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The Forensic Laboratory Needs Technology Working Group (FLN-TWG), formed by the National Institute of Justice (NIJ) in partnership with the Forensic Technology Center of Excellence (FTCOE) at RTI International, created this document in support of NIJ's mission to improve knowledge and understanding of federal, state, local, and tribal forensic science service providers' (FSSPs') technology needs.

TECHNICAL NOTE

Ultra-High Performance Liquid Chromatography Photo Diode Array Ultraviolet Single Quadrupole Mass Spectrometry (UHPLC-PDA UV-MS)

Introduction

Although gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) is considered the "gold standard" for seized drug analysis, it has several limitations that are especially applicable for the analysis of novel psychoactive substances (NPS). These shortcomings include similar retention times,[1] either very weak or a lack of molecular ions for several drug classes,[2,3] and similar fragmentation patterns for diastereomers and certain positional isomers.[4] Ultra-high performance liquid chromatography (UHPLC) coupled with photo diode array ultraviolet spectroscopy (PDA UV) detection and single quadrupole mass spectrometry (MS) detection in series provides a highly complementary technique to GC-EI-MS. Retention times generated by UHPLC are based on competing interactions for analytes between the mobile phase and stationary phase that are orthogonal to those obtained by GC, which are based on the analyte volatility and stationary phase interactions. PDA UV detection can distinguish between different classes of drugs and discriminate between positional isomers differing in substitution on a benzene ring, a problematic area for electron ionization (EI)-MS.[5] PDA UV detection is commonly referred to as PDA detection alone; however, in this technical

note we use PDA UV as a distinction from other spectroscopy techniques, such as near infrared spectroscopy (IR), that can also incorporate PDA detection. Finally, single quadrupole MS employing electrospray ionization (ESI) generates strong protonated or deprotonated molecules (e.g., $[M+H]^+$ or $[M-H]^-$) for most seized drugs.[6]

UHPLC separations are valuable as a primary screening technique. In contrast to GC, which may require derivatization for highly polar, thermally unstable, or nonvolatile analytes, UHPLC allows for simple dilute and shoot analysis.[7] The separation power of UHPLC in terms of resolving similar compounds (e.g., NPS) is comparable to GC, because of the selectivity imparted by mobile phase interactions. Although GC offers higher peak capacity than UHPLC, the carrier gas mobile phase of GC separation does not contribute to the separation selectivity. The inert GC carrier gas does not interact with the analyte and is only responsible for transport of the vaporized solute through the analytical column. An additional benefit of UHPLC-PDA UV-MS relative to seized drug screening with GC coupled to a flame ionization detector (FID) is the ability to identify the compounds present within each chromatographic peak because of the selectivity of the PDA UV and MS detectors relative to an FID detector. The utility of UHPLC-PDA UV-MS has been demonstrated throughout literature for seized drug screening, additional confirmation,[5, 6] or the generation of in-source collision-induced dissociation (IS-CID) fragment ions for increased specificity of detection.[8, 9] Specific examples include the analysis of cannabis,[10] synthetic cannabinoids,[5, 11, 12] synthetic cathinones,[5] fluoro-amphetamines,[5] and fentanyl analogs.[13] In addition, it is possible to employ PDA UV detection with much more expensive options such as tandem mass spectrometry (MS/MS)[14] and high-resolution mass spectrometry (HRMS) for even further increased specificity.[15]

Capabilities

UHPLC with PDA UV and single quadrupole MS detection is amenable to a wide variety of seized drugs including analytes that are thermally unstable, nonvolatile, and polar that provide poor chromatographic behavior. The use of UHPLC-PDA UV-MS is complementary to GC-EI-MS. UHPLC, which exhibits relatively high separation power, provides for uncorrelated retention times compared to gas phase separations. The generation of UV spectra "on the fly" provides complementary spectral information based on electronic transitions of chromophores, which can be particularly useful in distinguishing certain positional isomers for which EI-MS lacks the required specificity. Finally, single quadrupole MS detection, in contrast to EI-MS, provides strong protonated or deprotonated molecules (e.g., $[M+H]^+$ or $[M-H]^-$) for most classes of seized drugs. This difference in ionization behavior is because single quadrupole MS detection for UHPLC separation involves ESI, which is a soft ionization technique, resulting in only minimal analyte fragmentation during the ionization process. Exhibit 1 provides a summary of the approximate cost, analysis time, and cost-saving considerations to inform forensic laboratories that may be considering the adoption of this technology.

Exhibit 1. Summary of UHPLC-PDA UV-MS cost, analysis time, and cost-saving considerations.

Initial Cost	Cost/Analysis	Analysis Considerations	Cost-saving Considerations
Depends on the instrumental configuration and vendor (~$150,000-$230,000 for UHPLC-PDA UV-MS)	Depends on the mobile phase composition, flow rate, and column	The time for analysis is tied to the chromatographic method	Some vendors accept trade-ins or allow for coupling of detectors to existing instrumentation

Limitations

In contrast to GC, UHPLC provides significantly lower peak capacity. However, the interaction of the analyte with both the mobile phase and stationary phase, which provides enhanced selectivity, compensates for the reduced peak capacity. Another limitation is that UV spectra lack fine structure information because of the electronic excitation absorption process. This means that certain drug classes, such as phenethylamines (e.g., amphetamine, methamphetamine, and phentermine), provide identical UV spectra. Finally, although ESI-MS produces strong protonated or deprotonated molecules (e.g., $[M+H]^+$ or $[M-H]^-$), the lack of fragment ion information reduces the specificity provided by ESI-mass spectra. However, it is possible to generate IS-CID fragment ion spectra by manipulating the voltages within the source region as the ions transition into the high vacuum of the mass analyzer.[8]

Installation needs

The installation needs will be vendor- and instrument configuration-specific. The laboratory will need to consult with the individual vendor site requirements before installation. Examples of important installation requirements include sufficient space, adequate power plugs (e.g., 100-240 VAC, 50/60 Hz), nitrogen desolvation gas (> 95% purity and ~100 psi input), stable environmental conditions (e.g., 4-40 °C, 20-80% humidity), an appropriate exhaust mechanism, and necessary data systems (e.g., computer, monitor, and printer).

Vendor considerations

Multiple vendors offer versions of LC-UV-MS instrumentation. For example, Waters has a UPLC-PDA-MS system, Agilent offers an HPLC-DAD-MS instrument, Thermo Fisher Scientific sells an HPLC-DAD-MS system, and Sciex has an HPLC-PDA-MS instrument. Laboratories will have to assess which instrumental setup is most appropriate for their specific analytical needs and financial resources.

Space requirements

The space requirements for each instrument will be vendor- and configuration-specific. In general, several modules are associated with the chromatographic system, such as the solvent manager, autosampler, solvent degasser, column oven, and mobile phase reservoirs. Laboratories should also consider the space requirements for the detection systems, such as the PDA UV detector or single quadrupole mass spectrometer. The space requirements for each of these individual components or modules are vendor- and configuration-specific. In general, the combination of the chromatographic and detection systems will require ~36 in × 36 in × 36 in (height × length × depth). Finally, laboratories should consider the space requirements for any supplemental equipment such as a nitrogen generator. Nitrogen generators range in size based on the desired output, but a generator capable of supporting LC-UV-MS instrumentation is typically ~24 in × 24 in × 30 in (height × length × depth). Interested laboratories should consult vendors for specific space requirements.

User training and skill level

The training and skill level for UHPLC-PDA UV-MS is comparable to that required for GC-EI-MS and UV instrumentation. There will be a familiarization process learning to work with vendor-specific software. Analysts will also require general training on UHPLC principles, troubleshooting, and mobile phase preparation. The type of data derived from ESI-MS is very different than the classical EI-MS data with which most analysts have experience. It is important for analysts to understand differences in GC-EI-MS and UHPLC-PDA UV-MS instrumentation and data interpretation. Such training is available not only from vendors but also from individual experts within the field. An excellent venue for the latter training is at conferences. Additional training materials, references, validated methods, and standard operating procedures are also publicly available to assist with analyst education and familiarization.

Implementation needs

UHPLC-PDA UV-MS instrumental configurations have been implemented in casework by a few state and federal laboratories across the United States. See the laboratories in the early adopting laboratory section (Exhibit 2) for examples of where UHPLC-PDA UV-MS instrumentation has been successfully incorporated into casework analysis. The main implementation need that laboratories should consider for UHPLC-PDA UV-MS instrumentation is the generation of PDA UV and MS libraries. Because the PDA UV response is influenced by the solvent system during sample elution, laboratories will have to create their own internal PDA UV libraries that will be specific for a given chromatographic method. In comparison, ESI-MS libraries are independent of the solvent system during the sample elution and thus are capable of being more universal. The incorporation of IS-CID spectra collected at multiple voltages within the ESI-MS library provides spectra representing varying degrees of fragmentation that can assist with the identification of unknown compounds. Additional third-party mass spectral libraries, such as the NIST DART-MS Forensics Database, can also be used for searching unknown IS-CID fragment ion spectra generated with ESI-MS.[16] A nitrogen generator is also highly recommended because of

relatively high rates (~1200 L/h) of nitrogen used for the desolvation gas.

Validated methods

Even though UHPLC-PDA UV-MS instrumentation is growing in popularity thanks to the complementary nature of UV and MS detection, and the ability to provide orthogonal separation techniques when combined with classical GC-EI-MS, there are still only limited seized drug validation studies in peer-reviewed literature. For example, Li and Lurie using reverse phase chromatography (RPC) demonstrated the validation of a screening method for the detection of 62 seized drug analogs from the synthetic cathinone, phenethylamine, and cannabimimetic classes.[5] The method optimization included the stationary phase, mobile phase, flow rate, temperature, percent formic acid, and injection volume. This work demonstrated reproducible retention times with percent relative standard deviations (%RSD) of less than 1% over six months and repeatable retention times with %RSD less than 0.5% on a run-to-run basis. The MS limit of detection (LOD) for the representative synthetic cathinones, phenethylamines, and cannabimimetics was 0.4-10 µg/mL.[5] Given that the MS detection was based on the combination of ESI optimized to provide protonated molecules with a low-resolution single quadrupole mass analyzer, this method was limited as a confirmatory technique. However, the use of IS-CID with the developed method could provide additional selectivity through the formation of fragment ions.[8, 9]

Li and Quintero also developed and validated a UHPLC-PDA UV-MS method employing RPC for the detection for 29 common seized drugs encountered by the U.S. Drug Enforcement Administration (DEA).[6] The UV LOD was 0.2-5 µg/mL. In comparison, the MS LOD was 0.1-10 µg/mL for the untargeted analysis and 0.01-0.1 µg/mL for the targeted analysis using selected ion recording (SIR) to monitor only a single ion for each compound. The repeatability (intra-day, n=30) and reproducibility (3 replicates over 6 weeks) of the retention time and peak areas for the UV and MS detection systems for three selected compounds was evaluated. The retention time repeatability and reproducibility in %RSD was 0.03-0.06% and 0.2-1.3%, respectively. The UV peak area repeatability and reproducibility was 0.2-2.6% and 2.6-4.1%, respectively. In comparison, the full scan MS peak area repeatability and reproducibility was 3.4-7.3% and 8.5-13.6%, respectively. However, MS peak area repeatability and reproducibility improved with SIR and was 1.5-2.1% and 3.7-7.1%, respectively.

Finally, Agni et al. demonstrated the use of RPC UHPLC-PDA UV-MS for the differentiation of 20 fentanyl analogs, including positional isomers.[13] The method provided retention time run-to-run repeatability between 0.06-1.3% with the UV LOD ranging from 150 ppb-3 ppm and the MS LOD being 3 ppb for all fentanyl analogs using SIR detection.

Searchable libraries

The generation of a universal PDA UV spectral library is not feasible given that PDA UV spectra change depending on mobile phase conditions, such as the organic modifier composition, organic modifier concentration, and temperature. The PDA UV spectra also change with the number of diodes and resolution settings. Because of these limitations and unique software between different vendors, PDA UV libraries are only easily transferable within and between laboratories that employ the same vendor/model and use identical chromatographic conditions. In comparison, ESI-MS libraries are transferable and unaffected by differences in chromatographic conditions as long as the source voltages used for ionization and IS-CID fragmentation are kept constant between the unknown spectrum and known library spectrum. For example, the cone voltage can be manipulated on Waters single quadrupole instruments to collect spectra that contain both the protonated/deprotonated molecule and fragment ions formed through IS-CID as the ions transition from the source region to the high vacuum of the mass analyzer.

Data interpretation

The chromatographic and spectral data generated by UHPLC-PDA UV-MS are of a similar nature to GC, GC-EI-MS, and traditional UV instrumentation leading to similar data interpretation. The manufacture software typically includes data analysis tools to assist the forensic analyst. UHPLC-PDA UV-MS provides three points of comparison between any unknown and known samples, including the retention time, UV spectrum, and an ESI-mass spectrum. If additional selectivity for the ESI-mass spectrum is desired, analysts can explore the implementation of IS-CID for the generation of fragment ions, which has grown in popularity for the

detection of seized drugs with single-stage mass spectrometers combined with soft ionization sources.[8, 9]

Reporting and testimony

See Exhibit 2 for point of contact information from early adopting laboratories for specifics about reporting and testimony for UHPLC-PDA UV-MS instrumentation for the analysis of seized drugs. Given the combination of chromatography, spectroscopy, and spectrometry, the reporting and testimony follows that of instrumentation such as GC-FID, GC-EI-MS, and UV-Vis. Each sample provides a retention time and ideally a UV and ESI-mass spectrum. The analyte must contain a chromophore to be detected with the PDA UV detector, whereas the analyte must be ionizable under ESI conditions for detection with mass spectrometry. PDA UV and MS detection work well for most seized drugs, which provides both UV absorption information and molecular mass information about the analyte. Although the lack of universal PDA UV libraries is a drawback for unknown screening, the comparison of an unknown and known analytical reference standard can be used for spectral comparisons. IS-CID can also be used to gain fragment ion information by changing the voltages applied to the source region as the protonated/deprotonated molecules exit the ionization source. The use of IS-CID with soft ionization techniques and single-stage mass spectrometers provides increased selectivity without the associated increase in cost for tandem mass spectrometry instrumentation[8] and enables the use of third-party mass spectral libraries for searching unknown IS-CID fragment ion spectra. For unknown analytes, PDA UV detection can provide drug class information based on similar chromophores, while MS detection can provide the molecular mass of an unknown analyte.

Consumables

The consumables for UHPLC-PDA UV-MS are similar to those required for GC-EI-MS. Sample vials/caps, analytical columns, and sample syringe filters are required for both techniques and have comparable costs. Both GC and UHPLC have comparable mobile phase costs, considering the cost of the carrier gas for GC and the mobile phase solvents, organic solvent modifiers, and acidic/basic additives for UHPLC. The only consumable for the PDA UV detector is the light source, which should be stable for at least a year depending on instrument utilization. For GC additional consumables are liners and septa. Finally, for the mass spectrometer, replacement items such as the ESI probe assembly, lens associated with sample introduction, and source gas plumbing depending on the vendor and configuration could be required. Interested laboratories should consult with specific vendors to learn more about the consumables for each instrumental configuration.

Early adopting laboratories

Exhibit 2. Point of contact information from early adopting laboratories that have implemented UHPLC-PDA UV-MS into casework. This list may not be exhaustive but is intended to highlight points of contact for those interested in UHPLC-PDA UV-MS implementation.

Laboratory	Point of Contact	Email	Phone Number
Drug Enforcement Administration Special Testing and Research Laboratory	Li Li	Li.Li2@dea.gov	703-668-3300 (Main)
Texas Department of Public Safety Crime Laboratory Division	Jennifer Hatch	jennifer.hatch@dps.texas.gov	512-424-2015 (Office of the Chief)

References

1. Carnes, S., O'Brien, S., Szewczak, A., Tremeau-Cayel, L., Rowe, W. F., McCord, B., and Lurie I. S. (2017) Comparison of Ultra High Performance Supercritical Fluid Chromatography, Ultra High Performance Liquid Chromatography, and Gas Chromatography for the Separation of Synthetic Cathinones. *J. Sep. Sci.* 40: 3545-3556 DOI: 10.1002/jssc.201700349.

2. Tanen, J. L., Lurie, I. S. and Marginean, I. (2020). Gas Chromatography with Dual Cold Electron Ionization Mass Spectrometry and Vacuum Utraviolet Detection for the Analysis of Phenylethylamine Analogues. *Forensic Chem. 21* : 100281 DOI: 10.1016/j.forc.2020.100281

3. Buchalter, S., Marginean, I., Yohannan, J., and Lurie, I. S. (2019). Gas chromatography with tandem cold electron ionization mass spectrometric detection and vacuum ultraviolet detection for the comprehensive

analysis of fentanyl analogues. *J. Chromatogr. A* 1596: 183-93 DOI: 10.1016/j.chroma.2019.03.011.

4. Brandt, S. D., and Kavanagh, P. V. (2017). Addressing the challenges in forensic drug chemistry. *Drug Test. Anal.* 9(3): 342-46 DOI: 10.1002/dta.2169.

5. Li, L., and Lurie, I. S. (2014). Screening of seized emerging drugs by ultra-high performance liquid chromatography with photodiode array ultraviolet and mass spectrometric detection. *Forensic Sci. Int.* 237: 100-111 DOI: 10.1016/j.forsciint.2014.01.018.

6. Li, L., and Quintero, X. (2021). Rapid orthogonal screening of forensic drugs using a compact UHPLC-PDA/SQD; a complement to GC-MS. *Forensic Sci. Int.* 318:: 110565 DOI: 10.1016/j.forsciint.2020.110565.

7. Steiner, A., and Lurie, I. S. (2021) Applicability of liquid and supercritical fluid chromatographic separation techniques with diode array ultraviolet detection for forensic analysis. *Forensic Chem.* 26: 100359 DOI: 10.1016/j.forc.2021.100359 .

8. Sharp, J., Do, D., and Davidson, J. T. (2022). Assessment of the similarity between in-source collision-induced dissociation (IS-CID) fragment ion spectra and tandem mass spectrometry (MS/MS) product ion spectra for seized drug identifications. *Forensic Chem.y* 30: 100441 DOI: 10.1016/j.forc.2022.100441.

9. Davidson, J. T., Sasiene, Z. J., and Jackson, G. P. (2021). Comparison of in-source collision-induced dissociation and beam-type collision-induced dissociation of emerging synthetic drugs using a high-resolution quadrupole time-of-flight mass spectrometer. *J. Mass Spectrom.* 56(2): e4679 DOI: 10.1002/jms.4679.

10. Wang, Y. H., Avula, B., ElSohly, M. A., Radwan, M. M., Wang, M., Wanas, A. S., Mehmedic, Z., and Khan, I. A. (2018). Quantitative determination of Delta9-THC, CBG, CBD, their acid precursors and five other neutral cannabinoids by UHPLC-UV-MS. *Planta. Med.* 84(4): 260-66 DOI: 10.1055/s-0043-124873.

11. Nakajima, J., Takahashi, M., Seto, T., Kanai, C., Suzuki, J., Yoshida, M., and Hamano, T.. (2011). Identification and quantitation of two benzoylindoles AM-694 and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone, and three cannabimimetic naphthoylindoles JWH-210, JWH-122, and JWH-019 as adulterants in illegal products obtained via the Internet. *Forensic Toxicol.* 29(2): 95-110 DOI: 10.1007/s11419-011-0108-3.

12. Ichikawa, Y., Nakajima, J., Takahashi, M., Uemura, N., Yoshida, M., Suzuki, A., Suzuki, J., Nakae, D., Moriyasu, T., and Hosaka, M. (2017). Identification of (1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (DP-UR-144) in a herbal drug product that was commercially available in the Tokyo metropolitan area." *Forensic Toxicol.* 35(1) : 146-152 DOI: 10.1007/s11419-016-0327-8.

13. Angi, C., Lurie, I. S., and Marginean, I. (2019). Analysis of fentanyl derivatives by ultra high performance liquid chromatography with diode array ultraviolet and single quadrupole mass spectrometric detection. *J.Sep. Sci.* 42(9) : 1686-1694 DOI: 10.1002/jssc.201801098.

14. Jankovics, P., Váradi, A., Tölgyesi, L., Lohner, S., Németh-Palotás, J., and Balla, J.. (2012). Detection and identification of the new potential synthetic cannabinoids 1-pentyl-3-(2-iodobenzoyl)indole and 1-pentyl-3-(1-adamantoyl)indole in seized bulk powders in Hungary. *Forensic Sci. Int.* 214 (1) : 27-32 DOI: 10.1016/j.forsciint.2011.07.011.

15. Sultana, N., Williams, K., Ankeny, M., and Vinueza, N. R. (2019). Degradation studies of CI Reactive Blue 19 on biodegraded cellulosic fabrics via liquid chromatography-photodiode array detection coupled to high resolution mass spectrometry. *Color. Technol.* 135(6): 475-483 DOI: 10.1111/cote.12440.

16. Couch, A. N., Sharp, J., and Davidson, J. T. (2023). Assessing the effectiveness of the NIST DART-MS Forensics Database and Data Interpretation Tool for designer drug screening with alternative instrumentation. *Int. J. Mass Spectrom.* 483: 116964 DOI: 10.1016/j.ijms.2022.116964.

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