K35 The Advantages and Limitations of MRM vs. Full Scan MS/MS for Drug Confirmation Utilizing LC/MS/MS

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After attending this presentation, attendees will become familiar with two methods of drug screening using liquid chromatography tandem mass spectrometry (LC/MS/MS) and will understand the pros and cons of using full scan MS/MS versus two multiple reaction monitoring (MRM) transitions for drug confirmation.

This presentation will impact the forensic community by adding data to the debate of what constitutes a confirmation of drug presence in LC/MS/MS.

Introduction and Hypothesis: GC/MS has been the analytical technique of choice for drug confirmation in forensic toxicology labs. However, the use of LC/MS/MS for screening and confirmation has been increasing and this technique continues to be adopted by a rising number of labs. When any new confirmatory technique is implemented, debates arise regarding what constitutes a confirmation. Although it has been established that three ions are necessary for GC/MS SIM confirmation, the criteria for an LC/MS/MS confirmation is still a highly debated topic. In this work, confirmation using two MRM transitions is compared and contrasted with confirmation using full scan MS/MS spectra. The advantages and limitations of both techniques are presented and discussed. The goal of the study was to investigate which LC/MS/MS method was more robust and which had the largest dynamic range for drug confirmation.

Methods: Standards of various drug compounds were spiked into drug free urine and diluted 10x with mobile phase. Analysis was performed on an LC interfaced to a hybrid triple quadrupole/linear ion trap (LIT) mass spectrometer (Applied Biosystems 3200 QTrap). All compounds were analyzed using positive mode electrospray ionization.

For the MRM only method, two MRM transitions per analyte were monitored with the second transition functioning as a qualifier ion. The ratio of the peak areas of the target MRM to the qualifier MRM was calculated. For confirmation, it was required that the ratio be within +/- 20% of the standard.

When full scan MS/MS spectra were used for confirmation, an MRM survey scan was used to detect the presence of an analyte. If an analyte was detected, the system automatically acquired a full scan MS/MS spectrum of the compound using Q3 operating in LIT mode. The resulting spectrum could be searched against a library for identification and confirmation. A purity match of about 70% or higher was required for confirmation.

The precision for the two methods was also compared since it was expected that the full scan method may compromise precision due to switching between MRM and full scan modes.

Preliminary Data: Preliminary results from analysis of amphetamines showed that a full scan spectrum using LIT was more robust in confirmation than using a ratio of two MRM transitions. In 15 samples at 100 ng/mL, amphetamine and methamphetamine passed every time in both methods, MDA failed in both methods, and MDMA passed in the full scan method but failed 13 out of 15 times in the MRM with qualifier method. Also, there was no significant compromise in precision with the full scan MS/MS method: the within run and between day precision was 4.6 and 6.0, respec- tively, compared to 3.8 and 5.8 for the MRM with two transitions method.

Conclusion: To conclude, initial findings indicate that both the full scan method using a LIT and the MRM with two transitions method are robust and precise in performing amphetamine confirmations. However, the full scan method was more robust at the low end of the dynamic range. This study will be expanded to include drugs from other classes to determine if the initial trends are observed across most compounds and to determine how well each method functions in situations of co-eluting drugs at large concentration ranges.

LC/MS/MS, Drug Screening, Drug Confirmation

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