NIJ Forensic Laboratory Needs Technology Working Group (FLN-TWG)

IMPLEMENTATION STRATEGIES:

LC-MS-Based Forensic Toxicology Screening

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The Forensic Laboratory Needs Technology Working Group (FLN-TWG) developed this Implementation Strategy. The FLN-TWG is an activity administered under the National Institute of Justice (NIJ) Forensic Technology Center of Excellence (FTCoE) program. RTI International leads the FTCoE, which is supported through an NIJ Cooperative Agreement (2016-MU-BX-K110), Office of Justice Programs, U.S. Department of Justice (DOJ). Any opinions or points of view expressed in this white paper are those of the FLN-TWG and do not necessarily reflect the official position or policies of NIJ or the DOJ.

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Introduction

This paper provides guidance for the implementation and utilization of liquid chromatography-mass spectrometry (LC-MS)–based toxicological screening as a more advanced alternative to traditional immunoassay. LC-MS gives a multi-targeted, highly selective and sensitive screening capability compared with the screening of limited drug classes or target analytes offered by traditional immunoassay. Various LC-MS approaches have been used as an alternative, including LC-MS/MS (liquid chromatography-tandem mass spectrometry), LC-TOF-MS (liquid chromatography-time of flight mass spectrometry), and LC-QTOF-MS (liquid chromatography-quadrupole time of flight mass spectrometry).

These techniques are amenable to toxicological screening using routine postmortem or antemortem specimens. MS-based screening has received significant attention because of the proliferation of novel psychoactive substances (NPS) and the need for increased and rapidly adaptable testing scope and sensitivity. These benefits are an important consideration for NPS (e.g., novel synthetic opioids, benzodiazepines, cathinones, and cannabinoids), the use of which is commonly transient and dominated by geographical trends, requiring flexibility and agility with respect to analytical testing.

Although the most significant challenge associated with LC-MS–based screening is cost associated with obtaining, validating, and implementing this technology, costs must be weighed against the potential opportunity costs, such as the considerable public safety and criminal justice consequences associated with failures to identify a substance or mis-identifications.

Anticipated Implementation Costs

Because introducing new instrumentation into the laboratory is a costly and time-consuming endeavor, laboratory management must carefully weigh the resource needs and expected analytical outcomes prior to implementation. The costs of instrumentation, facility modifications, and personnel resources should be considered.

Within the industry, there are several instrument manufacturers that supply various types of LC-MS instruments suitable for forensic toxicology. Although pricing will vary based on the quotations received from different manufacturers, LC-TOF-MS and LC-QTOF-MS are generally the most expensive and carry a significant price tag for the one-time acquisition cost, starting at ~$400,000 (LC-TOF-MS) and over $500,000 (LC-QTOF-MS) for one-time acquisition. However, leasing equipment may be a viable option for some laboratories. In addition to the instrument acquisition costs, ancillary pieces of equipment may also be needed such as a nitrogen generator (~$25,000–$35,000), an uninterrupted power supply (~$25,000), and ongoing costs associated with a maintenance contract (~$30,000–40,000/year), consumables, and additional data storage for high-resolution mass spectrometry (HRMS).

LC-MS/MS is somewhat less expensive (~$350,000) but still requires a significantly increased capital outlay compared with immunoassay-based screening.

Updates or upgrades to the laboratory facility may also be required to house an LC-MS instrument. Modifications to facility infrastructure (e.g., reagent gas, power, exhaust, temperature, and humidity control) may be necessary before instrument installation. The particular requirements of each instrument may limit the locations where an instrument can be installed. With the exception of a few floor LC-QTOF-MS models, the instruments generally sit on a benchtop, but their footprints require significant square footage (~10–15 ft2) and a standalone nitrogen generator (~4–6 ft2). In addition, many instruments can interface with a laboratory's information management system (LIMS); however, the LIMS provider will most likely need to work with the instrument manufacturer for the initial configuration.

As with any new technology, there are costs associated with training on the new platform and validating methods. Costs for certified reference materials will also be higher than for immunoassay because of the expanded scope. To defray costs and support broader NPS testing capabilities, the Centers for Disease Control and Prevention began offering opioid reference materials free to qualifying labs in 2019.[1] Even though the initial training and validation costs are higher, new drugs can be incorporated and re-validated more readily than with immunoassay once the new instruments are implemented.

Personnel resources should also be considered. The complexity of this instrumental platform may require a lead scientist with experience or an advanced degree in analytical chemistry to oversee method development and validation and quality assurance task performance. Routine operation may be accomplished by a bachelor's-level scientist.

Specialized training on hardware and software is necessary and is often vendor-specific. Therefore, training and technical support from the vendor should be considered during the initial purchase of equipment.

Potential Funding Sources

Funding may be acquired via numerous granting opportunities and local/state funding.[2,3,4] Consideration may be given to setting up partnerships or regional centers where multiple jurisdictions pool funds and resources to provide this instrumental platform to a number of laboratories. There are instances when vendors will work with state and local authorities to establish themselves in a forensic laboratory. They will often provide equipment at low cost to do this.

[1] Centers for Disease Control and Prevention. (2020, November 3). *Traceable opioid material kits to improve laboratory detection of synthetic opioids in the U.S.* https://www.cdc.gov/nceh/dls/erb_opioid_kits.html.
[2] Bureau of Justice Assistance (BJA). (2020, September 9). *Paul Coverdell Forensic Science Improvement Grants Program*. U.S. Department of Justice, Office of Justice Programs. https://bja.ojp.gov/program/coverdell/overview.
[3] Centers for Disease Control and Prevention (CDC). (2019, September 3). *Overdose data to action.* National Center for Injury Prevention and Control. https://www.cdc.gov/drugoverdose/od2a/index.html
[4] National Highway Traffic Safety Administration (NHTSA). (n.d.). *Highway Safety Grant Programs*. U.S. Department of Transportation. https://www.nhtsa.gov/highway-safety-grants-program

Considerations Regarding Providing this Analytical Service

There are many advantages associated with LC-MS–based screening, including the following:

- Increased testing scope (i.e., larger number of drugs);
- Ability for retrospective data analysis (to identify new or emerging drugs) with some modes of data acquisition (i.e., TOF-MS, QTOF-MS);
- High sensitivity;
- New drugs readily incorporated into existing methods;
- Ability to target both free and conjugated drugs if needed;
- Multiple LC-MS platforms now available (improved selection of instruments to choose from);
- Multiple acquisition modes available, including data-dependent acquisition and data-independent acquisition (lending themselves to multiple criteria for decision-making, e.g., library searching, isotope ratios, retention times, mass accuracy); and
- Reduced consumable costs relative to immunoassay-based techniques.

Disadvantages include the following:

- Increased capital outlay for instrumentation;
- Need for personnel with specialized training/skills;
- Multiple instrument configurations and data acquisition modalities that can further complicate implementation/validation;
- Requires personnel with specialized training/experience;
- Possibly more extensive sample preparation than immunoassay (to avoid excessive maintenance/matrix effects);
- More routine maintenance (and is potentially less robust) than immunoassay;
- Cost considerations for redundancy (e.g., during equipment failure or maintenance);
- Possible need for negative and positive ionization to achieve requisite sensitivity for some drugs (may require more than one injection per sample);
- Increased analysis time per sample; and
- Increased data storage requirements for HRMS.

Considerations for Instrument Selection

LC-MS/MS is widely employed for confirmatory analysis and its use for toxicological screening is also increasing. Although the widespread use of and familiarity with LC-MS/MS may facilitate implementation for screening purposes, it does not lend itself to retrospective data analysis and is considered "targeted" screening.

HRMS (e.g., LC-TOF-MS, LC-QTOF-MS, LC-Orbitrap) approaches are more expensive than triple quadrupole LC-MS/MS but are amenable to non-targeted screening and retrospective data analysis. Vendors use various nomenclature to describe similar acquisition modes. Using this approach, data-independent acquisition—for which vendor-specific nomenclature may include All Ion Fragmentation (AIF), MSALL, MSE, or SWATH (sequential window acquisition of all theoretical fragment-ion spectra)—can be highly advantageous, although data analysis is considerably more complex. Data storage for HRMS must also be considered. For example, depending on how the mass spectra are stored (e.g., centroid data, profile data), a batch file of 48 samples could range from 10 GB to 100 GB.

Considerations when Conducting Method Development and Validation

Initial method development and implementation is more time-consuming than immunoassay depending on laboratory protocols and available resources. However, once the technology is in place, new compounds can be included fairly swiftly (if required), or at regular intervals in "batches" to improve efficiency (e.g., twice annually).

Laboratories should carefully consider the scope and sensitivity of testing required during initial method development. This may vary depending on the type of investigation (i.e., medicolegal death investigation,[5] impaired driving,[6] drug-facilitated crimes[7]). Standards for scope and sensitivity for various investigation types have been proposed in addition to standards for mass spectral data acceptance[8] and identification criteria.[9]

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[5] Academy Standards Board. (n.d.). *Standard for the analytical scope and sensitivity of forensic toxicology testing for medicolegal death investigations* (ASB 119 draft). Organization of Scientific Area Committees for Forensic Science. https://www.nist.gov/system/files/documents/2019/05/10/chemsac-tox_-_scope_sensitivity_for_postmortem_toxicology_-_for_asb_and_website.pdf

[6] Academy Standards Board. (n.d.). *Standard for the analytical scope and sensitivity of forensic toxicology testing for impaired driving investigations* (ASB 120 draft). Organization of Scientific Area Committees for Forensic Science. https://www.nist.gov/system/files/documents/2019/05/10/chemsac-tox_-_scope_sensitivity_for_duid_-_for_asb_and_website.pdf

[7] Academy Standards Board. (n.d.). *Standard for the analytical scope and sensitivity of forensic toxicology testing for drug-facilitated crimes* (ASB 121 draft). Organization of Scientific Area Committees for Forensic Science. https://www.nist.gov/system/files/documents/2019/05/10/chemsac-tox_-_scope_sensitivity_for_dfc_-_for_asb_and_website.pdf

[8] Academy Standards Board. (n.d.). *Standard for mass spectral data acceptance in forensic toxicology* (ASB 098 draft). Organization of Scientific Area Committees for Forensic Science. https://www.nist.gov/system/files/documents/2019/03/20/standard_for_mass_spec_spectral_data_acceptance_-_asb.pdf

[9] Academy Standards Board. (n.d.). *Standard for identification criteria in forensic toxicology* (ASB 113 draft). Organization of Scientific Area Committees for Forensic Science.

Although LC-MS is reported to be more amenable to limited or primitive sample preparation, this approach is taken at the expense of increased routine maintenance. A variety of sample preparation techniques are available for routine biological matrices (including blood and urine) ranging from dilution, protein precipitation, filtration, phospholipid removal, liquid-liquid extraction, supported liquid extraction, Quick Easy Cheap Effective Rugged Safe (QuEChers), and solid-phase extraction. If multiple biological matrices will be used for screening purposes, sample preparation requires careful consideration.

Validation Parameters Based on Scope of the Method

Standard practices for method validation in forensic toxicology have been published.[10] At a minimum, interference studies, limit of detection, matrix effects, and processed sample stability (if applicable) should be assessed during the validation of LC-MS–based screening. Scope and sensitivity (described previously) for the various sub-disciplines must be considered when establishing decision points.

Because of the expanded number of drugs (frequently >200) that can be identified using LC-MS–based screening, the initial validation can be time-consuming. Beginning with a method that targets a smaller number of drugs (~10) and a few prevalent NPS might be an efficient way to gain confidence with the technology before expanding to a broader screening method.

Interpretation of Data

A "targeted" screening approach includes a dynamic list of target compounds that limit the scope of the screening process. The list of target compounds should be available to customers/stakeholders and reviewed and updated on a regular basis. The frequency of review for the purpose of updates should be determined by laboratory protocols and documented.

Unlike traditional immunoassay, multiple criteria can be used for decision-making (e.g., library searching, isotope ratios, retention times, mass accuracy) using some LC-MS systems. Algorithmic approaches to reporting allow multiple identification criteria to be considered. Although this can be automated and custom reports can be generated, data review could potentially become labor-intensive.

Non-targeted screening is a more comprehensive approach that potentially does not limit the list of compounds but is considered to be more computationally intensive. Identification criteria using HRMS approaches may include retention time, accurate mass, isotope patterns/spacing, product ion spectra, and library searching. Commercial or shared databases and models may improve the performance of non-targeted approaches. However, effective screening workflows are critical for data analysis.

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https://www.nist.gov/system/files/documents/2019/04/22/chsac_-_tox_-_identification_in_forensic_toxicology_-_for_asb_and_website_1.pdf

[10] ANSI/ASB. (2019). *Standard practices for method validation in forensic toxicology* (Standard 036; 1st edition). http://www.asbstandardsboard.org/wp-content/uploads/2019/11/036_Std_e1.pdf

Resources in the Field

Although not an all-inclusive list, the following laboratories are known to be investigating or to have implemented LC-MS–based drug screening for toxicology:

- Alabama Department of Forensic Sciences, Toxicology
- Albany Medical College, Toxicology
- California Department of Justice, Bureau of Forensic Services, Toxicology
- Idaho State Police Forensic Services, Toxicology
- New York City Office of the Medical Examiner Laboratory, Toxicology
- Orange County Crime Laboratory, Toxicology
- University of Miami Department of Pathology and Laboratory Medicine, Toxicology
- Virginia Department of Forensic Science, Toxicology
- Washington State Patrol, Toxicology
- Wisconsin State Laboratory of Hygiene, Toxicology

Increasingly, some laboratories share procedures, training manuals, and validation plans on their websites, which are valuable resources. Links are provided above where known.

Further Reading

Allen, D. R., & McWhinney, B. C. (2019). Quadrupole time-of-flight mass spectrometry: A paradigm shift in toxicology screening applications. *The Clinical Biochemical Reviews, 40,* 135–145. https://doi.org/10.33176/AACB-19-00023

Ambroziak, K., & Adamowicz, P. (2018). Simple screening procedure for 72 synthetic cannabinoids in whole blood by liquid chromatography–tandem mass spectrometry. *Forensic Toxicology*, *36*(2), 280–290. https://doi.org/10.1007/s11419-017-0401-x

Boxler, M. I., Schneider, T. D., Kraemer, T., & Steuer, A. E. (2018). Analytical considerations for (un)-targeted metabolomic studies with special focus on forensic applications. *Drug Testing and Analysis*. https://doi.org/10.1002/dta.2540

Chindarkar, N. S., Wakefield, M. R., Stoneh, J. A., & Fitzgerald, R. L. (2014). Liquid chromatography high-resolution TOF analysis: Investigation of MSE for broad-spectrum drug screening. *Clinical Chemistry, 60*(8),1115–1125. https://doi.org/10.1373/clinchem.2014.222976

Colby, J. M., Thoren, K. L., & Lynch, K. L. (2018). Suspect screening using LC-QqTOF is a useful tool for detecting drugs in biological samples. *Journal of Analytical Toxicology, 42*(4), 207–213. https://doi.org/10.1093/jat/bkx107

Fagiola, M. (2019). Current and future directions of high resolution and tandem mass spectrometry in postmortem and human performance toxicology. *Legal Medicine (Tokyo), 37*, 86–94. https://doi.org/10.1016/j.legalmed.2019.02.004

Gundersen, P. O. M., Spigset, O., & Josefsson, M. (2018). Screening, quantification and confirmation of synthetic cannabinoid metabolites in urine by UHPLC-QTOF-MS. *Drug Testing and Analysis*. https://doi.org/10.1002/dta.2464

Helfer, A. G., Michely, J. A., Weber, A. A., Meyer, M. R., & Maurer, H. H. (2017). Liquid chromatography-high resolution-tandem mass spectrometry using Orbitrap technology for comprehensive screening to detect drugs and their metabolites in blood plasma. *Analytica Chimica Acta*, *965*, 83–95. https://doi.org/10.1016/j.aca.2017.03.002

Kahl, K. W., Seither, J. Z., & Reidy, L. J. (2019). LC-MS-MS vs ELISA: Validation of a comprehensive urine toxicology screen by LC-MS-MS and a comparison of 100 forensic specimens. *Journal of Analytical Toxicology, 43*(9), 734–745. https://doi.org/10.1093/jat/bkz066

Kimble, A. N., & DeCaprio, A. P. (2019). Systematic analysis of novel psychoactive substances. II. Development of a screening/confirmatory LC-QqQ-MS/MS method for 800+ compounds and metabolites in urine. *Forensic Chemistry, 16*, 100189. https://doi.org/10.1093/jat/bky050

Krotulski, A. J., Mohr, A. L. A., & Logan, B. K. (2020). Emerging synthetic cannabinoids: Development and validation of a novel liquid chromatography quadrupole time-of-flight mass spectrometry assay for real-time detection. *Journal of Analytical Toxicology, 44*(3), 207–217. https://doi.org/10.1093/jat/bkz084

Krotulski, A. J., Varnum, S. J., & Logan, B. K. (2020). Sample mining and data mining: Combined real-time and retrospective approaches for the identification of emerging novel psychoactive substances. *Journal of Forensic Sciences, 65*(2), 550–562. https://doi.org/10.1111/1556-4029.14184

Marin, S. J., Sawyer, J. C., He, X., & Johnson-Davis, K. L. (2015). Comparison of drug detection by three quadrupole time-of-flight mass spectrometry platforms. *Journal of Analytical Toxicology, 39*(2), 89–95. https://doi.org/10.1093/jat/bku134

Maurer, H. H. (2010). Perspectives of liquid chromatography coupled to low-and high-resolution mass spectrometry for screening, identification, and quantification of drugs in clinical and forensic toxicology. *Therapeutic drug monitoring, 32*(3), 324–327. https://doi.org/10.1097/FTD.0b013e3181dca295

Maurer, H. H. (2013). What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening? *Journal of Chromatography, 1292*, 19–24. https://doi.org/10.1016/j.chroma.2012.08.069

Maurer, H. H., & Meyer, M. R. (2016). High-resolution mass spectrometry in toxicology: Current status and future perspectives. *Archives of Toxicology 90*(9), 2161–2172. https://doi.org/10.1007/s00204-016-1764-1

Ojanperä, I., Kolmonen, M., & Pelander, A. (2012). Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping

control. *Analytical and Bioanalytical Chemistry, 403*(5), 1203–1220. https://doi.org/10.1007/s00216-012-5726-z

Partridge, E., Trobbiani, S., Stockham, P., Scott, T., & Kostakis, C. (2018). A validated method for the screening of 320 forensically significant compounds in blood by LC/QTOF, with simultaneous quantification of selected compounds. *Journal of Analytical Toxicology 42*(4), 220–231. https://doi.org/10.1093/jat/bkx108

Rosano, T. G., Ohouo, P. Y., LeQue, J. J., Freeto, S. M., & Wood, M. (2016). Definitive drug and metabolite screening in urine by UPLC-MS-MS using a novel calibration technique. *Journal of Analytical Toxicology, 40*(8), 628–638. https://doi.org/10.1093/jat/bkw050

Strathmann, F. G., Lynch, K. L., Krotulski, A., Negri, P., Cichelli, J., & Meyer, M. R. (2020). Challenges of high-resolution mass spectrometry for detecting designer drugs. *Clinical Chemistry, 66*(7), 868–874, https://doi.org/10.1093/clinchem/hvaa118

Vaiano, F., Busardò, F. P., Palumbo, D., Kyriakou, C., Fioravanti, A., Catalani, V., Mari, F., & Bertol, E. (2016). A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC–MS/MS and application to real cases. *Journal of Pharmaceutical and Biomedical Analysis*, *129*, 441–449. https://doi.org/10.1016/j.jpba.2016.07.009.

Wu, A. H., Gerona, R., Armenian, P., French, D., Petrie, M., & Lynch, K. L. (2012). Role of liquid chromatography–high-resolution mass spectrometry (LC-HR/MS) in clinical toxicology. *Clinical Toxicology, 50*(8), 733–742. https://doi.org/10.3109/15563650.2012.713108

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