Quantitation and Mass Spectrometric Data of Drugs and Isotopically Labeled Analogs

Ray H. Liu
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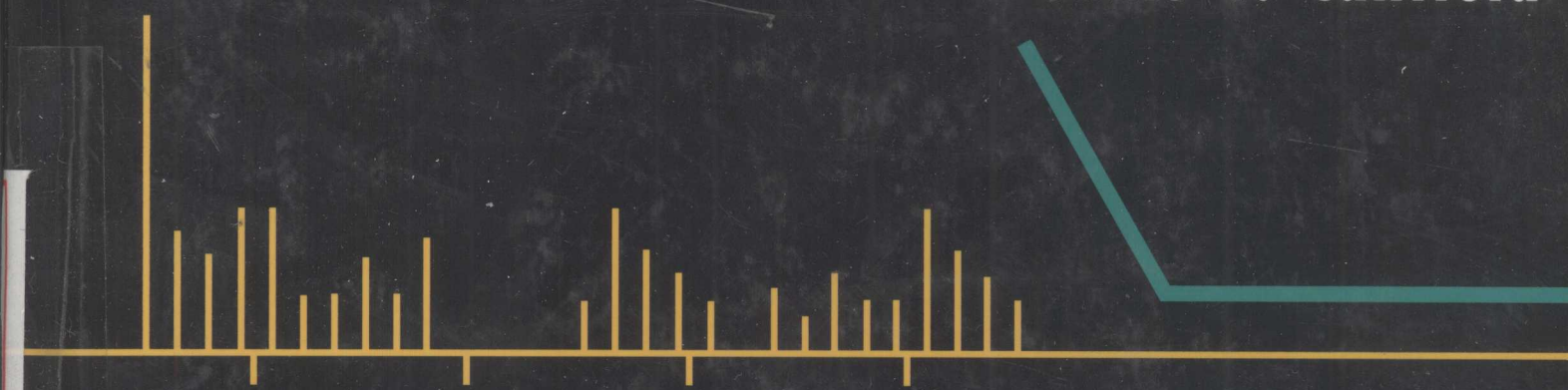

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Foreword

The whole is more than the sum of its parts. — Aristotle
The sum of all sums is eternity. — Lucretius

To say that mass spectrometric analysis of drugs in biological media is similar to archeology may be a bit of a stretch to some, but consider the parallels. The archeologist looks at fragments and sees patterns suggesting whole structures. A pottery shard becomes the intact vessel that in turn reveals cultural aspects of past generations. Likewise, when the forensic toxicologist is presented with a biological specimen, they perform an archeological "dig" for evidence of drug residues. Instead of a shovel or trowel, mass spectrometry becomes the tool for uncovering remains. Pattern analysis of the evidence, a technique used in virtually all fields of scientific endeavor, becomes essential in drug interpretation. Comparisons to standards of known purity are essential. Bodily processes frequently alter pharmaceutical products and illicit drugs to metabolites more suitable for elimination. The "remains" of biological analysis are the analytical report that identifies and provides quantitative information on what was present in the specimen.

The first and foremost goal of the analyst is to provide accurate and precise drug identifications and measurements. The power of chromatographic separation coupled with mass spectrometry allows this modern miracle to occur on drug residues that cannot be seen with the naked eye. The analytical report, thus, provides evidence of drug exposure based on what was present and identifiable and how much was present in the specimen. In many cases, the outcome of drug analysis is not a trivial issue and may be used in many circumstances such as guiding therapeutic outcome, accident, death and criminal investigations, and as a requirement in securing or continuing employment. Consequently, the analyst has to get it right! The results must be inconvertible. That is what this book is all about. One of the authors (RHL) and I have discussed the need for documentation of mass spectrometric data on drugs for many years. This work by the authors represents years of work compiling mass spectra of the many forms and derivatives of drugs and their metabolites and their isotopically labeled counterparts. This compilation should well serve those involved in drug analyses of biological specimens and those involved in interpretation of results.

Edward J. Cone, Ph.D.

Preface

The analysis of drugs and their metabolites in biological media are now expected to routinely achieve ±20% accuracy in the ng/mL concentration level. This is possible mainly because of the incorporation of the internal standard method, using isotopically labeled analogs of the analytes as the internal standards into the analytical protocols. The availability of various isotopically labeled analogs for a wide variety of drug analytes from commercial sources is also a helpful contributing factor.

Using isotopically labeled analogs of the analytes as the internal standards, the most important issue affecting the accuracy of the quantitation results and the achievable linear calibration range is the cross-contribution to the intensities of ions designating the analytes and their isotopically labeled analogs serving as the internal standards. Thus, the availability and the selection of quality ion-pairs designating the analytes and their isotopically labeled analogs (internal standards) are crucial matters. Quality ion-pairs come from careful selections of the isotopically labeled analogs to serve as the internal standards and the derivatization groups for the analyte/internal standard pairs that require chemical derivatization and amenable to chromatography-mass spectrometry methods of analysis.

With these understandings in mind, this book is prepared in three parts. Part One of this book includes two descriptive chapters illustrating crucial issues related to quantitative analysis using isotopically labeled analogs as the internal standards in the analytical protocols. Part Two of this book is a systematic compilation of full-scan mass spectra of drugs and their isotopically labeled analogs in various derivatization forms. Part Three of this book is a systematical compilation of cross-contribution data for ion-pairs, derived from various combinations of isotopically labeled analogs and chemical derivation groups that are potentially useful for designating the analytes and their internal standards. One hundred and three drugs along with 134 isotopically labeled analogs included in this study are grouped into 7 categories and accordingly presented in Parts Two and Three. Information included in these three parts should be of routine reference value to individuals and laboratories engaged in the analysis of drugs in biological media.

The preparation of this book was conceptualized during the summer of 1990 when one of the authors (RHL) was on an Intergovernmental Personnel Assignment serving as a visiting scientist at the U.S. Addiction Research Center's Laboratory of Chemistry and Drug Metabolism (Baltimore, Maryland), where Dr. Edward J. Cone then served as the Chief of the Laboratory. A major portion of the laboratory data was collected in 2004 under a contract (DTFAAC-04-C-00012) in the laboratory of Aeromedical Research Division, Civil Aerospace Medical Institute, U.S. Federal Aviation Administration (Oklahoma City, Oklahoma). Additional data collection, data preparation, and writing were completed at Fooyin University (Kaohsiung Hsien, Taiwan) with the support of a 3-year (2004–2007) grant from the Taiwanese National Science Council (NSC 93-2745-M-242-003-URD, NSC 94-2745-M-242-003-URD, NSC 95-2745-M-242-002-URD).

In addition to the financial supports mentioned above, the following colleagues have also made invaluable contributions to the completion of this book project: Chief Toxicologist Dr. Dong-Liang Lin of the Institute of Forensic Medicine (Taipei, Taiwan), Professor Dr. Wei-Tun Chang of Central Police University (Taoyuan Hsien, Taiwan), Principal Scientist Dr. Shiv Kumar of ISOTEC™

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Ray H. Liu, Ph.D.
Sheng-Meng Wang, Ph.D.
Dennis V. Canfield, Ph.D

About the Authors

Ray H. Liu received a law degree from Central Police University (then Central Police College, Taipei, Taiwan) and a Ph.D. degree in chemistry from Southern Illinois University (Carbondale, IL) in 1976. He is currently a professor in the Department of Medical Technology, Fooyin University (Kaohsiung Hsien, Taiwan), and professor emeritus in the Department of Justice Sciences, University of Alabama at Birmingham.

Before pursuing his doctoral training in chemistry, Dr. Liu studied forensic science under the guidance of Professor Robert F. Borkenstein at Indiana University (Bloomington) and received internship training in Dr. Doug Lucas's laboratory (Centre of Forensic Sciences in Toronto, Canada). Dr. Liu has worked as an assistant professor at the University of Illinois at Chicago, as a chemist at the U.S. Environmental Protection Agency's Central Regional Laboratory (Chicago, IL), and as a center mass spectrometrist at the U.S. Department of Agriculture's Eastern Regional Research Center (Philadelphia, PA) and Southern Regional Research Center (New Orleans, LA). He was a faculty member at the University of Alabama at Birmingham for 20 years and retired in 2004 after serving for more than 10 years as the director of the University's Graduate Program in Forensic Science.

Dr. Liu's works have been mainly in the analytical aspects of drugs of abuse (criminalistics and toxicology), with a significant number of publications in the following subject matters: enantiomeric analysis, quantitation, correlation of immunoassay and GC-MS test results, specimen source differentiation, and development of analytical methodologies. He has authored (or co-authored) several books and book chapters; more than 100 articles in refereed journals; and approximately 150 presentations in scientific meetings. He is qualified by the New York State Department of Health to serve as a laboratory director in forensic toxicology and he has served as a technical director in a U.S. drug-testing laboratory that held major contracts with military, federal, local, and private institutions.

Dr. Liu has been an active member of the following professional organizations for more than (or close to) 30 years: the American Chemical Society, Sigma Xi—The Scientific Research Society, the American Academy of Forensic Sciences (fellow), and the American Society for Mass Spectrometry. He is also a member of the Society of Forensic Toxicologists and the American Society of Crime Laboratory Directors (academic affiliate). Dr. Liu consults with several governmental and nongovernmental agencies, including serving as a laboratory inspector for the U.S. and the Taiwanese workplace drug-testing laboratory certification programs. He is the editor-in-chief of *Forensic Science Review* (www.forensicsciencereview.com) and serves on the editorial boards of the following journals: *Journal of Forensic Sciences (1998–2008)*, *Journal of Analytical Toxicology*, *Journal of Food and Drug Analysis* (Taipei), *Forensic Toxicology* (Tokyo), *Forensic Science Journal* (Taoyuan, Taiwan), and *Fooyin Journal of Health Sciences* (Kaohsiung, Taiwan).

Sheng-Meng Wang received a B.S. degree in forensic science from Central Police University (Taoyuan, Taiwan) in 1988 and a Ph.D. degree in chemistry from National Tsing Hua University (Hsingchu, Taiwan) in 1997. Dr. Wang is currently professor of forensic science and director of scientific laboratories, Central Police University.

Dr. Wang has been a visiting associate professor at the Graduate Program in Forensic Science, University of Alabama at Birmingham, and conducted research at the U.S. Federal Aviation Administration's Civil Aerospace Medical Institute (Oklahoma City, OK). Dr. Wang has been working in various areas of forensic toxicology and his current research activities include: evaluation of various chemical derivatization approaches in the sample preparation process, application of

solid-phase microextraction to the analysis of drugs in biological fluids, and the characterizations of drug depositions in various biological specimens.

Since 1988, Dr. Wang has been serving as a laboratory evaluator for the Drug Testing Laboratory Accreditation Program under the auspices of the (Taiwanese) National Bureau of Controlled Drugs. He has also been serving as the executive secretary for the Taiwan Academy of Forensic Science since 2006.

Dennis V. Canfield received a B.S. degree in biology from Lynchburg College (Lynchburg, VA) in 1971. He completed an M.S. degree in forensic science at John Jay College of Criminal Justice, City University of New York (New York, NY), in 1976. He earned a Ph.D. in forensic chemistry in 1988 at Northeastern University (Boston, MA). For the past 19 years, Dr. Canfield has been the manager of the Bioaeronautical Sciences Research Laboratory at the U.S. Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) in Oklahoma City, OK, conducting research into forensic toxicology, biochemistry, radiobiology, functional genomics, and bioinformatics.

Before joining CAMI, Dr. Canfield was a senior forensic chemist for the New Jersey State Police Crime Laboratory (Little Falls, NJ) for 5 years and worked as the director of forensic science at the University of Southern Mississippi (Hattiesburg, MS) for 10 years in a tenured associate professor position. Dr. Canfield has worked primarily in the areas of drug identification and toxicology, starting in 1971 at the New Jersey State Police Crime Laboratory, and has continued to the present. He has published numerous peer-reviewed articles on drug identification and toxicology and testified on numerous occasions in federal, state, and local courts as an expert in forensic science. Dr. Canfield has participated as an editor and author in *Selected Powder Diffraction Data for Forensic Materials*, and *Carbon Monoxide and Human Lethality: Fire and Non-Fire Studies*.

Dr. Canfield is a fellow in the American Academy of Forensic Sciences, a member of the Society of Forensic Toxicologists, Sigma XI Research Society, and the Executive Board of the National Safety Council's Committee on Alcohol and Other Drugs.

PART TWO
MASS SPECTRA OF COMMONLY ABUSED DRUGS AND THEIR ISOTOPICALLY LABELED ANALOGS IN VARIOUS DERIVATIZATION FORMS

PART THREE
CROSS-CONTRIBUTIONS OF ION INTENSITY BETWEEN ANALYTES AND THEIR ISOTOPICALLY LABELED ANALOGS IN VARIOUS DERIVATIZATION FORMS

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PART ONE

ISOTOPICALLY LABELED ANALOG AS INTERNAL STANDARD FOR DRUG QUANTITATION — METHODOLOGY

Chapter 1

Quantitation of Drug in Biological Specimen — Isotopically Labeled Analog of the Analyte as Internal Standard —

INTRODUCTION

The detection of drugs and their metabolites (collectively referred to as *drugs* hereafter) in biological tissues and fluids (collectively referred to as *biological media* hereafter) has always been an important component in clinical diagnostic analysis, forensic testing, pharmacological research, and drug discovery study. With advances in *analytical instrumentation* and a greater understanding of metabolism, we can now analyze drugs at a much lower concentration that was previously undetectable. Recent emphasis on monitoring illegal drug use in the workplace calls for massive testing of urine specimens, which has inspired the development and significant advances in *specimen pretreatment* technologies.

Newer instrumentation, such as GC-MS/MS or LC-MS/MS, capable of providing greater *specificity* and *signal-to-noise* ratio, are advantageous for identifying unknown metabolites at low concentrations. On the other hand, robust GC-MS methods are routinely used under therapeutic drug monitoring, emergency room drug screening, and workplace drug testing settings, in which the drugs of interest have previously been well characterized and often present at higher levels.

Analytical instrumentation and specimen pretreatment technologies are "*hardware*" aspects of the analytical sciences; the development and implementation of complementary "*software*" components help reach the full potential made possible by hardware advances. For example, the development of the "internal standard" method [1,2], especially the adaptation of isotopically labeled analogs (ILAs) as the internal standards (ISs) [3,4], has greatly improved the accuracy in the quantitation of drugs in biological media. Developments related to the use of ILAs as the ISs for accurate quantitation are based on GC-MS technology and readily adapted into GC-MS/MS, LC-MS, and LC-MS/MS applications. While many GC-MS/MS, LC-MS, and LC-MS/MS studies utilize ILAs as ISs, they do not generate better quantitative results than GC-MS and we know none that was devoted to better understanding the methodology itself.

Significance of Accurate Quantitation

Recent government regulations in workplace drug testing activities include monitoring quantitative data [5]; thus, making quantitation an important aspect of quality control practices in the analysis of drugs in biological media. Furthermore, specific "cutoff" value has been adapted as one of the essential criteria for defining whether a specific test specimen is "positive" or "negative" for a targeted drug. Accurate quantitation has now become an essential part of the routine testing protocol; it has, in addition to being a scientific pursuit, evolved into a legal issue.

In many non-routine analytical settings, emphasis may be placed on the *detection of a drug at very low concentrations* and *interpretation of quantitative data with small inter-specimen drug concentration differences*. Furthermore, sample preparation approaches often result in a final aliquot with hundred- or thousand-folds concentration in drugs' content; raw analytical result derived from the measurement step are then multiplied by a factor (of two or three orders of magnitude), thereby grossly magnifying any inaccuracies embedded in the raw data. Thus, proper interpretation and utilization of analytical findings rely heavily on the accuracy of the raw analytical data. This is especially critical in circumstances where drugs are present at a very low concentration level and interpretations are based on small inter-specimen differences, e.g., in hair-related studies where the objectives are on:

a. Differentiating drugs derived from external contamination from incorporation through active ingestion [6];
b. Determining racial bias due to the drug incorporation process or drug recovery in the sample pretreatment step [7]; or
c. Assessing variation in susceptibility to environmental contaminations due to differences in race origin [8] or hair treatments [9].

Preferred Calibration Method

Accurate quantitation requires a proper calibration (standardization) procedure to fully account for artifacts derived from variations in specimen matrix, specimen preparation, and instrumental conditions. Three most commonly used calibration techniques are the *analytical* or *working curve*, *standard additions*, and *internal standard* methods [1,2].

Mass spectrometric methods have proven to be one of the most sensitive and specific methods for drug assay. In particular, selected ion monitoring (SIM) approach has been used for several decades to achieve better accuracy and precision in ion intensity measurements. This approach is still an integral part of the quantitation protocol involving various forms of mass spectrometric methods in where an internal standard method is used. A typical protocol involves monitoring several selected corresponding ions (referred to as "ion-pairs" hereafter) designating the targeted drug and the ILA adapted as the IS. One or several calibration standards, containing known amounts of the drug, are processed in parallel with test specimens throughout the entire analytical protocol. All calibration standards and test specimens are spiked with the same amount of the IS. Quantitation is achieved by comparing a selected drug-to-IS ion-pair intensity ratio observed in the *test specimen* with the same ratio observed in the *calibration standard(s)*.

With practically identical chemical property and mass spectrometric fragmentation characteristics, an ILA is a preferred IS because it offers the following advantages:

a. Errors derived from (i) incomplete recovery of the drug in the sample preparation process or (ii) varying gas chromatographic and mass spectrometric conditions are compensated for; and
b. The presence of interfering materials (or mechanisms) affecting the detection (or quantitation) of the drug will result in the absence of the IS in the final chromatogram [10] or altered response and ion intensity ratios [11]; thus, alerting the analyst to conduct further investigation.

I. INTERNAL STANDARD AND QUANTITATION IONS

Under low resolution measurement conditions, the intensities of ions designating the drug and the IS are representative of these compounds' concentrations only if the following conditions are met:

a. The ILA is *isotopically pure* (an extrinsic factor); and
b. An adequate number of the *labeling isotopes are positioned at appropriate locations* in the molecular framework, so that, after the fragmentation process, ions meeting the following requirements are present (an intrinsic factor): (i) with high-mass and significant intensities; (ii) retaining at least three labeling isotopes; and (iii) without (or with insignificant) *cross-contribution* or CC (*see* Section II in Chapter 2 for full description on this phenomenon) between the ions designating the drug and the IS.

A. Inadequate Isotopic Purity — An Extrinsic Factor

If the ILA is not manufactured with sufficient *isotopic purity*, the addition of the IS, especially when a high concentration of the ILA is used, will result in the observation of a significant amount of the drug in a truly negative specimen. For a truly positive specimen, the resulting quantitative data will include systematic errors.

This problem has been well illustrated by a benzoylecgonine (BZ) study [12] in which a high concentration of ILA IS (1,500 ng/mL BZ-d_3) was adapted.

At the time of the study, a high concentration of IS (BZ-d_3) was commonly used by laboratories engaged in testing workplace specimens. Since the concentration of BZ encountered in positive samples are typically high (>5,000 ng/mL), adapting a high IS concentration can minimize the following problems:

a. To reduce the intensities of the ions designating the analyte (BZ), solvent volume used to reconstitute the extract may be so large that the resulting IS become too dilute to generate adequate ion intensity for reliable quantitative determination; and
b. The contribution of the isotopic ions, derived from the naturally abundant ^{13}C-atoms in the analyte, to the intensity of ions designating the IS may become very significant when the concentration of the latter is disproportionally low.

This study [12] examined two lots of 0.1 mg/mL BZ-d_3 in methanol. With the addition of 4.5 µg BZ-d_3 IS into 3 mL of urine samples (corresponding to 1,500 ng/mL), followed by solid-phase extraction, derivatization, and concentration down to 100 µL for GC/MS analysis, ions designating BZ (*m/z* 331, 272, and 210) were observed in truly negative test specimens. For a negative specimen, the concentration (X) of the observed BZ caused by the addition of these two lots of IS were 7.080 and 28.99 ng/mL as calculated by **Equation 1-1**.

$$X / (1{,}500 - X) = (\text{Ion intensity of } m/z\ 210) / (\text{Ion intensity of } m/z\ 213) \quad (1\text{-}1)$$

(where ions *m/z* 210 and 213 were used to designate BZ and the IS.) These concentrations correspond to 0.472% and 1.87% impurity of BZ in these two lots of BZ-d_3 IS provided by that specific manufacturer in 1988.

Isotopically impure ISs also introduced systematic errors embedded in the quantitative data derived from positive specimens. Data shown in **Table 1-1** demonstrate the systematic errors exhibiting the following characteristics:

a. No error is introduced if the concentration of the BZ in the test specimen is at the exact level of the BZ in the calibration standard.
b. A higher apparent result will be observed if the concentration of the BZ in the test specimen is lower than the concentration of the BZ in the calibration standard, and vice versa.
c. The degree of the above deviations increases as the isotopic impurity in the adapted IS increases.

Table 1-1. Quantitation error as a function of the isotopic impurity level of the internal standard and the difference between the analyte concentration in the calibration standard and the test sample

Isotopic impurity[a,b]	Apparent concentration[a,c]	True concentration[a,c]	% Error
	83.82	80.70	+3.87
	141.1	140.7	+0.284
	146.0	145.7	+0.206
7.080	147.1	147.0	+0.0680
	147.3	147.2	+0.0679
	148.1	148.0	+0.0676
	252.1	256.9	–1.87
	84.19	72.28	+16.5
	154.1	154.8	–0.453
27.99	155.5	156.6	–0.702
	156.8	158.0	–0.759
	161.7	163.9	–1.34
	273.5	296.6	–7.79
	288.9	314.6	–8.17
	292.5	319.1	–8.34
	293.9	320.7	–8.36
	298.3	326.0	–8.50

[a] Concentration in ng/mL. The concentration of the IS is 1,500 ng/mL. The analyte's (benzoylecgonine) concentration in the calibration standard is 150 ng/mL.
[b] 7.080 and 27.99 ng/mL of benzoylecgonine are included in the 1,500 ng/mL of benzoylecgonine-d_3 IS.
[c] *See* the original reference [12] for the calculation of the apparent and true analyte concentrations.

B. Cross-Contribution Derived from Ion Fragmentation Mechanism — An Intrinsic Factor

Under typical GC-MS analytical conditions, the drug and the IS are chromatographically inadequately resolved; thus, a proposed ILA IS must generate at least one (preferably two or three) ions relatively free from CC by the drug. There must also be at least one ion designating the drug that is relatively free from CC by the proposed ILA IS. (Current practice requires at least three "interference-free" ions derived from the drug allowing monitoring two ion-intensity ratios as an important criterion for drug confirmation.)

To make this possible, the *labeling isotopes in the ILA must be positioned at appropriate locations* in the molecular framework, allowing the fragmentation process to generate a sufficient number of high-mass ions (with significant intensities) that (a) retain the labeling isotopes; and (b) will not interfere with the intensity measurement of ions derived from the drug. Otherwise, the [M + n] ion (derived from the drug) may, because of the naturally occurring isotope abundance, make a significant contribu-

tion to the intensity of the ion designating the ILA that corresponds to the [M] ion of the drug. ("M" is the mass of the ion derived from the drug and selected for monitoring; "n" is the nominal mass difference of the ions designating the drug and the ILA serving as the IS.)

If deuterium, as in most currently available commercial products, is used as the labeling isotope, a difference in three mass units (n = 3) between the drug and the ILA is sufficient under normal circumstances. (If the concentration of the analyte is disproportionally higher than the concentration of the IS included in the assay process, the intensity of the [M + 3] ion originated from the analyte may become significant enough to require an additional analysis using a diluted aliquot.)

Secobarbital/$^{13}C_4$-secobarbital (SB/$^{13}C_4$-SB) data shown in **Figure 1-1** [13] illustrate how CC (of the intensities of ions designating SB and the IS) affects the accuracy in quantitation. In this example, CCs between the first pair of ions (*m/z* 196/200) are so insignificant (*see* CC data shown in the legend of the figure) that the linearity of the "SB/IS ion-pair intensity ratio" versus "SB concentration" plot (Figure 1-1-a) extends through a wide analyte concentration range. On the other hand, CCs between the two ions in the second ion-pair (*m/z* 181/185) are much more significant. In this latter case, significant errors can occur if the ion-pair intensity ratio generated from the test specimen is used directly to determine the analyte's concentration using a linear calibration model. The error can become very serious if the drug's concentration in the test specimen is significantly higher or lower (Figure 1-1-b) than the drug concentration adapted in the calibration standard (*see* further discussion in the next section — Fitting Calibration Data).

II. FITTING CALIBRATION DATA

Series of ions, [M – H_n], are typically seen in the EI fragmentation process [14,15]. The [M – H_n] processes, the presence of the naturally abundant ^{2}H-atoms in the drug and the ^{1}H-atoms in the ^{2}H-labeled IS, the isotopic effect of the [M – H_n] processes [16,17], and varying conditions in each sample (test specimen or standard) prohibit quantitations based on direct comparison of intensities of ions derived from the drug and the corresponding ion of the IS. The effects of these phenomena are minimized by comparing the drug/ILA IS ion-pair intensity ratio observed in the test sample against those observed in one or a set of calibration standards.

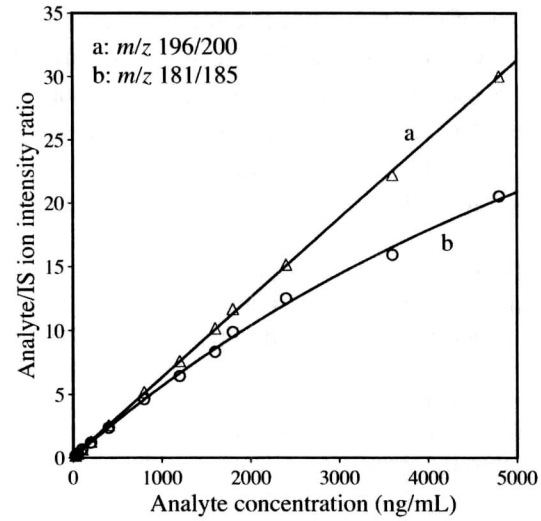

Figure 1-1. Fitting calibration data adapting linear (a) and hyperbolic (b) models using ion-pairs with different degrees of cross-contribution from the secobarbital/secobarbital-$^{13}C_4$ system. (a) *m/z* 196/200: 0.23% of the measured intensity of *m/z* 196 (designating secobarbital) is contributed by secobarbital-$^{13}C_4$; while 0.017% of the measured intensity of *m/z* 200 (designating secobarbital-$^{13}C_4$) is contributed by secobarbital. (b) *m/z* 181/185: 1.6% of the measured intensity of *m/z* 181 (designating secobarbital) is contributed by secobarbital-$^{13}C_4$, while 0.29% of the measured intensity of *m/z* 185 (designating secobarbital-$^{13}C_4$) is contributed by secobarbital [13].

A typical quantitative GC-MS protocol usually involves monitoring several selected ions from the drug and the ILA IS. Quantitation is achieved by comparing a selected drug-to-ILA ion-pair intensity ratio observed from the *test sample* against the same ratio observed from the *calibration standard*. The calibration standard contains the same amount of the IS (as those added to the test specimens) and a known amount of the drug, and is processed in parallel with the test specimens. The drug's concentration in the test specimen can be calculated using a one-point calibration approach as shown **Equation 1-2**.

The *one-point calibration* approach, in fact, is a *two-point linear calibration* method using only one empirical data point with the assumption that:

a. The drug-to-ILA ion-pair intensity ratio is zero when the drug's concentration in the test specimens or the standards is zero, i.e., the ILA IS will not contribute to the intensity of the ion monitored for the drug; and
b. The drug-to-ILA ion-pair intensity ratio will truly reflect the drug/ILA IS concentration ratio in the test specimens (and the standards), i.e., the drug will not contribute to the intensity of the ion monitored for the IS, and vice versa.