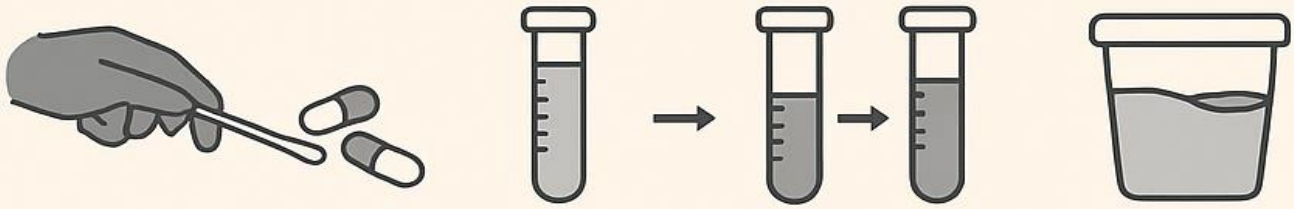
Integrated Framework for Forensic Profiling of Drug Samples

Sample Collection Strategy



Swabs exterior surfaces of capsules and tablets

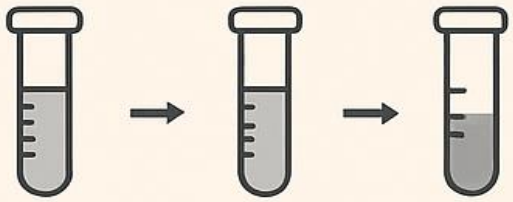
ExtractDNA using valid-dated kits; E.113

Quantification writh Quantifller® Trio

Powder samples into stenla. DNA-free tabex for

Use gloves, contamination controls, and documentation throughout all stages

DNA Profiling Workflow



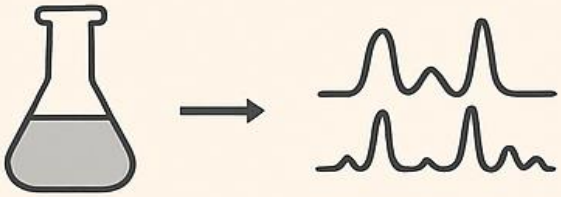
ExtractDNA using valid-ated kits

Quantificat using STR multiplex kit

Amplifidan with STR multiplax kits

Analyce STR profiles for quality, source attribution, and mixture interpretation using tools like STRmix®

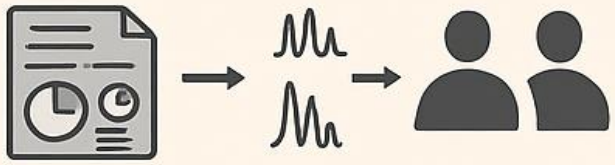
Chemical Profiling Workflow



Dissolve a pection of the drug sample in appropriate solvents, to analyzis via GC-MS; LC-MS to Identify chemical sig-

Compare chromatographic profiles (retention times and mass spectraa to reference standards and other seizures-

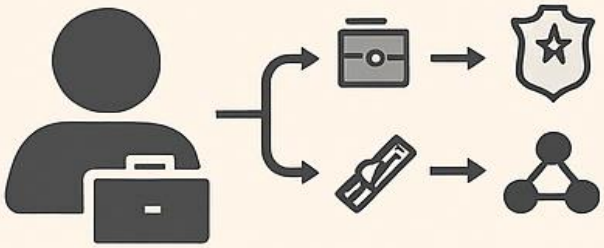
Reporting and Interpretation



Align DNA and chemical data jointly in forensic reports with statistical weight, (ilkelihood ralios, classification accuracy)

Clearly indicate limitations of partial/ mixed DNA proflies and variability in chemical signatures

Applications in Casework



- Identity handlers and manufacturers, not just distributors
- Link saparate- seizures based on buth physical evidence and biologi-cal trace signatures

📘 Supplementary Methodology: Integrated DNA and Chemical Profiling Workflow for Drug Evidence

S1. Sample Collection and Handling

S1.1 Surface DNA Recovery

- Capsules and tablets were sampled using **Copan 150C cotton swabs**, pre-moistened with 100 µL sterile distilled water.
- Swabs were applied with firm pressure, rotating over the full surface for 5–7 seconds to maximize contact.
- For powders, ~10–20 mg was transferred directly into sterile DNA-free microtubes using disposable spatulas.
- All tools and tubes were pre-screened for DNA contamination. Negative controls were collected at every batch.

S1.2 Contamination Controls

- Collection took place in a **dedicated low-template DNA workstation**.
- Personnel wore full PPE, including masks, gloves (double-layered), and disposable gowns.
- Chain of custody was documented for each handling step. Surfaces and equipment were cleaned with DNA-degrading agents between samples.

S2. DNA Profiling Workflow

S2.1 Extraction and Quantification

- DNA was extracted using the **PrepFiler Express™ Kit** (Thermo Fisher) on an **AutoMate Express™ system**.
- Whole swab heads or powder aliquots were processed and eluted in 50 µL volumes.
- DNA quantification was performed using the **Quantifiler® Trio Kit** on a **QuantStudio 5 Real-Time PCR System**.
- Extracted samples were checked for degradation and inhibitor presence using the IPC curve threshold (Ct < 31).

S2.2 Amplification and STR Analysis

- DNA amplification used the **GlobalFiler™ STR Amplification Kit** with 29 cycles on a **GeneAmp® 9700** thermal cycler.

- Amplified products were analyzed via **ABI 3500 Genetic Analyzer**, 36-cm capillary array, and POP-4™ polymer.
- STR profiles were interpreted using **GeneMapper® ID-X v1.5**, with a 75 RFU detection threshold.
- Mixed profiles were deconvoluted using **STRmix™ v2.8.0**, and likelihood ratios were calculated.

S3. Chemical Profiling Workflow

S3.1 Sample Preparation

- Tablets were ground using a sterile mortar and pestle. Powders and crushed tablet material (~10 mg) were dissolved in methanol, acetonitrile, or 0.1% formic acid solution.
- Samples were vortexed and sonicated (5–10 min), then filtered through 0.22 μm PTFE syringe filters.

S3.2 Instrumental Analysis

- **GC-MS** was performed using a capillary column with a temperature ramp (100–300°C) for compound separation.
- **LC-MS** used a reverse-phase column and binary gradient elution with detection in both positive and negative ion modes.
- Retention times and fragmentation spectra were used to construct chemical fingerprints for comparison.
- Batch attribution was made using similarity in peak profile patterns and mass spectral match scoring.

S4. Data Integration and Classification

S4.1 Combined Profiling Logic

- A **sample was classified as a match** if either DNA or chemical profile matched a reference seizure or known contributor.
- Classification accuracy was defined as the percentage of correct source attributions across 20 replicates per sample type.
- **Capsules, tablets, and powders were compared across DNA-only, chemical-only, and integrated methods**.

S4.2 Statistical Evaluation

- Comparative accuracy across methods was evaluated using **Kruskal–Wallis test with post hoc Dunn's tests**.
- Classification gain from integration was assessed against DNA- and chemical-only baselines.
- All evaluations were conducted using **Python (SciPy, Pandas)** and **R (stats, ggpubr)** environments.

S5. Operational and Forensic Implications

- Combined profiling allows differentiation between handlers (surface DNA) and manufacturers (embedded DNA).
- Integration improves attribution confidence in samples with degraded DNA or ambiguous chemistry.
- Framework aligns with modern forensic needs for **multi-modal, intelligence-driven evidence strategies**.