Chemical Derivatization for Forensic Drug Analysis by GC- and LC-MS

D.-L. Lin[1], S.-M. Wang[2], C.-H. Wu[3*], B.-G. Chen, R. H. Liu[4]

[1]Department of Forensic Toxicology
Institute of Forensic Medicine, Ministry of Justice
New Taipei City
Taiwan

[2]Department of Forensic Sciences, Central Police University
Kuei-Shan Hsiang, Taoyuan City
Taiwan

[3]Department of Safety, Health, and Environmental Engineering
National Yunlin University of Science and Technology
Douliou, Yunlin County
Taiwan

[4]Department of Justice Sciences
University of Alabama at Birmingham
Birmingham, Alabama
United States of America

TABLE OF CONTENTS

* Corresponding author: Dr. Chih-Hung Wu, Department of Safety, Health, and Environmental Engineering, National Yunlin University of Science and Technology, Douliou, Yunlin County, Taiwan; +886 9 2120 7635 (cell phone); g9710813@gmail.com.

1042-7201/28-1/Jan. 2016/17–35/$28.50 •

Chemical Derivatization for the Analysis of Drugs by GC-MS and LC-MS

REFERENCE: Lin D-L, Wang S-M, Wu C-H, Chen B-G, Liu RH: Chemical derivatization for the analysis of drugs by GC-MS and LC-MS; *Forensic Sci Rev* 28:17; 2016.

ABSTRACT: Utilizing chemical derivatization (CD) to improve gas chromatographic (GC) and GC-mass spectrometric (MS) analysis of drugs has been abundantly studied and widely practiced, while in liquid chromatography (LC) and LC-MS, application of CD approaches is still at an early stage. Silylation, acylation, and alkylation are common CD reactions, long adopted by GC and GC-MS (including GC-MS/MS) methodologies, to improve analytes' stability and/or to optimize their extraction/separation and detection efficiencies. Highly polar and nonvolatile analytes are not amenable to GC-MS analysis without the CD step; however, CD can improve LC-MS analysis of highly polar analytes, especially those with low molecular weights. Many CD reagents developed for GC and GC-MS applications are also effective in LC-MS. Other CD reagents are developed for LC-MS to enhance analytes' performance under electrospray and atmospheric pressure ionization sources. Certain CD reagents are designed to facilitate analytes' fragmentation (upon collision-induced dissociation) in generating intense product ions for sensitive MS/MS detection. In this review, various CD reagents, reaction types, and application examples are presented and discussed, with emphases on GC-MS and LC-MS analysis of drugs of abuse.

KEYWORDS: Acylation, alkylation, chemical derivatization, drug analysis, enantiomeric separation, GC-MS, GC-MS/MS, LC-MS, LC-MS/MS.

INTRODUCTION

Application of Chemical Derivatization (CD) in Gas Chromatography (GC) and GC-Mass Spectrometry (MS)

A series of sample preparation steps are often applied to a test specimen (typically with complex matrix) to prepare the analyte for analysis by the instrumental method of choice. One potential step is the conversion of the analyte to a more suitable form (for analysis) through a well-designed CD route. This CD option, thereby incorporated, may inadvertently increase the analytical cost; it may also complicate data interpretation caused by uncertainty on the completeness of an analyte's conversion process and other interfering factors, such as the introduction of impurities. However, drugs are often derivatized prior to their GC methods of analysis to improve their analytes' (a) volatility and stability (e.g., in the GC injection port); (b) chromatographic property and/or separation efficiency; (c) functional group characterization; and (d) analysis by non-mass spectrometric selective detection methodologies (e.g., electron capture and nitrogen-phosphorus detection) [10]. With MS detection in GC-MS (including GC-MS/MS) methodologies, the CD step can also (a) generate favorable mass shift in mass spectra; (b) modify fragmentation pattern; and (c) facilitate the chemical ionization methodology [10].

Application of CD in Liquid Chromatography (LC) and LC-MS

One commonly cited advantage of the LC and LC-MS (including LC-MS/MS) methodologies is that highly polar and/or low volatile analytes can be directly analyzed without the CD step. It was soon recognized, however, that CD can significantly benefit the LC-MS methods for the analysis of certain categories of analytes, e.g., highly polar short-chain acids [60] and steroid hormones [7]. Still at an early stage of development, to what extent CD approaches can benefit the LC-MS methodology is yet to be fully realized; nevertheless, numerous studies have already demonstrated that CD can improve stability, optimize recovery and separation, and enhance the detection of many analytes [12].

Scope and Relevance of This Review

Figure 1 [16] illustrates the approximate ranges (in terms of polarity and relative molecular mass) over which GC-MS and electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) LC-MS can be successfully applied to the analysis of selected compound classes. CD has the potential to favorably alter the ionization properties of analytes. For example, organic acids can be derivatized to reduce their polarity for electron impact (EI) GC-MS analysis or derivatized to increase their polarity, making them more amenable to analysis by positive ESI LC-MS.

Having noted this expanded role played by CD, we wish to widen the scope of an earlier review [27] to include CD's applications in LC-MS. Since scientists from the bioanalytical, pharmaceutical, environmental, and food-safety evaluation communities have been mainly responsible for these advances, most analytes included in their studies are not of particular interest to forensic

ABOUT THE AUTHORS

D.-L. Lin; S.-M. Wang
C.-H. Wu; B.-G. Chen; R. H. Liu

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 US 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).

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 (Birmingham, AL), and conducted research at the US 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 served as the executive secretary for the Taiwan Academy of Forensic Science since 2006.

Chih-Hung Wu received B.S. and M.S. degrees from the Da-Yeh University (Changhua, Taiwan) in 2002 and 2004, respectively. In 2015, he also received a Ph.D. degree from the National Yunlin University of Science and Technology (Yunlin County, Taiwan). Dr. Wu is currently a postdoctoral fellow in the Department of Safety, Health, and Environmental Engineering, National Yunlin University of Science and Technology.

Dr. Wu's research interests are environmental biotechnology, microbial fuel cell technology, and wastewater treatment. After receiving his master's degree, Chih-Hung worked in the mass spectrometry laboratory, Department of Medical Technology, Fooyin University, as a research assistant from January 2005 to June 2007. He has been the key persone for several derivatization articles published during this time. These articles were published in *Journal of Chromatography A, Journal of Analytical Toxicology, Clinica Chimica Acta,* and *Journal of Food and Drug Analysis*.

Bud-Gen Chen received a bachelor's degree in applied chemistry and a master's degree in medical technology, both from Fooyin University (Kaohsiung City, Taiwan). Ms. Chen is currently taking a break from her professional pursuits, raising two children at home.

Following the completion of her undergraduate education, Ms. Chen started working in the mass spectrometry laboratory, Department of Medical Technology, Fooyin University, first as a graduate student, then as a research assistant. She has since become very skillful in various aspects related to this analytical technology and is the key author of several articles, with focuses on derivatization approaches, quantification, and compounds of diagnostic values for diabetic patients. These articles were published in *Journal of Mass Spectrometry*, *Journal of the American Society of Mass Spectrometry*, *Analytical and Bioanalytical Chemistry, Journal of Analytical Toxicology, Journal*

of Chromatography A, *Analyst*, and *Journal of Neurochemistry*. The article published in *Journal of Neurochemistry* derived from a joint project with researchers at the Max Planck Institute of Psychiatry (Münich, Germany).

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 serving as the editor-in-chief of *Forensic Science Review* and professor emeritus in the Department of Justice Sciences, University of Alabama at Birmingham (Birmingham, AL).

Before pursuing his doctoral training in chemistry, Dr. Liu studied forensic science under the guidance of Professor Robert F. Borkenstein at Indiana University (Bloomington, IN) 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 (Chicago, IL), as a chemist at the US Environmental Protection Agency's Central Regional Laboratory (Chicago, IL), and as a center mass spectrometrist at the US 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 each of 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 coauthored) several books and book chapters; more than 120 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 US 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 approximately 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 (SOFT) and the International Association of Forensic Toxicologists (TIAFT). Dr. Liu consults with several governmental and nongovernmental agencies, including serving as a laboratory inspector for the US and the Taiwanese workplace drug-testing laboratory certification programs. He is the editor-in-chief of *Forensic Science Review* and serves on the editorial boards of the following journals: *Journal of Analytical Toxicology*, *Journal of Food and Drug Analysis* (Taipei), *Forensic Toxicology* (Tokyo), and *Forensic Science Journal* (Taoyuan, Taiwan).