Matrix Effects in the Liquid Chromatography-Tandem Mass Spectrometry Method of Analysis

H.-C. Liu*, D.-L. Lin
Institute of Forensic Medicine
Zhonghe, New Taipei City
Taiwan

H. H. McCurdy
eLab Solutions, Inc.
Atlanta, Georgia
United States of America

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* Corresponding author: Dr. Hsiu-Chuan Liu, Ministry of Justice Institute of Forensic Medicine, 123 Min'an Street, Zhonghe, New Taipei City 235, Taiwan; +886 2 2226 6555 x 701 (voice); hcliu@mail.moj.gov.tw.

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ABSTRACT: Matrix effects are dependent on biological fluid, ionization type, and sample preparation method. Although matrix effects are observed for both ionization types, ESI is especially susceptible, while APCI has proved to be less vulnerable. Sample preparation method has a clear influence on matrix effects as does, in particular, the choice of internal standard. When matrix effects result in severe ion suppression or enhancement of the target analyte by co-eluting residual components, they are typically located in isolated regions of the chromatogram. Postcolumn infusion and postextraction addition methods have been developed for the assessments of matrix effects. Approaches used for eliminating, minimizing, or compensating for matrix effects include improved sample preparation and chromatographic separation, sample dilution, and the utilization of internal standards. Matrix effects may not always be fully circumventable because a perfectly consistent matrix does not exist, but they can be significantly minimized and largely compensated for by various approaches, such as standard addition, matrix-matched calibration, and the use of isotopic analogs of the analytes as internal standards.

KEY WORDS: Ion suppression, LC-MS/MS, matrix effects, stable-isotope-labeled internal standards.

INTRODUCTION

Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) has been demonstrated to be a powerful technique for the quantitative analysis of drugs and metabolites in biological fluids. This technique is now widely applied to toxicological analysis and human pharmacokinetic studies. The ever-increasing demands for high-throughput bioanalysis have often resulted in LC-MS/MS methods with minimum sample preparation and chromatography, where large amounts of endogenous matrix components may potentially co-elute with the target analyte. These co-eluting components — often invisible to the mass spectrometric detector when multiple reaction monitoring (MRM) is employed for the detection of analyte and the internal standard (IS) — may significantly affect (usually attenuating) the efficiency and reproducibility of the ionization processes occurring in the ion source.

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the most commonly used soft ionization sources in mass spectrometry. Both ESI and APCI are susceptible to errors in quantification caused by matrix ion suppression or enhancement effects due to co-elution of matrix components [9,44,47,48]. This undesirable phenomenon, termed "matrix effects" in LC-MS/MS bioanalysis, is generally neither reproducible nor repeatable between sample batches or even samples; thus, it compromises the quality of the quantitative data derived from the assay process. The presence of a matrix effect can dramatically decrease the response of the analyte, thus affecting sensitivity, or it can adversely affect the accuracy/precision of a bioanalytical method by affecting the analyte to IS response ratio.

Even more dismaying is that matrix effects, despite whatever precautionary steps are taken, may occur at any given point even with the most rigorously validated analytical procedure. This is simply because whatever matrix is being analyzed is almost never consistently homogenous, i.e., always the same and never varying. For the forensic toxicologist, heavily variable forensic blood samples are often the norm. Even commercially supplied human plasma samples have been shown to have such different characteristics as to cause ion depression varying from one lot to the other [9,52].

I. MECHANISM OF MATRIX EFFECTS

Based on different ion formation mechanisms as illustrated in **Figure 1** [50], both ESI and APCI induce preferential formation of the protonated or deprotonated molecule without fragmentation. Kebarle and Tang [23] first reported the ion signal suppression phenomenon, showing that ESI responses of organic bases decreased with increases in concentrations of other organic phases. In the presence of a variety of co-eluting matrix components from biological samples usually procured from different sources, the MS/MS response signal can vary significantly for the analyte(s) of interest. The matrix-effect presence or absence is highly dependent on the degree of sample cleanup and the degree of chromatographic separation [35].

A. Formation of Matrix Effects

Matrix effects were thought to originate from the competition between an analyte and the co-eluting,

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ABOUT THE AUTHORS

H.-C. Liu; D.-L. Lin; H. H. McCurdy

Hsiu-Chuan Liu received a B.S. degree from the China Medical University (Taichung, Taiwan) in 1996. In 2005 and 2010, she also received M.S. and Ph.D. degrees from the Taipei Medical University (Taipei City, Taiwan), respectively. Dr. Liu is currently an associate researcher in the Toxicology Division, Institute of Forensic Medicine, Ministry of Justice (MOJ) of the Republic of China (Taiwan) in New Taipei City, Taiwan.

Dr. Liu was a pharmacist at Taipei Veterans General Hospital from 1996 to 2001. From 2001 to 2010, she served as a toxicologist in the forensic toxicology laboratory of the Institute of Forensic Medicine. She was promoted to her current position in 2010 as a supervisory forensic toxicologist. Dr. Liu has been actively working on research projects supported by the (Taiwanese) National Science Council and the MOJ. Dr. Liu's main research interests are application of liquid chromatography with tandem mass spectrometry and quadrupole time-of-flight mass spectrometry in postmortem forensic toxicology, with emphasis on systematic toxicological analysis. She has published more than 10 articles in peer-reviewed journals.

Dr. Liu's contributions and accomplishments are widely recognized in the forensic science community in Taiwan. She has been granted several awards, including the Research Article Award from the Taiwan Academy of Forensic Sciences in 2006, 2008, and 2009.

Dong-Liang Lin received B.S. and M.S. degrees from the China Medical University (Taichung, Taiwan) in 1982 and 1984, respectively. In 1995, he also received a Ph.D. degree from the Taipei Medical University (Taipei City, Taiwan). Dr. Lin is currently the head of the Toxicology Division of the Institute of Forensic Medicine, Ministry of Justice (MOJ) of the Republic of China (Taiwan), serving as the chief toxicologist for the Institute.

Through a competitive examination system, Dr. Lin entered government service in 1987, working in the laboratory division of the MOJ's Bureau of Investigation. He was transferred to his current position in 2001. Dr. Lin has received forensic toxicology and related training from several US institutions, including the Cook County Medical Examiner's Office (Chicago, IL), the New Jersey State Medical Examiner's Office (Newark, NJ), and the

U.S. Fish and Wildlife Service Forensics Laboratory (Ashland, OR). Dr. Lin has been actively working on research projects supported by the (Taiwanese) National Science Council, the Council of Agriculture, and the MOJ. He has published more than 30 articles in peer-reviewed journals.

Dr. Lin is a member of the American Academy of Forensic Sciences (AAFS) and the International Association of Forensic Toxicologists (TIAFT). He is also a member of the Taiwan Society of Forensic Medicine and the Taiwan Academy of Forensic Sciences (TAFS).

Horton H. McCurdy received his undergraduate degree in chemistry from Berry College (Mount Berry, GA) and a Ph.D. degree in medicinal chemistry from the School of Pharmacy, University of Mississippi (University, MS). Dr. McCurdy is currently the laboratory director of eLab Solutions, a CLIA- and COLA-certified clinical laboratory, in Atlanta, GA.

Dr. McCurdy has more than 30 years' experience as a forensic, analytical, and research toxicologist and has served as research and technical director at the Georgia Bureau of Investigation Division of Forensic Science (Atlanta, GA).

Dr. McCurdy is a member of several forensic toxicology organizations and has served as president of the Society of Forensic Toxicologists and as chairman of the Toxicology Section of the American Academy of Forensic Sciences. He has been a diplomate of the American Board of Forensic Toxicology for more than 20 years and also served two terms as a director for the American Board of Forensic Toxicology. Dr. McCurdy is a recipient of the prestigious American Academy of Forensic Sciences R. N. Harger Award in recognition of his outstanding contributions to forensic toxicology.