TRANSLATIONAL CHEMISTRY
AN INTERFACE JOURNAL
HTTPS://WWW.TRANSLATIONALCHEMISTRY.COM/

REVIEW ARTICLE | DOI: 10.5584/translationalchemistry.v2i1.252

Synthetic Cathinones: Analytical Strategies, Pitfalls and Priorities for Forensic and Clinical Toxicology

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Received: December 2025 **Accepted:** January 2026 **Available Online**: January 2026

Abstract

Synthetic cathinones are β-keto analogues within the broader class of β-phenethylamine amphetamine-type stimulants and represent a persistent analytical challenge due to their structural variability and rapid analogue turnover. Their frequent misrepresentation as other stimulants, together with the limited clinical and toxicological data available for many compounds, complicate both interpretation and risk assessment. This review examines the pharmaco-toxicological context of synthetic cathinones, with a primary focus on analytical strategies for their detection and interpretation in clinical and forensic settings. Methodological considerations are discussed across blood/plasma, urine, oral fluid and hair, highlighting the strengths and limitations of current screening, confirmatory and quantitative approaches. Attention is given to issues affecting analytical reliability, including compound instability, matrix effects, availability of reference materials and the impact of evolving sampling and microsampling formats on specimen handling. Emphasis is also placed on the interpretive integration of analytical data with patterns of use and potential co-exposures. Overall, this paper aims to bridge analytical methodology and translational application, supporting robust and adaptable testing practices in response to changing stimulant profiles.

Keywords: Cathinone analogues, Liquid chromatography, High-resolution mass spectrometry, Metabolism-based markers, Microsampling, Stability.

1. Introduction

Over the past fifteen years, synthetic cathinones (β-keto analogues of amphetamine-type stimulants, ATS) have become a defining challenge for forensic and clinical toxicology. Their market dynamics are characterised by rapid analogue turnover after scheduling actions, regional heterogeneity in what is circulating at any given time and misrepresentation (*i.e.*, products sold or consumed as 'classic' stimulants that contain distinct cathinone analogues) [1-5]. This situation strains routine testing procedures and creates coverage gaps between what laboratories monitor and what is actually present in casework, emergency department samples, post-mortem specimens and population indicators such as wastewater-based epidemiology (WBE) data [6]. A suitable response could be based on three pillars. First, HRMS-based suspect screening (triage-level matching against curated suspect lists, with defined confidence tiers) can expand scope and allow retrospective mining as new analogues appear [7,8]; second, shared criteria for reporting-grade LC-MS/MS confirmation can help solve isomer-related ambiguities and include metabolite targeting, especially in urine [9]; third, matrix-aware pre-analytical steps (sampling, storage, sample treatment) are critical to analysis success, also because β-keto stability tends to be analogue- and matrix-specific and can bias quantitative and even qualitative outcomes if unmanaged [10,11]. The scientific rationale behind this strategy is clear: relatively small structural changes (ring substitutions, side-chain homologation, amine cyclisation) preserve stimulant pharmacology while changing chromatographic retention behaviour, MS fragmentation and metabolic patterns, thereby invalidating common routine conditions when reused uncritically [12-14]. Complementing clinical and casework evidence, WBE and event-focused investigations can help identify the analogues that will later dominate analytical findings, making them valuable foresight tools for suspect lists and library updates [15-17].

This review outlines a practice-oriented approach, organised around: chemical space with analytical consequences; screening and confirmation strategies that can be applied to tackle changes in illicit market compound availability; matrix-specific approaches to solve identification/quantitation problems and to mitigate analyti-

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cal pitfalls. Throughout, we highlight open questions that remain critical for analytical success, such as immunoassay cross-reactivity, cut-off transfer across matrices (liquid / dried blood; blood / urine / oral fluid), reference material availability and clinical correlations for new analogues. This review is primarily written for routine and reference analysis laboratories operating in forensic and clinical toxicology. The decision frameworks are also applicable to public health and research laboratories involved in early-warning activities and population indicators (*e.g.*, WBE), where analytical governance and menu refresh are critical.

2. Chemical space and its analytical importance

Cathinone analogues represent a relatively large group of structurally closely related compounds and isomers sharing the β-keto phenethylamine scaffold. For analytical method developers and toxicologists, the most important subgroups are:

- **Pyrrolidinophenones** (*e.g.*, MDPV, α-PVP, MDPHP, α-PBP, α-PHP, α-PiHP, 5-PPDi, α-PCyP, 3,4-EtPV, α-PVT, **Figure 1a – 1j**).

These tend to be potent and rich in isomers, with lots of near-isobaric compounds and fragments, and in-source artefacts; their analysis relies largely on accurate-mass MS and metabolite confirmation in urine, but could also benefit from the orthogonality of GC-EI fingerprints and chiral LC to confirm identification in difficult cases [7,18-29].

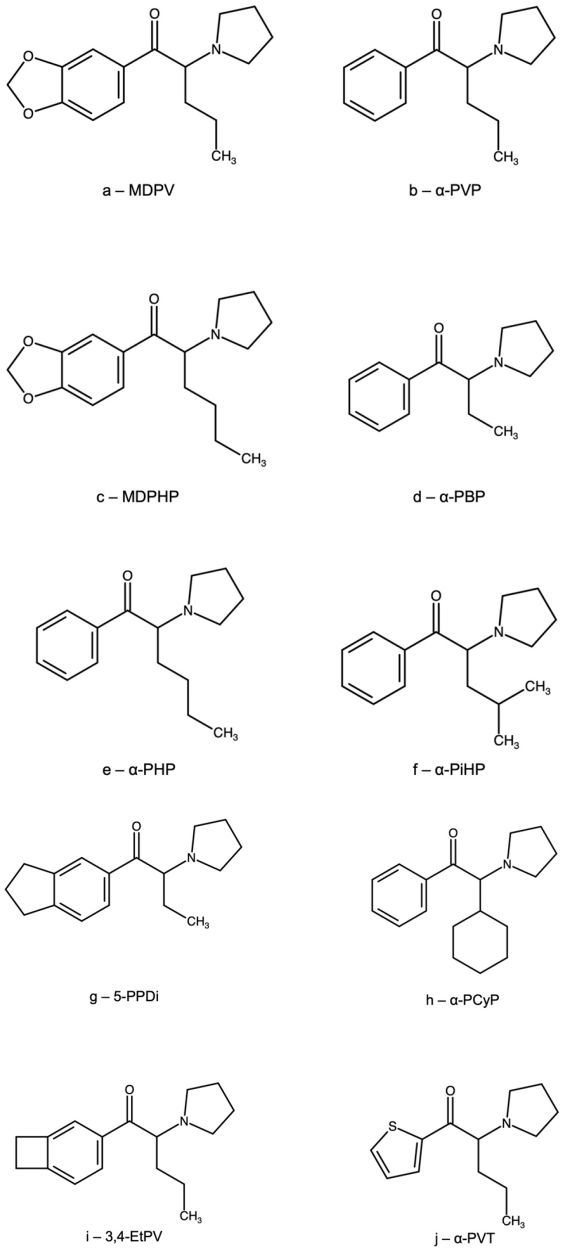


Figure 1 (1a - 1j) | Chemical structures of 10 representative pyrrolidinophenone cathinones.

Minimum ID Criteria

LC–MS/MS: two or more MRM transitions (quantifier + qualifier) with ion-ratio tolerance ≤20% absolute; RT window ≤±0.1–0.15 min vs calibrators; IS co-elution if labelled standard available [30-34]. HRMS: exact mass tolerance (*e.g.*, ≤5 ppm) on precursor + two or more diagnostic product ion; isotope fit within method bounds [7,35-39]. Urine metabolite co-evidence (recommended default for α-PVP/α-PHP families): include at least one phase-I metabolite established by hepatocytes/HRMS or case series; accept metabolite-only positives as screening-level, mandating HRMS re-interrogation for parent compounds [23,25,40-49]. Orthogonality triggers: Borderline ion ratios or co-eluting interferences → GC-EI spectrum with retention index match [9].

Positional/side-chain isomer ambiguity at reportable levels → GC-FTIR (solid-deposition if needed) or chiral LC when evidentiary defensibility is required [11,40,50-52]. An example of FTIR application is reported in **Figure 2**.

- **Methcathinones** (*e.g.*, 3-CMC/4-CMC; 2/3/4-MMC, 4-BMC, **Figure 3a - 3f**).

Positional isomerism is the main problem; coupling LC-(HR)MS retention orthogonality with diagnostic MS/MS fragments is the accepted best practice, with solid-deposition GC-FTIR providing additional information when necessary (*post-mortem*; contested forensic interpretation) [9,24,35,37,53–58].

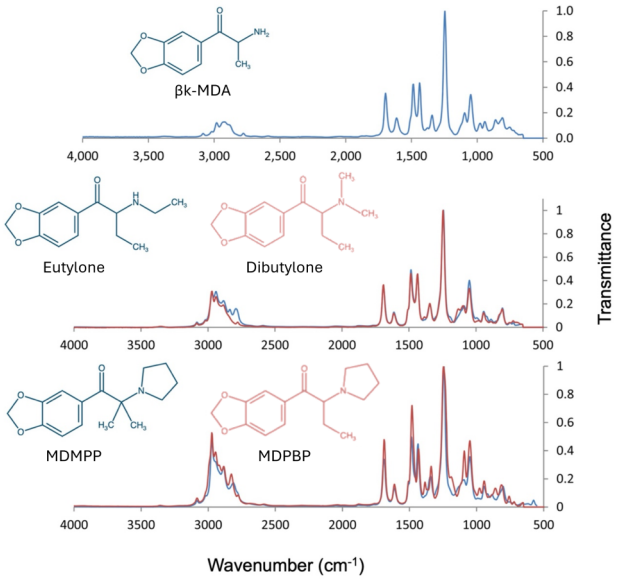


Figure 2 | FTIR spectra of some representative cathinone derivatives. Adapted with permission [17].

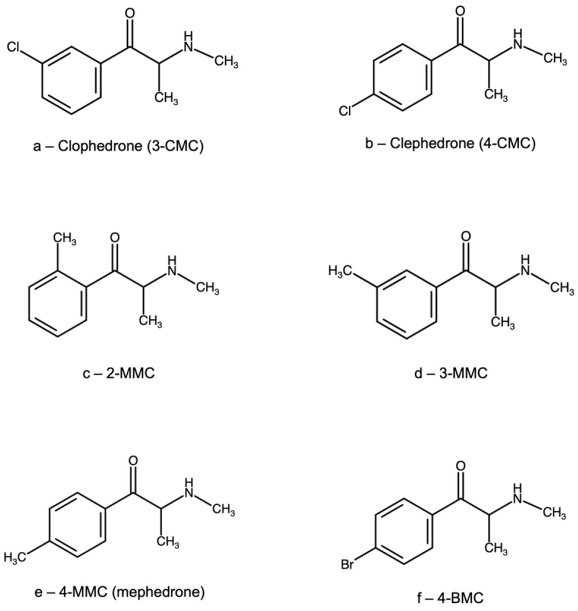


Figure 3 (3a - 3f) | Chemical structures of 6 representative methcathinones.

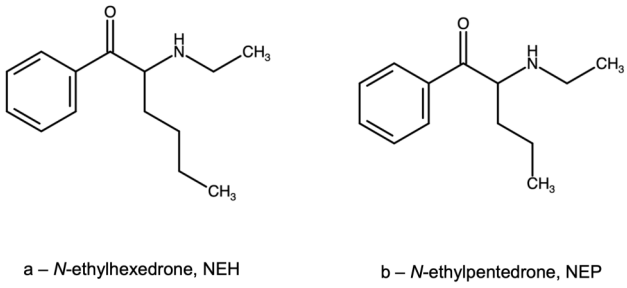
Minimum ID Criteria

Tight RT separation (optimise stationary phase and gradient) + two or more diagnostic transitions; where ΔRT <0.05-0.08 min, do not report without orthogonal evidence [30-34]. HRMS diagnostic product ions and fragment ratio patterns documented in method files for each positional isomer [7,35–38]. Orthogonality triggers (practically mandatory in contentious contexts): GC-FTIR with reference library for MMC/CMC resolution (document solid-deposition parameters if used) [15,37,55,57,67]. Chiral LC is optional if jurisdiction or question requires stereochemical assignment [34, 59-62].

- **N-ethylated derivatives** (*e.g.*, *N*-ethylhexedrone, NEH; *N*-ethylpentedrone, NEP, **Figure 4a - 4b**). Parent detectability can be limited; selected metabolites identified in hepatocytes/microsomes or controlled studies should be integrated into routine analytical panels to increase detection rates [8,14,39,40,46,48,53,57,63].

Minimum ID Criteria

Parent + metabolite confirmation preferred; where parent is absent, two complementary metabolites (distinct biotransformation routes) may justify confirmation if method validation anticipates this logic [31-34,59-61,64-66]. HRMS suspect tiers include metabolite formulas; store raw data for retrospective queries [7,35-39]. Orthogonality triggers: Any discordance between expected and actual metabolite pattern → targeted HRMS library matching; if still ambiguous, seek GC-EI on parent in fortified extracts for structural corroboration [15,32-39,46].



a – *N*-ethylhexedrone, NEH

b – *N*-ethylpentedrone, NEP

Figure 4 (4a - 4b) | Chemical structures of representative *N*-ethylated cathinones.

- **'One-Series' / newer homologues** (*e.g.*, eutylone, dipentylone, 4-MPD, 4-MEAP, 3,4-Pr-PipVP, **Figure 5a - 5e**). Minor side-chain modifications can produce significant chromatographic retention and fragment intensity ratio changes. For these reasons, frequent HRMS library and method updates must be applied, as well as metabolite inclusion where available [8,60,65-72].

Minimum ID Criteria

Refresh transitions and RT windows upon first WBE or casework signal; lock in diagnostic HRMS fragments in library before routine reporting [6,7,35-38]. Include at least one metabolite if available to confirm identifications in urine [32-34,49,59,60,62,64,66]. Orthogonality triggers: First 10-20 authentic positives post-onboarding → random GC-EI verification and second-operator HRMS review to qualify the menu update (documented in change-control log) [6,15,17, 35-37].

3. Screening and confirmation strategies

3.1. Screening

Immunoassays offer throughput and triage value but show uneven cross-reactivity across positional/side-chain isomers; negative immunoassays must not overrule clinical/investigative context [73-77]. This is particularly relevant when case history, setting (*e.g.*, nightlife/festival), or WBE intelligence suggest a newly rotated analogue. Modern screening workflows thus prioritise broad-scope MS-based screening (triage-level detection):

- HRMS (QTOF/Orbitrap) used for suspect screening with defined confidence tiers enables with suspect tiers enables retrospective mining when early-warning systems or seizures flag newcomers [16,35,36,78].
- Ion-mobility MS (when available) adds a gas-phase separation dimension that can reduce false positives/negatives for near-isomers at low levels [79].
- Matrix-tailored rapid MS (*e.g.*, multi-residue LC-MS for blood; CE-HRMS for urine) keeps throughput high while preserving essential selectivity [39].

Population indicators (WBE; pooled venue urinals; venue oral-fluid swabs) consistently anticipate analogue shifts, informing which suspects to add next and where to tighten ID criteria [17, 80-84]. In practice, labs that formally feed WBE and seizure intelligence into quarterly suspect refreshes report fewer surprise gaps downstream.

3.2. Confirmation

For reportable identifications (confirmation), targeted LC–MS/MS is foundational, provided that identification criteria (co-elution with IS; ion ratios within tolerance; S/N thresholds) are followed

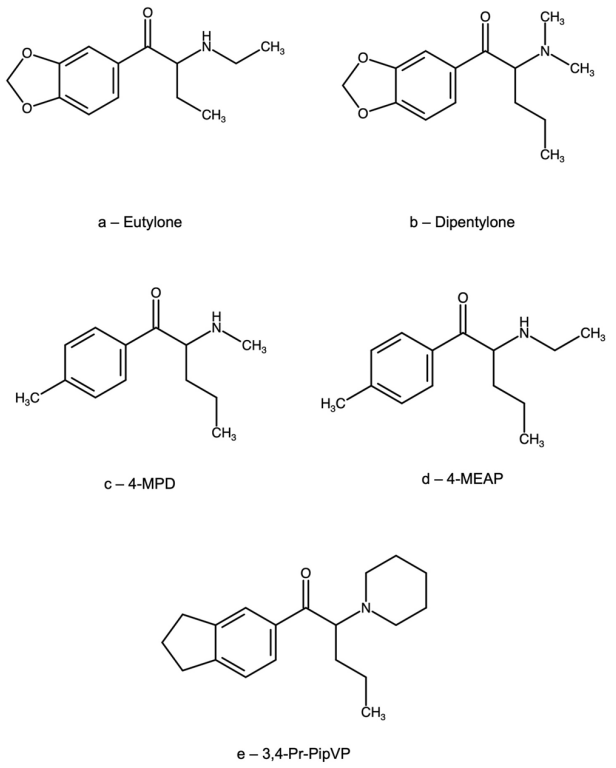


Figure 5 (5a - 5e) | Chemical structures of 5 representative 'one-series' and newer cathinones.

and library panels are renewed to reflect illicit market innovation [30,33,35,85]. When extreme selectivity is needed (positional isomers; near-isobars; low-level co-exposure), orthogonal evidence should be applied:

- GC-MS (EI) and GC-FTIR provide structural fingerprints and retention indexing; solid-deposition GC-FTIR markedly improves spectral quality and has proven decisive for MMC/CMC isomer resolution [9,16,86,87].
- Accurate-mass MS confirmation (exact mass + diagnostic product ions) resolves isomer-dense families and, importantly, allows retrospective re-interrogation of stored files when suspect lists change [57,88,89].
- Chiral LC is warranted for enantioselective questions and when evidentiary defensibility demands stereochemical resolution (selected ATS/cathinones) [51,61,62,90,91].

3.3. Metabolite-linked confirmation

Urine confirmations that include diagnostic metabolites substantially extend detection windows and increase specificity (*e.g.*, NEH/NEP; α-PVP; 3-CMC), reducing the risk of false negatives when parent compounds are unstable or poorly excreted [23,24,49,60,66,92,93]. Where metabolite hierarchies remain unsettled, method architecture should be modular (easy addition of new MRM transitions and cut-offs with limited re-validation), and suspect tiers in HRMS should be curated explicitly for new metabolites arising from hepatocyte and microsome models [25,55,94-96].

4. Matrix-specific approaches

4.1. Blood/plasma: strong and weak points

Strengths

Blood and plasma provide proximity to the effect site and are integral to clinical interpretation, pharmacokinetics, and evidentiary timelines. Validated LC-MS/MS methods demonstrate selective quantification with matrix-effect characterisation, carry-over control and well-defined ion-ratio tolerances [57,60,65,66]. When analogue density or low concentration challenges selectivity, accurate-mass confirmation and orthogonal EI/retention-index evidence enhance identifications [92,97,98].

Weaknesses

For β-keto stimulants, stability is not a class constant. Benchtop time, pH, temperature, preservatives (*e.g.*, NaF) and freeze-thaw cycles can compromise analyte integrity; re-injection windows matter (carry-over; adsorption; degradation) [10,66,71,99]. Parent levels can be transient, while conjugation/redistribution and post-collection metabolism continue in sub-optimally handled specimens.

Policy implication

Pre-analytical steps should be well-described and rigorously respected in SOPs, with the following critical points: (i) immediate cooling and pH control where indicated; (ii) limited benchtop-time; (iii) documented freeze-thaw behaviour; (iv) short, justified re-injection windows; and (v) mandatory QC acceptance rules that

discard batches showing drift in IS response consistent with instability. When parent decay is plausible, consider urine metabolite evaluation as a possible confirmation strategy.

4.2. Miniaturised dried blood matrices (DBS/VAMS) for cathinones

Dried formats shorten liquid residence time, potentially improving stability for labile cathinones, and simplify shipment/logistics (ambient shipping; reduced biohazard footprint) [51,57,60,100-103]. They can be especially helpful when serial sampling or field collection is required (*e.g.*, clinical toxicology in non-hospital settings; public health surveillance).

What to validate

- Device-specific calibration (do not borrow calibrations from liquid matrices).
- Extraction efficiency and substrate carry-over; check whether analyte/substrate interactions produces bias in recovery or ionisation.
- Haematocrit effects and spot inhomogeneity (DBS), or volumetric tip fill behaviour (VAMS) with real blood.
- Cut-off translation from liquid to dried matrices must be empirically verified with parallel collections in the same individuals/cases.

Analytical architectures

Methods for dried matrices include UHPLC–HRMS–QTOF for broad new psycoactive substance (NPS) panels and UPLC for targeted cathinone sets; both require recovery-focused validation and carefully designed IS panels [51,57,101]. When the target analogue is suspected to be labile (*e.g.*, α-PVP family), adding urine metabolite confirmation keeps overall case sensitivity high even if dried-blood parent is near LOQ.

4.3. Urine

Urine affords longer detection windows and generally higher concentrations of diagnostic metabolites, providing redundant axes when parent cathinones are unstable, transient, or weakly excreted (**Figure 6**).

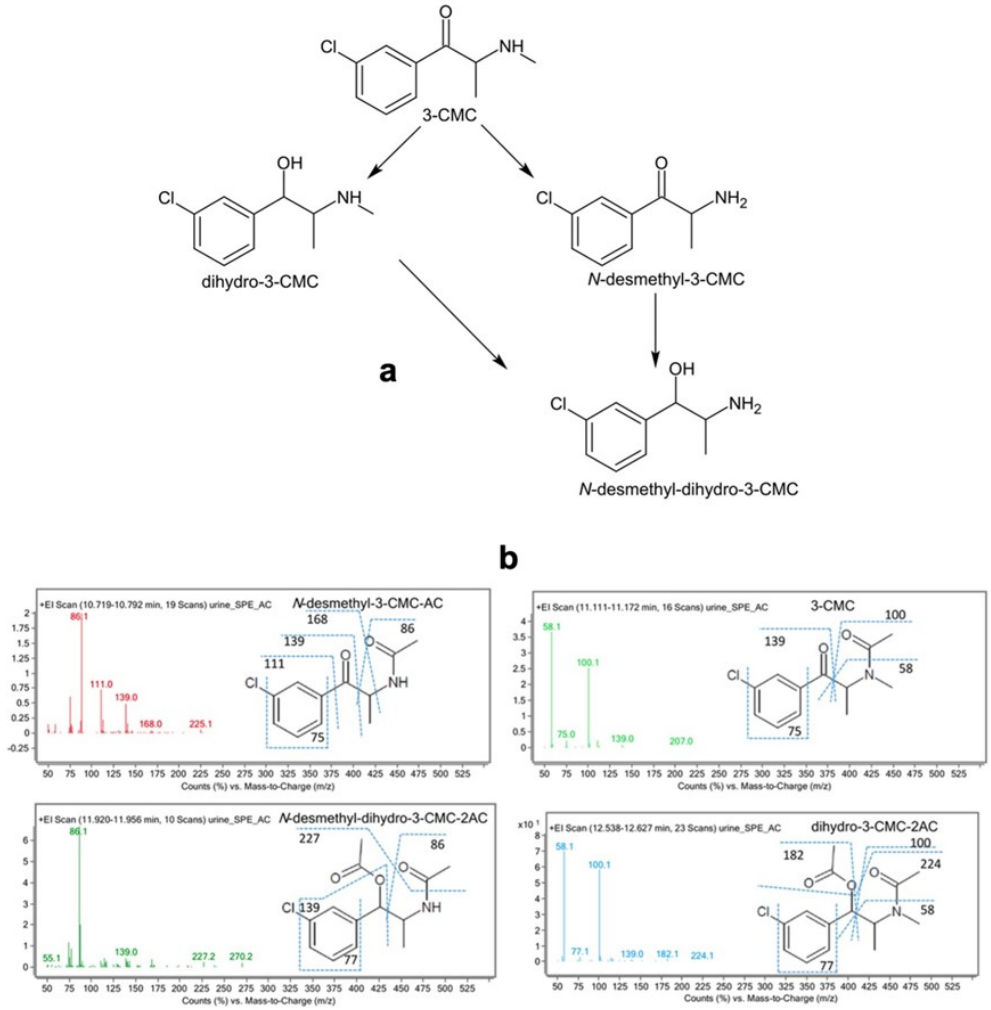


Figure 6 | (a) Proposed metabolism of 3-CMC and (b) experimental mass spectra of the acetyl derivatives of 3-CMC and its metabolites (acetylation was carried out to make the compounds suitable for GC separation). Adapted with permission [118].

For multiple families (*e.g.*, NEH/NEP, α-PVP/α-PHP, 3-CMC/4-CMC), determination of the parent drug plus at least one metabolite markedly increases specificity and practical sensitivity in real-world cases [32,44,46,49,77,100,104-106]. When blood/plasma show borderline parent levels or re-injection issues, a metabolite-linked urine confirmation increases the accuracy of case interpretation [63]. Actionable rule: for families with poor or variable parent excretion, reportable confirmation requires co-evidence of either parent + metabolite or two complementary metabolites with appropriate ion-ratio and retention windows.

4.3.1. Pre-analytical stability in urine

β-keto stability in urine depends on pH, temperature, preservatives and storage time. Conjugation/redistribution can shift profiles during storage. Several studies demonstrate compound-specific degradation and significant benefit from pH control and cold chain, including the use of NaF when justified by validation [47,66]. Re-injection windows and autosampler conditions also matter (carry-over; adsorption; hydrolysis). Suggested SOPs:

- Collection/Preservation: immediate cooling; pH documentation; add NaF if validated to slow degradation.
- Stability Files: per-analogue stability tables (benchtop, refrigerated, frozen; short- vs long-term; freeze-thaw cycles) referenced in batch plans; fail batch if IS drifts or QC trend suggests degradation.
- Re-injection Policy: specify maximum interval from extraction to analysis; specify autosampler temperature; require QC re-checks after delays.

4.3.2. Urine sample preparation

Urine method architectures span dilute-and-shoot (fast triage), SPE/μSPE, DLLME, PALME/EME and CE–HRMS screening; choice is based on throughput, matrix load and the need for orthogonality [35,42,107,108].

- Dilute-and-shoot LC-MS/MS: maximal throughput, acceptable for targeted sets with robust ion-ratio windows and good IS coverage; best when paired with HRMS suspect screening for scope and GC-FTIR fallback for isomers [16,57,63].
- SPE / μSPE: improved cleanliness/selectivity, compatible with metabolite panels; micro-formats (MIP–μSPE) add selectivity for problem families [45]. An example of advanced material that has been applied to MIP-μSPE for synthetic cathinone analysis in urine is shown in **Figure 7**, together with the corresponding sample preparation procedure.
- DLLME / PALME / EME: green(er) options with strong preconcentration; PALME is attractive for polar targets when coupled to LC-MS/MS [9,65,70].
- CE–HRMS: powerful for broad screening (including anionic metabolites) with high orthogonality; use as screening tier to precede LC-MS/MS confirmations [73].

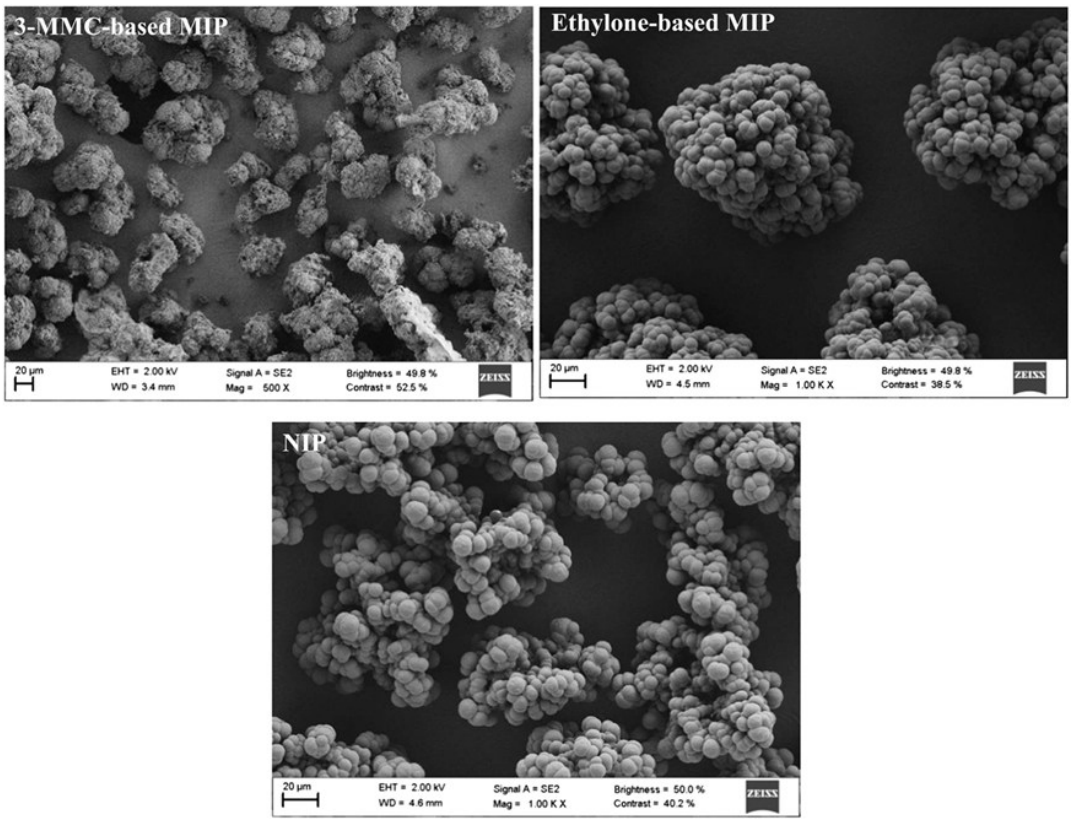


Figure 7 | Scanning electron microscope (SEM) images for, 3-MMC-based MIP, ethylone-based MIP and non-imprinted polymer (NIP). Used with permission [45].

Pragmatic SOP suggestion: use dilute-and-shoot (or µSPE) for daily throughput; route isomeric conflicts to GC-FTIR or HRMS supported by diagnostic fragments; embed metabolites for families with weak parent excretion.

4.4. Oral fluid

Oral fluid collection is overseen, non-invasive, and operationally compatible with rapid LC-MS/MS confirmation, making it valuable for time-proximal stimulant assessment (DUID, venue screening, ED intake) [78]. However, device chemistry, stimulation status, salivary flow, and oral contamination can skew concentrations and must be documented for interpretation [109,110]. SOP datapoints to capture: device brand/lot, stimulated vs unstimulated, collection time relative to alleged use/event, recent oral exposures (smoking, vaping, mouthwash), and internal volume controls where the device provides them [44,74,100].

4.4.1. Oral fluid extraction and screening options

Validated approaches include dilute-and-shoot LC–MS/MS with robust ion-ratio controls, µSPE or QuEChERS-style extractions (micro-QuEChERS shows a strong balance of speed and cleanup), and broad HRMS for non-targeted screening and presumptive identification [111]. Device eluates may contain salts and buffers that impact ionisation; matrix-matched calibration and IS mapping are mandatory.

4.4.2. Reporting and defensibility

- Cut-offs: align with device recovery properties and the lab's validated LLOQ/decision limits; report device brand and any collection-buffer dilution assumptions used in quantitation.
- Isomer issues: for positional isomers detected in oral fluid at low ng/mL, require HRMS diagnostic product ions or route to GC–FTIR confirmation if challenged.
- Contamination control: where recent oral use is suspected, pair with urine metabolite evidence to distinguish contamination from systemic exposure.

4.5. Hair

Hair provides long detection windows and information on patterns of use when segmental analysis is appropriate. It is especially useful for retrospective investigation and for contexts where frequent liquid sampling is impractical [6,22,30,56,112]. As an example, the frequency of NPS detected in postmortem hair samples per year between January 2008 and December 2020 are summarized in **Figure 8**.

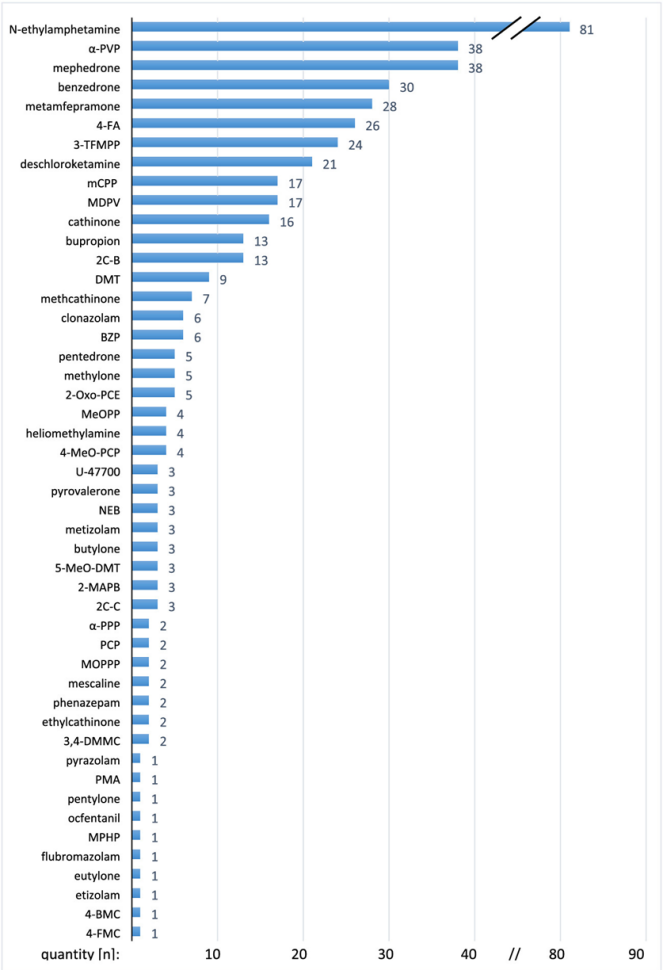


Figure 8 | Total number of detected NPS in postmortem hair samples (January 2008 - December 2020). Used with permission [115].

4.5.1. Challenges and opportunities of hair analysis

External contamination (smoke, powders), cosmetic treatments (bleaching, dyes), and hygiene impact interpretation. The literature converges on:

- Validated decontamination protocols (multi-solvent or aqueous/organic sequences) with procedural blanks [113].
- IS panels and matrix-matched calibrations.
- Segmental analysis guided by growth assumptions but reported conservatively due to inter-individual rate variation.

Key defensibility tactic: whenever parent compounds are detected in hair, seek metabolites in the same segment (where available) to strengthen the case for systemic exposure vs external contamination [114]. If metabolite presence is not supported by known biotransformation or isomeric doubt remains, escalate to GC-MS/TOF or HRMS with diagnostic product ions for structural confidence [115].

4.5.2. Reporting policy

- Decontamination statement (protocol steps, controls) is part of the analytical result.
- Cosmetic history (when available) should be documented in the case record.
- Matrix caveats: clearly separate pattern-of-use inference from dose/intoxication claims; avoid pharmacokinetic over-reach in hair unless supported by controlled studies.

5. Stability, Pre-Analytical Variables, and Degradation Pathways

Stability is one of the defining analytical challenges in the detection of synthetic cathinones. The β-keto substitution that characterises this class influences chemical reactivity across biological matrices, making these compounds more susceptible to degradation than amphetamines. This instability is neither uniform across analogues nor predictable from structural similarity alone. As a result, pre-analytical handling becomes integral to analytical validity, and even well-designed chromatographic and mass-spectrometric protocols can fail if sample integrity is compromised prior to extraction or analysis. Much of the instability observed in cathinones stems from the lability of the β-keto moiety. In aqueous media, this functional group undergoes reduction, hydrolysis, and rearrangement, often at rates that increase dramatically with temperature, pH variation, or prolonged exposure to enzymes [33,66,99]. Blood and plasma exemplify this vulnerability: degradation begins almost immediately after collection and can progress substantially within a few hours if samples remain at room temperature [116]. Several pyrrolidinophenones, as well as mephedrone- and methylone-type analogues, are particularly affected [41]. Their degradation not only reduces parent concentrations, but can lead to the appearance of transformation products that may interfere with interpretation, especially in clinical settings where early concentration data guide management decisions. Pre-analytical variables are bound to amplify or mitigate degradation. Temperature exerts the strongest influence; immediate cooling and prompt freezing slow decomposition substantially. pH exerts matrix-dependent effects. Acidification may stabilise some analogues but accelerate decomposition of others, and the addition of preservatives such as sodium fluoride (NaF) provides partial protection but cannot fully prevent β-keto reduction or oxidative pathways. Benchtop time is another critical determinant: even short delays between collection, centrifugation, and freezing can alter analyte profiles. Autosampler residence presents a further, often overlooked, risk. Some cathinones degrade measurably during overnight autosampler sequences, leading to downward drift in quality control results or unexpected discrepancies between first and last injections in a batch [88].

Urine offers improved stability for certain metabolites but not necessarily for parent compounds. In this matrix, pH once again plays a central role. Degradation accelerates in alkaline conditions, and repeated freeze-thaw cycles can modify both parent and metabolite concentrations. Because metabolites often serve as the primary targets for confirmation, mismanaging these pre-analytical steps may distort the relative abundance of phase I products and thereby complicate interpretive judgments. The risk is not limited to quantitative shifts: for some compounds, metabolite formation may occur ex vivo, leading to apparent 'metabolic signatures' that do not accurately reflect in vivo biotransformation [66]. Dried matrices such as DBS and VAMS offer partial mitigation against aqueous instability but introduce their own complexities. Drying typically slows degradation yet does not eliminate it, as the β-keto group may still undergo slow conversion even in the absence of liquid water [59]. Moreover, substrate interactions and differences in drying kinetics across devices can lead to inconsistent measurable concentrations. The effect of haematocrit on spot homogeneity and analyte distribution remains a notable concern, particularly for cathinones that partition unevenly between plasma and red blood cells [60]. Recently, dried urine spots (DUS) have been evaluated and showed good analyte stability (**Figure 9**) [117].

A further dimension of stability focus arises in processed samples. Extracts prepared for LC-MS/MS analysis may degrade in autosampler vials, especially at ambient temperatures or when stored for extended periods. Buffer composition, solvent ratio, and the presence of formic acid all influence degradation rates. Choosing appropriate storage conditions and limiting injection delays are essential to avoid artefactual decreases in analyte signal [71]. These pre-analytical and stability challenges extend beyond routine operations and directly affect confirmatory workflows. When parent compounds degrade rapidly, the detection window narrows, and metabolite-based confirmation becomes crucial. In some cases, such as with *N*-ethyl analogues or pyrrolidinophenones, the apparent absence of the parent alongside strong metabolite signals is not a paradox but an expected consequence of instability. Analytical frameworks must therefore integrate stability profiles into interpretation, ensuring that qualitative and quantitative findings are harmonised within the known degradation behaviour of each analogue [118]. Collectively, these observations emphasise that stability is not a peripheral concern but a central determinant of analytical success. Accurate measurement of cathinones requires a pre-analytical policy that codifies acceptable time windows, temperature controls, preservative use, and storage conditions for each matrix.

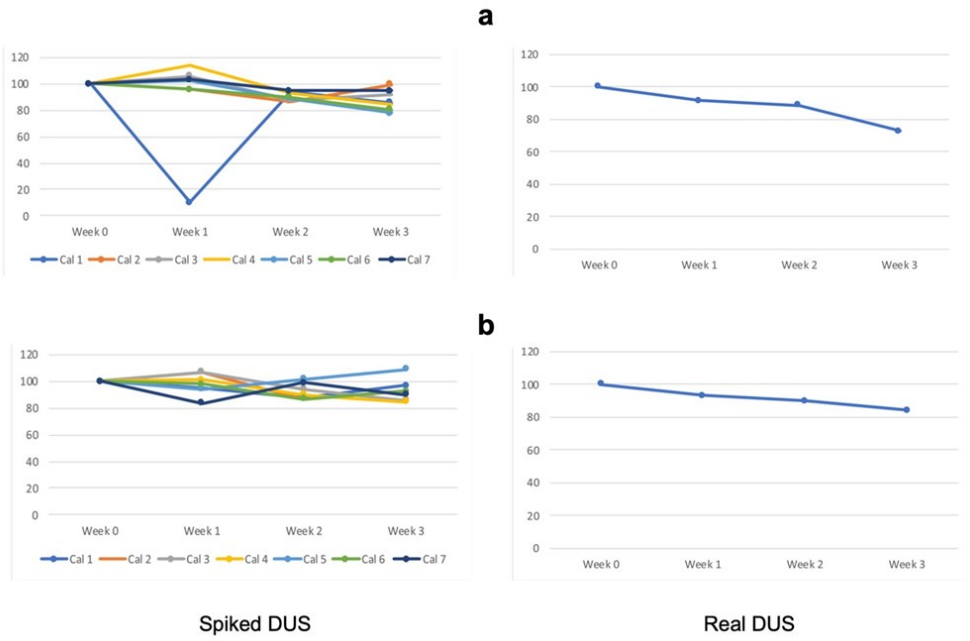


Figure 9 | Three-week stability of (a) MDPHP and (b) MDPV in spiked dried urine spots (DUS) and in a real DUS from a user. Used with permission [117].

Without such measures, degradation may outpace detection, compromising not only quantitative reliability but also the fundamental ability to confirm exposure. As new analogues continue to emerge, establishing compound-specific stability data (preferably harmonised across laboratories) will be essential for maintaining the integrity of cathinone testing in both forensic and clinical domains. A graphical summary of this section is shown in **Figure 10**.

6. Population Indicators, Early-Warning Systems, and Menu Refresh Strategy

Surveillance of synthetic cathinones increasingly depends on a constellation of population-level indicators that together offer a view of emerging substances far earlier than traditional casework alone. These external information streams, such as seizure intelligence, clinical toxicology alerts, poison centre enquiries, wastewater -based epidemiology (WBE), and coordinated early-warning systems, form a valuable counterpart to laboratory analytics.

In a drug class defined by rapid structural evolution, they allow laboratories to anticipate analogue turnover, update methods before widespread circulation, and validate analytical priorities with real -world evidence [119,120]. Among these indicators, seizure monitoring remains the most immediate source of intelligence on cathinone emergence. National forensic laboratories frequently record abrupt shifts in seized materials, with one analogue replacing another in a matter of weeks. This pattern has been well documented in Europe, North America and Asia, where cathinones regularly appear as substitutes in counterfeit MDMA or cocaine products or as the dominant component of powder mixtures sold through local networks [60,121,122]. Seizure data often demonstrate geographical specificity: certain metropolitan regions exhibit high circulation of pyrrolidinophenones, while others experience waves of methcathinone isomers or eutylone-type compounds [1,83]. Such heterogeneity reflects both regional supply lines and the operational behaviour of illicit manufacturers. From an analytical perspective, these observations show the limitations of fixed LC-MS/MS panels or static target lists; instead, laboratories require dynamic, evidence-driven menus shaped by current circulation rather than historical prevalence [123]. Clinical and emergency department presentations add a complementary dimension to this picture. Hospitals frequently detect new cathinones in symptom-driven testing before they appear in formal seizure datasets. The clinical profiles associated with sympathomimetic toxicity (tachycardia, agitation, hyperthermia, psychosis) are not specific to cathinones, yet temporal clustering of such cases can signal the introduction of a new analogue into local drug markets [6,17]. Poison centre enquiries play a similar role: spikes in calls related to unusual stimulant-like symptoms often precede systematic toxicology confirmation. These early clinical signals guide laboratories toward compounds that may require immediate expansion of HRMS suspect lists or targeted method development, particularly when reference standards are not yet available. In practice, laboratories that integrate clinical intelligence into their menu strategy are better positioned to detect analogues that initially circulate in low volumes or within specific user communities [36,84]. WBE further strengthens this surveillance ecosystem by providing near-real-time evidence of stimulant use at the population level. Several studies demonstrate that cathinone concentrations in wastewater can rise sharply within days following the introduction of a new analogue, often well before its identification in seized materials or clinical samples [54]. Because WBE reflects aggregate consumption rather than the behaviour of isolated individuals, it serves as a sensitive barometer for market shifts.

Peaks, declines and analogue replacements observed in wastewater frequently correspond to the manufacturing cycles that drive cathinone availability. By feeding these insights into analytical workflows, laboratories can identify which analogues merit inclu-

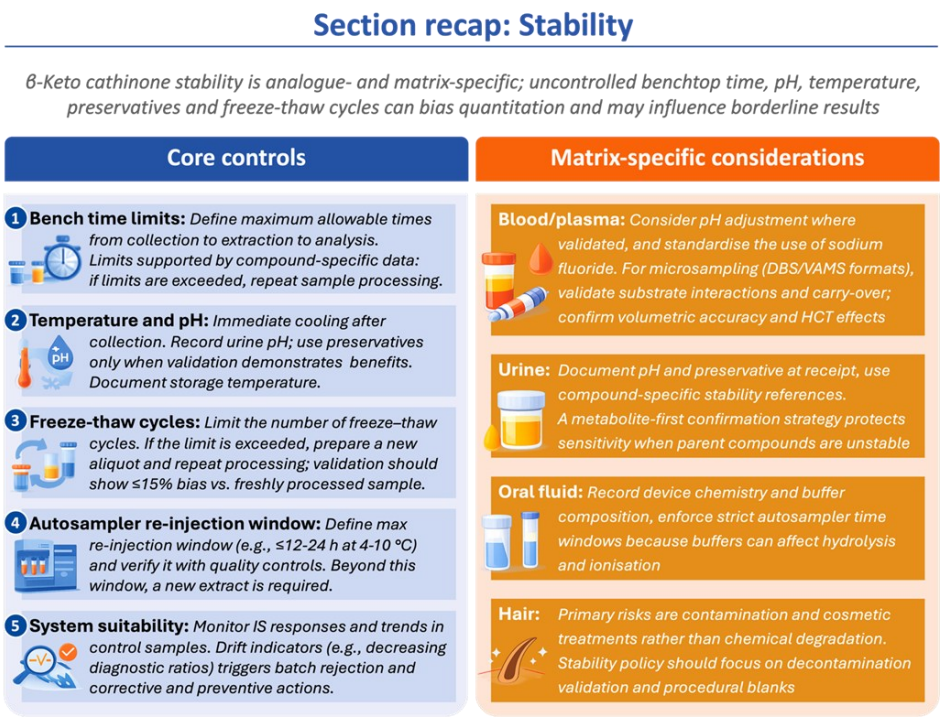


Figure 10 | Graphical recap of Section 5. Stability.

sion in suspect lists, which require new transitions or metabolite targets, and which have fallen below relevant prevalence thresholds [38]. Coordinated early-warning systems such as those operated by the EUDA (formerly EMCDDA), UNODC and national public-health agencies unify these disparate streams into structured alerts. These systems issue formal notifications when new analogues are detected, when toxic clusters emerge, or when severe adverse events occur. Importantly, early-warning bulletins often precede the availability of certified reference materials; therefore, HRMS laboratories must rely on predicted exact masses, class-informed fragmentation patterns, and retention-time heuristics to achieve preliminary detection [39,124]. This underscores the need for broad-scope HRMS acquisition rather than narrow targeted panels, as suspect-screening workflows allow laboratories to identify new analogues even before standards arrive. Retrospective data mining is particularly valuable in this context: once reference spectra or standards become available, LC-HRMS datasets collected weeks or months earlier can be interrogated again for previously unassigned features, thereby accelerating analytical readiness. The integration of these population indicators into routine toxicology practice directly informs the concept of menu refresh. In contrast to classical drug-testing paradigms where analyte lists remain stable for years, cathinone panels require continuous maintenance. A rational refresh strategy combines three elements: prevalence, risk and analytical feasibility. Prevalence is dictated by population indicators such as seizures, WBE and clinical case clusters [16,78,125]. Risk is informed by documented toxicity, frequency of severe presentations, and evidence of adulteration or substitution in high-use drug markets. Analytical feasibility reflects whether a compound can be detected and confirmed reliably with available technology, including the availability of standards, validated transitions and metabolic markers. When these three criteria intersect, a compound should be incorporated into LC–MS/MS confirmation panels and added to HRMS suspect screens. Conversely, analogues that disappear from circulation may be deprioritised but retained in retrospective-analysis libraries to ensure interpretive completeness. This population-driven approach has several advantages. It allows laboratories to keep pace with structural innovation in cathinone synthesis, reduces the risk of false negatives caused by outdated menus, and aligns analytical capacity with real-world harm. It also creates a feedback loop: toxicology findings contribute to early-warning alerts, which in turn shape the analytical priorities of laboratories across regions. In this cyclical framework, cathinone surveillance becomes a collaborative process between forensic laboratories, clinicians, epidemiologists and regulatory agencies. A graphical summary of this section is shown in **Figure 11**.

7. Conclusions and Expert Opinion: Future Directions and Analytical Priorities

Synthetic cathinones represent one of the most rapidly shifting and analytically demanding domains in forensic and clinical toxicology. Their ongoing structural diversification and 'chemical plasticity' ensure that new analogues will continue to emerge, often designed to exploit analytical blind spots and to challenge existing workflows. Consequently, laboratories must move from reactive method updates to a posture of continuous, intelligence-informed stewardship, combining structural awareness, predictive screening, and rapid implementation of fit-for-purpose confirmation routes. A recurring message across the evidence reviewed is that no single strategy is sufficient in isolation. Broad-scope HRMS remains crucial to detect emerging cathinones before reference materials are available and to support retrospective data mining, while targeted LC-MS/MS still provides the quantitative and confirmatory robus-

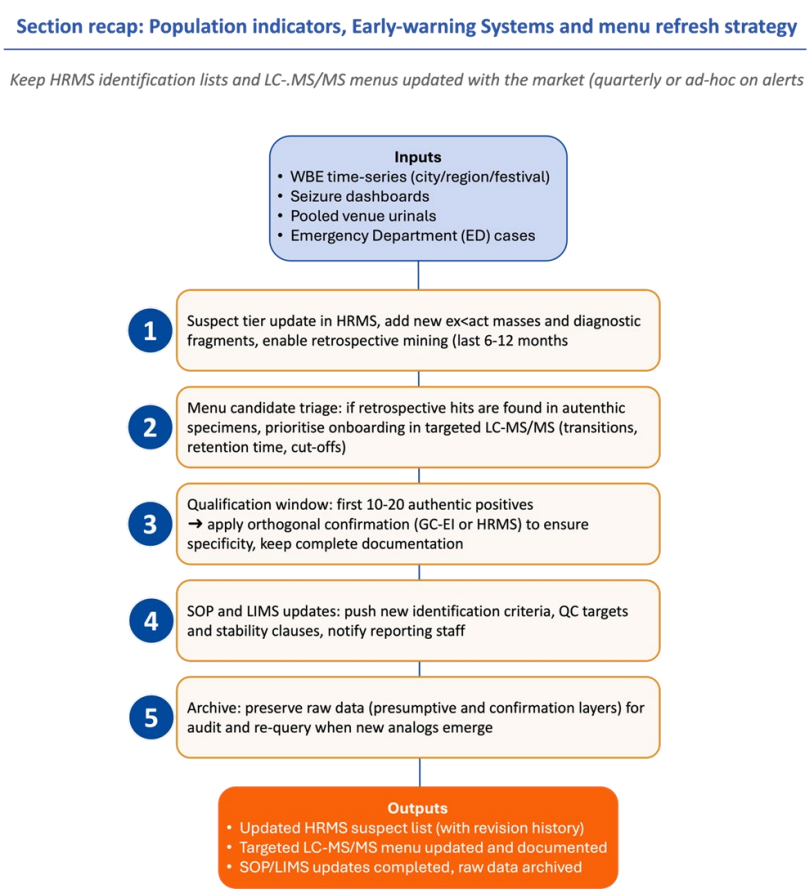


Figure 11 | Graphical recap of Section 6. Population Indicators.

tness required for defensible reporting. Orthogonal approaches (*e.g.*, GC-EI, GC-FTIR, accurate-mass MS/MS and, where appropriate, chiral LC) remain necessary whenever isomerism, interpretive ambiguity, or evidentiary thresholds require higher structural certainty. Rather than being viewed as 'specialty' options, these orthogonal tools should be embedded into decision trees with predefined triggers for escalation in high-suspicion or high-impact contexts. Matrix-specific constraints fundamentally shape detectability and interpretation and cannot be handled with legacy assumptions borrowed from classical stimulants. Blood/plasma often carry the highest interpretive value but are frequently most vulnerable to degradation; urine extends detection windows but increasingly shifts confirmation logic from parent compounds to metabolites; oral fluid narrows temporal coverage and introduces device- and contamination-related variability; hair provides longitudinal insight but requires stringent control of contamination and incorporation pathways. Microsampling platforms offer clear logistical advantages, but their deployment at scale will require systematic resolution of substrate interactions, drying kinetics, haematocrit-related effects, and long-term stability in dried formats. Analytical conclusions are only robust when these matrix-dependent uncertainties are explicitly acknowledged and addressed. A concise practice-oriented summary of recommended workflows by matrix is provided in **Table 1**. Cathinone instability is a defining analytical variable rather than a secondary technical nuisance. The β-keto motif confers chemical lability across matrices, and temperature, pH, preservatives, freeze-thaw cycles, autosampler residence, and extract longevity can all introduce substantial bias in parent and metabolite signals. Harmonised stability protocols, aligned across laboratories and matrices, would markedly improve interpretive confidence, reduce the risk of artefactual negatives, and strengthen comparability of clinical and forensic datasets. A shared, compound-specific stability resource (even if incremental) would be particularly valuable for newer analogues where handling uncertainty is currently high. In parallel, systematic characterisation of cathinone metabolism remains a high-priority gap. For many newly emerging substances, comprehensive biotransformation data are still missing, yet metabolite-informed confirmation is increasingly necessary because parent-only strategies are already unreliable for several families. Coordinated use of hepatocytes, liver microsomes, and complementary in vivo or case-based evidence can accelerate the identification of robust metabolite markers that extend detection windows, clarify interpretive boundaries, and reduce false negatives. Where metabolite hierarchies remain unsettled, laboratories should adopt conservative reporting logic and explicitly state uncertainty rather than over-interpreting parent-metabolite discordance. Finally, cathinone toxicology increasingly depends on integrating external intelligence streams into analytical governance. Seizure intelligence, WBE, clinical clusters and coordinated early-warning networks can reveal market changes earlier than conventional casework and should directly inform rolling updates of targeted panels, HRMS suspect lists, metabolite targets and spectral resources. Static LC–MS/MS menus cannot keep pace with the turnover rate of cathinones; a defined schedule for 'menu refresh'

Table 1 | Recommended analytical workflows by matrix.

Matrix	Primary screening (typical)	Confirmation / escalation (typical)	Key pitfalls to control (high-impact)
Blood / plasma	Targeted LC-MS/MS for panel coverage; HRMS when analogue turnover is suspected	LC-MS/MS under validated reporting criteria; escalate to accurate-mass MS/MS and/or GC-EI / GC-FTIR when isomerism or low-level ambiguity occurs; consider metabolite-linked urine confirmation when parent decay is plausible	Pre-analytical stability (pH/temperature/preservatives), re-injection windows, carry-over/adsorption, post-collection metabolism
Dried blood (DBS / VAMS)	Targeted LC-MS/MS or UHPLC-HRMS for broad NPS panels	Confirm with validated LC–MS/MS criteria; use urine metabolite evidence to preserve sensitivity when parent is near LOQ or suspected labile	Device-specific calibration, extraction efficiency/substrate interactions, haematocrit (DBS) or volumetric fill behaviour (VAMS), comparability vs liquid matrices
Urine	HRMS suspect screening to track market turnover; targeted LC-MS/MS for routine reporting	Reporting is strengthened by parent + ≥1 diagnostic metabolite (family-dependent) and/or orthogonal confirmation for isomers/challenged cases	Pre-analytical and autosampler stability; choice of preparation (dilute-and-shoot *vs* SPE/µSPE *vs* microextractions); ion suppression control; metabolite selection
Oral fluid	Dilute-and-shoot LC-MS/MS for rapid time-proximal assessment; HRMS when needed	LC-MS/MS with robust ion-ratio/RT criteria; escalate to HRMS diagnostic ions and/or GC-FTIR if positional isomers are critical; pair with urine metabolites when contamination vs systemic exposure is uncertain	Device chemistry/buffers and dilution assumptions, oral contamination, stimulated vs unstimulated collection, documentation of collection conditions
Hair	Targeted LC-MS/MS after validated decontamination; segmental strategy when appropriate	Defensibility can be strengthened by seeking metabolites in the same segment when supported; escalate to HRMS or GC-MS/TOF when structural confidence is challenged	External contamination and cosmetic treatments, decontamination controls/blanks, conservative interpretation (avoid PK over-reach)

with documented versioning is becoming an essential quality element rather than an optional organisational practice. In summary, analytical resilience against synthetic cathinones will not be achieved through any single technology, but through coordinated evolution of detection tools, shared reference and spectral resources, metabolite and stability knowledge, matrix-aware interpretation and intelligence-driven method stewardship. Alignment of these pillars enables laboratories to address cathinone innovation while maintaining accuracy, timeliness and defensibility in forensic and clinical decision-making. A graphical summary of this section is shown in **Figure 12**.

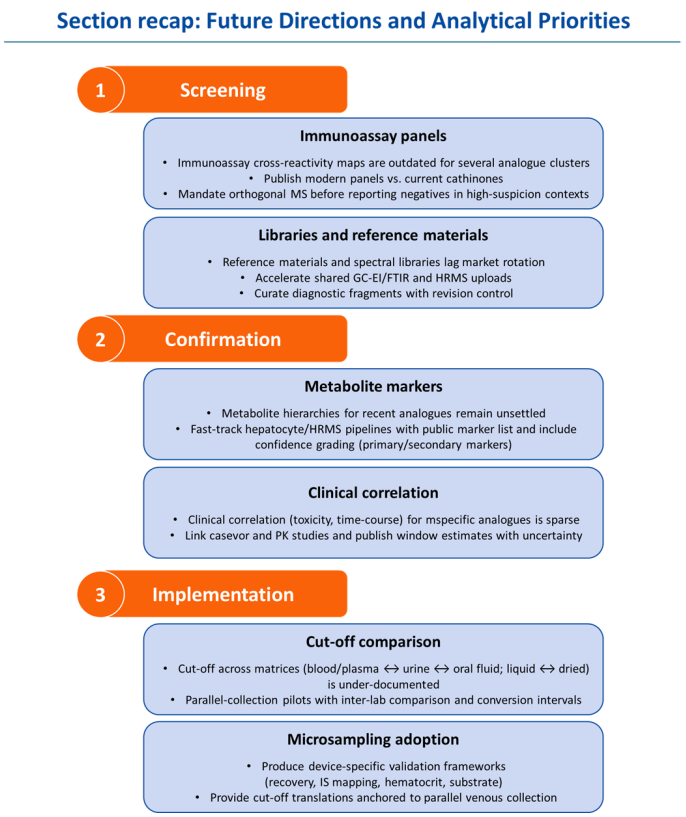


Figure 12 | Graphical recap of Section 7. Future Directions.

Acknowledgements

The Authors acknowledge the financial contribution from the Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum – University of Bologna through Fundamental Oriented Research (RFO) funds.

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