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# Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography

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Dedicated to
my wife, Gretta,
my daughters, Heather and Amanda,
and my parents

# FOREWORD

It has been just over 10 years since the technique of high-performance liquid chromatography took its first hesitant steps into the clinical laboratory. Since that time, it has deservedly become the technique of choice for many applications in therapeutic drug monitoring and toxicology. Modern liquid chromatography (LC) has made it possible for laboratorians to routinely measure many previously elusive analytes. Just as importantly, it has allowed the determination of highly polar, conjugated metabolites of therapeutic agents, the concentrations of which may be predictive of certain metabolic diseases or drug overdose. This latter capability of LC is not shared by currently available immunoassays. There is promise for the future: improved sensitivity, more specific detectors, and partial or full automation are now becoming available or are on the horizon.

This volume is an excellent representation of the many areas of active investigation in the clinical application of LC to drug analysis. Its editor and contributors are to be congratulated for their straightforward and pragmatic approach to this instrumental technique. I believe that their effort will promote and encourage the adoption of LC as a practical analytical technique by numerous clinical laboratories throughout the world.

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# PREFACE

Therapeutic drug monitoring (TDM), since its emergence in the 70s, has been established as a well-accepted subspecialty of clinical medicine. Undoubtedly, laboratory measurements of TDM and toxicology have been greatly enhanced by the advances in instrumentations such as immunoassay and chromatography. Liquid chromatography (LC), in particular, has played a very active and vital role, by providing fast, specific, cost-effective, and in selected cases (such as antidepressants, antihypertensives, and immunosuppressives), the preferred or only mode of analysis for drugs and metabolites, often before the immunoassay. Indeed, for comparison studies, the LC assay is often chosen as the reference method. In spite of numerous books, articles, and workshops dealing with various aspects of TDM and toxicology, a comprehensive and dedicated treatise of LC for these areas is still lacking. Furthermore, with the present emphasis on the new health-care economics, such as the Diagnostic Related Group (DRG) reimbursement policy, laboratories might want to reexamine the choice of instrumentation and methods. It is in light of these considerations that this book was conceived and finalized. The approach is to present a current account of the various LC instrumentations for TDM and toxicology drug assays, and to review the clinical pharmacology of major classes of drugs and their LC analyses. Each chapter is based upon its contributor's personal experience. The book should be useful to practitioners such as laboratory directors, toxicologists, and clinicians, and to interested nonpractitioners such as analytical chemists, immunologists, and instrument manufacturers.

The first part includes chapters on the principles of TDM, sampling techniques, and various instrumentation topics, such as the computer,

mass spectrometry, fluorescence, and electrochemical detection. The second part consists of chapters on six major classes of drugs, beginning with a review of their clinical pharmacology and LC analyses, followed by recommended LC procedures. Emphasis is placed on some of the recently introduced drugs and their LC analyses. The third part consists of chapters on medicolegal guidelines, LC analyses of miscellaneous groups of drugs, and various laboratory management considerations. It is hoped that through this updated and coordinated treatment, the roles of LC might be better defined to meet the present needs of the laboratory for TDM and toxicology drug measurements.

The editor gratefully acknowledges the effort of the contributors. He is indebted to his chairman, F. William Sunderman, Jr., M.D., for his encouragement and support; and his former mentor, Dr. Hans J. Ache, for introducing liquid chromatography to his graduate research. During the initial planning of this book, valuable suggestions were solicited from the following persons: Drs. Richard P. Spencer, Jack Cazes, Charles Pippenger, R. P. W. Scott, B. L. Karger, L. R. Snyder, and John Dolan. He also wishes to thank Drs. Dennis Hill, Carlos Santiago, Ralph Rodriquez, Mitchell Gandleman, and Nemat Marzouk for reviewing the manuscripts; and Mary Gagnon who undertook the laborious and important tasks of typing the manuscripts and correspondence. His family's understanding and support has inspired and enhanced the undertaking and completion of this project.

Steven H. Y. Wong

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# 1

# INTRODUCTION

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Therapeutic drug monitoring (TDM) has developed into a well-established subspecialty within the clinical laboratory. It deals with the optimization of the clinical response, as guided by the drug concentration in biological fluid such as plasma or serum. The rationale and the principles for TDM have been extensively reviewed [1-5] and updated in Chap. 2 by Pippenger. Toxicology deals with the identification/quantitation of known or unknown drugs and chemicals [6-9]. Traditionally, some of the modern TDM functions have been regarded as toxicology. Depending on the drug concentration and patient history (such as overdose), a TDM or toxicology measurement may be performed by the same procedure.

The evolution of TDM resulted from the increasing abundance of pharmacokinetic data [10], and from the introduction and/or improvement of instrumentations such as chromatography and immunoassay. With these instrumentations, laboratory personnel can measure the plasma concentration of drugs and metabolites within a reasonable amount of time (usually 1-4 hr), with predictable and acceptable precision (relative standard deviations are usually less than 10%) [11]. Among the various modes of chromatography used for TDM and toxicology, both gas and liquid chromatography have played vital roles. Applications of LC for drug measurement dated back to the early seventies with the advent of LC for drug identification and for pharmacokinetic studies [12]. Liquid chromatography, herein encompassing the following terms, "high-performance liquid chromatography (HPLC)," "high-pressure liquid chromatography (HPLC)," and "high-speed liquid chromatography," has already surpassed gas chromatographic applications for certain areas of TDM such as antidepressant monitoring,

according to a recent survey result of the College of American Pathologists, as shown by Table 1 for the measurement of nortriptyline [13]. Advantages of LC include its selectivity and sensitivity as a result of variety of separation and detection modes. Thus LC has enhanced TDM and toxicology as much as it has other areas of the biomedical and biochemical sciences. But perhaps the difference in regard to LC is the high frequency of daily routine LC clinical analysis—a testimonial to the remarkable state of the instrumentation and column technology.

In carrying out TDM/toxicology measurements, there are two primary considerations—personnel and instrumentation. A dedicated number of personnel (four to five technologists) would enhance the operation with the objective of becoming an active and integral part in the patient health care system. In view of the vast number of drugs and the increasing number of newly introduced drugs on the market, the technologists would have to gain a working knowledge of clinical pharmacology through meetings, discussions, and workshops. Then the personnel would be more proficient towards interpreting drug levels as well as detecting abnormal results or laboratory errors. The dosage regime and the clinical status of the patients would guide the laboratory personnel as to what to anticipate, and therefore to recommend the appropriate drug measurement/toxicology test. The recommendations would include sampling time, types of specimens, and others. Based on the above considerations, it is inherently difficult to train a large laboratory staff to be proficient in various aspects of clinical pharmacology and measurement.

For the measurement or identification of drugs and chemicals, the instrumentation ranges from the simple flame photometer used to measure lithium, to automated equipment such as the Automated Clinical Analyzer (Dupont, Wilmington, Delaware) used for Enzyme Multiplied Immunoassay Technique (EMIT) (Syva, Palo Alto, California), and TDX, for Fluorescence Polarization Immunoassay (FPIA) (Abbott, North Chicago, Illinois), to the more demanding chromatography. The immunoassay technology and its instrumentation are much more adaptable to automation and thus require less involvement of personnel. However, the chromatographic procedures usually require extraction and analysis performed with close personal attention. Depending upon the ability of the personnel, TDM/toxicology chromatographic equipment may range from simple TLC and basic gas or liquid chromatographs, to the more sophisticated chromatographs with various data handling capability such as GC-MS and its associated data bank. In order to utilize these instruments, it will be more efficient to train a dedicated group of technologists.

Among the whole range of instrumentation used by clinical laboratories for drug measurement, chromatography has always played a vital role. From the survey results of the College of American Pathologists [14], phenobarbital measurement (Table 2) is still performed by

Table 1 CAP Survey Results for the Measurement of Nortriptyline (ng/ml)

| Specimen/method | No. labs | Mean | S.D. | C.V. | Median | Low value | High value |
|---|---|---|---|---|---|---|---|
| Specimen Z-5 | Target value 100.0 | | | | | | |
| Gas-liquid chromatography | | | | | | | |
| Underivatized | 18 | — | — | — | 90 | 49 | 151 |
| High-performance liquid chrom. | | | | | | | |
| Solvent extraction | 42 | 98.7 | 37.2 | 37.7 | — | — | — |
| All HPLC results | 49 | 97.8 | 35.5 | 36.3 | — | — | — |
| All methods/all results | 74 | 102.6 | 43.0 | 42.0 | — | — | — |
| Specimen Z-6 | Target value 1200.0 | | | | | | |
| Gas-liquid chromatography | | | | | | | |
| Underivatized | 18 | — | — | — | 1243 | 207 | 1094 |
| High-performance liquid chrom. | | | | | | | |
| Solvent extraction | 41 | 1199.8 | 201.5 | 16.8 | — | — | — |
| All HPLC results | 48 | 1175.4 | 170.3 | 14.5 | — | — | — |
| All methods/all results | 72 | 1230.9 | 237.2 | 19.3 | — | — | — |

*Source*: Ref. 13.

Table 2 CAP Survey Results for the Measurement of Phenobarbital (mcg/ml)

| Specimen/method | No. labs | Mean | S.D. | C.V. | Median | Low value | High value |
|---|---|---|---|---|---|---|---|
| Specimen Z-2 | Target value 2.0 | | | | | | |
| The results listed below were reported as "Equal To" values. | | | | | | | |
| Photometry | | | | | | | |
| Spectrophotometry/colorimetry | 11 | — | — | — | 2.0 | 1.3 | 6.0 |
| All photometry results | 12 | — | — | — | 2.0 | 1.3 | 6.0 |
| Gas-liquid chromatography | | | | | | | |
| Derivatized | 11 | — | — | — | 2.0 | 0.0 | 2.4 |
| Underivatized | 14 | — | — | — | 2.3 | 1.2 | 6.1 |
| All GLC results | 24 | 2.12 | 0.95 | 44.7 | — | — | — |
| High-performance liquid chrom. | | | | | | | |
| Protein precipitation | 21 | 2.45 | 0.76 | 31.2 | — | — | — |
| Solvent extraction | 21 | 2.30 | 0.63 | 27.6 | — | — | — |
| All HPLC results | 45 | 2.38 | 0.69 | 28.8 | — | — | — |
| Enzyme immunoassay | | | | | | | |
| Syva | 105 | 2.40 | 0.88 | 26.6 | — | — | — |
| Other | 19 | — | — | — | 2.2 | 1.0 | 19.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fluorescent immunoassay | | | | | | | |
| Ames | 18 | — | — | — | 2.0 | 0.7 | 2.5 |
| Fluorescence polarization imm. | | | | | | | |
| Abbott | 84 | 1.44 | 0.30 | 21.0 | — | — | — |
| Immunonephelometry/immunoturbid. | | | | | | | |
| Beckman | 65 | 3.88 | 1.22 | 31.4 | — | — | — |
| Radioimmunoassay | | | | | | | |
| Clinical assays | 13 | — | — | — | 2.5 | 1.7 | 3.3 |
| All immunoassay results | 307 | 2.34 | 1.02 | 43.6 | — | — | — |
| All methods/all results | 401 | 2.34 | 1.01 | 43.1 | — | — | — |

The results listed below were reported as "Less Than" values.

| | No. labs | | | | Mode | Low value | High value |
|---|---|---|---|---|---|---|---|
| Photometry | | | | | | | |
| Spectrophotometry/colorimetry | 36 | — | — | — | 5.0 | 2.0 | 5.0 |
| Enzyme immunoassay | | | | | | | |
| Syva | 418 | — | — | — | 5.0 | 1.5 | 5.0 |
| Other | 118 | — | — | — | 5.0 | 3.0 | 5.0 |
| Fluorescence polarization imm. | | | | | | | |
| Abbott | 31 | — | — | — | 5.0 | 0.5 | 5.0 |
| Immunonephelometry/immunoturbid. | | | | | | | |
| Beckman | 21 | — | — | — | 8.0 | 3.8 | 8.0 |

*Source*: Ref. 14.