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Contents

A. Summary of Project

A.1 Statement of Need

Pennsylvania (PA) saw a 394 percent increase in the presence of fentanyl-related substances (FRS) and non-prescription opioids (NPSO) in drug related overdose deaths from 2015-2017.[1] Opioid-related fatalities comprised most overdose deaths (85%) in Pennsylvania (PA) in 2016 which had the fourth highest overdose death rate in the U.S.[2] Southwestern PA had the highest rates of overdose in 2016 (50.8 per 100,000) as compared to the entire state (3.6 per 100,000).[3] Nationally, overdose deaths rose from 17,415 in 2000 to approximately 92,000 by 2020.[2] An increase in demand for opioids directly contributed to the rise of synthetic opioids such as fentanyl, fentanyl analogs, and other novel compounds such as U-47700 and MT-45.[4] These substances continue to be detected in white heroin, black tar heroin, and pills and pose a serious public health threat.[4] As the most populous county in southwest PA, Allegheny County is a key region in PA for measuring these rapid changes in the illicit synthetic opioid supply. According to the National Forensic Laboratory Information System (NFLIS), Allegheny County reported 15 distinct fentanyl-related compounds (FRC) from 2017-2019, more than any other county in PA.[5] Not surprisingly, opioid-related fatalities continued to increase in the same period. In 2019, the PA Department of Health reported 3,728 opioid-related fatalities.[6] In Allegheny County, overdose deaths increased by 16 percent from 2018 to 2019.[6]

A.2 Major Goals and Objectives

Traditional data sources that are extensively relied on to inform overdose prevention activities in the U.S. include toxicology reports from medical examiners and coroners, overdose death rates from local and state health departments, and overdose spike reports from emergency medical services (EMS) and emergency departments (ED). While critical, these sources and others tend to be reactive and offer limited value in defining, describing, and tracking the fluctuating drug supply.[7]

Quantitative analysis of seized drugs confiscated by law enforcement entities and submitted to forensic drug chemistry laboratories is an underutilized data resource that may help to increase comprehension and implementation when applying multidisciplinary public health surveillance models to drug markets. The broad application of real-time quantitative forensic drug chemistry testing and analysis may better support drug intelligence and community surveillance goals, thus informing local overdose prevention efforts. First, quantitative testing offers a more comprehensive picture of the drug supply and localized trends within a jurisdiction than simple counts[8]; quantitative testing of drug seizures can paint a picture of the presence, potency, and various combinations of FRS and other illicit substances. Second, this type of analysis and data would allow for real time detection of the presence of FRS and novel synthetic opioids in a community supply prior to a large-scale overdose event. Third, results from quantitative drug seizure analysis can be leveraged and combined with overdose toxicology results to improve coordinated overdose prevention efforts and policies at the local, state, and federal level.

To meet this need, the University of Pittsburgh Program Evaluation and Research Unit (PERU) collaborated with the Allegheny County Office of the Medical Examiner (ACOME) on the Strategies for Coordinating Overdose Prevention Efforts (SCOPE) Drug Chemistry Surveillance for Public Health and Safety Project which aimed to meet the following objectives:

Objective 1: Use Allegheny County, Pennsylvania as a pilot county, to create a quantitative, real-time forensic drug chemistry workflow to identify responsive strategies aimed at supporting investigations, connecting cases, and improving drug intelligence and community surveillance goals.

- Objective 1A: Create a stakeholder steering committee to shape and improve the forensic drug chemistry workflow and dissemination system.

- Objective 1B: Develop an algorithm to determine the minimum number of samples of confiscated drugs that should be submitted for forensic drug chemistry to yield enough statistical power to assess drug surveillance trends within a defined geographic area (such as a county).

- Objective 1C: Create a protocol to efficiently add and sustain quantitative, real-time forensic drug chemistry capability into an existing workflow.

- Objective 1D: Develop, implement, and sustain a reporting mechanism to provide actionable information in real time, and larger trend analysis to designated public safety and health officials.

Objective 2: Assess the validity and reliability of the quantitative, real-time forensic drug chemistry workflow described in Objective 1, then evaluate the impact on case investigation and case connection, and in providing actionable drug intelligence and surveillance data through longitudinal and comparative studies.

- Objective 2A: Assess the validity and reliability of the quantitative, real-time forensic drug chemistry workflow.

- Objective 2B: Evaluate the impact of case investigation and case connection, and in providing actionable drug intelligence and surveillance data through longitudinal and comparative studies.

A.3 Research Questions

The overarching objective of this research project was to develop, implement, and evaluate a real-time quantitative forensic drug chemistry workflow in Allegheny County, PA. To meet this objective, three specific questions were asked and answered over the course of the project.

1. Is it possible to incorporate and standardize real-time quantified drug sample analysis into the existing case work protocols in a public forensic drug chemistry lab?

2. What types of drug supply trends can be identified comparatively and longitudinally using real-time drug sample analysis?

3. Is this information able to be communicated with public health and safety stakeholders in actionable ways that can impact case connections, investigations, and overdose fatalities?

A.4 Research Design, Methods, Analytical and Data Analysis Techniques

A.4.1. Research Design

The original research design consisted of a quasi-experimental evaluation to assess the validity, reliability, and impact of real-time quantitative forensic drug chemistry analysis in Allegheny County, PA on case investigations, case connections, and data user perception. Changes to the research design (described in section C.) occurred during the project. The project consisted of an exploratory case study, examining the feasibility of a quantitative, real-time forensic drug chemistry workflow and its impact on organizational change, case backlogs, drug supply trends, and distribution.

A.4.2. Systems Transformation Framework

The Systems Transformation Framework (STF) was developed to best support and implement any intervention in a complex adaptive system.[9] The STF improves the precision of implementation, performance measurement, and overall success of varying initiatives in healthcare and community settings. The strategies needed to transform these systems toward an intended outcome must involve a process to assess the functioning of specific levers against given principles and apply strategies to facilitate the levers' functioning toward these principles. The levers are components of a system that have great influence in facilitating the system's transformation toward its intended vision, such as optimal communication. The principles on which these levers are assessed are associated with their ability to better support the system's implementation of an intended innovation to improve criminal justice related activities. The system agents are the organization members who do the work of moving toward an intended vision. The following is a brief description of each lever contained in the STF:

- Vision/Greater Purpose: The "Ideal." This lever acts as the lens that guides the work conducted by the organization.
- Leadership: The agents within the system who see their role as providing the resources and tools to other agents, so they may continuously meet the organization's vision/greater purpose.

- Performance Measurement: Meaningful information collected during agents' work that is used by the internal learning system to determine how to best meet the system's vision/greater purpose.
- Internal Learning System: The systematic method of using performance measurement data to learn how to move toward the organizational vision/greater purpose.
- External Learning System: The identification of the precise knowledge or skill the system needs to acquire in order to address a specific performance gap and only engage this new knowledge or skill with the system when it is needed using learning principles that increase knowledge/skill absorption by the targeted system agents.
- Organizational Culture: The agents' attitudes, values, beliefs, and assumptions that influence how the work is completed.
- Organizational Behavior: The way the system behaves across five domains: (1) relationships; (2) decision making; (3) power; (4) conflict; and (5) learning.
- Organizational Structure: The way the system is structured so that optimal communication and innovation can occur among its agents.

A.4.3. Evaluation Methods

A mixed methods evaluation approach was used to assess the effectiveness of the project's quantitative protocol and study its impacts on the ACOME organization, the lab's case backlog, and tracking drug supply trends in the county. According to NIH, mixed methods are best used to embrace multiple perspectives, contextualize information, and effectively examine processes, experiences, and outcomes.[10] The quantitative and qualitative data collected via statistical tests, structured surveys, and semi-structured interviews were used to assess the effectiveness of the forensic drug chemistry pilot implementation.

A.4.3.1. Organizational Health Assessment (OHA)

The organizational health assessment (OHA) puts the STF theoretical framework into practice, assessing an organization's readiness for change through an analysis of the STF's eight levers (described in Section A.4.2). By analyzing an organization's health, recommendations can be identified to improve health, better inform and sustain implementation efforts, and measure organizational health improvements over time. The OHA was administered as a structured interview and an abbreviated survey for this project.

A.4.3.2. Key Informant Interviews

In addition to the OHA, PERU conducted key informant interviews (KII) with ACOME staff during the project; KIIs assess implementation barriers and facilitators. The KIIs are conducted using a semi-structured interview protocol (Appendix Item A.), with one team member conducting the interview while another acts as a note taker. The participants also completed a Qualtrics survey to collect identity characteristic information, in support of the KII.

A.4.3.3. Quantitative Methods

The quantitative analysis focused on process improvement efforts within ACOME and additional analysis of drug seizure sample data, specifically focused on relationships between drug presence, potency, packaging, and geographic location. The programming developed using R and R Studio software allowed for various quality control measures to harmonize and clean the sample data produced by ACOME. The resulting data set then generated automated reports and additional analysis, including descriptive statistics, spatial analysis, and tests of association.

The results of these analyses identified the measures and analyses most useful to include in a data dashboard resource intended for ACOME and key community partners.

A.5 Applicability of the Research

The opioid crisis has increased the workload of forensic and crime laboratories leading to challenges in organizational capacity, retention, and case back logs which are barriers to innovation and adoption of practices such as quantitative drug sample analysis.[11] This study adds to the growing body of multidisciplinary research that is needed to appropriately address these systemic barriers in the forensic community through interventions to improve organizational health, leadership, and performance.

The application of quantitative drug analysis for this project focused on a small subset of opioids that met criteria for study inclusion (Appendix Item B.). While limited in scope, this demonstrated the feasibility of integrating real-time quantitative analysis into a public forensic chemistry lab setting. Other forensic practitioners and researchers will be better suited to implement quantitative interventions using liquid chromatography (LC) in standard casework practices and make technical testing adjustments to monitor and respond to locally emerging drug threats in given jurisdictions. In terms of distribution, existing quantitative drug sample analysis that occurs at the federal level through the National Forensic Science Laboratory Information System (NFLIS) lacks real-time reporting capabilities which limits the effectiveness of the data to improve and inform locally coordinated overdose response activities. Through the creation of automated static reports and an interactive data visualization dashboard using Tableau, the project created a sustainable mechanism to house and communicate quantitative sample analysis and testing results to stakeholders in near real time.

B. Participants and other Collaborating Organizations

PERU collaborated with ACOME throughout the project. ACOME provides professional death investigations and forensic services to more than one million residents of Allegheny County. In addition to providing coroner/medical examiner death investigation services, ACOME provides state-of-the-art forensic and environmental laboratories and services to assist law enforcement, the judicial system, and health and environmental agencies in the county. The ACOME drug chemistry division led the validation and implementation efforts of the LC including subsequent quantitative analysis of drug seizure samples. During validation of the LC instrument, ACOME leadership collaborated with law enforcement entities across the county to ensure buy-in and provide education on the goals and data management protocols related to the project. PERU further collaborated with ACOME and Allegheny CountyStat to produce static reports and an interactive data dashboard (described in section E.1.4) to distribute quantitative drug sample results. ACOME consulted with other targeted stakeholders to gather their feedback during the dashboard development, including

the Pittsburgh Bureau of Police, the University of Pittsburgh Medical Center (UPMC) Emergency Medicine, the Drug Enforcement Administration (DEA), and Allegheny County Emergency Medical Services.

C. Changes in approach from Original Design and Reason for changes

The project experienced various challenges which resulted in changes to the original study design. The COVID-19 pandemic brought delays in accomplishing project goals, providing deliverables, and sharing data from the stakeholder committee, which consisted of local, state, and federal partners such as the Drug Enforcement Agency (DEA) and United States Postal Service (USPS) . Additionally, stakeholder committee members and partner organizations expressed capacity concerns regarding continued participation and the need to balance their efforts with other local initiatives such as Overdose Data to Action (OD2A) in Allegheny County. PERU responded by transitioning reporting efforts internally with ACOME, developing programming to harmonize incident and LC data, and creating monthly reports for distribution to ACOME leadership and chemistry staff. These monthly reports were reviewed and continuously improved, and supplied the development data for the distribution tools (described in section E.1.4)

Confidentiality concerns from participating law enforcement agencies limited the geographic specificity of the data from ACOME. Initially, PERU planned to gather geographic data at the street address level, but was unable to due to confidentiality concerns by law enforcement regarding active investigations. Similar challenges were experienced when efforts were made to gather zip code-level data. To address the case confidentiality concerns expressed by law enforcement stakeholders and submitting agencies, PERU focused their sample analyses and reporting on data submitted by law enforcement agencies. . The longitudinal analyses were performed on aggregated data at the county level to provide a suitable sample across the project period.

The project's narrow scope of opioids and stamp bags prevented access to the full record of drug seizure sample submissions. The scope of the written proposal by PERU and the interpretation of that proposal by ACOME suggested a more narrowly defined sample of seized drugs. This subset dictated that the case samples selected for analyses were required to (1) be contained in stamp bag packaging, (2) contain fentanyl, acetyl fentanyl, despropionyl fentanyl (4- ANPP), fluorofentanyl, and/or heroin, and (3) have been submitted by a participating law enforcement agency. These inclusion criteria limited the scope of the data collection, thus negating the ability to develop street address scale, stamp bag-based predictive models as described above. Due to the limited data sample obtained and delays related to COVID-19, PERU was unable to analyze the impact of the quantitative, real-time forensic drug chemistry workflow on case investigation, case connection, and its use by first responders and emergency department physicians (Objective 2B). While PERU and ACOME have established a sustainable data communication pathway, it was not in operation long enough to impact the community indicators described. PERU was able to investigate the possibility of predictive analytics to estimate variation in the potency of drug samples analyzed by ACOME over time and any relationship to fatal overdoses at the county level. The predictive models reflected a prediction of potency for those opioids present in samples that meet the project criteria (e.g., submitted in stamp bag, submitted by participating agency, etc.). The relationship between drug potency and stamp bag design and seizure location was also explored, but findings and analyses were limited by the sample size restrictions described above.

Due to political and social challenges in the City of Philadelphia and Philadelphia County, specifically with large-scale turnover in health department staff and leadership, there was limited ability for PERU or ACOME to create robust partnerships and develop a process for sharing data and collaborating on this project. This negatively impacted the proposed comparative analyses between Allegheny County and Philadelphia County. ACOME and PERU also experienced key leadership transitions during the project which created additional obstacles to relationship-building with potential stakeholders in Philadelphia. The relationship-building barriers and limitations were compounded by the shift of county public health priority and strategy related to the onset of the COVID-19 pandemic during the project period. As a result, PERU's analysis will not include Philadelphia County as a comparison group as originally proposed (Objective 2B), but focused on longitudinal samples for Allegheny County, described above.

D. Outcomes

D.1 Activities and Accomplishments

Objective 1: Use Allegheny County, Pennsylvania as a pilot county, create a quantitative, real-time forensic drug chemistry workflow that can be used to identify responsive strategies aimed at supporting investigations, connecting cases, and improving drug intelligence and community surveillance goals.

Key activities and accomplishments from Objective 1:

- Created a project vision and conducted multiple OHAs of ACOME to assess its organizational health.
- Purchased an ultra-performance liquid chromatography with photodiode array detector (UPLC-PDA).
- Developed and validated a quantitative method and sampling protocol used to analyze seized drug samples for the project.
- Produced sustainable reporting mechanisms, including an interactive dashboard and static reports, to distribute sample testing results.

Objective 2: Assess the validity and reliability of the quantitative, real-time forensic drug chemistry workflow described in Objective 1, then evaluate the impact on case investigation and case connection, and in providing actionable drug intelligence and surveillance data through longitudinal and comparative studies.

Key activities and accomplishments from Objective 2:

- Executed KIIs with site staff to evaluate implementation barriers and facilitators.
- Conducted longitudinal and exploratory analyses of quantified drug sample results and relationships across drug potency, stamp bag design, and seizure location/submitting agency.
- Constructed an implementation toolkit to aid practitioners in research replication efforts and drug quantification implementation.

E. Results and Findings

E.1 Objective 1: Use Allegheny County, Pennsylvania as a pilot county, create a quantitative, real-time forensic drug chemistry workflow that can be used to identify responsive strategies aimed at supporting investigations, connecting cases, and improving drug intelligence and community surveillance goals.

E.1.1. Project Vision and OHAs

For ACOME to attain their vision to improve surveillance of drug threats to the community, it was critical to evaluate their organizational health for readiness to implement innovation. PERU conducted two proprietary organizational health assessments (OHA), one in 2020 and one in 2022. The goal of the OHAs was to provide feedback on ACOME's strengths and areas for improvement and to recommend strategies for improving their organizational health to achieve their desired outcomes.

The OHAs conducted were based on the Systems Transformation Framework (STF), a model characterizing complex systems, developed internally at PERU by its founder. The STF is based on eight levers: vision; leadership; culture; behavior; performance measurement; structure; internal learning; and external learning, all of which interact to move a system toward a desired state and determine its organizational health.

E.1.1.1. 2020 OHA

In 2020, ten structured interviews were conducted across three levels of office staff: one executive leader; two laboratory managers; and seven analysts. The assessment focused on how leadership style, values, beliefs, and collective behavior influenced performance and outcomes. The questions on the OHA were separated into six sections: (1) demographics; (2) vision; (3) turnover; (4) leadership; (5) behavior and culture; and (6) structure, performance measurement, external learning, and internal learning (SPEI). The questions used a five-point Likert scale with the option for open-ended comments accompanying each response. The qualitative data was also collected for each lever with staff asked to think out loud to explain their ratings.

Each interview was scored using a rubric, in which a score from one to five was assigned to each of the STF levers based on the responses. Leadership was calculated by comparing leadership and frontline staff interviews resulting in a score between one and 12. Two trained raters scored the interviews separately with the final score determined by taking the average of those two scores for each lever. Finally, scores across levers were summed to provide an overall score. As the raters scored the interviews, common themes, trends, and details that emerged in interviewee comments were noted, and specific quotes illustrating these trends were extracted for inclusion in the report.

ACOME had a score of 24.75 out of a possible 46 points for the 2020 OHA. The results revealed several strengths and several opportunities for improvement. The strengths included low turnover, striving to do excellent work, expert decision-making, real-time data collection, and leaders trying to provide staff with resources and tools necessary for their job. The areas for improvement included vision, organizational behavior and culture, and SPEI.

E.1.1.2. 2022 OHA

In 2022, ACOME wanted to provide the opportunity to their staff to participate in the OHA. Additionally, ACOME requested that a section related to the influence of middle managers on organizational health be added. As a result, PERU converted the structured interview protocol into a survey format, duplicated the leadership section for middle managers, and included space for open-ended comments concerning each lever. Questions were separated into seven sections: (1) demographics; (2) vision; (3) turnover; (4) leadership; (5) middle management; (6) behavior and culture; and (7) SPEI. The responses used a four-point Likert scale.

A total rating score was calculated for each respondent by summing ratings across all survey items. The lever rating scores were calculated by averaging all responses for each item associated with that lever. The average domain score was then categorized into one of four categories: (1) strong opportunity for growth; (2) opportunity for growth; (3) strength with slight opportunity for growth; and (4) strength. The qualitative data analysis was completed by counting positive and negative comments associated with each lever and then noting common themes and trends while extracting specific quotes illustrating the trends for inclusion in the report.

ACOME had a score of 200 out of a possible 320 points for the 2020 OHA. The results revealed several strengths and several opportunities for improvement. The strengths included vision, striving to do excellent work, expert decision-making, use of up-to-date data to make decisions, and middle managers developing a culture where everyone feels valued. Some notable areas for improvement were addressing high frontline staff turnover, creating a culture were gossiping and backbiting are not tolerated, providing consistent and repeated training for staff, and engaging in continuous quality improvement cycles to improve the work.

E.1.1.3. OHA Comparison

The OHA results for 2020 and 2022 are not directly comparable because of changes to the sampling method and data collection format noted above. However, since the levers and general aim of the questions remained the same, some general statements can be made with caution. Between 2020 and 2022, ACOME appears to have addressed the opportunity for growth related to the organization's vision statement; most staff now believe that their work colleagues know there is a vision statement even if not everyone can recite it for memory. Additionally, staff anecdotally noted improvement in external learning opportunities and using performance measures to drive decision making and evaluate performance even though some areas for growth were still identified within SPEI. Additional opportunities for improving organizational health exist in addressing the destructive conflict reported and engaging in formal and deliberate continuous quality improvement cycles.

E.1.2. Purchase ultra-performance liquid chromatography with photodiode array detector (UPLC-PDA)

As part of the project, ACOME purchased an ultra-performance liquid chromatography with photodiode array detector (UPLC-PDA) to analyze and quantify drug samples. ACOME has historically and exclusively used gas chromatography (GC) for sample analysis but has discovered limitations in drug sample quantification capabilities with these methods. Ultra-high performance liquid chromatography (UHPLC), when compared to GC, may offer more flexible separation possibilities and the ability to handle any soluble substance regardless of volatility.[12] In addition, diode-array detection (DAD) or photodiode-array detection (PDA) is an analytical technique that can be used to determine the purity of an analyte or related impurity peak eluting during an HPLC

separation. DAD allows users to analyze a variety of wavelengths and has shown promise in differentiating specific fentanyl isomers. The ThermoFisher Scientific Vanquish UHPLC-DAD was the specific instrumentation selected, validated, and used for subsequent development of a quantitative method and associated analysis.

E.1.3. Development and validation of quantitative testing methods and protocol

The original LC validation was conducted off site due to the COVID-19 pandemic and completed in partnership with Dr. William Campbell, an assistant professor at Penn State University's Department of Biochemistry and Molecular Biology. Part one of the original and proposed scope of validation included completing separation, identification, and development of standard curves for key target compounds (at Phase Analytical Technology) which initially included: methamphetamine, tramadol, heroin, cocaine, acetyl fentanyl, fentanyl, and isotonitazene. Upon transfer of the method to the ACOME Drug Chemistry lab, not all the compounds in the original target set were available. The target set was modified to include the following set for validation: methamphetamine, cocaine, 4-ANPP, acetyl fentanyl, heroin, and fentanyl. Figure 1 provides detail on the column and conditions used during the original validation.

Figure 1. UHPLC-DAD Original Validation Column and Conditions

Column:

- IQC18 (Phase Analytical Technology)
- 120Å C18 / High load C18 with Extensive Endcapping
- 150 X 3.0mm; 3mm

Conditions:

- Mobile Phase A: Water
- Mobile Phase B: Acetonitrile
- Mobile Phase C: Water / 1% Trifluoroacetic Acid
- Flow Rate: 0.65mL/min; Detection: 200 to 225nm; Injection volume: 5mL

A gradient elution was used to achieve optimum separation and reproducible chromatography. A gradient elution refers to the technique of adjusting the composition of the mobile phase being introduced to the sample during the chromatographic run. As you can see below in Figure 2, the mobile phase starts strongly aqueous and slowly becomes more organic during the run. The mobile phase C (water / 1% TFA) remained consistent throughout the run.

Figure 2. UHPLC-DAD Gradient Elution

Time	Flow (mL/min)	%A	%B	%C
0.000	0.650	80.0	10.0	10.0
10.000	0.650	35.0	55.0	10.0
12.000	0.650	35.0	55.0	10.0
12.200	0.650	80.0	10.0	10.0
13.000	Stop Run			

Several columns were evaluated for this application. The IQC18 was ultimately chosen and is a custom column chemistry available from Phase Analytical Technology. Phenol was chosen as an internal standard as it is readily available, provides good peak shape, and fully separates from

compounds of interest. 10ug/mL to 100ug/mL was determined to be an adequate calibration range to evaluate street samples. In term of run conditions (mobile phases) compounds of interest all showed absorbance at / below 230 nm. This presented challenges because at these low wavelengths methanol and other mobile phase modifiers also absorb. Using substances that absorb in the same region reduces analyte sensitivity and causes baseline noise and distortions. Acetonitrile (ACN) was chosen as the organic mobile phase as it is transparent at low wavelengths. ACN also has low viscosity and will provide lower back pressure than methanol. Individual compounds were evaluated at wavelengths from 200nm – 350nm so the following wavelengths were selected: 215, 220, 225, and 230 nm. The wavelengths for individual compounds can be optimized since a DAD detector is being used. Remote validation and development of the method presented challenges. A lack of available standards and street samples limited the ability to robustly test and reproduce the method. Additional issues were encountered regarding co-elution, particularly with newly emerging drugs of interest (i.e., fluorofentanyl).

The original method that was developed served as a useful starting point. Over the course of the project, ACOME updated the UHPLC-DAD validation method on-site, making changes to better adapt to street samples. Two alterations were made to the original column and conditions (Figure 1), specifically decreasing the injection volume to 3ml, and adjusting the column compartment temperature to 70 degrees Fahrenheit. These slight changes allowed for better separation of co-eluting compounds and better overall chromatography.

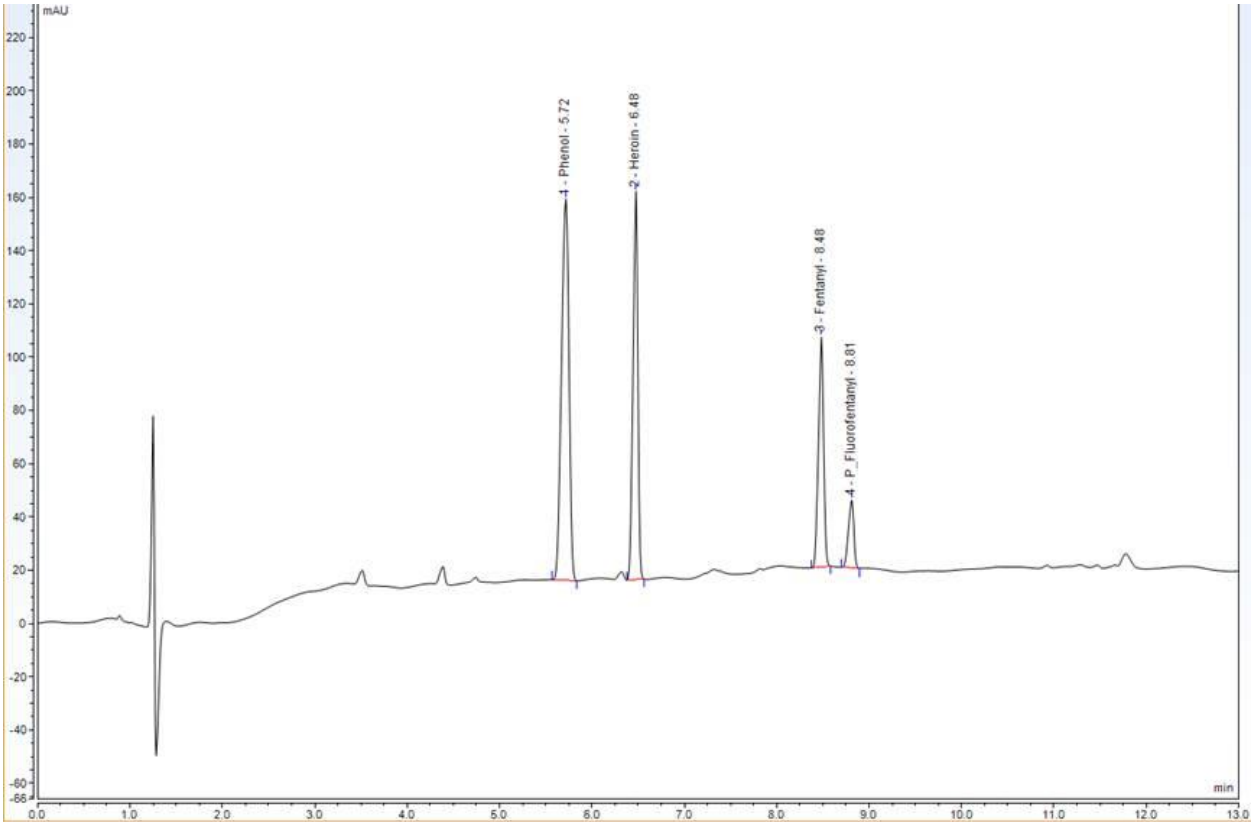
The updated UHPLC-DAD validation method did not include any changes to the column or internal standard. However, a completely new gradient was developed to address coelution issues that consistently occurred with street samples containing xylazine and tramadol. This change also allowed for improved compound separation. Figure 3 provides detail of the new gradient, that is slightly longer than the original. Percentages of mobile phase A and B changed along with a slight change in run time. Mobile C was consistent between both methods at 10%.

Figure 3. UHPLC-DAD Updated Gradient Elution

Time	Flow (mL/min)	%A	%B	%C
0.000	0.650	80.0	10.0	10.0
10.000	0.650	35.0	55.0	10.0
12.000	0.650	25.0	65.0	10.0
12.100	0.650	80.0	10.0	10.0
13.000	Stop Run			

Additional changes to the updated UHPLC-DAD validation method were made to the calibration range. The original calibration range (10ug/mL to 100ug/mL) was not comprehensive enough. The street samples fell below and above the lowest and highest calibrator. Additional calibrators were added to the curve, and 6.25ug/mL to 400ug/mL was determined to be a more realistic range to evaluate street samples. No changes were made to the original run conditions (mobile phases) or wavelengths used for the original UHPLC-DAD validation method. Figure 4 provides a diagram of a street sample analyzed by the updated UHPLC-DAD method.

Figure 4. UHPLC-DAD Updated Validation Method Sample Run



Using the updated UHPLC-DAD validation method, ACOME developed standard operating protocols and an associated sampling plan. The Work Instructions - LC-DAD Quick Reference Guide (Appendix Item C.) document and Liquid Chromatography Photo Diode Array Detector (LC-PDA): Sampling Plan (Appendix Item B.) acted as training aids across the ACOME Drug Chemistry Division to ensure its operational and sample selection fidelity. The UHPLC-DAD validation and resources referenced above allowed for the successful separation and quantification of all compounds of interest for the study.

E.1.4. Reporting Tools (Dashboard and Static Reports)

PERU and ACOME developed an interactive online dashboard titled "Real-Time Data Review of Focused Opioid Identification and Quantitative Analysis" and automated static reports. For the static reports, PERU developed an R markdown report that provides more detailed quantitative metrics, along with sample submission and completion trend information to ACOME. This report is automatically generated and distributed to ACOME via email, with the frequency determined by the data sharing plan between ACOME and CountyStat, the Allegheny County department responsible for the dashboard development and static report maintenance. For the duration of the project ACOME has used the static reports primarily for internal purposes and CountyStat has granted access and distribution of the reports to approved external recipients in the community, such as public health and safety officials. An example of a static report can be found in Appendix Item D.

In addition to the static reports, the "Real-Time Data Review of Focused Opioid Identification and Quantitative Analysis" dashboard was developed as a sustainable and easily accessible mechanism to report and visualize sample analysis in real-time. This tool provides users with a landing page (Figure 6) that outlines the sample submission protocols and a total of samples analyzed, drug detections, and participating agencies. On each proceeding page, users can toggle between drug type (fentanyl, heroin, flurofentanyl, acetyl fentanyl) and view updated analysis results based on that selection. A "Drug Identification" tab (Figure 7) provides a sample summary, including information on fentanyl combinations with other drugs (i.e., fentanyl and acetyl fentanyl). This page also includes a visual map detailing the number of drug detections by submitting agencies (i.e., municipal and city police) within specific jurisdictions. Based on the specific drug selected, an available "Quantitative Results" page (Figure 8) provides a detailed summary of reportable measures of that drug across all submitted samples, an average percentage of the drug by submitting agency, and the average percentage of drug detections annually and quarterly. Like the static reports, the dashboard is updated based on data sharing plans established between ACOME and CountyStat, and it can be disseminated externally with approved recipients.

Figure 6. Dashboard Landing Page

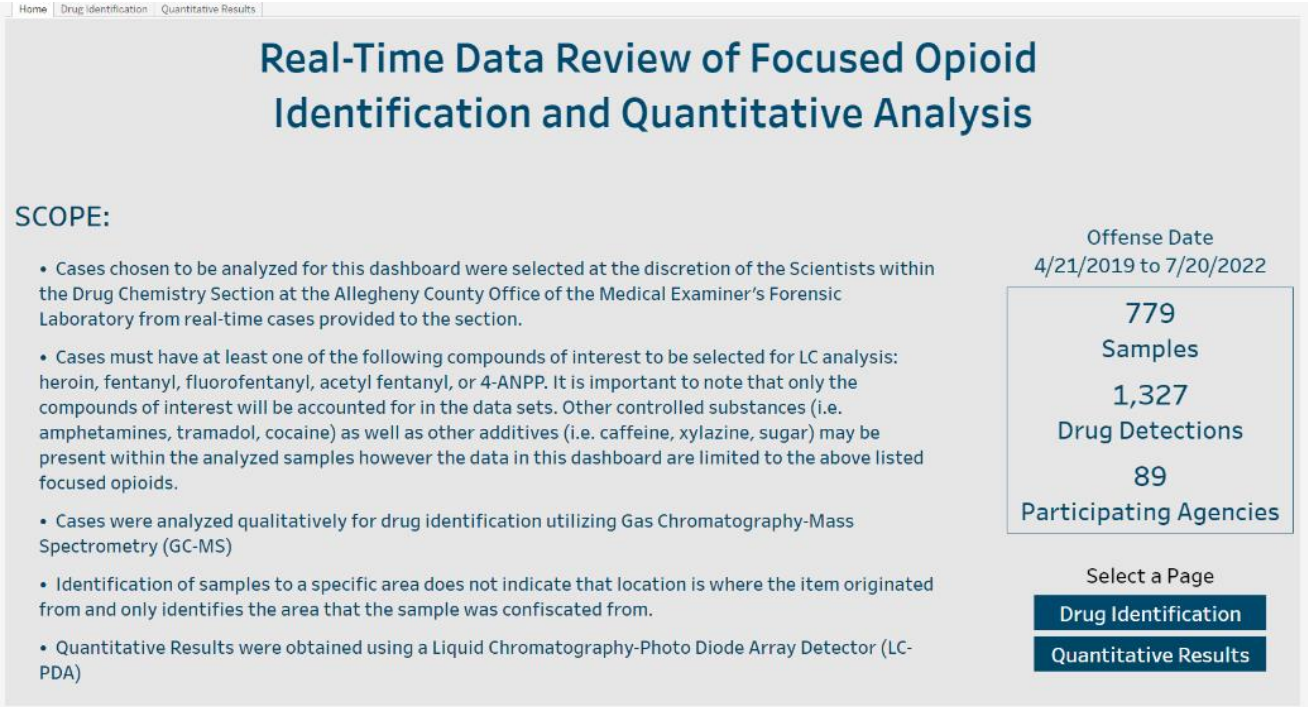


Figure 7. Drug Identification Page

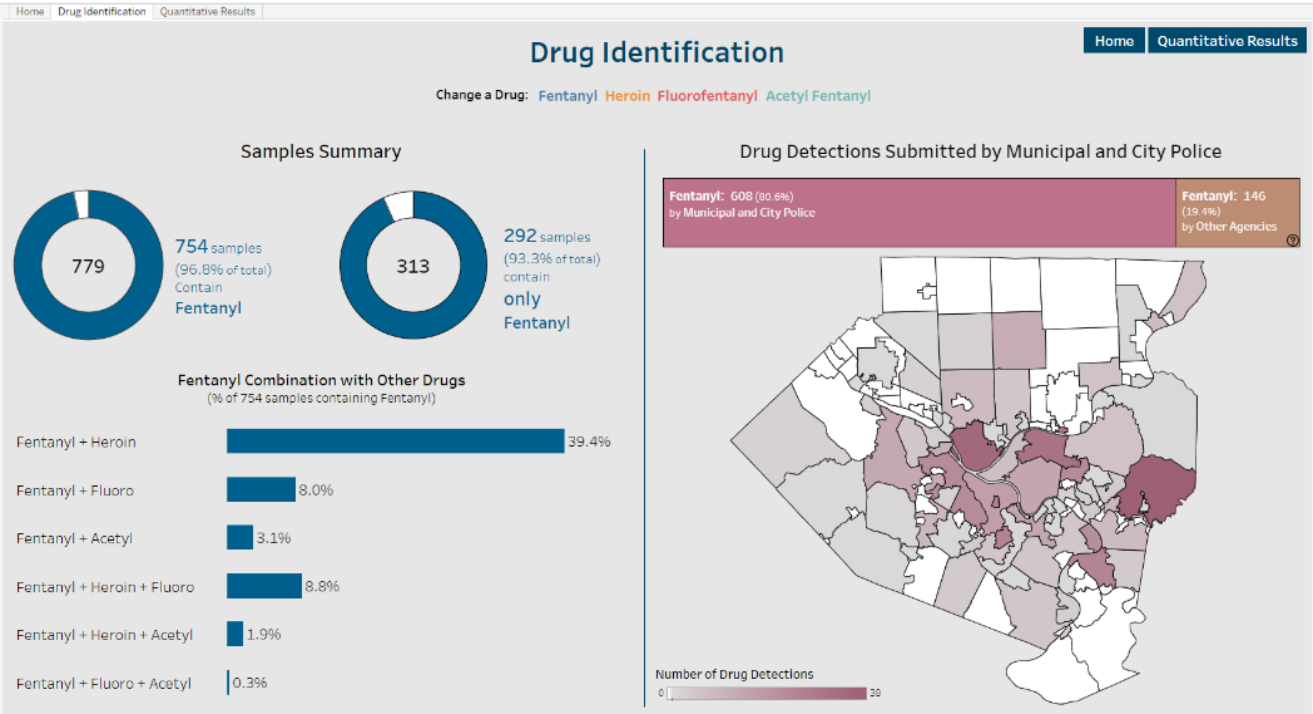


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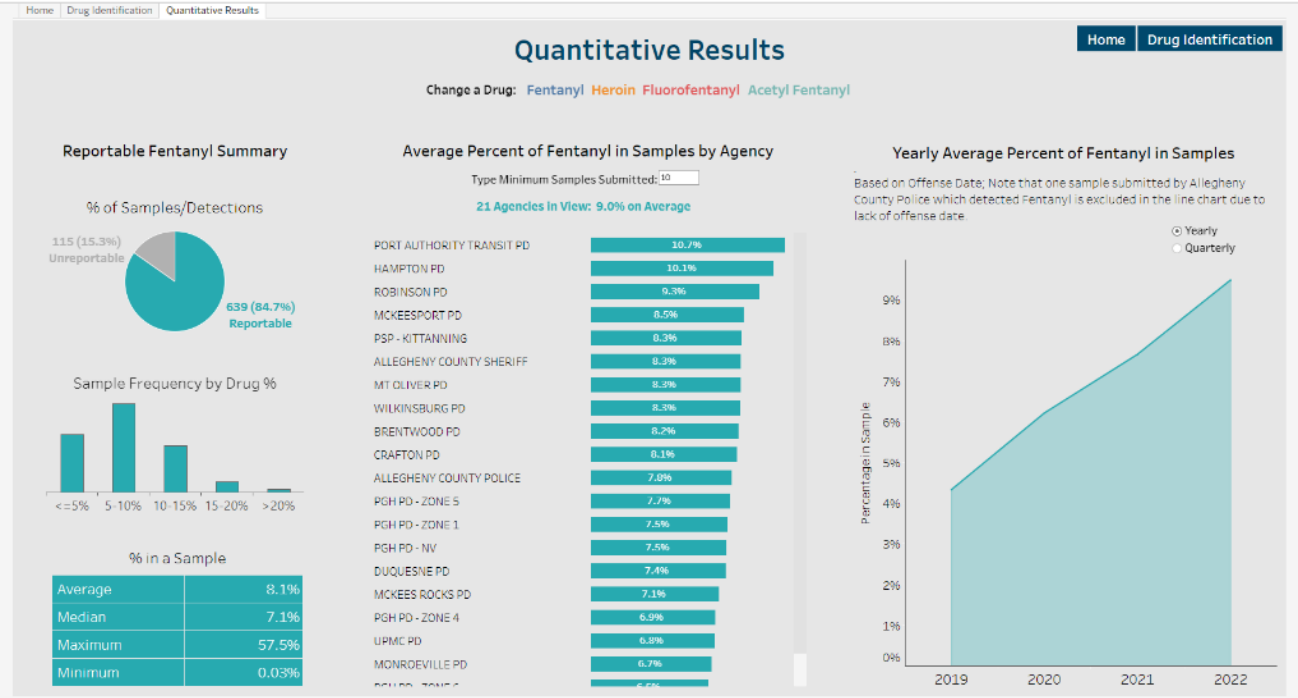
Figure 8. Quantitative Results Page



E.2 Objective 2: Assess the validity and reliability of the quantitative, real-time forensic drug chemistry workflow described in Objective 1, then evaluate the impact on case investigation, and case connection, and in providing actionable drug intelligence and surveillance data through longitudinal and comparative studies.

E.2.1. Key Informant Interviews

As part of the program evaluation, PERU conducted KIIs with ACOME staff to determine their understanding of the project goals and involvement with the project, describe the data entry and reporting process, and clarify the impact of COVID-19 on the project work. This section summarizes the findings.

Members of the Program Evaluation and Quality Improvement (EQI) team conducted eight interviews using a semi-structured interview guide. The interview data was analyzed through thematic content analysis with NVivo (Version 12Pro) using a combination of predetermined and emerging themes and sub-themes. The predetermined themes and sub-themes were based on the questions included in the interview guide. The emerging themes and sub-themes were related to the impact of COVID-19 on the goals and objectives of the grant, the role of overtime, and the challenges related to the project. After completion of coding, data from each theme or sub-theme were summarized into a comprehensive report.

Interviewed participants were mostly (75 percent) between 35-44 years of age, scientists (75 percent) who mostly identified as White (75 percent) female (50 percent). The length of time interviewees had worked in their position ranged from less than one year to almost 19 years.

E.2.1.1. Description of project goals and involvement

The participants differed in their understanding of the project goals. Some thought the project goal was to provide real-time information to emergency departments and law enforcement agencies based on the results of analyses, with agencies using the information as part of their emergency response to obtain additional doses of naloxone or additional support to clear a patient's body of the substance. Other interviewees mentioned the project goal was to further data collection efforts or improve the organizational and work structure which would lead to a reduction in the backlog of samples. Another participant mentioned that the project goal had changed from its conception. Though the initial focus was to track stamp bag information in Allegheny County, the project has created a platform for stakeholders to see trends and hotspots.

The participants had various responsibilities during the project such as transmitting data to PERU, communicating with PERU regarding the data, checking for eligible cases, grant writing, conducting investigations, developing laboratory standard operating procedures for the project, maintaining instruments, and validating instruments. For some participants, their responsibilities evolved or changed as they received promotions within their department or moved to a different department.

The participants mentioned working overtime in their responses regarding their involvement with the project. Other than administrators, participants could work on the project during overtime hours only; not all participants could work overtime, so some were not as involved as they wanted to be. The participants found the overtime provision useful, as they could separate their daily work from that of the project and some mentioned that without the overtime pay component they would not have engaged with the project. Thus, overtime work for the participants made the project sustainable made implementation easier, and offered them monetary compensation for their participation.

E.2.1.2. Data entry and reporting

The participants mentioned changes in data entry and reporting since the start of the project including what data was saved, how data was tracked, and results of analyses. In addition, the reporting values as the percentage of analogues found was included to make it easier for stakeholders to understand the data; scientists could continue to match case information using the data.

Despite the changes, most participants did not find data entry difficult. Using the liquid chromatography instrument made the work easier because the instrument did most of the work. Additionally, the report template developed for the project made it easy to transfer results to the spreadsheet since the software associated with the instrument automated the reporting process to a significant extent.

However, concerns were noted about how external stakeholders were interpreting the data since it was not presented by ACOME scientists. The participants felt that if stakeholders did not understand the findings, it could result in misinterpretation and misinformation. Monthly meetings with PERU helped resolve some of these data reporting concerns.

E.2.1.3. COVID-19 and the grant

Due to the COVID-19 pandemic, a work from home (WFH) policy was instituted. The WFH policy specified staff coming to the workplace two to three days per week to work in the laboratory on a rotating schedule to complete job responsibilities that could not be completed at home. The participants mentioned they reviewed cases, completed administrative work, wrote validation

studies, developed procedures, testified in court, and focused on evidence and crime centered work while working from home. This policy change resulted in positive and negative impacts on the project work.

Most of the participants reported the WFH policy made them more efficient and productive because it allowed the delineation of laboratory work from other duties. This meant that participants did not mix administration and laboratory duties throughout the day. The participants felt that the new work structure helped to reduce the backlog of cases. Additionally, management changes and not analyzing marijuana cases were also cited as reasons for the reduction in the backlog of cases. Each scientist working in the laboratory only on certain days also helped ACOME start analyzing samples in real-time. The participants believed that analyzing samples in real-time helps the community, law enforcement, and the courts.

A few of the participants mentioned they could not work overtime because of county rules, and this prevented scientists from completing grant-related tasks. Also, not all of the scientists were in the laboratory at the same time, so ACOME was unable to send data during many reporting periods because the scientists could not operate the liquid chromatography instrument.

The participants stated that their mental health improved when they were partially working from home due to the separation between laboratory work and other duties. However, others mentioned that mental health was negatively impacted because they felt isolated or experienced anxiety because of long work hours. In response to these concerns, one participant in a leadership position stated that leadership discussed with the staff what could be done to better support them. These discussions led to leadership changing their perspective and staff becoming more productive.

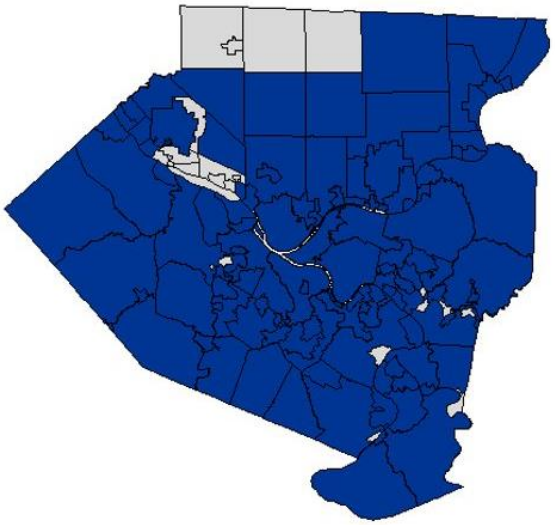
E.2.1.4. The ACOME staff ideas for improvement

The participants made suggestions for improving the project. They felt that communication pathways as they currently exist should continue so that staff at PERU and ACOME are aware of all aspects of the project work. One of the participants expressed concern that the platform for viewing trends and hotspots was not easy to use and wanted stakeholders to receive information that was easier to access and understand. Another participant wanted to know more about what happens to the data when PERU receives it. In addition, the participants wanted feedback on whether the laboratory was meeting expectations and if data sent to PERU was useful. Still another participant mentioned the need for a statistician to assist scientists in understanding the data and results generated by the liquid chromatography instrument.

E.2.2. Longitudinal and exploratory sample analysis

A total of 89 law enforcement entities at the local, state, and federal level participated in this study (see Figure 9 for a map of participating entities in Allegheny County). 767 samples from 571 cases were received and analyzed, resulting in 1,311 unique opioid detections. The samples included in the project had to be submitted by participating law enforcement entities and meet sampling plan inclusion criteria (Appendix Item B.).

Figure 9. Participating Municipal and City Police Departments in Allegheny County (N = 67)

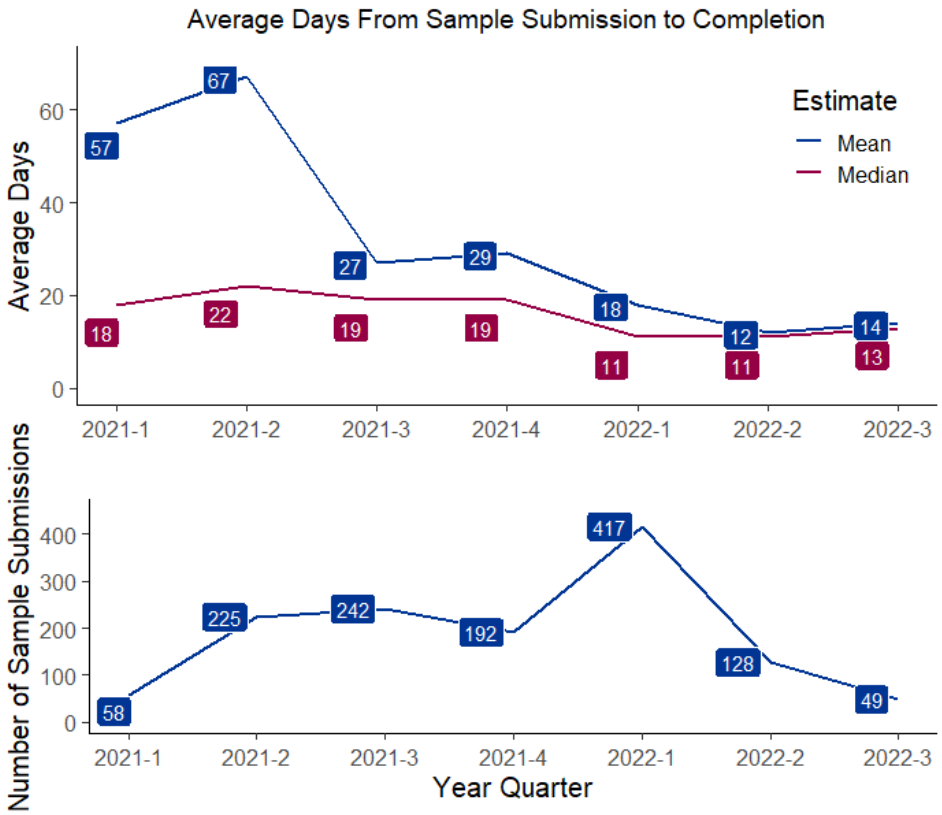


Note: Participating municipalities and city police departments are colored blue.

E.2.2.1. *Internal Processes*

Following the implementation of the UHPLC-DAD instrument, PERU monitored the average time between sample submission and completion of the LC analysis from the first quarter of 2021 to the third quarter of 2023. Sample submission was low at the beginning and end of the project period and peaked in the first quarter of 2022. The use of the LC and ongoing process improvement by the ACOME chemists resulted in a decrease in the average time from case submission to completion throughout the project period from an average high of 67 days to an average low of 12 days. PERU included the median estimate of days from sample submission to completion, which is less sensitive to outliers compared to the average estimate. Over the project period, the average estimate approaches the median, illustrating that there were fewer outlying samples with a relatively longer length of time between sample submission and completion (see Figure 10).

Figure 10. Average Days from Sample Submission to Completion



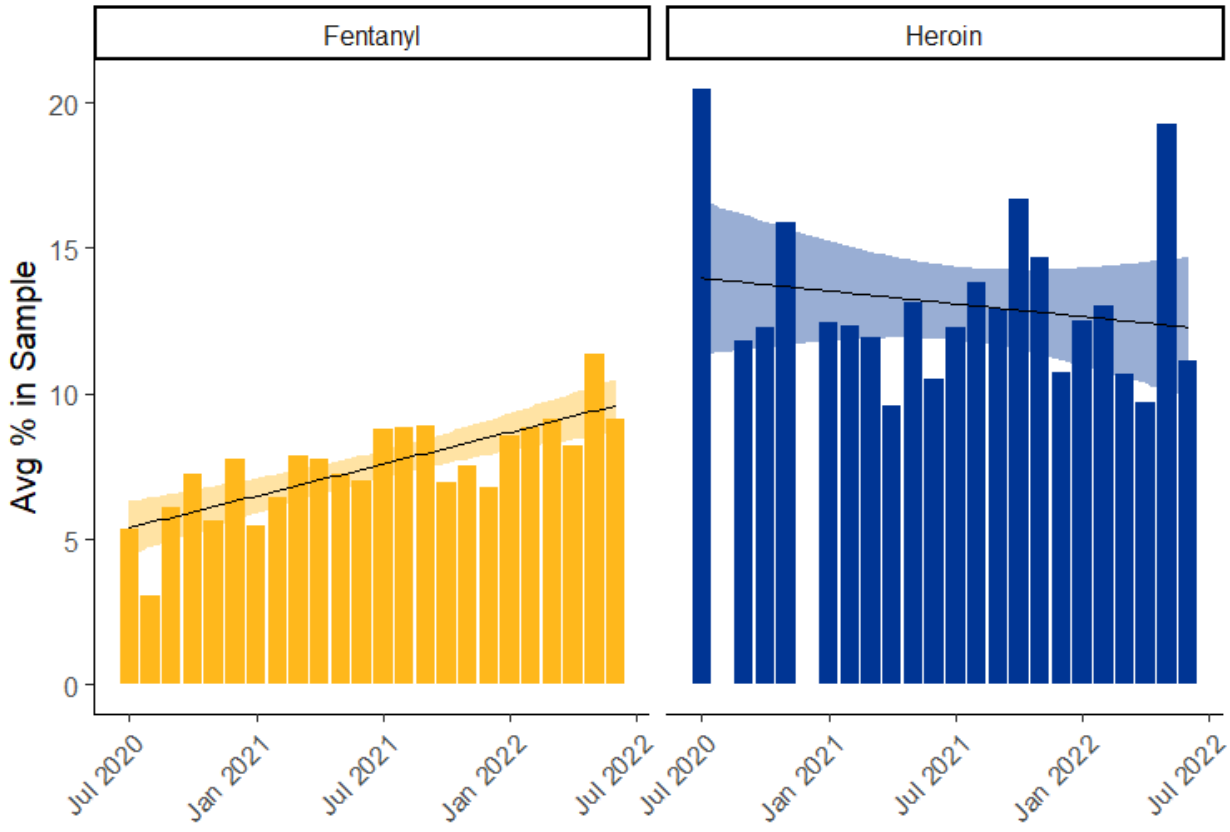
E.2.2.2. *Drug Quantitation*

There was an increase in the percentage of fentanyl in samples observed throughout the project period (r = 0.79, p < .001; Figure 11, left panel) . That same trend was not observed for heroin samples over the same period (r = -.14, p = .51; Figure 11, right panel). The increase in the percentage of fentanyl in the sample did not correlate significantly with overdose fatalities (r = -0.11, p = .61). Overdose fatalities showed a statistically non-significant decrease over time (r = -.19, p = .38).

There was, however, a statistically significant correlation between the number of samples per month and the average percent of opioids in samples in the samples (r = .45, p = .02). There was also a statistically significant correlation between the number of samples and time (r = .63, p < .001).

The results of these tests suggest that as time went on, there were more samples seized and analyzed, and a higher concentration of opioids in the samples. However, we found no support for the hypothesis that an increase in the percentage of fentanyl in the samples are associated with an increase in overdose fatalities.

Figure 11. Average Percent of Drug in Sample, by Month

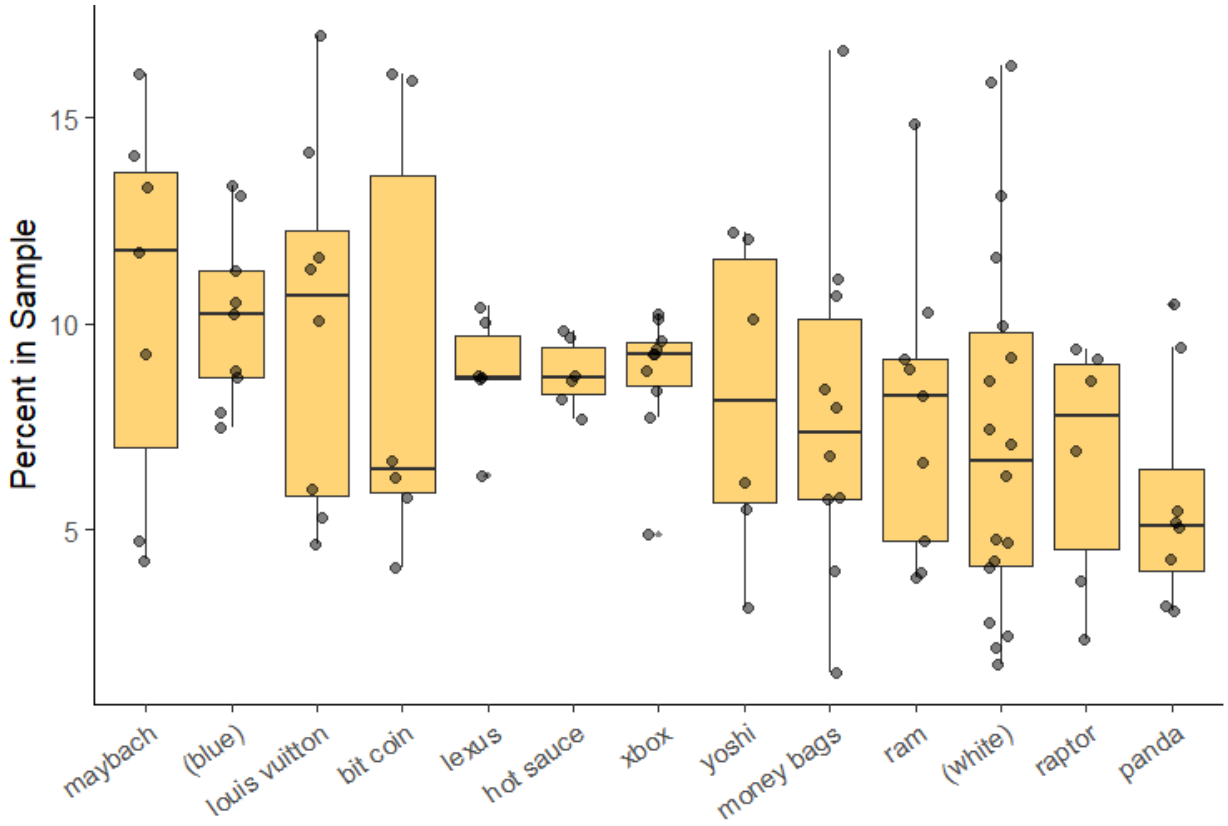


Note: The black line represents the line of best fit in a linear regression model. The shaded area around the black line represents the standard error.

E.2.2.3. *Stamp Bag Analysis*

An open text field was provided to the ACOME chemistry staff to collect stamp bag design information. This field was reviewed and cleaned by PERU staff prior to analysis. We identified 13 stamp bag designs associated with samples containing fentanyl (e.g., panda, hot sauce, and money bags). The reportable values of fentanyl provided 114 values for analysis after the exclusion of any stamp with fewer than six reportable observations of fentanyl. The normally distributed data were analyzed using an ANOVA (two-way, assuming equal variance). The text description of stamp design yielded no relevant trends in drug presence, potency, or seizure location. The difference in fentanyl percentage across stamps with more than five fentanyl observations was not significant ($p = 0.35$; see Figure 12). The other drugs included in the study did not provide sample sizes appropriate for statistical analysis.

Figure 12. Fentanyl Percentage in Sample by Stamp Bag Design

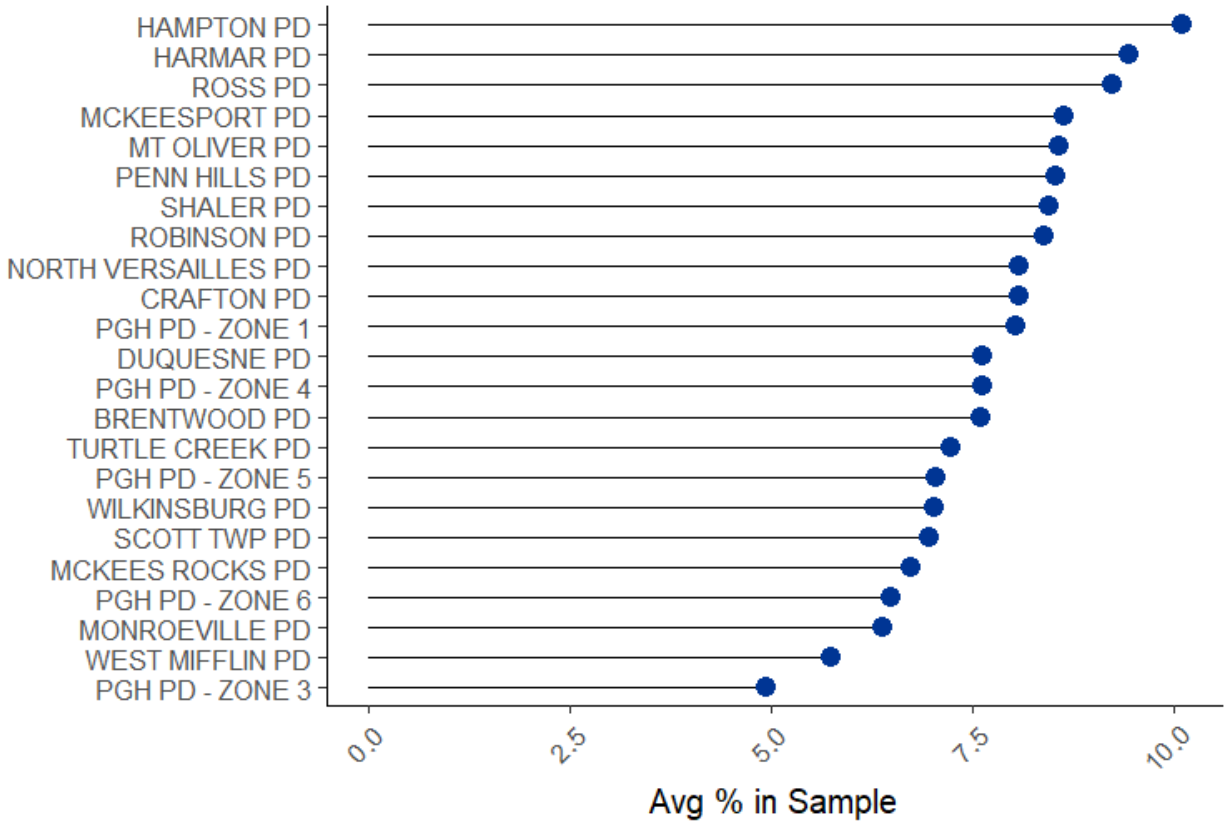


Note: The black points represent individual stamp bag samples.

E.2.2.4. *Drug Percentage and Location*

We assessed the relationship between drug percentage and sample seizure location. The samples were restricted to those submitted by municipal and city law enforcement departments that could be joined to a spatial polygon layer representing seizure locations. The jurisdictions with five or fewer submissions of the drug of interest were excluded, as anything below this number resulted in a highly skewed (or uneven) distribution of means. Due to non-normal data that failed correction through log transformations, analysts used the non-parametric Kruskal-Wallis test to evaluate the effect of geographic location on drug percentage in the sample. The samples included reportable observations of fentanyl (n = 330) and heroin (n = 68) submitted by municipal or city law enforcement departments. The results indicated no significant difference ($p_{fentanyl}$ = 0.24; p_{heroin} = 0.97) in drug percentage in the samples based on the seizure location (Figure 13).

Figure 13. Average Percent of Fentanyl in Sample, By Submitting Entity



E.2.3. Implementation toolkit

Previous research has demonstrated associations between crime laboratory drug sample seizure data and overdose fatalities, making it a useful yet underutilized public health tool that can inform overdose prevention efforts.[13] In the age of FRS, the reliance on qualitative-only testing methods in publicly funded drug chemistry laboratories creates additional blind spots. Without quantitative testing methods, differentiating specific drug percentages within samples (e.g., fentanyl and cocaine) is impossible to identify.[7] In response to this need, PERU and ACOME partnered to create a drug quantification toolkit. The toolkit is a checklist-style resource targeting forensic drug chemistry labs, medical examiner and coroner offices, researchers, and policy makers interested in leveraging quantitative drug seizure sample data to improve community drug surveillance. The structure and content of the toolkit is based on the Exploration, Preparation, Implementation, Sustainment (EPIS) Framework and the Template for Intervention Description and Replication (TIDieR). The toolkit titled, "Implementing Real Time Quantitative Drug Analysis" will be archived with NIJ as a grant product and be made publicly available.

F. Limitations

The study and associated activities experienced limitations that impeded the ability to fully answer the proposed research questions (described in section A.3) but provided insight and highlighted critical research aspects to target in future drug quantification studies. In terms of qualitative

analysis, due to changes in data collection format and sampling requested by the stakeholder, results from the 2020 OHA were not directly comparable to the 2022 OHA results. However, some general recommendations could be made based on the results of the 2022 OHA for ways to improve the health of the organization. The key informant interviews revealed several important factors related to ACOME's participation in the project. First, all project work was relegated to overtime work which was necessary to ensure that the regular analysis work was completed as specified. However, this resulted in the inability of some the ACOME analysts to participate in the project since they were unable to work overtime hours due to other commitments. Additionally, the COVID-19 pandemic and resultant hybrid work schedule reduced participants' ability to work overtime hours, directly impacting the ability of the data analysts to complete the work associated with the project; the overtime caveat applied to analysts, not to the administrative staff.

In terms of quantitative analysis undertaken on seizure samples, the narrow scope of sample inclusion criteria limited the number of eligible sample seizures used for quantitative analysis. This small sample size impacted the ability to produce generalizable knowledge on drug trends across the county and develop associated predictive models associated with drug concentration, geography, and stamp bag design. Future research should expand those criteria to include other major illicit drugs and allow for flexibility to change the included drugs and adapt instrumentation based on emerging drug trends. The sample adulterants should be included in future studies as they may reveal additional variants that could be used to predict sample drug characteristics. Also, the text description used for studying stamp bag design offered little analytical value and required additional data entry time for the ACOME staff. Future projects may benefit from a photo system and image analysis. The use of image analysis may provide a less time-intensive, more accurate data source for stamp bag analysis. Lastly, recruitment of law enforcement entities placed substantial burden on the ACOME staff and limited the amount of data available for the project. Future projects should identify points of contact and data sharing relationships with all law enforcement entities as early as possible. A long-term surveillance data program could be developed to allow for more adaptable data sharing that could be modified to include new initiatives.

G. Artifacts

G.1 List of Products

- Allegheny County Office of the Medical Examiner. "Liquid Chromatography Photo Diode Array Detector (LC-PDA): Sampling Plan."

- Allegheny County Office of the Medical Examiner. "LC-DAD Quick Reference Guide for ACOME Drug Chemistry."

- Allegheny County Office of the Medical Examiner and Allegheny CountyStat. "Real-Time Data Review of Focused Opioid Identification and Quantitative Analysis."

- University of Pittsburgh School of Pharmacy. "Implementing Real Time Quantitative Drug Analysis."

G.2 Data Sets Generated

The following datasets were generated as a result of the project. These datasets will be archived and made available through the National Archive of Criminal Justice Data (NACJD).

- "Drug Chemistry Analysis", Liquid Chromatography analysis of drug metabolites in seized samples (CSV). Spatial polygons associated with main analysis representing sample seizure location and participating municipal and city police departments (SHP/SHX/DBF). R analysis script (R). User guide with data dictionary (PDF).

- "Key Informant Interview Protocol," semi-structured interview script used to conduct stakeholder exit interviews at the conclusion of the project (PDF).

- "Key Informant Interview Roster and Notes," notes taken in real-time by notetaker during interview that provided text for content analysis for final report (PDF).

G.3 Dissemination Activities

- Auflick, D.; Baisden, F.; Caterino, J; Porter, E. Novel Quantitation Workflow for Improved Drug Surveillance. *Presentation given to the National Institute of Justice and pertinent stakeholders*, November 15, 2022.

- Auflick, D.: Novel Quantitation Workflow for Improved Drug Surveillance. *Presentation to be given at the Academy of Criminal Justice Sciences (ACJS) Annual Meeting*. March 2023 (future).

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I. Appendix

I.1 Appendix Item A. KII Protocol

NIJ Drug Chemistry

Key Informant Interview Questions

Revised December 9, 2021

Introduction (1 minute)

Thank you for agreeing to participate in the NIJ Novel Quantitation Workflow for Improved Drug Surveillance Project (aka the NIJ Drug Chemistry project) Key Informant Interview process.

My name is _____ and I work as a _____ at Pitt PERU. As a reminder for what this project is, the vision for the NIJ Drug Chemistry project is "To increase vigilance and surveillance of current and future drug threats to the community through quantitative analysis of drug samples". Before we begin, I want to just explain why we are doing these interviews and how the data from them will be used.

The purpose of this interview is to get your feedback about your experiences participating in the NIJ Drug Chemistry Project. These interviews are important because they will help us better understand the impact of the project and Pitt PERU's involvement.

Data gathered from these interviews and surveys will be de-identified and analyzed by the Evaluation and Quality Improvement team at Pitt PERU. A report will be generated that will summarize the outcomes of the interview process.

This interview should take us 30 minutes or less. You should have been given a short demographic survey via email before the meeting. Were you able to complete the survey? [If not, give them a link to the survey to take afterward.]

[Insert survey link]

Additionally, to make sure we can most accurately reflect your thoughts and opinions, we would like to audio record this interview. Is that ok with you? [If yes, start recording. If no, do not record]

[If recording] Ok, I've started the recording. Thank you.

Do you have any questions before we begin?

Demographic Information

What is your position / title within your organization? _____

Please indicate the number of years you have worked in this type of position:
Years:
Months:

Please indicate your age:
- ☐ 18-24
- ☐ 25-34
- ☐ 35-44
- ☐ 45-54
- ☐ 55-64
- ☐ 65-74
- ☐ 75 or older

How would you describe your gender identity? (Select all that apply)
- ☐ Man
- ☐ Woman
- ☐ Non-binary
- ☐ Prefer to self-describe: _____
- ☐ Prefer not to answer

Please indicate your race: (Select all that apply)
- ☐ American Indian or Alaska Native
- ☐ Asian or Pacific Islander
- ☐ Black or African American
- ☐ White
- ☐ Race not listed
- ☐ Two or more races
- ☐ Prefer to self-describe: _____
- ☐ Prefer not to answer

Please indicate your ethnicity:
- ☐ Hispanic or Latinx
- ☐ Not Hispanic or Latinx
- ☐ Prefer not to answer

Key Informant Interview Questions

FOR INTERVIEWERS

Ask the following questions during the interview.

Project Expectations

For all of the questions I ask today, I want you to draw on your professional experience as a member/employee of your organization.

First, we'd like to get a sense of what your understanding of the project is.

1. From your perspective, what were the goals of this project? (Encourage them to use their own words as opposed to trying to remember the formal Vision statement)

2. How do you see the work you do as contributing to the project's goals?

3. How would you describe your involvement with the project?
    a. Were you as involved as you think you needed to be?
    b. Do you think you were too involved? That is, did the project take up too much of your time? Was it intrusive to getting your work done?

4. Any other follow up question.

Data Reporting and Entry

Next, we'd like to understand your perspective on data reporting and data entry.

5. What does the data reporting process look like for you?

6. Are there any parts of the data reporting process you found particularly challenging?
    a. What made the data reporting process challenging?
    b. What do you think PERU could have done to reduce/address those challenges?

7. Are there any parts of the process that worked particularly well for you?
    a. What about that part made it work well?
    b. What other processes would benefit from what worked well in data reporting?

8. Are there any factors that made it more difficult to accurately enter data?

9. Are there any factors that made it easier to accurately enter data, or that helped you enter data more accurately?

10. Any other follow up question.

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Daily Work and COVID-19

Next, we'd like to understand the work you do and what impact, if any, the COVID-19 pandemic had on it.

12. What does a standard workday look like for you?

13. How does this compare to your workday before the COVID-19 Pandemic?

14. Which aspects of your work were made more difficult because of the pandemic?

15. Were any aspects of your work easier during the pandemic compared to before the pandemic?
    a. If so, which ones and how?

16. Any other follow up question.

Thank you so much for your time. Your responses will be used to create a report about this project.

Again, everything you have said during this interview will be kept confidential.

If you have any questions or comments, you are welcome to reach out to Rachel Rock at RAR178@pitt.edu.

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I.2 Appendix Item B. Liquid Chromatography Photo Diode Array Detector (LC-PDA): Sampling Plan

Liquid Chromatography Photo Diode Array Detector (LC-PDA): Sampling Plan

- Cases chosen for LC analysis will be chosen at the discretion of the section Scientists from the cases that are provided to the section.

- Case samples chosen must contain the following compounds: fentanyl, acetyl fentanyl, despropionyl fentanyl (4-ANPP), fluorofentanyl and/or heroin and **must be submitted by a participating agency.**

- If the case contains more than one stamp, all stamps should be analyzed
  - If there is a bundle / multiple stamp bags and a few bags do not have stamps, these unmarked bags do not have to be sampled. However, if there are multiple unmarked stamp bags in the population they should be sampled
  - Sampling is up to the discretion of the Scientist who is working the case

- **All samples will first be analyzed following the appropriate Drug Chemistry SOPs**
  - If a sample is not suitable for LC analysis, it will not be included in the study
    - Does not contain any of the method validated controlled substances
    - GC data shows very low concentrations mixed with large amounts of other additives (sugars, xylazine, acetaminophen, diphenhydramine, quinine, etc.) - will be Scientist discretion
    - If there is only one stamp bag in the case, the bag must contain at least 20 milligrams

- Powders and solids will be homogenized prior to selecting a representative sample.
  - **LC Sample will be removed from the same item as the GC Sample**

- Weigh out 1 milligram of sample using the Mettler Toledo Microbalance
  - Sample weight must fall within the range of 1.000 to 1.030 mg

- Powder will then be added to a LC vial, capped, and stored in the DC fridge in the appropriate sample tray
  - Sample tray will have a label reading "LC Samples"
  - Sample vials will be labeled with case info similar to how Scientists label MS/FID vials
    - Label will include "LC" on the vial to differentiate this sample from other samples that may have been previously prepared for GC analysis
    - Label can also contain the weight of the powder

- The assigned Scientist will be responsible for preparing the following:
  - Daily Standard Mix
  - Internal Standard Solution
  - Calibration Curve Standards
  - Sample Vials

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** Reference the LC Quick Reference Guide on how to properly prepare these samples if needed.

- The Scientist who runs the samples will also be responsible for checking the Daily Standard Mix to ensure the instrument ran properly, checking the calibrators / calibration curve, collecting data, calculating the quantitation values of the compounds of interest, and importing the data into the Excel file then uploading it into TEAMS
    - If LC data is not suitable for reporting and/or there is an issue with a quantitation value for one or more controlled substances of interest, please add the following comment to the Excel Data sheet:
        - If the chromatography is unacceptable / poor - "_____ was present in the sample but could not be quantitated due to poor chromatography"
        - If there is an item co-eluting with the internal standard - "Not Reportable - unknown compound co-elution w/ I.S. - data not used"
        - If concentrations are below the lowest calibrator "Not Reportable - _____ concentration below lowest calibrator."
        - If concentrations are greater than the highest calibrator - "Not Reportable - _____ concentration is greater than the highest calibrator."

I.3 Appendix Item C. LC-DAD Quick Reference Guide for ACOME Drug Chemistry

LC-DAD Quick Reference Guide for ACOME Drug Chemistry

This document will serve as a guide for usage of the LC-DAD instrument. This does not replace the information in the manufacturer provided user manual, this is a guide for usage specific to the needs of the drug chemistry section. This manual will be updated with major changes, but minor changes will be communicated to scientists through communication from the section manager. Deviations from this guide will not be considered a noncompliance unless the deviation results in a violation of the section or office documented quality standards.

- Scientists should coordinate run dates with each other to ensure the run goal for the month is being met
- The Scientist who oversees the LC run is responsible for all maintenance / duties necessary to run the LC for the day along with data processing / data uploading to TEAMS.
  - **Maintenance**
    - LC Column
      - Check for leaks
    - DAD (ensure it is not burnt out)
    - Autosampler
      - Needle
      - Sample Loop
  - Glassware located beside the LC is to be used for LC solvents only!
    - There is a separate beaker for the acetonitrile, water, and the column rinse waste

Daily Maintenance / Prior to running LC

- Check to make sure the waste bottle is empty or close to empty
- **Condition the column** – this is mainly done to prep the column since there is a decent amount of down time between runs
  - Disconnect the column from the line the runs to the detector (top of the column)
  - Flip the column so the free end is now facing down and the end of the column that is still attached is now on top
  - Clip the column in place and place a beaker underneath the column so that the mobile phases can be caught and properly disposed of
  - Click the first tab (labeled "Home") & go down to the section that reads "Vanquish Pump"
  - Change the mobile phase settings to the following:
    - Mobile Phase A – 40%
    - Mobile Phase B – 60%
    - Flow Rate: 0.350 ml/min
  - Once you hit "enter" the flow will automatically start, allow this to run for approximately 10-15 minutes

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- Change the mobile phase settings to the following:
    - Mobile Phase A – 20%
    - Mobile Phase B – 80%
    - Flow Rate will remain 0.350 ml/min
- Allow this to run for approximately 10-15 minutes
- Change the mobile phase settings back to the following:
    - Mobile Phase A – 40%
    - Mobile Phase B – 60%
    - Flow Rate will remain 0.350 ml/min
- Allow this to run for approximately 10-15 minutes
- Shut off the flow and re-attached the column
    - It helps to wipe off the column with a Kim Wipe so it doesn't drip down the column into the basin causing the leak detector to go off
- Dispose of the mobile phase in the beaker into the LC waste container

- Create new mobile phase / refill pump wash bottles if volumes are low

***ALL BOTTLES ARE LABELED AS TO WHAT THEY CONTAIN & WHAT THEY ARE USED FOR**

*** NEW MOBILE PHASE(S) <u>CAN NOT</u> BE ADDED TO EXISTING MOBILE PHASES!!!!**

- <u>Mobile Phase A</u> (Water): 600 mL
    - Needs to be changed prior to EACH run
- <u>Mobile Phase B</u> (LC Grade Acetonitrile): 600 mL
    - If less than 350 milliliters, then the mobile phase must be discarded and re-made fresh
- <u>Mobile Phase C</u> (Water w/ 1% TFA): 200 mL
    - Needs to be changed prior to EACH run
    - Fill bottle to with 200 mL of water, then add 2 mL TFA
    - Use the blue cap located beside the LC labeled "Mobile Phase C" – invert the bottle to ensure the TFC is mixed throughout
    - **TFA can evaporate over time & water can go bad therefore it needs to be made fresh prior to each run**
- <u>Seal Wash</u> (90:10 Water:MeOH): 1000 mL
    - If less than 250 milliliters, then the seal wash must be discarded and re-made fresh prior to running the instrument for the day
- <u>Needle Wash</u> (90:10 MeOH:Water): 200 mL

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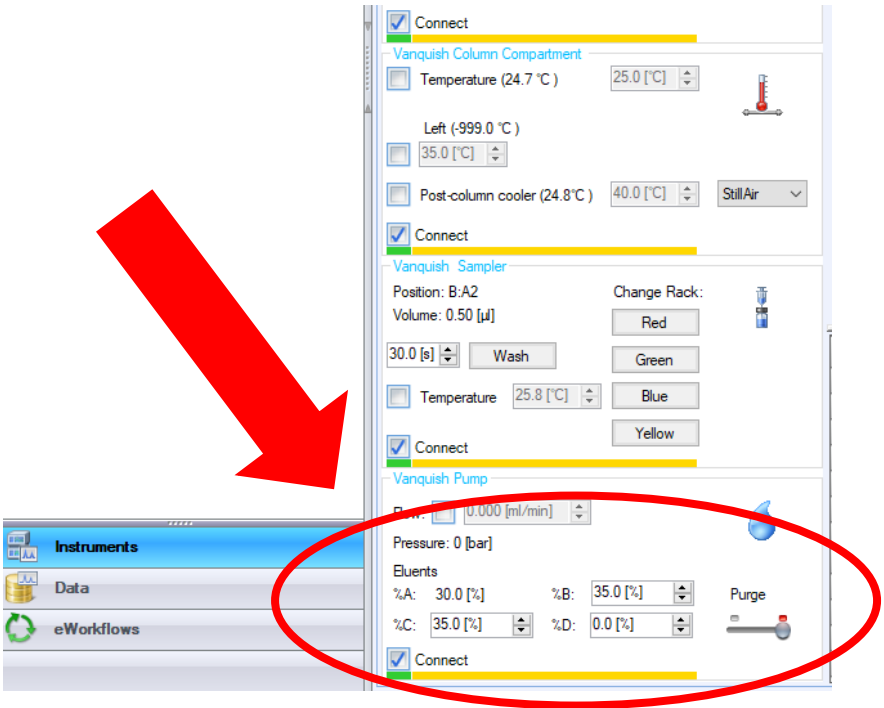
- If less than 50 milliliters, then the needle wash must be discarded and re-made fresh prior to running the instrument for the day

- **<u>Purge the lines</u>** – This is necessary to ensure there is no air present / no build up within the lines
  - Open the bottom door on the LC (labeled "Vanquish Pump")
  - Turn the **GOLD** metal knob approximately 1/2 turn (or until loose) to the left – you will feel the knob loosen up
  - Go to the Chromeleon Console, click Instrument on the left panel (will be colored blue if it's the active panel)
  - Click the first tab (labeled "Home") & go down to the section that reads "Vanquish Pump"
  - Change mobile phases to the following values:
    - Phase A – 30.0 %, Phase B – 35.0 %, and Phase C – 35.0 %
    - These values are set as is so that close to equal amounts of each mobile phase will be purged throughout the lines
    - Flow should automatically be set to 3.000 mL/min for the purging event



  - In that same "Vanquish Pump" section, toggle the purge over to ON (the toggle will move from RIGHT to LEFT & the light will change from RED to GREEN)

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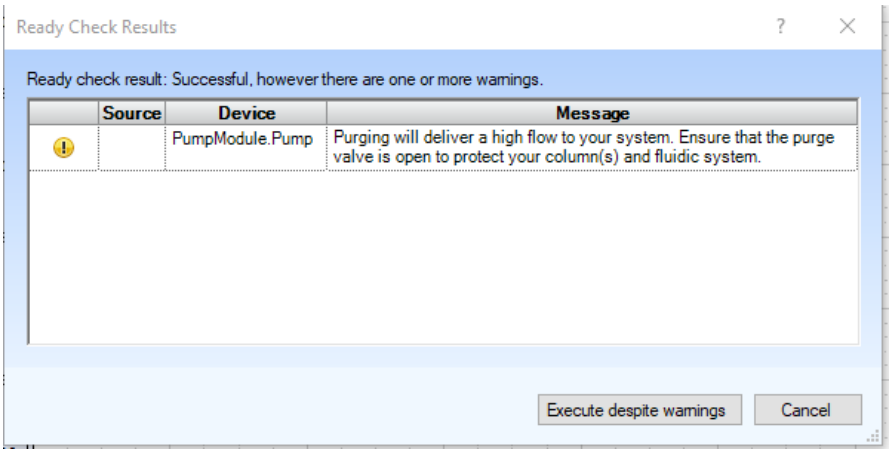
- After the toggle is moved a message box will appear on the screen



^The message is reminding the instrument operator to be sure that the purge valve is OPEN before moving forward

- **BE SURE TO CHECK THAT THE VALVE IS OPEN AT THIS TIME! If that value is open, click "Execute despite warnings"**
- **If the valve is not open, be sure to open it then click "Execute despite warnings"**

- Let instrument purge (it is programmed to purge for 3 minutes)
  - Be sure to observe the lines to make sure there are no air bubbles and that the mobile phases are freely moving throughout the lines
  - You will hear 3 consecutive clicking noises this is normal (a click per each line being purged)
- After 3 minutes, the purge will automatically shut off
- Turn the GOLD metal knob to the right until it is finger tight
  - **If the knob is not shut, all samples analyzed will run to the waste instead of through the column to the detector!**
- Shut the bottom door on the LC (labeled "Vanquish Pump")

- **<u>Leak Check / System Check & Prep</u>**
  - In the Instrument panel, click the box beside UV Lamp. Clicking this box will turn on the UV lamp.
    - It does take the lamp some time to turn on so double check the audit log within the program to ensure the button was indeed clicked

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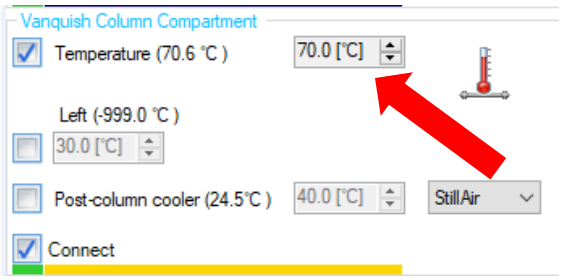
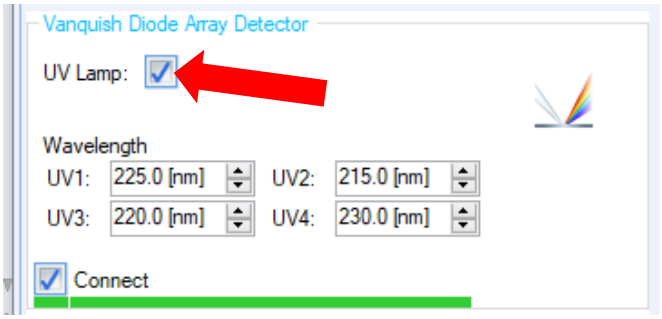
- Click the box beside Temperature and change the temperature to 70°C

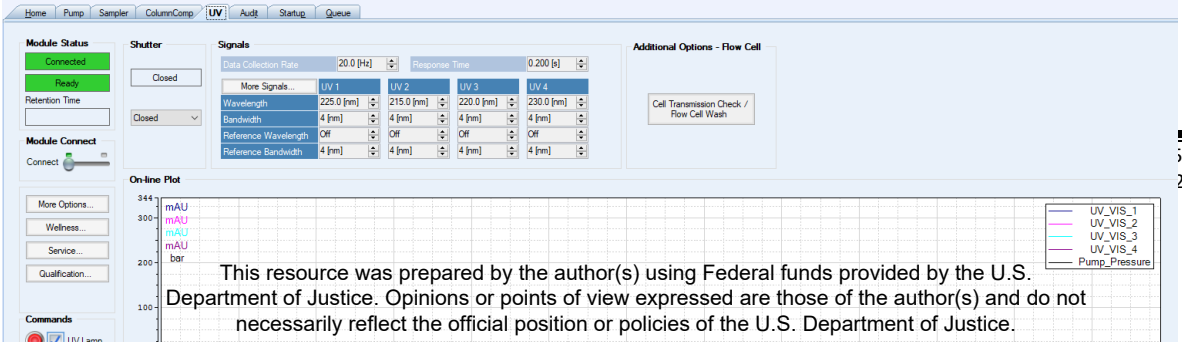


- Change flow rate to 0.650 mL/min and make Mobile Phases B and C 10% forcing Mobile Phase A to be 80%.
    - These mobile phase conditions mimic the beginning gradient of the method that will be used to condition the column

- Open the Column Compartment door to ensure that there are no leaks
    - There is a leak sensor located at the bottom of the Column Compartment that will beep if liquid is detected
        - To mute the alarm just push the button beside the red alarm light that reads “MUTE ALARM” & use a Kim Wipe to dry out the basin near the sensor.
        - Be sure the connectors into the column are finger tight
        - Reference Column Compartments Operating Manual if needed (pg. 138)

- **<u>Monitoring Detector</u>**
    - Click on the “UV” tab
    - Be sure that the UV Lamp box is checked indicating that the Lamp is on





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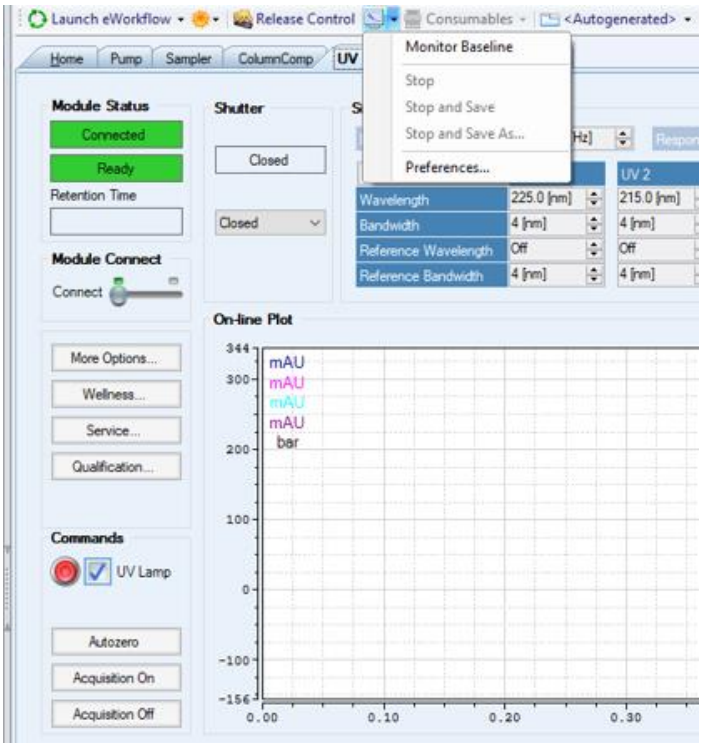
** The UV wavelengths that are listed at the top of the graph is indicative of the wavelengths being monitored along with the pump pressure (these are the same wavelengths that are used in the method)

- At the top of the page there is an icon that looks like a computer monitor, click on the down arrow that is to the right of that image



- Click on "Monitor Baseline" & the image below will appear

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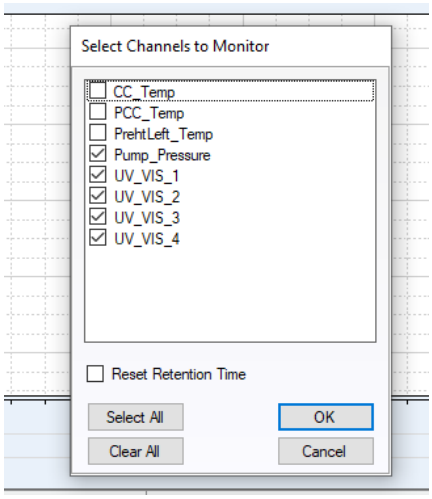
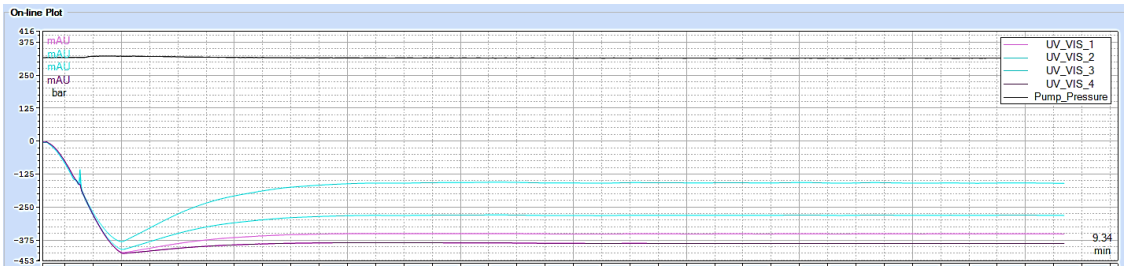
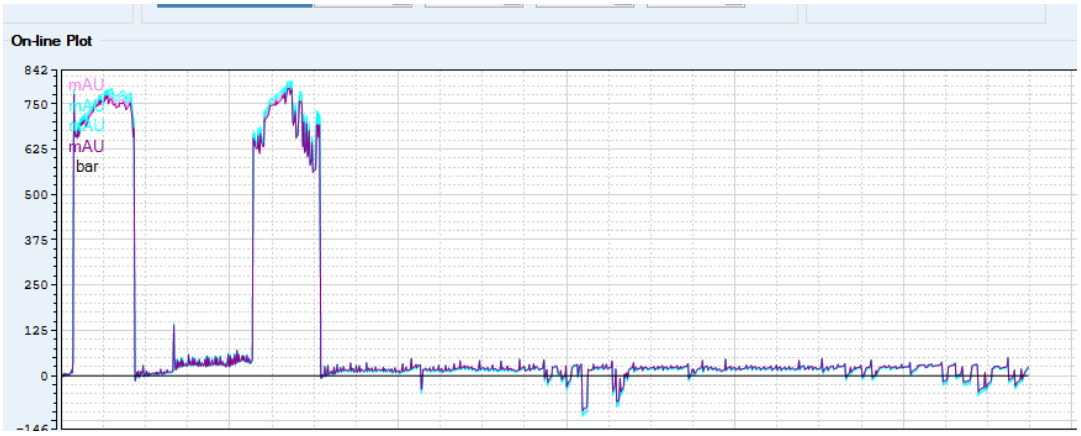


- Make sure Pump Pressure and UV_VIS 1-4 are all selected before clicking "OK"
- There should be 5 different colored lines that appear on the graph
- Let this run for 20-30 minutes – this step allows for the UV detector to warm up / to monitor that the detector is properly working
- If after that time the lines on the spectra are not straight / settled (image 1) this may be indicative that there is an issue with the system (image 2)





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- Click "STOP" beside the computer monitor – this will stop the monitoring only, it will not stop the flow

CALIBRATORS / INTERNAL STD.

- The Scientist on rotation is also responsible for creating the internal standard solution (if there is not enough left), the calibration curve mix (if there is not enough left), the calibration curve standards as well as the case samples
    - **Internal Standard Solution:**
        - Create a 1mg/mL internal standard solution of phenol/acetonitrile
            - May be some pre-made in the DC fridge; check on amount before making new
            - The amount needed per run will vary depending on how many case samples are in the tray for the run (DO NOT FORGET TO INCLUDE CALIBRATORS)
                - **Each sample requires 100 µL**
                - **Each calibrator requires 20 µL**
    - **Daily Standard Mix:**
        - Take a DSM vial from fridge, remove solution from vial insert, and place into the vial (dispose of insert)
        - Add approximately 500 µL of LC Grade water and invert
        - The purpose of running this mix is to just ensure that the instrument is running properly, not for any quantitation information!
    - **Calibration Curve Standards: 6.25, 12.5, 25, 50, 100, 200, 400 µg/mL**
        - Add 2 milligrams of the following standards into a LC vial: heroin, fentanyl, 4-ANPP, acetyl fentanyl, and fluorofentanyl
        - Add 2 milliliters of acetonitrile to the vial containing the standards then vortex
        - Label a LC vial for each calibrator (pre-made labels located in the drawer w/ the pipettes)
        - Place 460 µL of HPLC Grade Water into the vial labeled Cal. 7 and place 380 µL of HPLC Grade Water in the other calibration vials
        - Pipette 320 µL of the standard stock solution into the 460 µL of HPLC Grade Water in the Cal. 7 vial then vortex – **400 µg/mL (Cal. 7)**
        - Pipette 400 µL of the Cal. 7 solution into the 380 µL of HPLC Grade Water in the Cal.6 vial then vortex – **200 µg/mL (Cal. 6)**

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- Pipette 400 µL of the Cal. 6 solution into the 380 µL of HPLC Grade Water in the Cal. 5 vial then vortex – **100 µg/mL (Cal. 5)**
- Pipette 400 µL of the Cal. 5 solution into the 380 µL of HPLC Grade Water in Cal.4 vial then vortex. –**50 µg/mL (Cal. 4)**
- Pipette 400 µL of the Cal. 4 solution into the 380 µL of HPLC Grade Water in Cal.3 vial then vortex. –**25 µg/mL (Cal. 3)**
- Pipette 400 µL of the Cal. 3 solution into the 380 µL of HPLC Grade Water in Cal.2 vial then vortex. –**12.5 µg/mL (Cal. 2)**
- Pipette 400 µL of the Cal. 2 solution into the 380 µL of HPLC Grade Water in Cal.1 vial then vortex. –**6.25 µg/mL (Cal. 1)**
- **Discard 400 µL of Cal. 1!**
- Add **20 µL** of the internal standard stock solution **to each vial** & **vortex**
- Remove the cal. solution & place into a small volume insert using a disposal glass pipette

- **Sample Prep**:
  - Add 0.4 mL (400 µL) acetonitrile to 1 mg sample
  - Add 100 µL internal standard solution
  - Add 100 µL Mobile Phase C
  - Add Mobile Phase A (HPLC Grade Water) until the volume reaches the neck of the sample vial (approximately 1.4 mL)
  - Leave a space at the top so the liquid inside can be easily homogenized
  - Vortex vials to ensure the solution is uniform

** The final concentration of Internal Standard will be 50 µg/mL

Chromeleon / LC-DAD Instrumentation

- **Autosampler**
  - The Autosampler has four trays (red, green, yellow, blue)
  - The Autosampler can be rotated to view a new rack by pressing the "ROTATE" button on the Autosampler (last button on the right)
  - Each tray has 9 vial positions across and 6 vial positions down (labeled A-F)
  - Be sure that the tray is properly inserted into the Autosampler so that the writing on the front of the tray is facing you
  - When adding samples to the instrument run list, the vial position will be designated as follows: color of tray + vial position (i.e. G:A1, R:B9, B:C20, Y:D10, etc.)

- **Adding Samples**

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- Open the main page of the Chromeleon Console. This is where a daily sequence is created.
- Create a sequence by opening the last used daily sequence which will be named "DC_SEQUENCE_MM_DD_YY".
- Ensure that all injections in the opened sequence are labeled as "Finished" under the status column. If they are not, the injection may have not been completed.
- If the previous sequence was successfully completed **FIRST**, select "Save As" from the "File" menu and re-name the sequence for the current date.
- **ONLY AFTER USING THE "SAVE AS"** function and creating a new sequence, delete all "Finished" rows, which should now be set to "Idle" after making the new sequence,
- LEAVE THE INITIAL BLANKS, DAILY STANDARDS MIX, INTERAL STANDARD, CALIBRATORS, AND FINAL BLANK AFTER CAL. 4
- The blank runs will act as a way to equilibrate the column prior to running samples for the day
    - Blank Injection volume: 1.00 µL

- **Daily Standards Mix**
    - Update the date, user initials, and standard mix lot number (if needed) for the standards mix injection.
    - **Any additions or changes must be saved to ensure the sequence runs properly.**
    - The "Type" column in the sequence should be "unknown".
    - Enter the proper vial position
    - Injection volume: 1.00 µL
        - Unlike the GC, this parameter is NOT set in the method
    - Ensure "**PITT QUANT_2.0**" is selected for the instrument method. The instrument monitor(s) will ensure only updated methods are available in the daily sequence. **Do not add additional methods, which may exist, to the daily sequence.**
    - Select appropriate processing method: Daily Standard Mix
        - If the incorrect processing method is inadvertently selected, a correction can be made after data is collected. The sample does not need to be re-run.

- **Calibration Curve Samples**
    - Calibrators will be named in the following manner:
        - Cal 1_6.25µg/mL_INITIALS_DATE
        - Cal 2_12.5µg/mL_INITIALS_DATE
        - Cal 3_25µg/mL_INITIALS_DATE
        - Cal 4_50µg/mL_INITIALS_DATE

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- Cal 5_100µg/mL_INITIALS_DATE
- Cal 6_200µg/mL_INITIALS_DATE
- Cal 7_400µg/mL_INITIALS_DATE

➢ The initials will be of those who made the calibrators, and the date will be the date that the calibrators were made

- The "Type" column in the sequence should be "Calibration Standard".
- The "Levels" should be as follows:
  - Cal. 1 – Level 1
  - Cal. 2 – Level 2
  - Cal. 3 – Level 3
  - Cal. 4 – Level 4
  - Cal. 5 – Level 5
  - Cal. 6 – Level 6
  - Cal. 7 – Level 7
- Injection volume: 3.00 µL
- Be sure to enter the proper vial positions
- **Any additions or changes must be saved to ensure the sequence runs properly.**
- Ensure "**PITT QUANT_2.0**" is selected for the instrument method.
- Select appropriate processing method: **Quantitation_2.0**
  - If the incorrect processing method is inadvertently selected, a correction can be made after data is collected. The sample does not need to be re-run.

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Save | Studio | Print | Up | Insert Row | Fill Down | Lock | Filtering | Grouping | Custom Columns | Find Next

#	UV_VIS_2	Name	Level	Type	Position	Volume [ul]	Instrument Method	Processing Method	Status
1	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
2	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
3	None	DSM_10.07.2022_042722RTR1_PITTQUANT_EAP		Unknown	Y:A2	2.00	PITT QUANT_2.0	Daily Standards Mix	Idle
4	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
5	None	INT STD_EAP_10.07.2022		Unknown	Y:A3	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
6	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
7	None	CAL 1_6.25UG/ML_EAP_10.07.2022		Unknown	Y:A4	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
8	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
9	None	CAL 1_6.25UG/ML_EAP_10.07.2022	1	Calibration Standard	Y:A4	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
10	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
11	None	CAL 2_12.5UG/ML_EAP_10.07.2022	2	Calibration Standard	Y:A5	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
12	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
13	None	CAL 3_25UG/ML_EAP_10.07.2022	3	Calibration Standard	Y:A6	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
14	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
15	None	CAL 4_50UG/ML_EAP_10.07.2022	4	Calibration Standard	Y:A7	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
16	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
17	None	CAL 5_100UG/ML_EAP_10.07.2022	5	Calibration Standard	Y:A8	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
18	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
19	None	CAL 6_200UG/ML_EAP_10.07.2022	6	Calibration Standard	Y:A9	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
20	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
21	None	CAL 7_400UG/ML_EAP_10.07.2022	7	Calibration Standard	Y:B1	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
22	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle

- **<u>LC Quant Samples</u>**
  - The daily sequence may be edited as data is running by copying and pasting rows or by clicking the "Click here to add a new injection" at the bottom of the last row in the sequence.
    - The new line will duplicate the previous line

17	None	CAL 5_100UG/ML_EAP_10.07.2022	5	Calibration Standard	Y:A8	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
18	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
19	None	CAL 6_200UG/ML_EAP_10.07.2022	6	Calibration Standard	Y:A9	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
20	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
21	None	CAL 7_400UG/ML_EAP_10.07.2022	7	Calibration Standard	Y:B1	3.00	PITT QUANT_2.0	Quantitation_2.0	Idle
22	None	BLANK		Blank	Y:A1	1.00	Blank Run		Idle
		Click here to add a new injection							

Line 22 was added by clicking "Click here to add a new injection"

- As you begin to add a data file name, ensure your cursor is in the desired area before typing.

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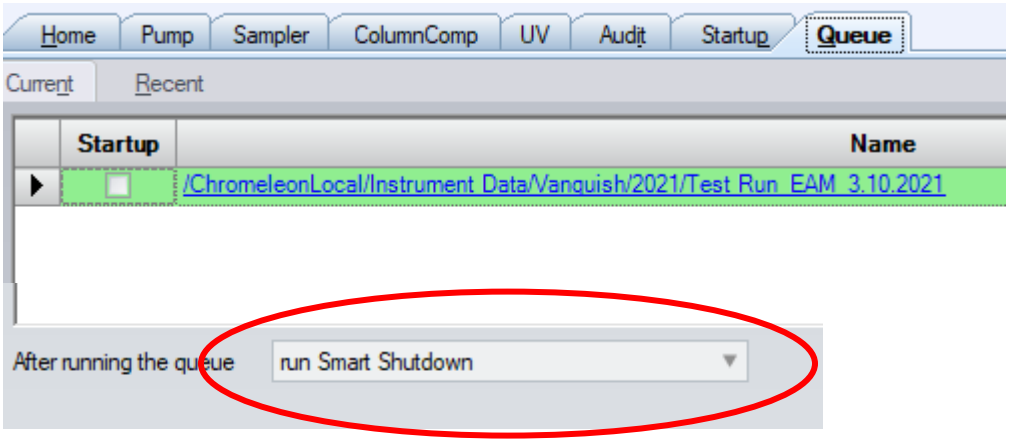
- **Any additions or changes must be saved to ensure the sequence runs properly.**
- The "Type" column in the sequence should remain as "Unknown".
- Enter the proper vial position
- Injection volume: 3.00 µL
- Select "**PITT QUANT_2.0**" for the instrument method.
- Select appropriate processing method: **Quantiation_2.0**
  - If the incorrect processing method is inadvertently selected, a correction can be made after data is collected. The sample does not need to be re-run.
- Data remains in the sequence and can only be accessed from the sequence; there will not be separate data folders for files belonging to each scientist. The sequence name containing the data file will need to be referenced in case notes in the event that hard copy is not available, and the data needs to be located.

- After the last sample is run, add a blank run (Blank Run Method) followed by another blank run using the method **COLUMN FLUSH**
  - The Column Flush method is more organic than the sample blank runs; therefore it will ensure that the column is cleaned prior to sitting until the next time the instrument is ran

- The instrument is set to do a Smart Shutdown after the last sample in the instrument queue runs. **BE SURE THAT THIS IS SELECTED PRIOR TO LEAVING FOR THE DAY / NIGHT!**



  - If the instrument does not go through the Smart Shutdown process properly then the mobile phases will continue to flow and the UV detector will remain on potentially causing harm to the instrument

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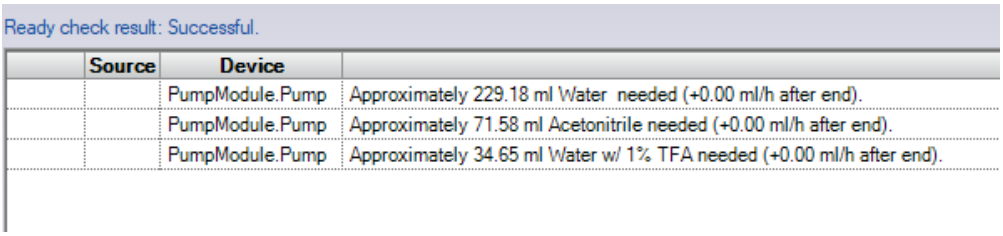
- There is an "Emergency Method" that was created, and it is supposed to automatically load if there is an error during the sequence that causes the sequence to abort. This is another safe-proof measure to ensure that if the sequence does not run to completion that the UV lamp will be turned off and the mobile phases will stop flowing.

- Check to see how much of each mobile phase is needed to complete the sequence that is loaded in the queue.
  - In the Queue tab, there is an option on the far right that reads "Ready Check". If you click that option, the box below appears at the bottom of the page

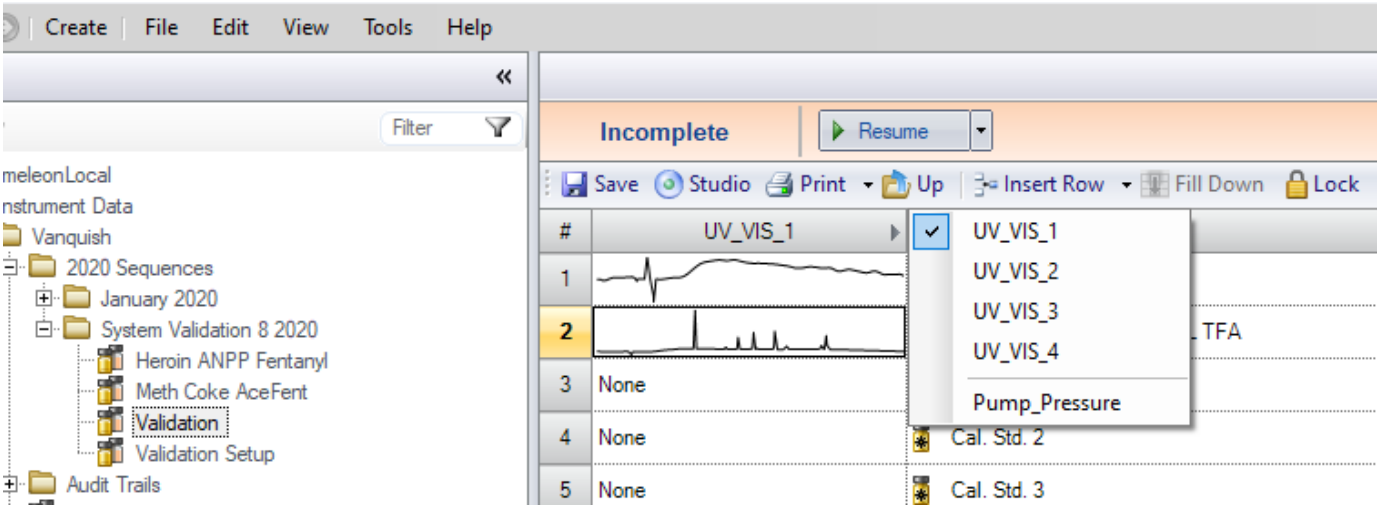


Ready check result: Successful.

	Source	Device	
		PumpModule.Pump	Approximately 229.18 ml Water needed (+0.00 ml/h after end).
		PumpModule.Pump	Approximately 71.58 ml Acetonitrile needed (+0.00 ml/h after end).
		PumpModule.Pump	Approximately 34.65 ml Water w/ 1% TFA needed (+0.00 ml/h after end).

  - Be sure that there is enough in each of the mobile phase bottles, as well as the seal wash and pump wash bottles, to complete the run.

- **<u>Checking Daily Standard Mix</u>**
  - In the sequence, double click on the chromatogram preview in the UV VIS column of the sequence. This will open the data with the selected processing method.
    - You can switch between the UV VIS Channels in this column as well to display the sample under a different UV wavelength. **BE SURE UV_VIS_2 IS SELECTED FOR THE STD MIX.**



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- In the Channels section, click UV_VIS_2 (wavelength 215nm)

- Ensure that the following components are present: Caffeine, Methamphetamine, Lidocaine, Heroin, Cocaine, Alprazolam, and Fentanyl
    - Like the GCs, the retention times for peaks may shift outside of the pre-programmed "acceptance criteria" range resulting in the peak not to be labeled by the software. If this happens, go to the "Data Processing" panel, then in the ribbon above the spectra, click the "Processing Method" option. This will open a new panel underneath the spectra. In this panel, the retention times can be updated. Once updated, the peaks will automatically be labeled.

        - **<u>If the time shifts are extensive, please let the instrument operator(s) know. The shifts should be minimal.</u>**

- **<u>Checking Calibration Curve Standards</u>**

    - Process the data using the "**Quantiation_2.0**" Processing Method
        - Evaluate the curves for each controlled substance - Calibration curves for each compound must show a coefficient of determination (r^2) of 0.98 or greater.
            - If the r^2 is not within acceptable range, then the calibration curve for that compound will be deemed unacceptable and therefore the compound cannot be used for analysis
        - Values of +/- 20% from the target concentration are acceptable however, the lowest calibrator can be +/- 30% from the target concentration.
            - If the calibrators are unacceptable, repeat analysis for case samples.
        - One point can be dropped if needed.
            - If the low calibration point is dropped for one or more compounds, this will change the LOQ for the compound(s) affected
            - If the high calibration point is dropped for one or more compounds, this will change the ULOQ for the compound(s) affected

- **<u>Checking Quantitation Samples / Calculations</u>**

    - Process the data using the Processing Method: **Quantiation_2.0**
    - Quantitated analytes are identified according to their retention times by the instrument software
        - The UV-Vis Spectra for each component needs to be investigated as well

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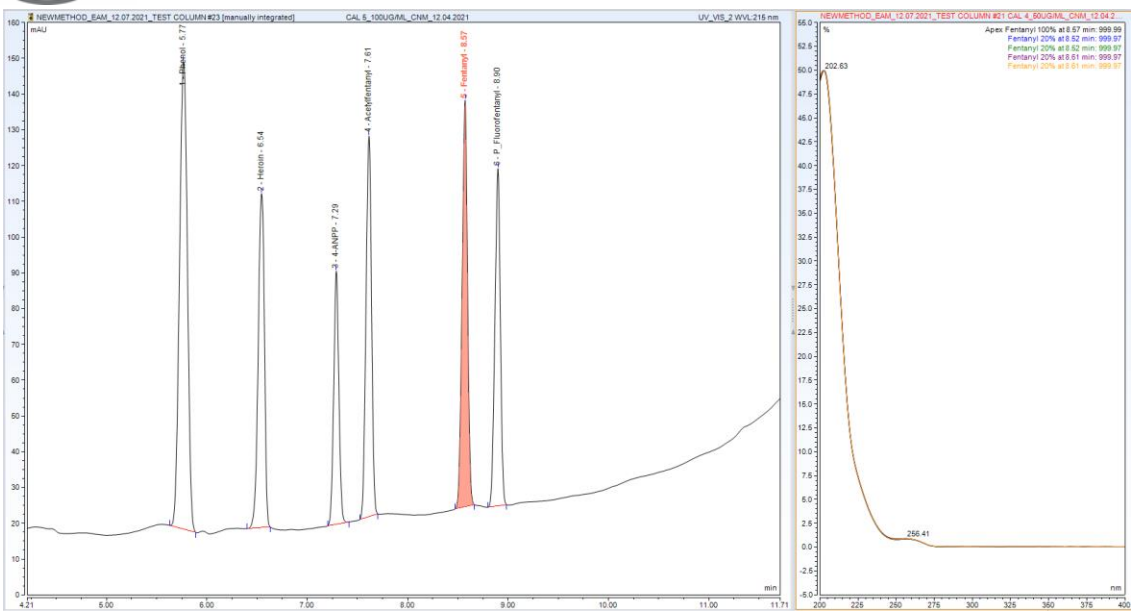
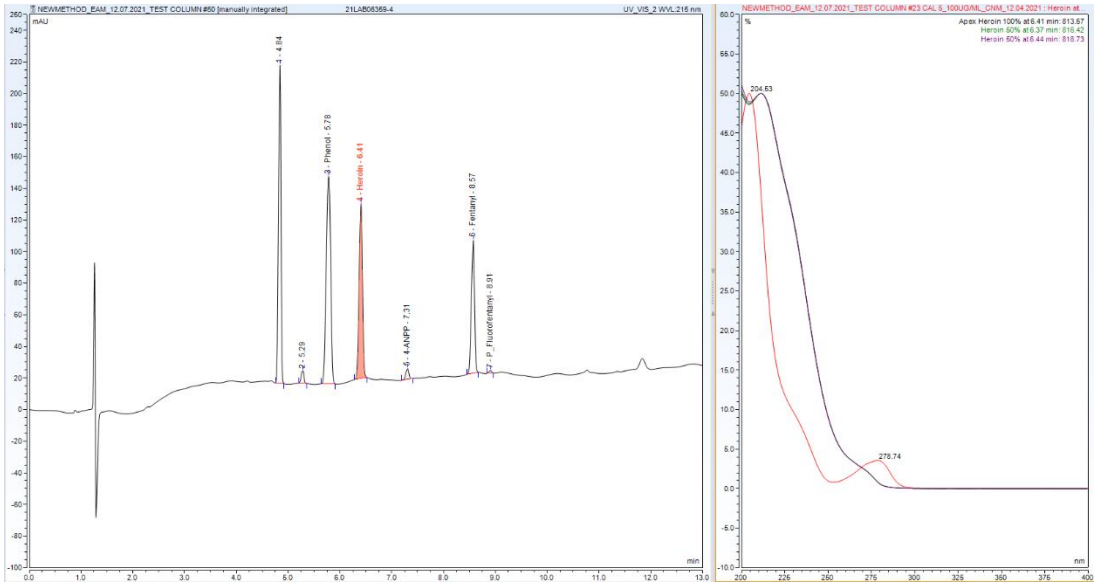


- Each component should be confirmed by reviewing both the RT and the UV-Vis Spectra although, RT should be the first confirmation tool
    - Two components may have similar UV-Vis spectra at the 215nm wavelength which may result in an incorrect UV-VIS match
    - Unknown components within the sample may also elute close to the RT of components of interest resulting in an incorrectly labeled peak (see below).



^ The processing method is labeling the peak at 6.41 as heroin; however, based on the UV-Vis spectra, it can be determined that the peak is not heroin. In this situation, the RT in the processing method for heroin is entered as 6.50 but needs to be edited so that the RT

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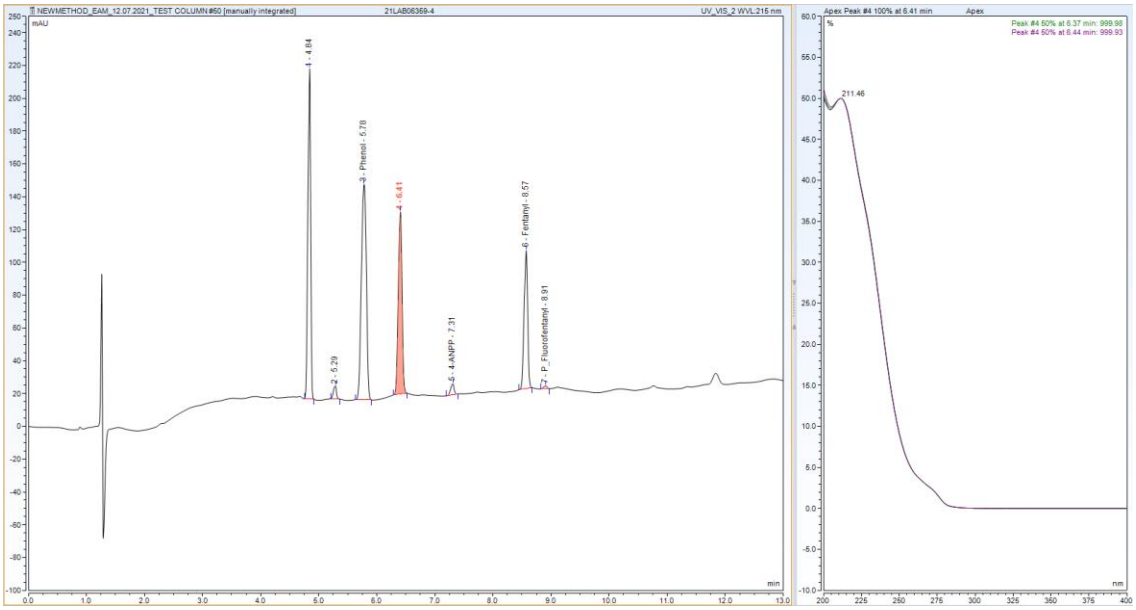
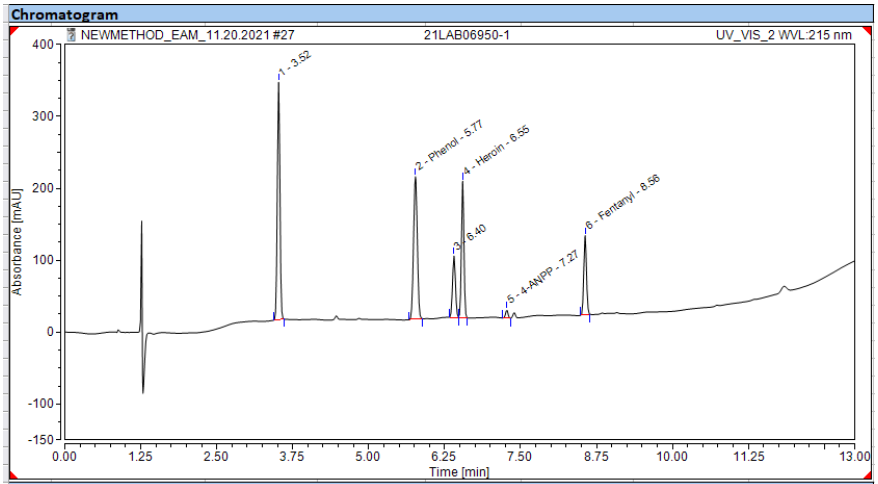
window is "tighter" preventing unknown peaks around the same RT to be incorrectly labeled as heroin. By editing the RT to 6.55 (which is close to the heroin RT in the calibration curve for this run), the label "heroin" is removed from the peak (see below).



- Analyte concentration is calculated by the instrument software by comparison of the area of the analyte peak to the area of the internal standard peak.
  - Analyte concentration is calculated in 'µg/mL' by the instrument software.
- **Component % is the value that will be reported to Pitt.**



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Amount ug/ml	Component %
2	Phenol	5.77	14.161	198.110	28.07	50	10.0
4	Heroin	6.55	8.961	189.986	17.77	42	8.4
5	4-ANPP	7.27	0.531	11.536	1.05	n.a.	n.a.
n.a.	Acetylfentanyl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Fentanyl	8.56	5.671	110.419	11.24	26	5.1
n.a.	P_Fluorofentanyl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**29.324**	**510.052**	**58.13**		

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- **Quality Control**
  - The following procedure will be used when the linear regressions curve produces an unacceptable r^2 value or quantitative control levels are outside of the acceptable ranges:
    - Calibrators will be re-made, and all samples will be disposed of (due to the sample being prepared in mainly water, there will be a decent amount of breakdown occurring)
      - In the Pitt Excel Data file, mark "N/A" for the LC Results and Component %
      - In the Pitt Excel Data File under comments write "Calibration Curve Failed"

- **Reporting**
  - Create new Excel page on LC Data file on LC flash drive & add in data
    - Add the date that the sample was analyzed on the LC and analyst initials who ran the samples
  - Add the component(s) that were detected
  - Add the component amount
  - Component percentage will be calculated via the calculation in the Excel column – scientist should double check this value against the value listed in Chromeleon to ensure there is not an error

    - If the amount is below or above the lowest / highest calibrator write "N/A" in the Component % column and change the font color to red
    - In the comment column use the following statements:
      - "Not Reportable -____ conc. is below the lowest calibrator"
      - "Not Reportable - ____ conc. is greater than the highest calibrator"
    - If the data cannot be used (i.e. – poor data, compound interfering with I.S., etc.), write "N/A" in the Component % column, change the font color to red, and use one of the following comments:
      - "Unknown compound co-elution w/ I.S. – data not used"
      - "Poor data – data not used

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** Reporting Example**

CASE	LC RESULTS	AMOUNT µg/mL	COMPONENT % IN SAMPLE	COMMENTS
21LAB06086-1A	heroin	92.1	18.4	
	4-ANPP	7.4	1.5	
	fentanyl	29.9	6.0	
21LAB06086-1B	heroin	103.5	N/A	Not Reportable - heroin concentration above highest calibrator
	fentanyl	50.3	10.1	
21LAB06086-1C	heroin	49.3	9.9	
	4-ANPP	3.7	N/A	Not Reportable - 4-ANPP concentration below lowest calibrator
	fentanyl	58.1	11.6	
21LAB06086-1D2	heroin	41.8	N/A	Not Reportable - Unknown compound co-eulting with Internal Standard
	fentanyl	53.2	N/A	
21LAB06086-1D6	heroin	99.4	19.9	
	4-ANPP	1.7	N/A	Not Reportable - 4-ANPP concentration below lowest calibrator
	fentanyl	56.6	11.3	

- Once data is added to the LC Flash Drive, copy the Excel Document and upload it to TEAMS (TEAMS → Pitt Research Grant → Files → Data)

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I.4 Appendix Item D. ACOME Drug Chemistry Opioid Surveillance (Static) Report

ACOME Drug Chemistry Opioid Surveillance Report

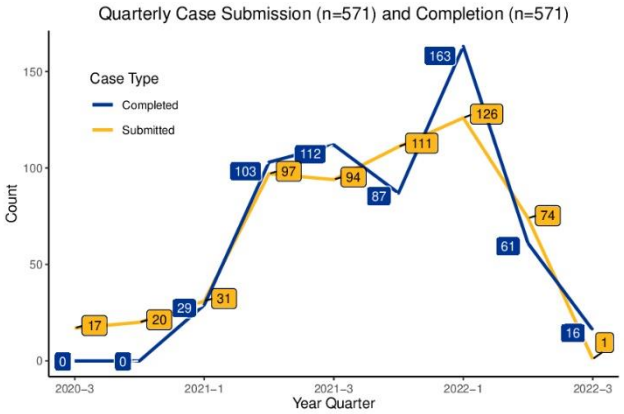
Thu October 06, 2022 01:44 PM

Report Includes Opioid Stamp Bag Submissions, 2020-08-10 to 2022-07-06

Interactive visualizations for drug detection and quantitative results are available on the ACOME Drug Chemistry Opioid Dashboard.

Case Completion

- ACOME chemists have reported the results of 767 opioid-related stamp bag samples processed on the LC instrument.
- This reflects samples from 571 cases submitted by 89 law enforcement entities in Allegheny County.
- A total of 0 cases have been completed in the past sixty days.

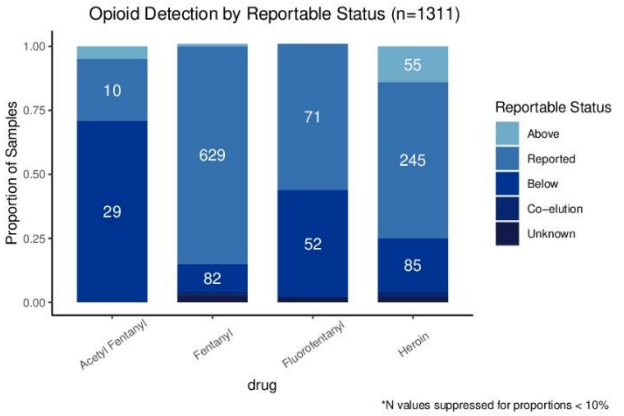


Drug Detection

- 72.85% of all opioid detections fell within the reportable range of the LC instrument.
- Opioid detections averaged 2.3 per case.
- This does not include non-opioid drugs and adulterants that may also be present in drug samples.

Table 1: Total Opioid Detections

Drug	Sum
Fentanyl	744
Heroin	401
Fluorofentanyl	125
Acetyl Fentanyl	41



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Single Drug Detections

- 303 (39.5%) of samples submitted to the ACOME drug chemistry lab included a single opioid in absence of other opioids of interest.
- Table 2 provides a summary of single opioid detection samples.
- Note: These cases may have included other non-opioid drugs or cutting agents not reflected in this report.

Table 2: Single Opioid Sample Detections

Drug	Samples	% of All Detections	Total Detections
Fentanyl	284	38.2	744
Heroin	16	4.0	401
Fluorofentanyl	2	1.6	125
Acetyl Fentanyl	1	2.4	41

Drug Detection Combinations

- 624 (81.4%) of samples submitted to the ACOME drug chemistry lab included at least two opioids of interest.
- Table 3 provides a summary of the two- and three-drug combinations present.
- Note: These cases may have included other non-opioid drugs or cutting agents not reflected in this report.

Table 3: Opioid Combination Sample Detections

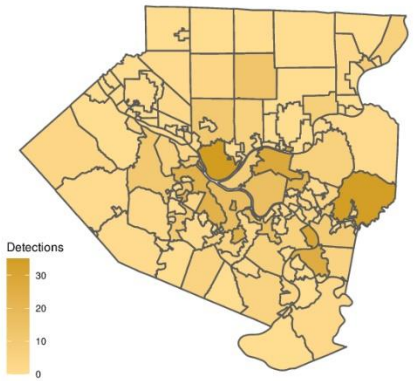
Drug Combination	Samples
Fentanyl + Heroin	381
Fentanyl + Fluorofentanyl	119
Heroin + Fluorofentanyl	68
Fentanyl + Acetyl Fentanyl	40
Heroin + Acetyl Fentanyl	14
Acetyl Fentanyl + Fluorofentanyl	2
Drug Combination	**Samples**
Fentanyl + Heroin + Fluorofentanyl	64
Fentanyl + Heroin + Acetyl Fentanyl	14

Geographic Distribution of Drug Detection

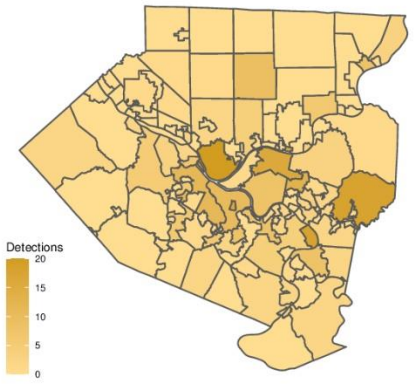
The following maps display the count of drugs detected in samples submitted by 113 municipal and city law enforcement entities, along with the proportion of samples submitted that included a given drug.

Map 1. Count of Drug Detections

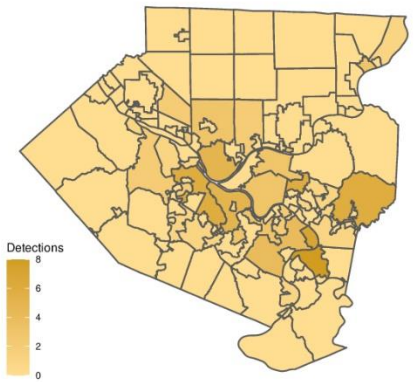
Count of Fentanyl Detections by Municipal and City Police



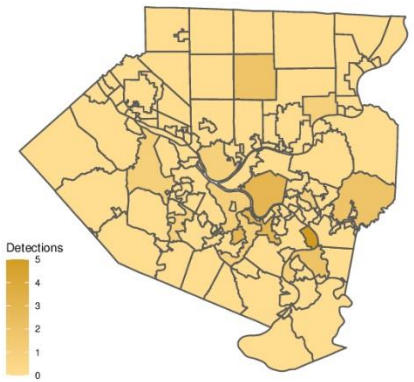
Count of Heroin Detections by Municipal and City Police



Count of Fluorofentanyl Detections by Municipal and City Police

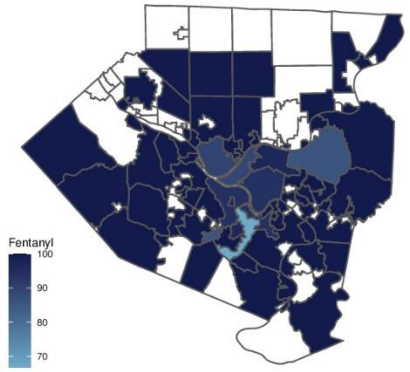


Count of Acetyl Fentanyl Detections by Municipal and City Police

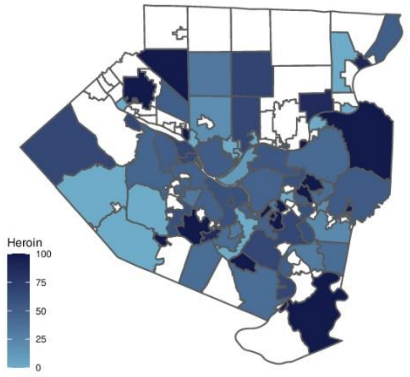


Map 2. Proportion of Drug Samples Submitted

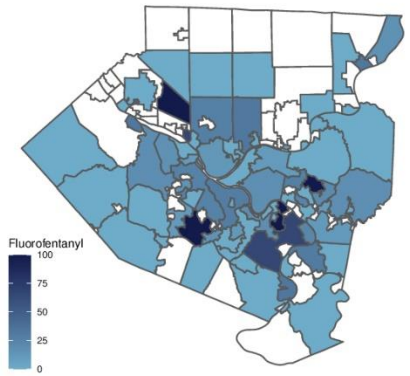
Proportion of Total Samples Including Fentanyl



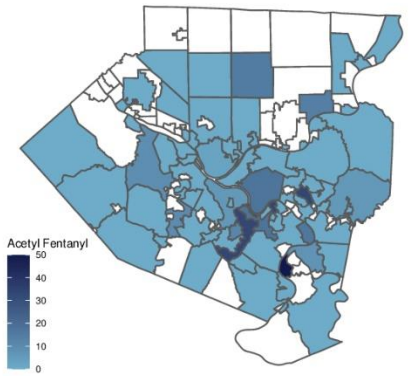
Proportion of Total Samples Including Heroin



Proportion of Total Samples Including Fluorofentanyl

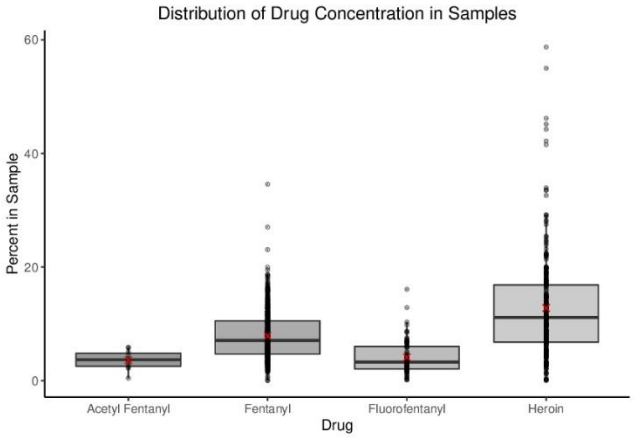


Proportion of Total Samples Including Acetyl Fentanyl



Quantitative Results

- A total of 664 samples fell within reportable ranges.
- This reflects samples from 507 (88.8%) of the 571 total cases submitted to ACOME.
- The figure at right displays the concentration of each drug of interest in reported samples, along with descriptive statistics (summarized in Table 4).



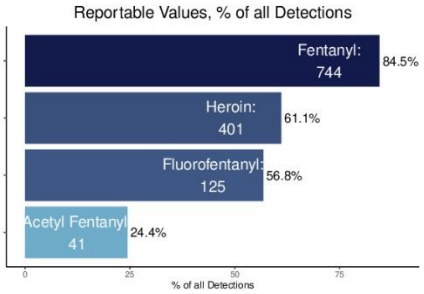
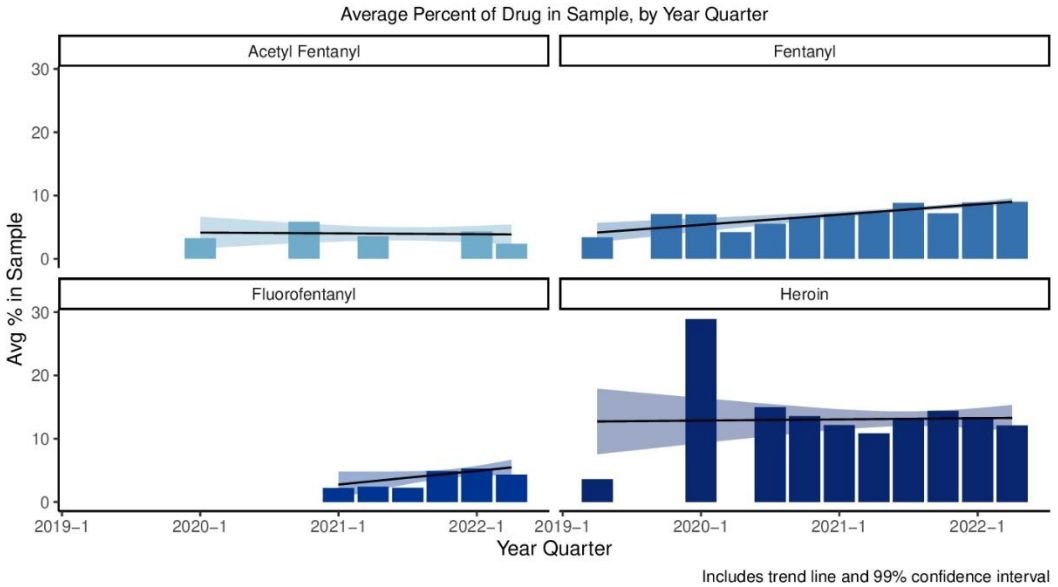


Table 4: Quantitative Drug Potency Result Summary

Drug	n	min	q1	median	mean	q3	max
Acetyl Fentanyl	10	2.20	2.88	3.78	3.95	4.97	5.86
Fentanyl	629	0.03	4.74	7.10	7.92	10.60	34.60
Fluorofentanyl	71	1.46	2.33	3.68	4.52	6.45	16.10
Heroin	245	1.18	7.24	11.30	13.16	17.20	58.74



Includes trend line and 99% confidence interval

Average Percent of Drug in Sample by Submitting Entity

Each figure below includes all **entities submitting more than the average number of samples for a given drug**. For example, entities have submitted an average of 7 samples with reportable amounts of fentanyl. Therefore, the fentanyl figure below only includes those entities submitting 7 or more samples with reportable fentanyl.