An Executive Summary

Applications of GC-MS-MS and LC-Ion Trap MS-MS in Postmortem Forensic Toxicology

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Introduction

The modern day postmortem forensic toxicologist is confronted with many new challenges today due to the emerging trends in the use of new legal and illegal drugs in our society. With the advent of new illegal stimulants, depressants, hallucinogens, synthetic fentanyls and opioids flooding the market over the last several years, forensic toxicologists are obligated to maintain the necessary techniques to properly identify and measure these substances in human tissues and fluids. The postmortem toxicologist becomes an investigator of sorts using the techniques of science to reveal the cause and manner of death. Forensic toxicologists, however, can no longer afford to operate in the dark. Information regarding the decedent, any related evidence collected at the scene, and the pathologist's findings, must all be considered in their search to identify lethal substances.

One of the more significant challenges in forensic toxicology is developing adequate screening methods to detect the broadest range of drugs and poisons possible from complex postmortem biological matrices. The data obtained must have enough detail to assure the compound has been properly identified, alleviating the possibility of false positives or negatives. Mass spectrometry (MS) becomes an obvious choice to address this issue. Additional challenges include the choice of sample introduction technology (GC or LC) suitable for the broadest range of compounds, as well as the choice of the right MS instrument to meet the sensitivity requirements necessary to detect low concentrations in blood and tissue samples. This manuscript discusses the value of GC–MS/MS and LC-Ion Trap MS/MS when used as quantitative and qualitative screening tools to address these challenges in postmortem forensic toxicology analyses.

The Analytical Approach to Post Mortem Forensic Toxicology

The most difficult task of any postmortem forensic toxicology laboratory is to provide accurate identification of a wide range of toxins in human tissues and fluids. The current trends in drug use require postmortem forensic laboratories to be able to provide a comprehensive and highly sensitive screening protocol to identify abused as well as prescription type drugs and poisons. Such a screening protocol must be applied to complex matrices to include a large number of different compounds. These compounds can be pharmaceutical drugs, illegal drugs, household chemicals, gases or other types of compounds, and the testing protocols used must provide conclusive identification. MS technology provides the best ability for identification and quantitation in this application. Qualitative analysis must be conclusive and quantitative procedures must be sensitive and provide identification certainty. The methods must also be robust and able to meet strict QC guidelines. Postmortem laboratories do not always get quality samples, so analytical methods must be adaptable to a wide range of materials or bodily tissues and fluids.

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The approach most forensic toxicology laboratories use involves an initial urine and blood screening technique, such as Enzyme Linked Immunosorbent Assay (ELISA) or Enzyme Multiplied Immunoassay Technique (EMIT). Considered presumptive in their results due to the generalized nature of the screen, this initial testing process, if done properly, must be followed by more concrete confirmation methods. Consequently laboratories follow this initial screening procedure with some type of MS analysis such as GC-MS and LC-MS to expand their screening and confirm substances found in the presumptive screens. LC Ion Trap MS/MS is such a type of technology used to confirm drugs and other toxins in blood, urine and tissue samples. To better understand the impact of the toxin on the decedent the screening/confirmation process is often followed by a quantitative analysis to measure the concentration of a substance in specific fluids and tissues. Both triple quadrupole LC-MS/MS and GC-MS/MS are often used for this purpose.

There are many advantages to using an LC-Ion Trap MS/MS system for screening including higher sensitivity at the ppb or even ppt levels and better compound identification using full spectral analysis. The ability to perform full spectral analysis allows analysts to be adaptable to all of the new trends in illegal drugs, while instrument robustness and software simplicity minimizes the learning curve, enabling analysts to get systems up and operating fast.

Similarly, there are many advantages to using GC-MS/MS for quantitation. At ppb or ppt levels, the ability to distinguish targets from background or other compounds is superior to GC-SIM-MS and the ability to use deuterated internal standards improves accuracy and precision. GC-MS/MS is more selective in its identification and provides higher S/N for most analyses, improving peak detection and integration. Once optimized, the method is very robust and reproducible.

The sensitivity and the spectral detail provided by LC-Ion Trap MS/MS makes it a superior technology to other forms of MS/MS analyses, both for small and large molecule drugs in postmortem fluids and tissues, especially when utilized in MS/MS or MSn mode. Also, with GC-MS/MS, quantitation is far superior to any other GC-MS technique. When properly implemented, it provides the highest quality in accuracy and precision when applied to compounds conducive to GC analysis.

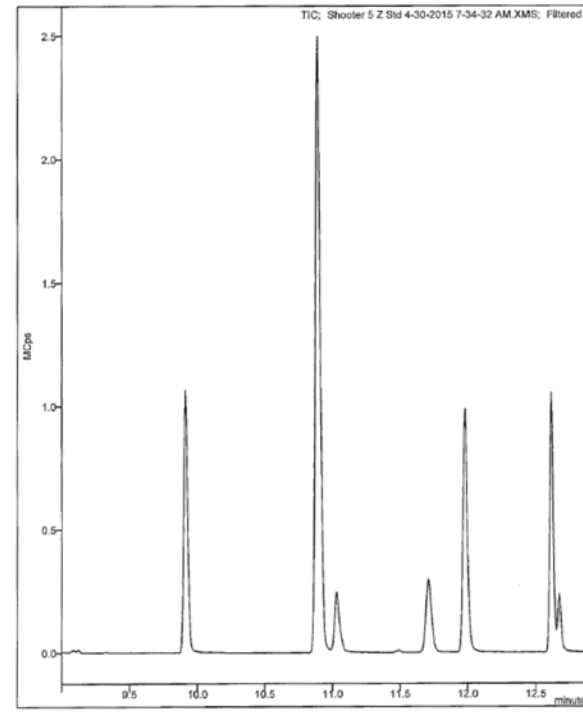
The current challenge in postmortem forensic toxicology includes identifying and quantifying many different types of compounds from a wide variety of sources: e.g. synthetic designer stimulants, including cathinones, amphetamines, and tryptamines, bath salts or analogues of MDMA or ecstasy, all kinds of tryptamines or DMT analogues, more than 400 different types of synthetic cannabinoids, such as K2-Spice, and synthetic analogues of fentanyl (acetyl fentanyl, beta-hydroxythiofentanyl, and butyryl fentanyl), which are of particular concern because of their contamination in heroin. Synthetic opioids are also now emerging, as well as synthetic hallucinogens known as N-BOMes. Meanwhile, the pharmaceutical industry is coming up with new drugs to treat anxiety, cardiac conditions, and new anti-psychotic drugs. In a modern day postmortem forensic toxicology laboratory such as the Miami-Dade Medical Examiner Department, many cases reveal a mixture of legal and illegal drugs acting in concert as a lethal cocktail and requiring each be identified and measured to better understand their role in the cause of death.

Applications of GC–MS/MS in Postmortem Forensic Toxicology

Legacy GC systems and methods that rely on nitrogen phosphorus detection (NPD) depend solely on retention time for identification and are not very specific. Meanwhile, traditional single quadrupole GC-MS systems have evolved into more modern triple quadrupole instruments, like the Bruker EVOQ GC-Triple Quad. The EVOQ GC-TQ was designed to reliably quantify samples in the fastest sample-to-report time possible while delivering exceptional sensitivity, precision, accuracy, and linearity over a wide dynamic range for multiple reaction monitoring (MRM) assays. Legacy methods can be easily converted to the EVOQ system, with most using the same exact extraction procedure and analyte specific deuterated internal standards.

Figure 1 is an example of a EVOQ GC-TQ MS total ion chromatogram (TIC) of the commonly encountered opiates hydrocodone, codeine, oxycodone, hydromorphone, 6-monoacetylmorphine, morphine, and oxymorphone along with their

Figure 1



deuterated internal standards. **Figure 2** illustrates the extracted ion chromatograms for hydromorphone, hydrocodone, codeine and 6-mono-acetylmorphine (clock-wise starting in the upper right of **Figure 2**) along with a quantifier ion on the top, as well as two qualifier ions that are specific for each of these analytes. These chromatograms were obtained from the analysis of solid phase extracted blood samples at 5 ng/mL using deuterated internal standards.

Another commonly run method is for the analysis of cannabinoids. Using the EVOQ GC-TQ MS system and derivatized samples (so that they are amenable to GC analyses), acceptable S/N can be obtained at the 0.1 ng/mL level from blood following protein precipitation and solid phase extraction. **Figure 3** shows THC at 0.1 ng/mL, hydroxy-THC at 0.1 ng/mL, and carboxy-THC at 1 ng/mL (the established LODs for these three analytes).

Applications of LC–Ion Trap Screening in Postmortem Forensic Toxicology

LC-Ion Trap screening methods can be used to detect a wide range of drugs. A typical workflow using the Bruker amaZon system, incorporating Toxtyper is presented in **Figure 4**. As seen in **Figure 4**, the complete Toxtyper workflow including sample preparation, measurement and reporting requires approximately 30 minutes. Serum, plasma, blood or urine samples are cleaned-up by either Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE), or simple Protein Precipitation (PP). The subsequent LC-MSn analysis including sample analysis, data processing, and report generation takes about 11 minutes.

In one study, legacy GC-MS screening procedures were compared to the Toxtyper LC-MS system workflow for 80 cases chosen at random, including whole blood, liver, brain, and drug paraphernalia samples all prepared using solid phase extraction. Surprisingly, eighty percent of the cases contained additional compounds consistent with case history and prescription medication detected by the LC-MS, which were not detected by the GC-MS system. Never-before GC-MS detected compounds included metformin, risperidone (an anti-psychotic), sildenafil, and various antibiotics like azithromycin.

Another application involving a drug paraphernalia case

Figure 2

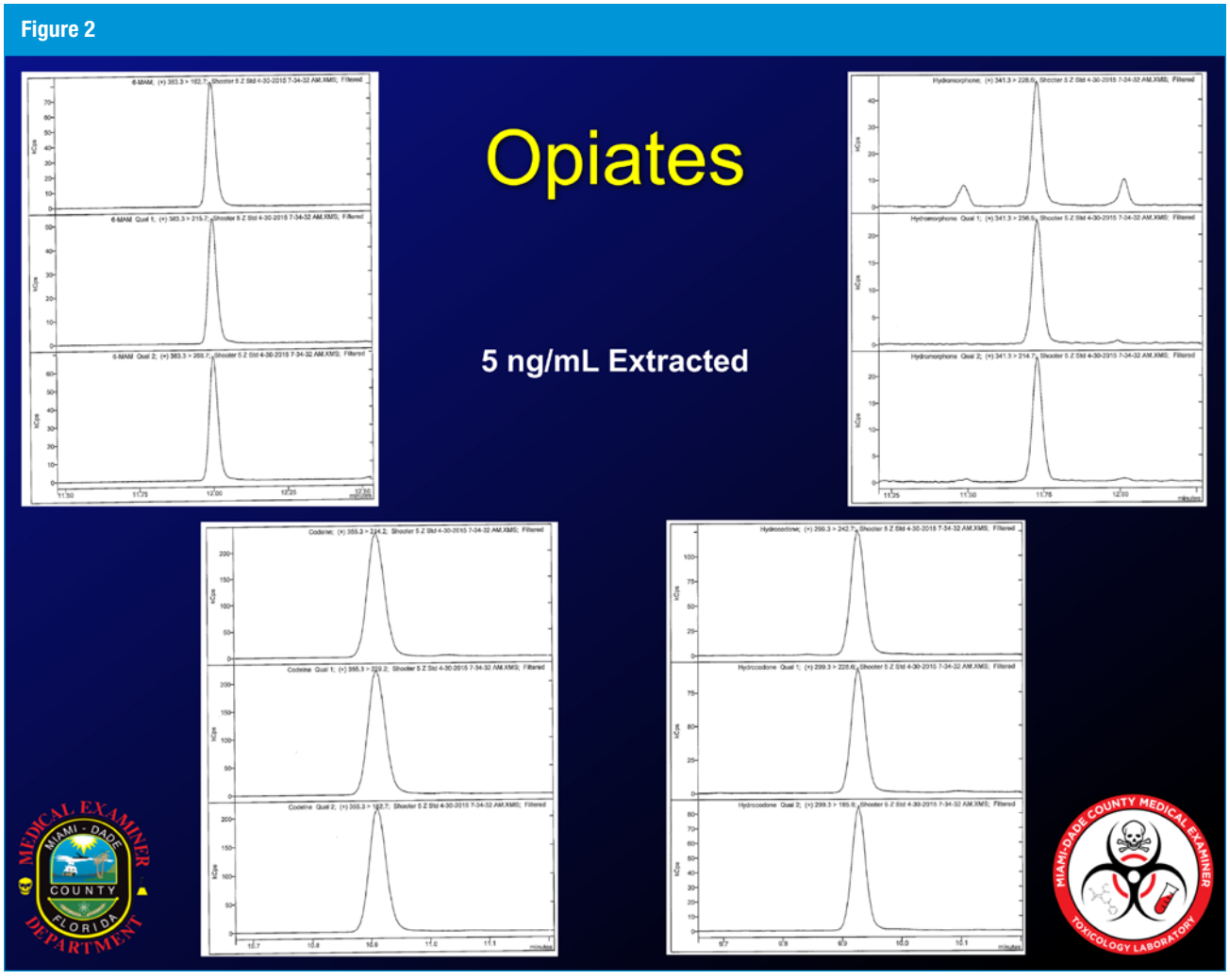
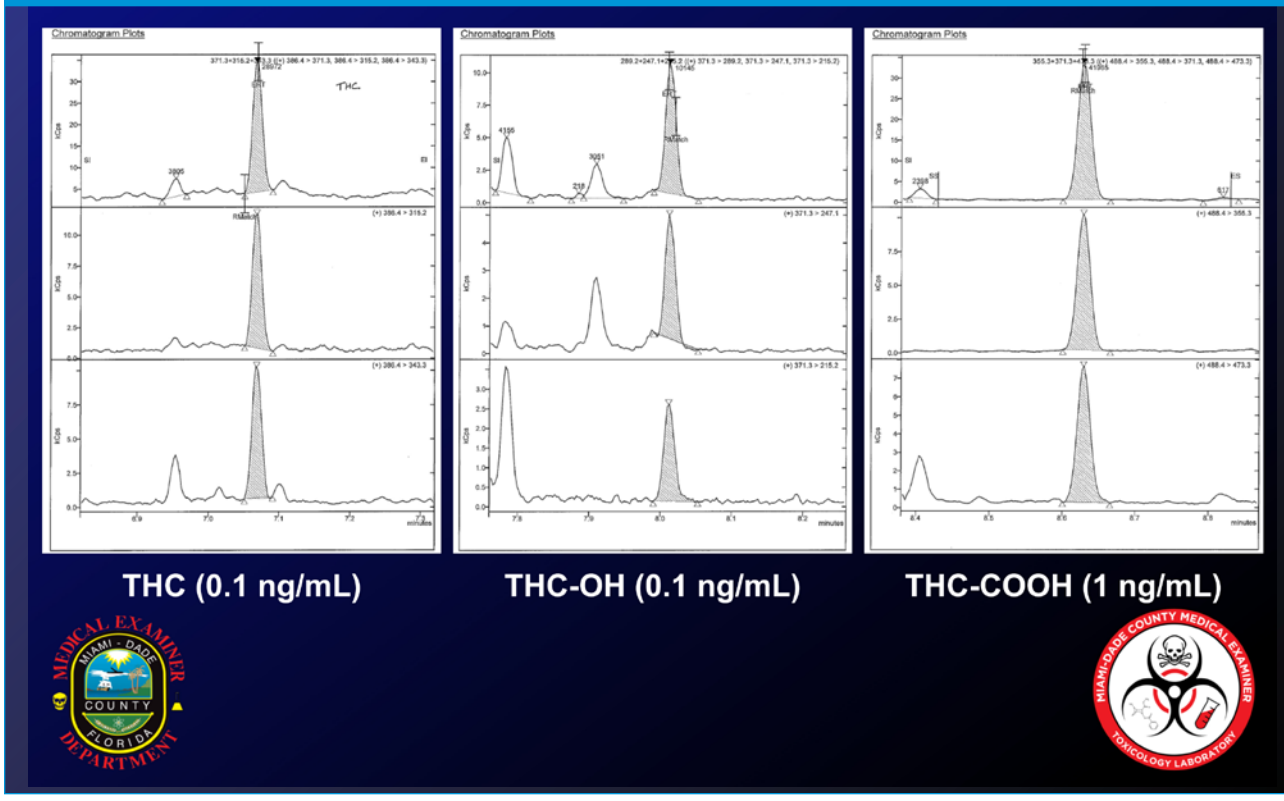
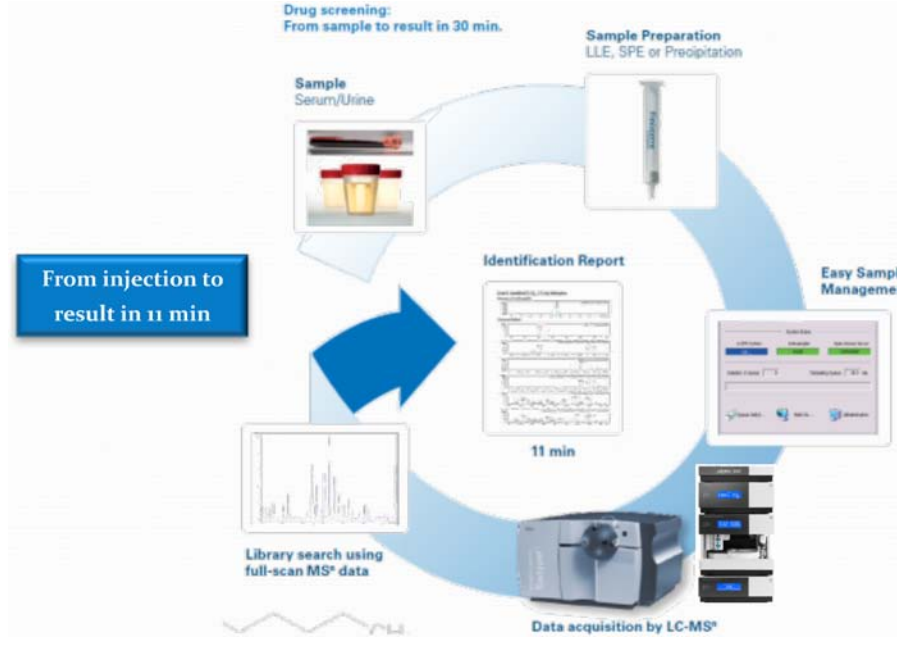


Figure 3



resulted in the identification of a previously unseen fentanyl derivative. Using the GC-MS system, only fentanyl was observed. However, using the Toxtyper system, in addition to fentanyl, diphenhydramine, cocaine, 6-mono-acetylmorphine, THC, a compound called beta-hydroxythiofentanyl were observed. Beta-hydroxythiofentanyl is a fentanyl analogue sold on the black market in the 1980's and was never detected at the Miami-Dade Medical Examiner Department previously.

Figure 4



Currently, there are five LC-Ion Trap MS/MS screening methods in use at the Miami-Dade County Medical Examiner Department Toxicology Laboratory: benzodiazepine screen, a pain panel screen, a psych panel screen, a designer stimulant screen, and a general screen. The general screen uses the Toxtyper system as-is for unknown cases, scanning for over 900 compounds in 11 minutes. The benzodiazepine method targets 50 different compounds, ranging from benzodiazepines and their metabolites to sedatives and sleep aids like zolpidem and zopiclone. Twenty-seven of the compounds

in this 50-compound screen are validated using guidelines recommended by the Scientific Working Group for Forensic Toxicology (SWGTOX), with limits of detection (LODs) for these compounds between 1 to 10 ng/mL. **Figure 5** lists the LODs observed for a few of the benzodiazepines broken down from 1 ng/mL to 10 ng/mL well-below that required to determine whether or not they have contributed to the cause of death. **Figure 6** is an example chromatogram of a benzodiazepine screen extracted in whole blood at the 2ng/mL level. LODs for these compounds can range as low as 0.5 ng/mL.

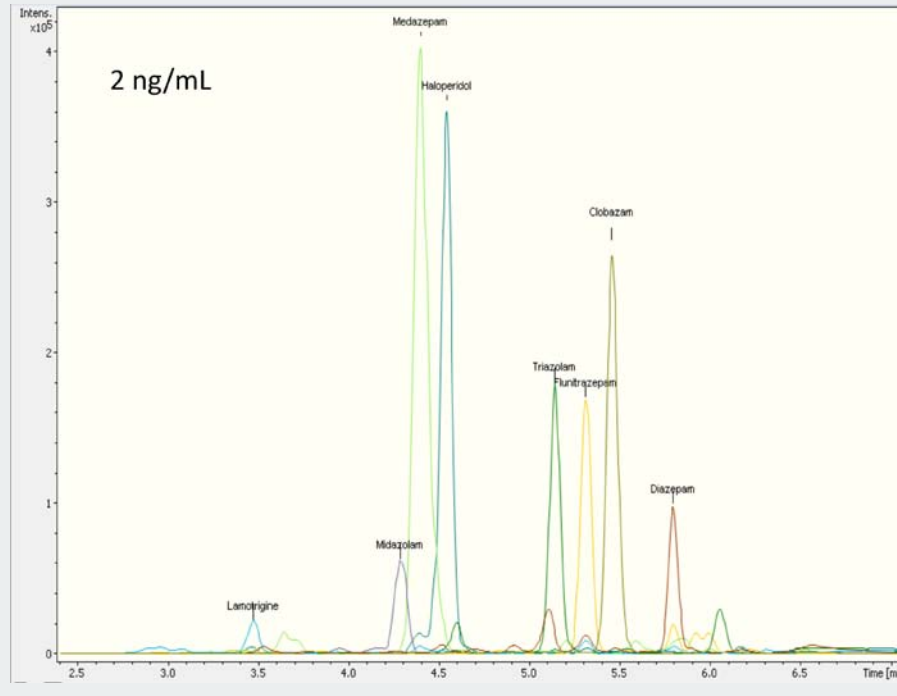
The psych panel consists of 22 compounds, including anti-psychotics like quetiapine, risperidone and other antidepressants, with LODs on the same level as the pain drugs (0.5-10 ng/mL).

The designer drug method is a 64-compound method, 35 of which were added in-house, as they were not previously in the Toxtyper library. Using the Toxtyper system, the LOD is reduced by an order of magnitude for many compounds compared to the amaZon system.

Figure 5

1ng/mL	2ng/mL	5ng/mL	10ng/mL
Chlordiazepoxide	Alprazolam	Alpha-Hydroxyalprazolam	7-Aminoclonazepam
Haloperidol	Flunitrazepam	Clobazam	Bromazepam
Medazepam	Flurazepam	Clonazepam	Estazolam
Temazepam	Midazolam	Desalkylflurazepam	Lorazepam
Zolpidem	Oxazepam	Diazepam	N-Desmethyl flunitrazepam
Zopiclone	Triazolam	Flumazenil	Nordiazepam
		Lamotrigine	
		Nitrazepam	

Figure 6



Conclusion

The use of GC-MS/MS technology compared to GC-SIM-MS or GC-NPD allows laboratories to quantify compounds at lower concentrations with a higher level of selectivity, accuracy, and precision. Ion trap mass spectrometry has many significant advantages when used as a screening tool in postmortem forensic toxicology analyses, covering a broad range of drugs and poisons. Detailed spectral data assures that compounds have been properly identified, alleviating the possibility of false positives or negatives. The utilization of the triple quadrupole and ion trap mass spectrometry technology, used in the Bruker EVOQ GC-TQ and the amaZon Toxtyper LC-Ion Trap MSn systems, results in a robust and automated drug testing solution to reliably and efficiently identify and quantify thousands of real compounds from complex postmortem biological matrices.