Comparison of automated and manual sample preparation for detection of various chemical warfare agent metabolites

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Introduction

Chemical warfare agents (CWAs) have been used in terroristic attacks, posing grave threats to public safety and global security. Detecting these intact agents in biological samples is particularly challenging, as many CWAs are either rapidly metabolized into stable metabolites or bind to macromolecules, both of which serve as critical biomarkers for exposure. There have been screening methods created to detect SBMSE (sulfur mustard metabolite), PMPA (soman acid), and EMPA (VX acid) using liquid chromatography (LC) tandem mass spectrometry (MS/MS) (Rodin et al., 2014). Hamelin et al. compared detection sensitivity on high-resolution (HRMS) and Triple Quad (MS/MS) mass spectrometers but used different metabolites (2014). However, conventional methods of screening individual samples for specific metabolites or bound agents are often labor-intensive and inefficient, highlighting the pressing need for innovative solutions such as automated sample preparation or advanced, unified mass spectrometry techniques that enable rapid and comprehensive detection.

This study sought to refine CWA exposure screening protocols, maximize detection sensitivity, and conclude the most effective sample preparation technique including automated and manual.

Methods and Materials

The study utilized three metabolites (SBMSE, EMPA, and PMPA), their internal standards (SBMSE-d6, EMPA-d3, and PMPA-d3), human urine, and various solvents. Samples were analyzed using LC-MS/MS on a SCIEX Triple Quad 6500+ system paired with an Agilent 1290 Infinity I LC system optimized to maximize sensitivity and linearity as seen in Figure 1.

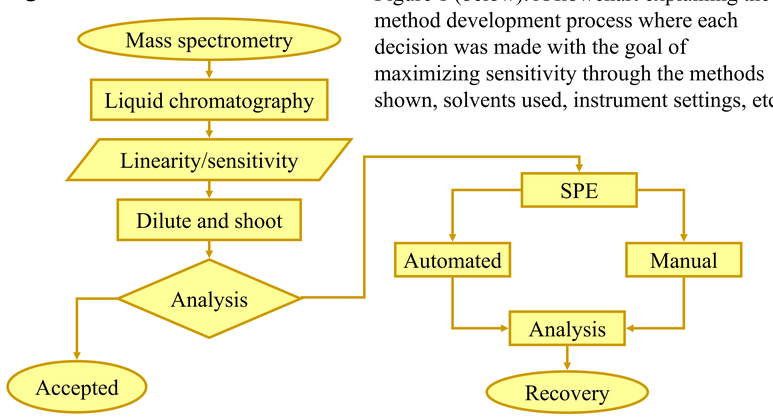
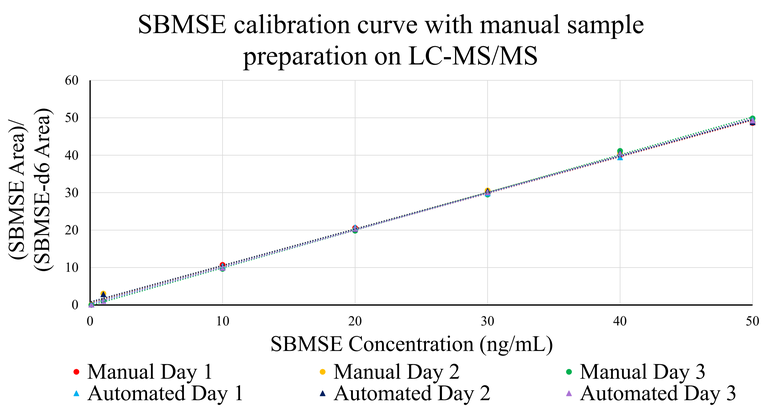


Figure 1 (below): A flowchart explaining the method development process where each decision was made with the goal of maximizing sensitivity through the methods shown, solvents used, instrument settings, etc.

Methods and Materials (continued)

Sample preparation first used simple dilution or 'dilute-and-shoot' as described by Rodin et al. (2014) but failed to achieve satisfactory limits of detection. Accuracy analysis was performed by spiking human urine with each metabolite and their internal standard for seven calibrators (0.1, 1, 10, 20, 30, 40, and 50 ng/mL) and three quality control (QC) standards (0.3, 25, and 35 ng/mL). These results give the limit of detection (LOD) which is the lowest concentration with accurate quantification. The lowest limit of quantification (LLOQ) is the lowest concentration where the signal ratio was not 0. The LOD was calculated by multiplying 2.776 by the standard deviation of the LLOQ. Solid phase extraction (SPE) was employed to isolate and concentrate the metabolites for better sensitivity. SPE was performed using Oasis HLB 96-well plates as detailed by Liu et al. (2017) for SBMSE and Strata SI cartridges described by Hamelin et al. (2014) for PMPA and EMPA. Methods were conducted both manually and automated with the Biomek i7 Hybrid Workstation. The QC standards were extracted both manually and with automation to determine if there is a difference in compound recovered to assess the feasibility of utilizing this automation.

Results



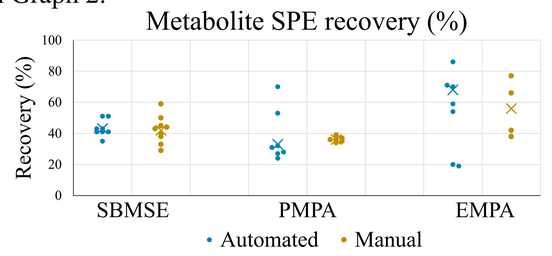
Graph 1 (above): This graph shows the average calibration curve for EMPA with manual sample preparation ran on LC-MS/MS. The equations' average R^2 = .9990 and ranges from .9980 to .9997, which is acceptable.

The R^2 values of each calibration curve are above 0.99 indicating a strong linear and replicable relationship as seen for SBMSE (Graph 1), which suggests a replicable method. The LOD for SBMSE on MS/MS was found to be 0.0099 ng/mL, which is adequately sensitive.

Results (continued)

Percent recovery determines the amount of metabolite retained isolation of the target analyte through SPE. Mean percent recoveries were similar between automated (SBMSE: M = 43%, n = 8; PMPA: M = 33%, n = 6; EMPA: M = 68%, n = 8) and manual (SBMSE: M = 42%, n = 9; PMPA: M = 36%, n = 6; EMPA: M = 56%, n = 4) sample preparation as seen in Graph 2.

Graph 2 (right): Univariate scatterplot comparing the mean percent recoveries of all tested metabolites for automated and manual sample preparation. Means are denoted by ×.



Conclusion

This study optimized and validated methods to detect CWA metabolites (SBMSE, EMPA, and PMPA) in human urine using LC-MS/MS. Strong linear calibration curves (R^2 > .99) and a low limit of detection demonstrates the sensitivity and reliability for the tested techniques. Automation resulted in a comparable percent recovery due to limiting human error and is more time-efficient. The findings support using automated sample preparation for accurate, sensitive, and efficient metabolite detection expanding forensic and public health response capabilities for potential CWA exposure. Further studies could be conducted comparing sensitivity for HRMS to MS/MS for testing feasibility of a unified screening method.

References

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