS/MS system commonly utilized for analyzing drugs. Matrix components identified by different analytical techniques are shown in Table 1.1. Major classes encountered in plasma consist of inorganic ions, proteins and/or macromolecules, small organic molecules, and endogenous materials.[51–56]

Mass spectrometry is the most preferred technique employed during high-throughput screening. It provides specificity based on its capability to monitor selected mass ions, sensitivity because it affords enhanced signal-to-noise ratio, and speed due to very short analysis times that allow analysis

TABLE 1.1
Interferences Identified in Human Plasma

Classification	Components	Concentration (mg/L)	Reference
Inorganic ions	Sodium [Na^+]	3.2×10^3 to 3.4×10^3	51
	Potassium [K^+]	148.6 to 199.4	
	Calcium [Ca^{2+}]	92.2 to 112.2	
	Magnesium [Mg^{2+}]	19.5 to 31.6	
	Chloride [Cl^-]	3.5×10^3 to 3.8×10^3	
	Hydrogencarbonate [HCO_3^-]	1.5×10^3 to 2.1×10^3	
	Inorganic phosphorus [P], total	21.7 to 41.6	
	Iron [Fe] in men	1.0 to 1.4	
	Iron [Fe] in women	0.9 to 1.2	
	Iodine [I], total	34.9×10^{-3} to 79.9×10^{-3}	
	Copper [Cu] in men	0.7 to 1.4	
	Copper [Cu] in women	0.9 to 1.5	
Proteins/Macromolecules (g/L)	Prealbumin	0.1 to 0.4	51
	Albumin	42.0	
	Acid-α_1-glycoprotein	0.2 to 0.4	
	Apolipoproteins (globulins)	4.0 to 9.0	
	Haptoglobin (α_2-globulin)	1.0	
	Hemopexin (β_1-globulin)	0.7	
	Transferin (β_2-globulin)	2.9	
	Ceruloplasmin (α_2-globulin)	0.4	
	Transcortin (α_1-globulin)	0.04	
	Transcobalamin	94.0×10^{-8}	
	α_2-Macroglobulin	2.5	
	α_1-Antitrypsin	2.5	
	Protein-binding metal (α_1-globulin)	0.06	
	Antithrombin III (α_2-globulin)	0.2	
	Fibrinogen	4.0	
	Immunoglobulins (γ-globulins)	15.0 to 16.0	
Endogenous components (small organic molecules)	**Amino Acids**		52
	Alanine	NA	
	Valine	NA	
	Leucine	NA	
	Serine	NA	
	Threonine	NA	
	Methionine	28.8 μM	
	Aspartate	NA	
	Glutamate	43.5 μM	
	Phenyl alanine	55.8 μM	
	Glycine	NA	
	Lysine	127.3 μM	
	Tyrosine	NA	
	Proline	289.1 μM	
	Cystine	NA	
	Tryptophan	55.7 μM	

TABLE 1.1 (CONTINUED)
Interferences Identified in Human Plasma

Classification	Components	Concentration (mg/L)	Reference
	Fatty Acid Derivatives		
	2-Hydroxybutyrate	NA	
	3-Hydroxybutyrate	NA	
	3-Methyl-2-hydroxybutyrate	NA	
	Palmitate	125.8 μM	
	Oleate	NA	
	Stearate	NA	
	Laurate	NA	
	Linoleate	NA	
	Other Small Organics		
	Urea	NA	
	Glycerate	NA	
	Creatinine	106.5 μM	
	Glycerol phosphate isomer	NA	
	Citrate	318.6 μM	
	Ascorbic acid	NA	
	Carbohydrate Derivatives		
	Glucose	NA	
	Myoinositol	24.5 μM	
	Inositol phosphates	NA	
	Purine Derivatives		
	Urate	331.5 μM	
	Nucleosides	NA	
	Steroids		
	Cholesterol	2109.7 μM	
Endogenous phospholipids	Phosphatidylcholine Lysophosphatidylcholines (18:2, 16:0 and 18:0)	NA	53
Prostaglandins	Prostaglandin D_2 and F_2	NA	54
Hormones	Melatonin	NA	55
Polysaccharides	Glycosaminoglycans	NA	56
Unseen endogenous matrix components (dosing excipients)	Hydroxypropyl-β-cyclodextrin	NA	
	Polyethyleneglycol 400	NA	
	Propyleneglycol	NA	
	Tween 80	NA	

of dozens of samples per hour. One important factor affecting the performance of a mass detector is ion suppression, with the sample matrix, coeluting compounds, and cross talk contributing to this effect. Operating conditions and parameters also play a role in inducing matrix effects that result in suppression of the signal, although enhancement is also observed occasionally. The main cause is a change in the spray droplet solution properties caused by the presence of nonvolatile or less volatile solutes. These nonvolatile materials (salts, ion-pairing agents, endogenous compounds,

drugs, metabolites) change the efficiency of droplet formation or droplet evaporation, affecting the concentrations of charged ions in the gas phase reaching the detector.

The literature clearly reviews how plasma constituents and endogenous materials adversely affect the quantitation of drugs and their metabolites in these matrices.[57–70] It follows that when drugs or metabolites in biological matrices are analyzed, a thorough purification step must be invoked to eliminate (or at least minimize) these adverse effects. In the context of high-throughput screening of ADME (or DMPK) samples, the following discussion elaborates on protocols popularly employed for the high-throughput clean-up of biological matrix components and/or endogenous materials.

1.2.3 Solid Phase Extraction (SPE)

Application of SPE to sample clean-up started in 1977 with the introduction of disposable cartridges packed with silica-based bonded phase sorbents. The *solid phase extraction* term was devised in 1982. The most commonly cited advantages of SPE over liquid–liquid extraction (LLE) as practiced on a macroscale include the reduced time and labor requirements, use of much lower volumes of solvents, minimal risk of emulsion formation, selectivity achievable when desired, wide choices of sorbents, and amenability to automation. The principle of operation consists of four steps: (1) conditioning of the sorbent with a solvent and water or buffer, (2) loading of the sample in an aqueous or aqueous low organic medium, (3) washing away unwanted components with a suitable combination of solvents, and (4) elution of the desired compound with an appropriate organic solvent.

With increasing popularity of the SPE technique in the 1980s and early 1990s, polymeric sorbents started to appear to offset the two major disadvantages of silica based sorbents, i.e., smaller surface area resulting in lower capacities and instability to strongly acidic or basic media. Around the mid-1990s, functionalized polymers were introduced to overcome the shortcomings of the first generation polymers such as lower retention of polar compounds and loss of performance when the solvent wetting them accidentally dried. Tables 1.2 and 1.3 list some of the popular polar functionalized neutral and ion exchange polymeric SPE sorbents, respectively, along with structure and

TABLE 1.2
Functionalized Neutral Polymeric Sorbents

Source	Sorbent	Chemistry	Mode of Interaction	Examples from Literature (Plasma Samples Only)
Waters (see 2006–2007 Catalog, SPE products)	Oasis HLB	Divinylbenzene-N-vinyl-pyrrolidone copolymer	Reversed phase with some hydrogen bond acceptor and dipolar reactivity	Rosuvastatin (71); NSAIDs (72); fexofenadine (73); catechins (74); valproic acid (75)
Phenomenex (see 2006 Catalog, SPE products)	Strata-X	Polar functionalized styrene-divinylbenzene polymer	Reversed phase with weakly acidic, hydrogen bond donor, acceptor, and dipolar interactions	Cetirizine (76); pyridoxine (77); omeprazole (78); mycophenolic acid (79); 25-hydroxy-vitamin D_3 (80)
Varian (see Catalog, SPE products)	Focus	Polar functionalized styrene-divinylbenzene polymer	Reversed phase with strong hydrogen bond donor, acceptor, and dipolar character	Fluoxetine, verapamil, olanzapine, tramadol, loratidine, and sumatriptane (81); verdanafil (82)
Varian (see Catalog, SPE products)	Bond Elut Plexa	Highly cross-linked polymer with hydroxylated surface	Hydrophobic retention of small molecules and hydrophilic exclusion of proteins	See catalog