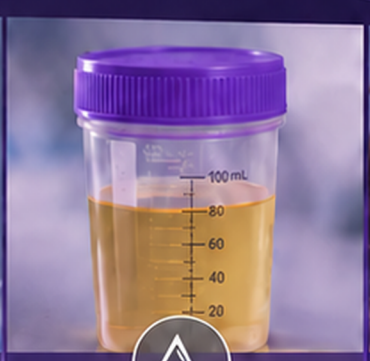
Recommended procedures
for the determination of
drugs of abuse and new psychoactive substances
in biological matrices.

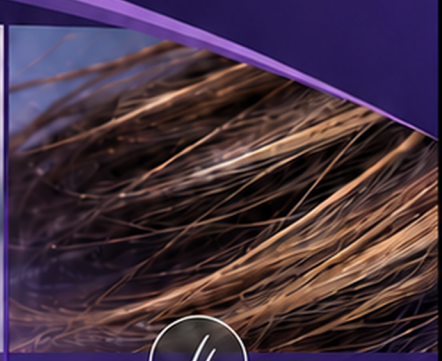
Guidance for clinical, forensic and epidemiological toxicology.


BLOOD

ORAL FLUID



URINE


HAIR

- Sample collection, storage and chain of custody
- Screening and confirmatory analytical methods
- Method validation and quality assurance
- Interpretation and reporting for reliable results

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Recommended procedures for the determination of drugs of abuse and new psychoactive substances in biological matrices.

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Abstract

This document provides guidance on the determination of drugs of abuse and New Psychoactive Substances (NPS) in the main biological matrices, including blood, oral fluid, urine and hair. The clinical, forensic, and epidemiological objectives of toxicological analyses are outlined, highlighting the need for sensitive and specific analytical approaches to address the rapid evolution of the NPS market. For each biological matrix, the procedures for collection, storage, management of non-compliance, and analysis are described, together with documentation requirements, including informed consent, chain of custody, and reporting. The document details analytical methodologies, distinguishing between screening tests and confirmatory analyses based on chromatographic techniques coupled with mass spectrometry and provides reference *cut-off* values. Emphasis is placed on method validation, internal and external quality control, and the correct interpretation and communication of results. The overall aim is to harmonize laboratory procedures, ensuring consistency, reliability, and the medico-legal validity of analytical findings.

Key words: Biological matrices; Drugs of abuse; New psychoactive substances; Analytical toxicology

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PRESENTATION

The detection of substances of abuse and new psychoactive substances in biological matrices is one of the most complex and significant challenges currently encountered in the fields of clinical and forensic toxicology.

Changing patterns of substance use, the rapid emergence of new substances and the expanding fields in which it is necessary to ascertain substance use (clinical, healthcare, social care, workplace and judicial) requires rigorous analytical tools and standardised procedures capable of ensuring the reliability, comparability and forensic validity of the results.

Scientific advances currently enable targeted testing for illicit substances in various biological samples, confirming substance misuse, identifying the type of substance – whether traditional or emerging – and determining the timing and duration of use.

These recommended procedures have been developed with the aim of providing healthcare practitioners and professionals in the sector with a clear, up-to-date and scientifically sound guide for managing the entire diagnostic process: from the selection of the biological matrix to sample collection, from sample storage to reporting, right through to the integrated use of screening and confirmatory methods in accordance with international standards.

This document combines technical knowledge, legal aspects and operational recommendations, suggesting procedures that can be adapted to different clinical and forensic contexts. The growing complexity of the psychoactive substances market and the continuous introduction of new molecules require a response based on multidisciplinary expertise, advanced methodologies and harmonised protocols.

It is hoped that these recommendations will help to support the day-to-day work of professionals, ensuring the quality, transparency and traceability of the analytical process and promoting a consistent approach throughout the country.

The accuracy of the results and their correct interpretation are not only a technical requirement, but also an essential element in safeguarding public health, ensuring public safety and the proper administration of justice.

ABBREVIATIONS

6-MAM	6-Monoacetylmorphine
11-OH THC	11-Hydroxy-Δ9-tetrahydrocannabinol
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BEG	Benzoylecgonine
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
CBD	Cannabidiol
CDT	Carbohydrate-deficient transferrin
CV%	Coefficient of variation (%)
DBS	Dried Blood Spots
DFC	Drug-Facilitated Crime
DSS	Dried Saliva Spots
DUS	Dried Urine Spots
EDDP	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EQA	External Quality Assessment
EtG	Ethyl glucuronide
EtPa	Ethyl palmitate
EtS	Ethyl sulfate
EWDTS	European Workplace Drug Testing Society
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GDPR	General Data Protection Regulation
GTFI	Gruppo Tossicologi Forensi Italiani
HCl	Hydrochloric acid
HRMS	High-resolution mass spectrometry
HS-GC	Headspace gas chromatography
IQC	Internal Quality Control
LC	Liquid chromatography
LC-HRMS	Liquid Chromatography-High-Resolution Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MBDB	3,4-Methylenedioxy-N-methyl-α-ethylphenethylamine
MBTFA	N-Methyl-bis(trifluoroacetamide)
MCV	Mean corpuscular volume
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxy-N-ethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
MS	Mass spectrometry
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoroacetamide
NaOH	Sodium hydroxide
NSP	New Psychoactive Substances
PEth	Phosphatidylethanol
PLD	Phospholipase D
PTCA	1H-Pyrrole-2,3,5-tricarboxylic acid
PTeCA	1H-Pyrrole-2,3,4,5-tetracarboxylic acid
QC	Quality Control
SoHT	Society of Hair Testing
SOP	Standard Operating Procedure(s)
THC	TetraHydroCannabinol
THC-COOH	11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol
EQA	External quality assessment

INDRODUCTION

The growing diffusion of psychoactive substance use constitutes a major challenge for public health and modern society. The history of drugs of abuse shows a continuous evolution reflecting cultural, social and scientific change, from ancient cultures that used natural substances for ritual and therapeutic purposes to the laboratory synthesis of increasingly potent and diversified active compounds (1-4).

In particular, the market for New Psychoactive Substances (NPS) has recorded an unprecedented increase in recent decades, characterized by the introduction of synthetic compounds with variable chemical structures, often undocumented and difficult to detect with conventional methods (4-10). These scenarios require healthcare, forensic and institutional structures to develop innovative analytical strategies capable of ensuring sensitivity, specificity and accuracy in identifying substances across different biological matrices (5, 10, 11).

The continuous variability of NPS and consumption behaviours, together with the speed with which new compounds are placed on the market, require ongoing training of operators, data sharing between laboratories, and the adoption of rigorous, standardized methodological protocols (12-16).

Analyses for psychoactive substances are generally performed on preferred biological matrices, such as blood, urine, saliva and hair. These matrices are not equivalent but provide complementary information relating to substance intake and metabolism, allowing different exposure time windows to be assessed, ranging from acute (blood and saliva) to chronic/past use (hair) (17-19). The chemical-physical complexity of the matrices, combined with the wide variability due to different individual consumption behaviours, requires the adoption of advanced analytical strategies. These are based on the use of highly sensitive and specific separation and detection techniques, such as liquid and/or gas chromatography coupled with mass spectrometry, and on strict adherence to validation protocols that must comply fully with international standards (17-24).

This document aims to outline a shared and scientifically rigorous methodological framework for the analysis of classic drugs of abuse and NPS. The objective is to provide detailed operational guidance on the analytical pathway: from the correct selection of the biological matrix, to sampling and storage methods, through chain of custody and sample preparation, analysis execution, and interpretation of results.

Through this standardization, the intent is twofold: to promote the harmonization of analytical methods adopted at national level, and to significantly improve inter-laboratory comparability.

The application of these guidelines is essential to support the scientific and technical-operational quality of monitoring and research activities in the field of analytical toxicology.

The increasing use of psychoactive substances has led to a significant increase in requests for analytical testing across different biological matrices. Toxicological investigations aimed at detecting psychoactive substances are an essential tool in both clinical and forensic settings, making it possible to identify, quantify and monitor the intake of drugs and new NPS in different biological matrices. Artificial intelligence, and in particular machine learning—a discipline that enables systems to learn from data and identify patterns without explicit instructions—is emerging as a valuable tool in the identification and characterization of drugs of abuse and NPS, thereby facilitating the continuous updating of analytical methods in response to the rapid evolution of the drug market (25, 26).

Toxicological analysis of drugs of abuse and NPS in biological matrices is applied in various settings, with differentiated objectives depending on the field of investigation, which may be clinical, forensic, or aimed at public health monitoring. This methodology makes it possible to identify and quantify substance intake precisely and reliably, contributing both to the therapeutic management of patients and to judicial investigations and social prevention strategies. Table 1 summarizes the main objectives of such analyses across different areas of application.

Table 1. Main objectives of the toxicological analysis of drugs of abuse and new psychoactive substances in human

Objectives	Description	Action
Clinical	Diagnosis of acute intoxication	Rapidly identify the substance(s) responsible for an acute symptomatic picture (overdose, poisoning, confusional state). This is crucial for initiating supportive treatment or specific antidote therapy, where available.
	Therapeutic drug monitoring	Assess patient compliance in pharmacological treatment programmes (e.g. substitution therapy for drug addiction) or rehabilitation programmes.
	Assessment of severity and prognosis	Correlate the concentration of the substance (or its active metabolite) in blood with the severity of intoxication and the risk of complications, helping to define prognosis.
	Adverse event investigations	Determine whether substance use may have contributed to trauma, accidents, or apparently natural deaths in a hospital setting.

Legal and forensic medicine	Assessment of fitness to drive	Determine whether substances that may have impaired psychophysical capacity in relation to driving have been taken (Art. 187 of the Italian Highway Code).
	Assessment of substance use/abuse	In cases of child custody or international adoption.
	Evidence in judicial proceedings	Detect substances in biological samples taken from victims or suspects to investigate crimes such as sexual assault or forced administration.
Public health and monitoring	Surveillance	Epidemiological: collect data on substance use (particularly NPS and their diffusion patterns) to understand emerging trends and support the planning of health and legislative interventions.
	Environmental monitoring	Although not directly performed on biological matrices, wastewater-based monitoring (wastewater-based toxicology) is often correlated with clinical data and is used to estimate large-scale community consumption.
Occupational medicine and safety	Workplace safety	Verify the absence of drugs of abuse in workers performing safety-critical duties, in accordance with current regulations.

1. BIOLOGICAL MATRICES

Biological matrices are fundamental tools for the analysis and detection of drugs of abuse and NPS. The choice of biological sample must be closely linked to the information sought and/or the question underlying the investigation. Consequently, the biological matrix used will vary according to the aim of the investigation: for example, assessing an individual's psychophysical state at a given moment requires different samples from those used to ascertain possible dependence on specific substances.

One of the main differences between the various biological matrices concerns the time window during which the presence of the substance can be detected, usually expressed in hours for blood and saliva, days for urine, and up to several months for keratin matrices (hair). When choosing the matrix, the ease and invasiveness of collection, analysis costs, the reliability and reproducibility of results, the presence of possible interferences, and any analytical difficulties should also be considered.

Knowledge of the specific characteristics of each matrix is essential for the appropriate application of the guidelines, ensuring reliable and comparable results in toxicological analyses.

Table 2 summarizes the main biological matrices used in toxicological analysis, classifying them according to their ability to detect current use, recent use, and chronic or past use of drugs of abuse and NPS.

Table 2. Classification of the main biological matrices used in toxicological analyses

Category	Biological matrix	Purpose of toxicological analysis
Current use	Blood / Saliva	Assessment of current use and correlation with psychophysical state
Recent use	Urine	Assessment of substances and metabolites with a detection window of days
Chronic or past use	Keratin matrix (hair)	Temporal reconstruction of intake and assessment of chronic use

1.1 Blood Matrix

The blood matrix (whole blood and plasma) plays a fundamental role in toxicological analysis, in both clinical and forensic settings (27). The use of this matrix makes it possible to detect drugs of abuse and/or NPS very shortly after intake, providing precise information on current use and on the possible presence of active pharmacological effects.

1.1.1 Collection Procedures

Blood sample collection (Table 3), performed exclusively by qualified and authorized personnel, is a fundamental procedure in clinical diagnostics, research, and forensic toxicology. Correct technique and adherence to these guidelines are essential to ensure sample quality, patient safety, and the reliability of laboratory results (28-31).

Key steps include pre-sampling preparation (patient identification, informed consent), the sampling procedure itself (site selection, use of closed collection systems, order of draw), and post-sampling management (labelling, transport, chain of custody). For skin disinfection, the use of non-alcoholic solutions is recommended, particularly when sampling for blood alcohol determination, in order to avoid any legal doubt or challenge (12, 13), notwithstanding the absence of demonstrated contamination (32). The choice of collection tube (colour-coded for specific tests) and correct mixing are essential to prevent coagulation or contamination.

The amount of blood required may vary according to the analytical protocol and the requirements of the test, but generally at least 5-10 mL of whole blood is collected, in duplicate tubes in the case of forensic testing.

Table 3. Kinds of blood collection for the analysis of psychoactive substances

Type of collection	Advantages	Disadvantages	Recommendations
Venipuncture	Provides the largest volume, essential for performing screening, confirmatory analyses and the review aliquot.	Risk of external contamination, e.g. from improper use of alcohol-based disinfectants (e.g. ethanol) before collection.	Disinfect only with non-alcoholic products (e.g. aqueous chlorhexidine).
Capillary sampling	Non-invasive. Useful in emergency situations or for	Limited sample volume. The concentration of some analytes	Disinfect the puncture site. Use only suitable lancets and collection

	critical patients (neonates, burn victims).	(e.g. ethanol) may differ from venous blood.	materials (capillary tubes or Dried Blood Spots cards).

Specifically, the blood collection kit for the determination of drugs of abuse and NPS must contain:

- collection tubes for blood aliquots for screening, confirmatory and review analyses (respectively A, B and C), with known and constant volumes (at least 5 mL) per sample, and anticoagulant. For blood alcohol determination, dedicated tubes with anticoagulant (potassium oxalate) and preservative/stabilizer (sodium fluoride) are mandatory to prevent degradation/neoformation of ethanol, particularly if the tubes must be stored for an extended period;
- a sampling report documenting the details of the person responsible for the collection, of the person undergoing testing, the unique sample identification, the list of medicines taken or administered to the person tested, and the signatures of both parties;
- a chain-of-custody form recording every transfer of the sample and the name/signature of everyone who has had custody of it, specifying the place, date and time of collection;
- an informed consent form signed by the person undergoing testing;
- a privacy notice and consent form in accordance with Regulation (EU) 2016/679 (General Data Protection Regulation, GDPR);
- adhesive labels with an alphanumeric or bar code, identical for the report and the containers, to ensure unique identification;
- tamper-evident seals.

The Italian Highway Code and the law on vehicular manslaughter (Law 41/2016) introduced the possibility, in the event of a driver's refusal to undergo testing and after informing the judicial police officer, of carrying out compulsory blood sampling ordered by the judicial authority, even verbally.

If the subject undergoing testing is unable to give consent to sampling, biological samples are nevertheless collected by healthcare personnel, with the judicial authority subsequently assessing their legitimacy and usability (12, 13, 27).

1.1.2 Storage Conditions

Proper storage of blood samples (Table 4) should be ensured in order to preserve the integrity of the analytes and guarantee the reliability of analytical results in clinical, research and forensic applications. Storage conditions, particularly temperature, duration and mode of storage, directly influence the stability of the substances, affecting the quality and reproducibility of analytical determinations.

Table 4. Effects of temperature on the stability of drugs of abuse and NPS in blood

Storage condition	Effect on stability	Notes
Freezing (-20 °C or below)	Minimizes degradation over long periods.	Standard method for long-term storage and for the legal review aliquot.
Refrigeration (4 °C)	Slows degradation, but is less effective than freezing.	Suitable only for short-term storage (e.g. immediate transport or analysis within 2-3 days).
Room temperature	Degrades rapidly (high instability).	To be avoided for storage; unacceptable in forensic settings.
Dried Blood Spot (DBS)	Good long-term stability at room temperature.	Convenient for collection and transport; recommended to store in the dark in a dry environment; useful for long-term storage.

Stability studies have shown that storing blood samples at room temperature leads to significant degradation of the analytes, whereas storage at -20 °C or -80 °C preserves their stability for several months (33-39).

The presence of additives, such as sodium fluoride at 2% w/v, is essential to prevent microbial growth (37, 39). To prevent blood coagulation in tubes intended for the collection of samples for toxicological analysis, anticoagulants such as potassium oxalate are also used, either alone or in combination with sodium fluoride.

Storage of the blood sample is a critical step in the chain of custody and must be managed in access-restricted environments. Aliquots B and C of samples that test positive on screening analysis (aliquot A), in a medico-legal context, must be stored at -20 °C for a minimum period of 12 months.

It is also essential that documentation (such as chain-of-custody records) relating to sample storage be retained for a period of no less than three years, or as established by current regulations. Documents and samples must be kept in locked cabinets, refrigerators or freezers, with constant temperature monitoring for sample storage.

DBS technology, up to now used mainly in research settings, allows samples to be stored stably at room temperature or under refrigeration for long periods (weeks or years) with minimal degradation of analytes. The use of DBS is particularly useful for transport and for reducing biological risk (40-42).

1.1.3 Non-conformities

The most frequent cause (Table 5) that render a blood sample unsuitable or unacceptable for forensic-toxicological and medico-legal analyses mainly concern compromise of the integrity and authenticity of the sample, failure to comply with the chain of custody and cold chain, and insufficient sample quantity. Blood samples should be intact and free of alterations, in particular without signs of haemolysis, coagulation, or contamination. Their collection, storage, and handling should be carried out in full compliance with Standard Operating Procedures (SOPs), with particular attention to the choice of containers, anticoagulants and storage conditions (Appendix A).

Any non-conformity at any of these stages may compromise the analytical reliability of the sample, leading to altered, non-reproducible, or invalid results.

Table 5. The main non-conformities that make blood samples unacceptable for analytical purposes

Non-conformity	Impact on analysis
Haemolysis	Analytical interference and inaccurate results
Insufficient volume	Incomplete, invalid or non-repeatable analysis
Incorrect container or additive	Incompatibility with the test; sample to be rejected
Coagulation	Loss of analytes and invalid quantification
Contamination	Falsified results or instrumental interference
Inadequate storage or handling	Degradation of analytes, false negatives

1.2 Oral fluid

Oral fluid is a biological matrix of growing interest in toxicological analysis for the determination of drugs of abuse and NPS (17). Its non-invasive, simple and rapid collection makes it particularly suitable for screening analyses, roadside checks, workplace settings, or clinical and forensic contexts in which current substance use needs to be ascertained (43). In many cases, the concentration of active compounds in oral fluid is correlated with, and sometimes comparable to, that detectable in blood, allowing for a timely assessment of the presence of specific analytes within a few hours of intake (44-46). However, the result depends on several factors, including the dose, the time elapsed since intake, and the individual's physical condition.

1.2.1 Collection Procedures

The collection of oral fluid, which must be carried out by qualified and authorized personnel, represents a critical stage of the analytical process, as the quality of the sample and the reliability of analytical results depend to a large extent on the collection method and the device used.

For this reason, samples should be obtained exclusively using certified devices, such as swabs or collectors with a volume indicator, compliant with current regulations and validated for toxicological applications (47, 48).

The choice of method (Table 6) and device for oral fluid collection is fundamental to the reliability of drug tests.

Table 6. Kinds of oral fluid collection for the analysis of psychoactive substances

Collection method	Advantages	Disadvantages	Recommendations

Direct collection (stimulated/unstimulated)	Allows physiological assessment of oral fluid.	Risk of contamination; difficulty standardizing volume.	Avoid salivary stimulants and disinfectants before sampling.
Absorbent swabs	Non-invasive, easy to use, rapid collection.	Limited volume; possible adsorption of the substance onto the swab material.	Use only certified devices; avoid contamination from beverages or smoking.
Devices with stabilizing buffer	Improves analyte stability; facilitates transport.	Possible sample dilution; buffer interference with some immunochemical tests.	Record the exact volume of saliva and type of buffer.

A sample collected for legal and forensic medicine purposes should be divided into two aliquots, designated A (for screening analysis and, if positive, for confirmatory analysis) and B (for any review analysis requested by the subject) (16, 49).

1.2.2 Storage Conditions

Appropriate storage of oral fluid samples is essential to preserve analyte integrity and ensure reliable results. Temperature conditions and the time elapsed between collection and analysis significantly affect the stability of many psychoactive substances, in particular cocaine, synthetic cathinones and cannabinoids.

The presence of stabilizing solutions in commercial kits can prolong analyte stability (Table 7), but storage conditions have to always comply with the manufacturer's instructions (50-53).

Table 7. Effects of temperature on the stability of psychoactive substances in oral fluid

Storage condition	Effect on stability	Notes
Freezing (-20 °C or below)	Maximum stability; minimal degradation for weeks/months.	Preferred method for extended storage and legal review.
Refrigeration (4 °C)	Moderate stability for analysis within a few days.	Suitable only for transport or rapid analysis.
Room temperature	Rapid degradation; risk of false negatives.	Unacceptable for forensic purposes.
Dried Saliva Spots (DSS)	Good long-term stability at room temperature for many analytes, reducing the need for refrigeration.	Convenient for collection and transport; useful for long-term storage.

1.2.3 Non-conformities

Oral fluid samples planned for the determination of psychoactive substances should be collected, stored, and handled according to standardized protocols, ensuring the representativeness and stability of the sample. The main non-conformities, listed in Table 8, that may compromise analytical reliability include food or environmental contamination, insufficient volume, use of non-validated devices, or improper storage.

Table 8. The main non-conformities that make oral fluid samples unacceptable for analytical purposes

Non-conformity	Impact on analysis
Insufficient sample volume	Incomplete or invalid analysis.
Unsuitable or non-validated device	Incompatibility with the test; risk of unsuitability.
Failure to add stabilizing buffer	Degradation of analytes, false negatives.
Inadequate storage (temperature or time non-compliant)	Chemical or microbiological alteration of the sample.
Improper handling or cross-contamination	Distorted results, loss of traceability.

1.3 Urine

Urine is the most widely used biological matrix for the detection of psychoactive substances in forensic, clinical, and occupational settings. Its wide detection window, ease of collection, and high concentration of metabolites make it particularly suitable for monitoring the use of such substances (54, 55).

Unlike blood and oral fluid, urine mainly reflects the elimination of substances and their metabolites, providing information on intake that occurred in the hours or days preceding sampling, without, however, allowing an accurate determination of the exact time of use. Despite its advantages, critical issues remain related to possible sample adulteration, variations in pH and density, and analyte stability.

These factors require careful interpretation of results and awareness of the intrinsic limitations of the urinary matrix, as well as individual metabolic variability (56).

Complexity increases further in the case of NPS, which are often characterized by rapid structural evolution, poorly known metabolites, and limited availability of analytical standards. These peculiarities make urine a useful matrix for detecting psychoactive substances, while at the same time requiring the use of sensitive and constantly updated methods (24, 57).

1.3.1 Collection Procedures

Urine collection (Table 9) is a well-established procedure but requires attention to ensure sample integrity and prevent tampering (58). Samples should be collected in sterile, sealed containers compliant with current regulations. Direct supervision of collection or the use of control systems to reduce the risk of adulteration or dilution of the sample is recommended, always maintaining respect for privacy, and may be supplemented by additional checks such as urinary creatinine, osmolality, density, and immediate temperature measurement, in order to guarantee the reliability of the analytical result (56, 59, 60).

Standardization of collection protocols is essential to ensure reliable and reproducible results. For example, a urine sample collected for legal and forensic medicine purposes should be divided into three distinct aliquots: aliquot A, intended for preliminary screening tests; aliquot B, reserved for confirmatory testing; and aliquot C, retained for any review or control analysis.

Table 9. Types of urine collection for the analysis of psychoactive substances

Collection method	Advantages	Disadvantages	Recommendations
Uncontrolled individual collection	Non-invasive, simple, allows targeted analyses.	Risk of adulteration, short detection window, privacy issues.	Use certified containers; check temperature and creatinine for authenticity.
Controlled individual collection	Non-invasive, simple; reduces the risk of substitution or adulteration; ensures traceability.	Intrusive; ethical and logistical issues.	Apply only in high-risk contexts and forensic investigations; comply with privacy and consent protocols.
Pooled sampling	Higher analyte concentration (less dilution), anonymous, allows monitoring of groups.	Does not identify individuals; possible gender bias; qualitative data only.	Use for population investigation (e.g. festivals, public events); define the collection period.

1.3.2 Storage Conditions

Proper storage of urine samples is essential to ensure the accuracy of analyses, as many drugs of abuse and their metabolites can degrade rapidly under suboptimal conditions (Table 10). Factors such as temperature, pH, and storage time significantly affect analyte stability, making controlled conditions necessary. In particular, pH control or the use of preservatives can help reduce the loss of particularly labile compounds (24).

Among storage methods, freezing is the most effective procedure for preserving the integrity of most substances and their metabolites, while refrigeration is suitable only for short periods (17, 61-63). To facilitate the transport and storage of samples, alternative formats such as *Dried Urine Spots* (DUS) are also becoming more widespread, particularly in research settings (64), although this requires further assessment of their long-term stability.

This information highlights the importance of implementing rigorous and appropriate storage protocols, which are essential to ensure the reliability of analytical results and their forensic sustainability.

Table 10. Effects of storage conditions on the stability of psychoactive substances in urine

Storage condition	Effect on stability	Notes
Freezing (-20 °C or below)	Maximum stability; minimal degradation for up to 12 months.	Recommended for extended storage and legal review.
Refrigeration (4 °C)	Moderate stability for analysis within 14 days.	Check pH; add preservatives for labile compounds.
Room temperature	Rapid degradation; risk of false negatives.	Not recommended for forensic purposes.
Dried Urine Spots (DUS)	Good short-term stability (24 h) at room temperature.	Useful for short-term sampling, storage and transport.

1.3.3 Non-conformities

Several factors can compromise the integrity of the urine sample or the validity of results (Table 11). Among the main critical issues are inappropriate collection, contamination, insufficient volume, inadequate storage, failure to comply with the chain of custody, and physiological variables that affect the concentration of substances and their metabolites (12, 13, 15, 57). It is therefore essential to strictly comply with collection, handling, and analysis protocols in order to ensure reliable determinations. Appropriate sample management reduces the risk of unreliable results or samples being declared unacceptable, ensuring the validity and reproducibility of the toxicological analyses performed.

Table 11. The main non-conformities that make urine samples unacceptable for analytical purposes

Non-conformity	Impact on analysis
Contamination (e.g. water, detergents, foreign substances)	Dilution or masking of substances; false negatives.
Insufficient volume	Incomplete analysis or inability to repeat the test.
Unsuitable or non-validated container	Risk of sample misidentification and legal challenges.
Inadequate storage	Degradation of analytes; unreliable results.
Improper handling or cross-contamination	Unreliable results, loss of traceability.

1.4 Keratin Matrix

Keratin matrices, particularly hair, are widely used in clinical and forensic toxicology for the identification of drugs, toxic compounds, narcotics, psychotropic substances, and various biomarkers, including those indicative of ethanol consumption. Hair analysis is particularly useful as it significantly extends the time window for detecting a substance in the preceding months, compared with other biological matrices (65-67).

Furthermore, segmental analysis of the sample allows, even though with a margin of uncertainty, the reconstruction of the history and pattern of use based on the average rate of hair growth, estimated at approximately 1 cm per month. The proximal portion of the hair, close to the scalp, reflects recent exposure, while moving towards the distal end it is possible to identify intake dating back to more distant periods (66, 68).

This makes hair particularly useful for documenting chronic or past substance use, confirming self-reported history, and supporting clinical or forensic investigations (24, 68-71). Analysis of hair from other areas of the body, such as the axilla, chest, or pubic region, can be useful for documenting past use or exposure to substances, although it does not allow for segmental chronological assessment (67, 72, 73).

In the case of pubic hair, interpretation of results requires particular attention, as the presence of drugs of abuse or their metabolites may be influenced by contamination from the subject's own urine. In this context, quantitative assessment is more complex and must be accompanied by a thorough analysis of the collection conditions and possible sources of contamination (74).

1.4.1 Collection Procedures

The collection of hair samples for the analysis of psychoactive substances, as with all matrices of clinical-forensic interest, is a critical phase as it directly affects the reliability and interpretability of results. Collection, handling, and documentation of the sample are fundamental factors in preventing contamination and ensuring accurate analysis of substances.

Hair samples are generally taken from the posterior vertex region, preferred for the uniformity of growth and reduced intra-individual variability, allowing a more accurate correlation between sample length and periods of exposure. At least 200 mg of hair is collected as close as possible to the scalp, with the proximal end appropriately marked to allow chronological reconstruction.

The amount of sample collected should be sufficient to perform both screening and confirmatory analyses (aliquot A), as well as to retain part of the sample for any verification analysis (counter-analysis, aliquot B). In the absence of hair, sample from other body regions may be used, with due respect for the privacy of the person undergoing testing (14, 75-76). Figure 1 shows an example of keratin matrix sampling.

When hair analysis is requested to investigate a *Drug-Facilitated Crime* (DFC) involving unknowing consumption of drugs or medicines by the victim (77-81), sample collection have to follow the general recommendations, supplemented by the additional key points listed in Table 12**.**

Roll up and secure with a piece of string a lock of hair (o more, if necessary) about the width of a thin pencil from the back of the head.

Cut the hair just above the skin, as close to the scalp as possible, and note the length of the hair.

Put the hair lock(s) into the separate aluminium foils.
The part of the seal corresponding to the roots should extend beyond the foil.

Figure 1. Instructions for Hair Collection (75)

Fold each aluminium foil once as shown above.

Insert the aluminium foil into the envelope. Seal the envelope.

The envelope should be accompanied by:
the sample collection report,
the informed consent;
the chain of custody form

Table 12. Recommendations for hair sample collection in cases of DFC

Aspect	Details and recommendations
Collection	The hair sample should be collected between 30 and 45 days after the alleged crime. This period is adequate to ensure that the hair shaft, having incorporated the drug, emerges from the bulb area of the follicle at a height sufficient for collection above the skin surface.
Number of samples	Collection of a quantitatively adequate sample is recommended, to be divided for any confirmatory analyses in case of a positive result.
Analysis	Segmental analysis is recommended owing to the possible differentiation of substance concentration along the hair shaft.
Hair care	The victim should avoid cosmetic treatments or haircuts from the time of the criminal event until the time of sampling.

1.4.2 Storage Conditions

To ensure the reliability of analyses (Table 13), it is essential to adopt, as with all matrices used in clinical-forensic settings, appropriate storage procedures. Hair samples should be stored in a dry, dark environment at room temperature. Direct exposure to sunlight should be avoided. Storage at low temperatures and in humid environments is not recommended, as it can cause hair swelling, mould growth, and loss of substances. Storage in plastic bags could affect analytical results, as some plastic materials contain additives that may be absorbed by the sample; moreover, plastic may extract lipophilic substances. It is therefore advisable, where possible, to prefer other types of material. A simple storage procedure consists of wrapping the hair sample in aluminium foil and sealing it in a paper envelope.

Table 13. Effects of storage conditions on the stability of psychoactive substances in the keratin matrix

Storage condition	Effect on stability	Notes
Freezing (-20 °C or below)	Can cause hair swelling, mould growth and loss of the substance.	Storage not recommended.
Refrigeration (4 °C)	Over time can cause hair swelling, mould growth and loss of the substance.	Not recommended.
Room temperature	Maintains good stability if stored appropriately.	Avoid direct sunlight; wrap in aluminium foil and place in a paper envelope.

1.4.3 Non-Conformities

The main non-conformities (Table 14) that make keratin matrix (hair) samples unacceptable for the analysis of psychoactive substances mainly relate to external contamination, cosmetic or chemical treatments, and degradation of the matrix. Strongly oxidative hair treatments such as bleaching and certain dyes, and the use of substances such as sodium bicarbonate or salicylic acid, can significantly reduce the concentration of psychotropic substances in hair without visibly damaging the keratin matrix, complicating interpretation and potentially leading to false negatives (60)

Furthermore, an insufficient sample quantity, unacceptable collection, or degradation due to environmental exposure can compromise the integrity of the sample itself. It is advisable to include in the report information relating to elements useful for identifying possible adulterating treatments (e.g. colouring of the extraction solution, positivity for 1H-pyrrole-2,3,5-tricarboxylic acid (PTCA) and 1H-pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA).

Table 14. Main non-conformities that make keratin matrix samples unacceptable for analytical purposes

Non-conformity	Impact on analysis
Unacceptable collection (insufficient quantity, unsuitable area, contamination)	Inability to perform analysis, inaccurate or unrepresentative results.
Unsuitable storage (light, humidity, plastic, low temperatures)	Degradation or loss of the substance, alteration of the analytical profile, unreliable results.

2. CURRENT USE AND PAST/CONTINUOUS ETHANOL CONSUMPTION

Ethanol consumption represents a public health issue, being associated with a wide range of adverse effects on the liver, cardiovascular system, nervous system, and psychosocial wellbeing (82). Assessment of ethanol intake in clinical and forensic-toxicological settings requires the combined use of direct and indirect biomarkers, each characterized by specific detection time windows and different interpretative meanings (Table 15). The aim is to distinguish between:

- current use (intake within the last few hours);
- recent use (last few days);
- past and/or continuous use (weeks/months).

Table 15. Assessment of ethanol consumption

Clinical/forensic objective	Main biomarkers	Time windows
Current use	Blood/breath/urine ethanol + questionnaires/interviews	Hours
Recent use	EtG/EtS in urine + questionnaires/interviews	1-3 days (up to ~80 h)
Past use	PEth in blood + questionnaires/interviews	2-4 weeks
Chronic use	hEtG, FAEE, clinical history + questionnaires/interviews	Months/years

Abbreviations: EtG: Ethyl glucuronide; EtS: Ethyl sulfate; FAEE: Fatty Acid Ethyl Esters; hEtG: hair ethyl glucuronide; PEth: Phosphatidylethanol

Although blood remains the most widely used matrix for laboratory testing in clinical practice and primary care (83), the choice of matrices and related biomarkers (84-86) is nevertheless determined by regulatory requirements (e.g. the Highway Code, safety-critical duties), clinical needs (therapeutic monitoring, follow-up) and forensic needs (fitness assessment, administrative and criminal proceedings). Finally, for a correct clinical-forensic assessment of ethanol use, it is useful to integrate questionnaires/interviews, medical history information, and biomarkers.

2.1. Current Use: Blood Alcohol Concentration

Whole blood is the reference matrix for determining blood alcohol concentration for forensic-toxicological purposes, as it directly and reliably reflects the subject's psychophysical state at the time of sampling. Ethanol determination must be performed in accordance with rigorous procedures aimed at ensuring the analytical and medico-legal validity of the result (12, 13).

Forensic measurement of ethanol must be performed exclusively on whole blood; analysis of blood derivatives such as plasma or serum is not permitted in regulated settings, as it results in a systematic overestimation of values, on average between 12 and 18%. Such determinations may be used exclusively for diagnostic-clinical purposes and must not be used for regulatory assessments (12, 13).

Blood sampling must be preceded by skin disinfection using products free of ethyl alcohol and must be performed by a single venipuncture, simultaneously collecting at least two blood aliquots (samples A and B) in separate vacuum tubes. The tubes must contain a suitable preservative and anticoagulant and must comply with chain-of-custody requirements. In particular:

- the tubes should contain sodium fluoride as a preservative and potassium oxalate as an anticoagulant;
- the tubes should be equipped with tamper-evident systems and adequate identification labelling;
- the samples should be inverted several times after collection to prevent serum separation;
- the samples should be stored, together with the counter-sample, according to the time and temperature conditions specified in the reference protocols.

During the pre-analytical phases, the main sources of interference must be prevented, in particular:

- sample contamination due to the use of skin disinfectants containing ethyl alcohol;
- in vitro neoformation of ethanol;
- evaporation of ethanol during or after sampling.

Instrumental determination of blood alcohol concentration must be performed using validated, specific and reproducible analytical methods. The reference method of choice in forensic toxicology is headspace gas chromatography (HS-GC), coupled with flame ionization detection (FID) or mass spectrometry (MS) (12, 13). The analytical method must be validated in compliance with the acceptance criteria set out in Table 16.

Table 16. Acceptance parameters for validation of the method for determining blood alcohol concentration

Parameter	Requirement	Acceptance criterion
Calibration range	Validation range	0.05-3.0 g/L ethanol
Precision (CV%)	Repeatability	≤ 10%
Accuracy (Bias, E%)	Systematic error	≤ 10%
Limit of detection (LOD)	Method sensitivity	< 0.05 g/L

For regulatory purposes, blood alcohol concentration[1] values must be interpreted with reference to the limits established by current legislation (e.g. the Highway Code) and reported clearly, completely and traceably in the analytical report. For interpretative purposes, the measurement uncertainty must always be subtracted from the determined value when it is compared with the various threshold values set out in Article 186 of the Highway Code (87).

Determination of blood alcohol concentration documents only recent alcohol intake and does not allow a reliable assessment of past consumption or medium- to long-term drinking habits. For a comprehensive assessment of the subject's drinking behaviour, integration with other biological matrices and specific biomarkers should be considered.

2.2 Recent Use: Ethanol, Ethyl Glucuronide and Ethyl Sulfate in Urine

Urine is a matrix of choice for assessing recent ethanol consumption, allowing identification of intake that occurred in the hours or days prior to sampling, even in the absence of detectable ethanol in blood. Urinary ethanol determination is influenced by variables such as hydration status, bladder emptying time, and possible sample contamination. Furthermore, the presence of ethanol in urine does not allow a direct estimate of blood concentration at the time of intake. Generally, urinary ethanol falls below the limit of detection within approximately 6.5 hours of intake (84, 88).

The direct metabolites of ethanol, ethyl glucuronide (EtG) and ethyl sulfate (EtS), are highly specific biomarkers of ethanol intake and are detectable in urine for a longer period than unmetabolized ethanol, generally up to 24-72 hours after intake, depending on the amount ingested and the individual characteristics of the subject (88).

The combined use of EtG and EtS is recommended in clinical and forensic-toxicological settings because:

- it reduces the risk of false-negative results, since EtG can undergo bacterial degradation in the urine sample, for example in the presence of urinary tract infections, with the possible occurrence of false negatives, whereas EtS is more stable and is not subject to such phenomena, allowing confirmation of ethanol intake even in the absence of EtG;
- it reduces the risk of false-positive results, since it allows the discrimination of actual ethanol intake from possible bacterial neoformation of ethanol in the urine sample, distinguishing real consumption from *in vitro* bacterial synthesis;
- it allows the identification of involuntary exposure to ethanol or conditions characterized by residual effects of ethanol intake, even in the absence of a positive blood alcohol result, which could potentially compromise safety, particularly in contexts related to driving and hazardous activities.

Nevertheless, results should be interpreted with cautious owing to high interindividual variability and the influence of external factors. Urinary concentration can be drastically reduced by forced diuresis (ingestion of large amounts of water), which lowers EtG and creatinine levels. In addition, low-level positivity may result from the consumption of certain foods or beverages (88).

[1] Annex 2 to circular note prot. no. 11280 of 11/04/2025. The result of tests performed on biological samples is considered positive for blood alcohol when ethanol concentrations equal to or exceeding those set out in Article 186, paragraph 2, letters a), b) and c) of the Highway Code are detected for the relevant categories; and equal to or exceeding 0.1 g/L in relation to Article 186-bis of the Highway Code

2.3 Past and Continuous Use

Assessment of past and continuous ethanol use requires the use of biomarkers characterized by an extended time window, capable of documenting drinking habits sustained over time. In this context, analysis of EtG in hair (hEtG) represents, together with other direct and indirect biomarkers, such as fatty acid ethyl esters (FAEE) in hair, phosphatidylethanol (PEth) in blood, and carbohydrate-deficient transferrin (CDT) in serum, the reference approach in clinical and forensic-toxicological settings (86).

2.3.1 hEtG

Hair is a privileged biological matrix for the retrospective assessment of ethanol use, as it allows reconstruction of intake occurring over weeks or months, depending on the length of the segment analysed. EtG, a direct and specific metabolite of ethanol, is incorporated into the hair shaft during the growth phase, remaining relatively stable over time (89, 90).

Analysis is generally conducted on a 0-3 cm segment, corresponding to an observation period of approximately three months. Interpretation of results is based on internationally shared cut-off values (91), which allow the distinction of:

- abstinence or occasional use (hEtG ≤ 5.0 pg/mg);
- moderate consumption (5.0 < hEtG < 30.0 pg/mg);
- chronic excessive consumption (hEtG ≥ 30.0 pg/mg).

It is essential to consider potentially interfering factors, such as aggressive cosmetic treatments (e.g. bleaching), individual hair characteristics, and the correctness of sampling procedures (84).

2.3.2. FAEE in Hair

FAEE are direct biomarkers of ethanol produced through a non-oxidative pathway via the reaction of ethanol with endogenous fatty acids or triglycerides. Among the main compounds monitored, ethyl palmitate (EtPa) is considered the most relevant analytical reference (91). Table 17 shows the internationally shared cut-off values.

Table 17. Cut-off values for ethyl palmitate (EtPa) (in pg/mg) in hair segments: 0-3 cm and 0-6 cm

Hair segment	Abstinence	Repeated consumption	Chronic excessive consumption
0-3 cm	≤ 120	> 120	≥ 350
0-6 cm	≤ 150	> 150	≥ 450

Determination of FAEE in hair is indicated in particular for identifying chronic and excessive ethanol consumption and, where possible, should be supported by EtG and other clinical/forensic data (86).

2.3.3. PEth in Blood

PEth is a direct biomarker of alcohol consumption that forms exclusively in the presence of ethanol in red blood cell membranes, through a reaction mediated by phospholipase D (86). The various PEth homologues differ in their fatty acid composition; among these, PEth 16:0/18:1 is the most abundant and is the reference homologue for clinical-forensic interpretation.

PEth forms rapidly after ethanol intake, reaching whole blood levels within approximately 8 hours, and degrades slowly, making it a reliable indicator of past ethanol consumption. It is detectable in blood for 3-12 days after a single intake and has a detection window of 2-4 weeks, with an average persistence of up to 28 days in cases of moderate or repeated consumption (85, 92-94). Its exclusive formation in the presence of ethanol confirms its high specificity as a direct biomarker, with no known false positives (86).

Interpretation of results follows specific thresholds:

- Abstinence or low consumption: PEth 16:0/18:1 concentrations below 20 ng/mL.
- Moderate or social consumption: values between 20 and 200 ng/mL.
- Chronic excessive consumption: values above 200 ng/mL, suggestive of chronic exposure, defined as average daily consumption equal to or greater than 60 g of ethanol for men and 40 g for women.

In the context of clinical-forensic assessment of ethanol consumption, blood PEth represents an ideal complement to hair EtG analysis and to indirect biomarkers, contributing to a more robust and integrated assessment of past and continuous consumption (86).

2.3.4 Indirect Biomarkers of Chronic Ethanol Consumption

Indirect biomarkers reflect the biological consequences of prolonged ethanol intake rather than the direct presence of the substance or its metabolites. Among the most widely used are:

- CDT;
- γ-glutamyltransferase (γ-GT);
- aspartate aminotransferase (AST) and alanine aminotransferase (ALT);
- mean corpuscular volume (MCV).

CDT is the most well-established biomarker for identifying chronic ethanol consumption. It is used in clinical and forensic settings, for example for assessing fitness to drive or to perform safety-critical duties, and in custody proceedings or civil disputes (86). CDT is internationally standardized (IFCC), with defined clinical *cut-offs*: 1.7% (CDT% relative to total transferrin) in clinical settings and 2.0% (CDT% relative to total transferrin) in forensic settings (95). It can be determined by LC/HPLC or capillary electrophoresis (CE), techniques that allow the different glycoforms of transferrin to be distinguished and improve specificity compared with immunochemical methods (96, 97).

Indirect biomarkers have lower diagnostic sensitivity and specificity than direct biomarkers, as they can also be altered in the presence of liver disease, metabolic conditions, or drug use. Their use is therefore recommended exclusively in a complementary role, in support of direct ethanol biomarkers, particularly in cases where the keratin matrix is unavailable or unsuitable.

3. DOCUMENTS

The documents required for the correct determination of drugs of abuse and NPS in biological matrices includes procedures such as informed consent for analytical testing, a privacy notice and consent form under Regulation (EU) 2016/679, the sampling report for sample identification and collection, the chain-of-custody form, and the reporting form. These are supplemented by detailed information on validated analytical methods, sample preparation procedures, and storage conditions. The entire body of documentation, supported by up-to-date analytical protocols compliant with standards, ensures the quality, traceability, reliability, and medico-legal value of the analytical process (12-16, 27, 49, 75, 98).

3.1 Informed Consent

The informed consent form is a fundamental and essential document, as it formalizes the patient's conscious and affirmative willingness to knowingly accept the sampling procedures and subsequent analyses to which they will be subjected, while also protecting the associated ethical and legal aspects.

The form must clearly and comprehensibly inform the subject about the type of biological matrix collected (blood, urine, saliva, hair, etc.), the purpose of the analysis, potential risks, and the implications of the results, including the possible transmission and disclosure of data to third parties in medico-legal or judicial contexts.

In the case of minors, informed consent must be given by the parents, by whoever exercises parental responsibility, or by the guardian, while nonetheless taking into account the wishes of the minor in relation to their age and level of maturity. Since such investigations are always accompanied by a specific request with an associated medico-legal question, in the event that consent is not given, it is necessary to promptly inform the party who submitted the request, in order to properly manage the resulting issue; for the same reason, consent to the transmission of analytical data to the requesting party must also be obtained in advance.

Correct and complete completion of the informed consent form is particularly important in clinical and forensic settings, in which legal validity, sample traceability, and the reliability of the analytical result are essential elements. An example of the form is provided in Appendix B1.

3.2 Privacy Notice and Consent under Regulation (EU) 2016/679

The personal and health data of the person should be processed in compliance with Regulation (EU) 2016/679.

Under the Regulation, the privacy notice must be concise, transparent, intelligible, and easily accessible to the data subject. It must therefore explain that data and samples will be used exclusively for healthcare, diagnostic, or research purposes, in a manner that guarantees the security, confidentiality, and protection of the information. Furthermore, data and samples will be retained only for the time necessary to achieve the purposes for which they were collected, except where otherwise required by law.

It must also be made clear that the subject has the right to access their own data, to request rectification, erasure, or restriction of processing, and to withdraw consent at any time, without prejudice to the lawfulness of processing carried out prior to withdrawal. An example is provided in Appendix B2.

3.3 Sampling Report

The sampling report, which should be drawn up in triplicate, is an essential document for ensuring the correct collection, identification, traceability, and storage of biological samples intended for the determination of traditional drugs of abuse and NPS.

The form have to include data relating to the person responsible for the collection and to the person undergoing analytical testing (personal details, address, identity document details); if the subject does not possess a valid identity document, identification may be carried out by an authorized supervisor or a witness in possession of an identity document, whereas in the absence of certain identification it is not possible to proceed with sample collection.

The report must necessary contain the data for the unique identification of the sample (identification code, facility or department where the collection is done), the type of biological matrix used (blood, urine, saliva, hair, etc.), the time

of collection, the purpose of the analysis, the list of any medicines taken or administered in the days prior to collection, as well as information relating to collection methods, storage conditions, and precautions taken to prevent contamination or alteration of the sample. In cases of DFC, the date and circumstances of the criminal event must also be added.

The form has to be signed both by the operator who performed the collection and by the person undergoing analytical testing, also attesting to the presence of informed consent, an essential element particularly in clinical and forensic settings. Of the three copies of the sampling report, one is transmitted, together with the samples and the chain-of-custody form, to the facility responsible for the analysis; one is retained by the facility or the person who performed the collection; and one is given to the person undergoing analytical testing. An example of the form is provided in Appendix B3.

3.4 Chain of Custody

The chain of custody form is an essential document for ensuring the traceability of every movement of the biological sample, from the time of collection to its arrival at the laboratory responsible for analysis, preventing contamination, tampering, or mix-ups, and ensuring the legal validity of results, particularly in clinical, forensic, and judicial settings. The form shows each stage of sample management in detail, including adhesive labels with a bar code or alphanumeric code identical to those affixed to the sampling report and to the collection containers of the three aliquots, as well as information relating to the type of biological matrix (blood, urine, saliva, hair, etc.), the identity of the subject, the place, date, and time of collection, and any notes on the sample.

The name, address, e-mail address, and telephone number of the testing laboratory should also be recorded, together with the place, date, and time the samples were received by the laboratory. The form must bear the name and signature of everyone who has had custody of the sample aliquots during transfer from the collection site to the final destination, thereby ensuring full traceability of responsibility. The adoption of standardized chain of custody forms facilitates sample management, reduces the risk of errors or legal disputes, and constitutes an essential requirement for the acceptance and reliability of analytical data. An example of the form is provided in Appendix B4.

3. Reporting

The reporting form has to provide a clear and complete picture of the analytical results, including the type of biological matrix analysed, the methods used, the list of substances tested for, those detected, and the respective detection levels with appropriate units of measurement (46). The report has to also include information on the clinical or forensic context, the date and time of collection, and the chain of custody, in order to ensure the legal validity of the results. The use of standardized forms facilitates communication between the laboratory, clinicians, and authorities, improving the accuracy and timeliness of decisions based on analytical data.

Finally, the report should be clear and accessible even to non-specialists, in order to effectively support medical treatment or forensic investigations. An example is provided in Appendix B5.

4. ANALYTICAL METHODS: SCREENING AND CONFIRMATORY ANALYSIS

The laboratory must use validated analytical methods described in documented procedures that include operational steps, acceptability criteria, and quality control (12-16).

Results obtained through enzymatic or immunochemical screening tests must always be confirmed using techniques based on different chemical-physical principles, such as mass spectrometry combined with chromatographic techniques including liquid or gas chromatography.

The analytical process is supported by quality control procedures, which include the analysis of control samples and a structured injection sequence to ensure the absence of interference and correct instrument function. All methods should be validated before routine use, and any modification or revision requires a new validation, with results documented, archived, and retained to ensure traceability and regulatory compliance.

Finally, ISO[2] accreditation for laboratories that determine drugs of abuse (drug testing, forensic and clinical toxicology) is essential to guarantee the accuracy, reproducibility, and legal validity of results.

4.1 Screening Methods

Screening methods are used in forensic and clinical toxicology to rapidly analyse large numbers of samples at low cost, mainly using immunochemical/enzymatic techniques or, in some cases, chromatographic techniques coupled with mass spectrometry. Immunochemical tests offer speed and automation but are characterized by reduced specificity and poor quantitative accuracy, providing only presumptive results, expressed as negative or "non-negative" relative to a threshold or cut-off value (Table 18).

Table 18. Screening threshold values for narcotic and psychotropic substances in different biological matrices as defined by law or scientific societies

Substance class	Urine (ng/mL)		Oral fluid (ng/mL)	Hair (ng/mg)
	DL.vo 81/2008*	EWDTS	EWDTS	EWDTS
Amphetamines	500	500	40	0.2
Cannabinoids (THC)	50	50	10	0.1
Cocaine and metabolites	300	150	30	0.5
Opiates	300	300	-	0.2
6-MAM	-	-	4	-
Morphine	-	-	40	-
Methadone (EDDP)	300	300 (100)	50	0.2
Buprenorphine	-	5	5	0.01
Ketamine	-	-	-	0.5

EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
EWDTS: European Workplace Drug Testing Society. * Agreement of 30 October 2007, State-Regions-Autonomous Provinces Agreement; procedures for ascertaining the absence of drug dependence

Detection of NPS represents a significant challenge for conventional screening methods: many NPS are not detectable by traditional immunochemical tests owing to their structural variability and the lack of specific antibodies (18). The use of chromatographic techniques coupled with mass spectrometry is therefore strongly recommended, as it allows simultaneous identification of a broad spectrum of NPS, including metabolites, even in the absence of specific commercial kits.

A positive screening result has no forensic validity unless it is confirmed by mass spectrometry coupled with chromatographic techniques on a new sample aliquot. Screening results must not be reported in quantitative form but, as noted, expressed as "non-negative". Although negative results are generally considered indicative of the absence of the substance tested, it is essential to minimise the risk of false negatives. If substance use is suspected, initially negative screening samples may also undergo confirmatory analysis.

Threshold or cut-off values defined by diagnostic kit manufacturers should not be adopted unconditionally, but must be adapted to the purpose of the analysis; if modified to meet laboratory needs, the method must be re-validated using calibrators specifically prepared for that purpose.

[2] UNI CEI EN ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories; UNI EN ISO 15189: Medical laboratories — Requirements for quality and competence

Chromatographic techniques allow simultaneous identification of numerous analytes, including NPS, but require specific instrumentation and expertise (13, 99). In any case, only results confirmed by chromatographic techniques coupled with mass spectrometry can have forensic-toxicological or medico-legal value.

Commercial immunochemical kits for screening drugs of abuse are mainly validated for conventional biological matrices, such as urine and oral fluid. The use of such systems on alternative matrices is less common; however, specific kits are available for the blood matrix. Similar approaches can also be applied to the keratin matrix.

4.2 Confirmatory Methods

Confirmatory analyses must ensure unambiguous identification and accurate quantification of the substances of interest, both parent compounds and their metabolites, with adequate sensitivity and specificity, producing an analytical result independent of that obtained at the screening stage, particularly if the latter was performed using enzymatic or immunochemical techniques. For this reason, it is necessary to use methods based on chemical-physical principles different from those used for screening, with superior selectivity and sensitivity, and a lower limit of quantification suited to the purpose of the analysis (e.g. differing sensitivity requirements under Legislative Decree 81/2008 vs. DFC investigations) (12-16).

In forensic toxicology, chromatographic separation is always necessary, and the use of mass spectrometry coupled with chromatographic techniques is recognized as the international standard. The use of internal standards, preferably deuterated, added before the preparative phase, is essential to ensure the reliability of the analytical data in both the extraction and instrumental phases.

Use of the same chromatographic technique employed at screening is acceptable only if the detection method differs. The threshold value for the confirmatory test (Table 19) must always be lower than that used for screening, and positive samples must be stored in dedicated aliquots for the period established by regulations or agreed with the requesting party, as indicated in the laboratory's SOPs.

Table 19. Confirmatory threshold values for narcotic and psychotropic substances in different biological matrices as defined by law or scientific societies

Substance	Urine (ng/mL)			Oral fluid (ng/mL)		Hair (ng/mg)		
	DL.vo 81/2008*	EWDTS	Sibioc	DL.vo 81/2008	EWDTS	DL.vo 81/2008	EWDTS	SoHT
Amphetamine	250	200	2	15	15	0.2	0.2	0.2
Methamphetamine	250	200	2	15	15	0.2	0.2	0.2
MDA	250	200	2	15	15	0.2	0.2	0.2
MDMA	250	200	2	15	15	0.2	0.2	0.2
MDEA	250	200	-	-	-	0.2	0.2	0.2
THC	15	-	1	2	2	0.1	0.05	0.05
THC-COOH	15	15	-	-	-	0.1	0.0002	0.0002
Cocaine	100	-	2	8	8	0.5	0.5	0.5
BEG	100	100	2	8	8	0.05	0.05	#
Morphine	100	300	2	15	15	0.2	0.2	0.2
Codeine	100	300	2	15	15	0.2	0.2	0.2
6-MAM	100	10	2	2	2	0.2	0.2	0.2
Methadone	100	250	2	20	20	0.2	0.2	0.2
EDDP	-	75	2	20	20	-	0.05	**
Buprenorphine	5	2	2	1	1	0.05	0.01	0.01
Norbuprenorphine	-	2	2	1	-	-	0.01	***
Ketamine	-	-	-	-	-	-	0.5	0.2
Norketamine	-	-	-	-	-	-	0.1	-

6-MAM: 6-monoacetylmorphine.
BEG: benzoylecgonine.
EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
EWDTS: European Workplace Drug Testing Society.
MDA: 3,4-methylenedioxyamphetamine.
MDEA: 3,4-methylenedioxy-N-ethylamphetamine.
MDMA: 3,4-methylenedioxymethamphetamine.
Sibioc: Italian Society of Clinical Biochemistry and Clinical Molecular Biology – Laboratory Medicine.

SoHT: Society of Hair Testing (consensus guidelines).
THC: delta-9-tetrahydrocannabinol.
THC-COOH: 11-nor-9-carboxy-delta-9-tetrahydrocannabinol.
*Agreement of 30 October 2007, State-Regions-Autonomous Provinces Agreement; procedures for ascertaining the absence of drug dependence.
**Confirmation of EDDP demonstrates methadone use.
***Confirmation of norbuprenorphine demonstrates buprenorphine use.
The presence of benzoylecgonine, norcocaine, cocaethylene, hydroxycocaine, or hydroxybenzoylecgonine should be considered to confirm cocaine use

For the blood matrix, reference should be made to the "minimum performance requirements" (Table 20), i.e. the analyte concentrations that the laboratory must be able to quantify, with the accuracy of the analytical method used, as indicated in the "Guidelines for the determination of narcotic and psychotropic substances in biological samples for forensic-toxicological and medico-legal purposes" produced by the Italian Group of Forensic Toxicologists (Gruppo Tossicologi Forensi Italiani[3]) (100).

Table 20. Confirmatory threshold values for drugs of abuse in the blood matrix

Substance	Concentration corresponding to minimum performance requirements (ng/mL)
Amphetamine	2
Methamphetamine	2
MDMA	2
MDA	2
MDEA	2
MBDB	2
THC	1
11-OH-THC	0.1
THC-COOH	2
Cocaine	2
BEG	2
Cocaethylene	2
Norcocaine	2
Morphine	2
Codeine	2
6-MAM	2
Methadone	2
EDDP	2
Buprenorphine	2
Norbuprenorphine	2

11-OH-THC: 11-hydroxy-delta-9-tetrahydrocannabinol
6-MAM: 6-monoacetylmorphine
BEG benzoylecgonine
EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
MBDB: 3,4-methylenedioxy-N-methyl-α-ethylphenethylamine
MDA: 3,4-methylenedioxyamphetamine
MDEA: 3,4-methylenedioxy-N-ethylamphetamine
MDMA: 3,4-methylenedioxymethamphetamine
THC delta-9-tetrahydrocannabinol
THC-COOH: 11-nor-9-carboxy-delta-9-tetrahydrocannabinol

[3] The minimum performance requirements and concentration values adopted by the Gruppo Tossicologi Forensi Italiani (GTFI), as well as the screening and confirmatory cut-offs set out in the main national and international technical-regulatory references (Legislative Decree 81/2008, EWDTS, SoHT), are reported in the tables of the full version of the "Guidelines for the determination of narcotic and psychotropic substances in biological samples for forensic-toxicological and medico-legal purposes"

5. VALIDATION OF ANALYTICAL METHODS

Validation of an analytical method consists of demonstrating that the method is fit for purpose and produces precise, accurate, and reproducible results under the intended operating conditions. Every analytical methodology routinely used by the laboratory must undergo prior validation, in accordance with national and international procedures and criteria (101-107). Immunoenzymatic screening methods are generally validated by the manufacturer and do not require further validation procedures. However, any modification to the conditions of use specified by the manufacturer requires full validation of the method or kit, to be carried out only in the absence of alternative methodologies (13).

The method validation procedure adopted by laboratories must consider a series of fundamental parameters that ensure reliability, reproducibility, and fitness for the intended use (Table 21).

Table 21. Key points of bioanalytical method validation

Parameter	Description	Acceptance criteria
Selectivity	Ability of the method to distinguish the analyte from endogenous interferents	-
Accuracy (bias)	Agreement between the measured value and the true value	±15% (±20% at LOQ)
Precision (CV%)	Reproducibility of measurements	≤15% (≤20% at LOQ)
LOD	Minimum detectable quantity	Determined experimentally
LOQ	Minimum quantifiable quantity	bias ±20%; CV ≤20%
Calibration curve	Concentration/response relationship	-
Linearity	Proportionality of response/concentration	Demonstrated within the validated calibration range
Matrix effect	Influence of matrix components	bias ±15%; CV ≤ 15%
Carryover	Cross-contamination between samples	≤ 20% LOQ (analyte); ≤ 5% IS
Recovery	Extraction efficiency	Reproducible and consistent (no fixed percentage value required)

IS: *Internal Standard*

Among the fundamental parameters for bioanalytical method validation are: accuracy, which indicates how close results are to the true value; precision, which assesses the repeatability and reproducibility of measurements; the limit of detection (LOD) and limit of quantification (LOQ), which respectively define the minimum quantity of analyte that can be detected and reliably quantified; linearity across the calibration range, which describes the proportionality of the analytical response to analyte concentration; and matrix effect, which allows identification of any interference arising from the components of the biological matrix.

Other essential parameters include carryover, analyte recovery, sample stability during storage and handling, and dilution integrity, which is necessary when samples should be diluted because the analyte concentration exceeds the calibration range. Evaluation of these parameters makes it possible to establish clear and uniform acceptance criteria, ensuring that the method can produce reliable results under different operating conditions. The specific values and acceptable limits for each parameter can be summarized in a reference table, useful for quality monitoring and for documenting the validations performed.

6. INTERNAL AND EXTERNAL QUALITY ASSESSMENT

Internal Quality Control (IQC) and External Quality Assessment (EQA) constitute the foundation of the quality assurance system in clinical and forensic toxicology laboratories (Table 22), ensuring the reliability, correctness, and consistency of analytical data in a critical context such as the determination of traditional drugs of abuse, NPS, and biomarkers of ethanol use in biological matrices (13, 106).

IQC is the set of procedures the laboratory uses to continuously monitor the performance and reliability of its own analyses. In the context of psychoactive substances in biological matrices, IQC ensures that each analytical batch is verified using controls of known concentration, a blank sample (matrix free of the analyte(s) of interest), and independent standards, ensuring the stability of instrumental and methodological performance. In this way, the laboratory can confirm the reproducibility of results over time, promptly identify any deviations, and apply the necessary corrective actions, thereby maintaining high standards of accuracy and compliance.

EQA is the periodic assessment of a laboratory's performance relative to other participants, through the analysis of unknown samples provided by an independent and/or accredited organizing body. EQA is fundamental for:

- determining the accuracy (bias) of the laboratory in an inter-laboratory comparison;
- ensuring the metrological traceability of the result, essential for its validity in medico-legal and forensic settings forense;
- promoting the harmonization of methods among laboratories.

Table 22. Summary of internal and external quality procedures in analytical processes

Quality system	Main role	Methodology and materials	Objective
Internal assessment	Daily monitoring of process stability	Internal Quality Controls and traceable calibrators	Ensure precision, accuracy, and analytical robustness for each analytical run
External assessment	Periodic comparison and verification of accuracy against consensus	Commutable control samples provided by a third-party body	Ensure the accuracy and metrological traceability of the result

The objective of participating in EQA programmes is to achieve total quality assurance, aimed at the continuous maintenance of validation and verification of the analytical methods adopted. Pursuing these objectives makes it possible to obtain results that are reliable, technically sound, and legally defensible, in compliance with the applicable international accreditation requirements and standards for clinical and forensic toxicology laboratories.

The laboratory is required to retain documentation relating to IQC and EQA for all analytical lines for at least three years, in order to guarantee the traceability and analytical quality of its performance.

7. REPORTING AND COMMUNICATION OF RESULTS

Reporting, the production of an analytical report containing the results of the tests performed by the laboratory, and the communication of analytical results constitute fundamental stages of the diagnostic process in clinical and forensic toxicology, as they represent the interface between the laboratory and the requesting party.

The report, in the context of the determination of traditional drugs of abuse and NPS in biological matrices, must be drawn up according to criteria of clarity, completeness, and compliance with quality standards. It is essential to distinguish screening results from confirmatory results, to report the methods used, and to indicate the analytical limits of the method. When screening and confirmatory analyses detect the presence of narcotic or psychotropic substances and/or their metabolites at concentrations above the defined analytical thresholds, the report must include identification of the substance(s) detected, the corresponding quantitative results, and the cut-off values applied, in order to guarantee the accuracy, traceability, and evidentiary value of the reported data. In forensic contexts, the document must be verifiable and legally defensible, with full traceability of the procedures adopted.

Results should be communicated within an appropriate timeframe and through secure channels, in compliance with data confidentiality regulations. In the case of positive results or results of particular clinical or medico-legal relevance, prompt transmission is required, accompanied by interpretative notes to facilitate understanding. Delivery may be made on paper to the requesting party (or a delegated person) and, with prior written consent, electronically using systems that guarantee the integrity and security of the document.

CONCLUSIONS

The analytical determination of narcotic and psychotropic substances in biological matrices is not merely a technical procedure: it represents a fundamental tool for the protection of public health and for the administration of justice.

In medico-legal, administrative, and criminal contexts, every piece of data produced should be reliable, accurate, and fully defensible, as it may have direct consequences for clinical decisions, legal proceedings, and individual accountability.

The procedures described in this report aim to promote uniformity, transparency, and quality in the activities of forensic laboratories[4], providing shareable operational procedures in order to guarantee consistent, traceable, and interpretable results.

Rigorous adherence to these principles is not only good practice but also an ethical and professional imperative.

Only through high standards and consolidated procedures is it possible to ensure that analytical data deliver real value, reliability, and forensic evidentiary weight

[4] It should be borne in mind that clinical cases can also become of legal interest (road traffic accidents, workplace accidents, covert administration of substances, etc.); all cases involving the search for xenobiotics should therefore be treated as potential forensic cases

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APPENDIX A
RECOMMENDED STANDARD OPERATING PROCEDURES

Introduction

These SOPs provide practical examples for the qualitative and quantitative analysis of psychoactive substances in various biological matrices, including blood, saliva, urine, and keratin matrices, and outline specific protocols applicable in clinical and forensic settings.

These SOPs are intended as guidance and should be adapted by qualified laboratory personnel according to sample characteristics, available technologies, and operational resources. Any modifications to the proposed protocols, made to meet specific needs, should not compromise the validity of results, which have to always meet requirements of accuracy, reproducibility, and traceability.

Each laboratory is responsible for the validation and/or verification of analytical methods before their routine use. The main objective is to standardize analytical and organizational procedures in order to guarantee results that are reliable and defensible in medico-legal contexts, and to provide methodological tools for clinical, forensic, and research applications

Preliminary Operations

The following operations systematically describe the sample preparation and analysis stages required to perform a qualitative and quantitative analytical method in a biological matrix. These procedures include the preparation of reference solutions and quality control samples, the preparation of matrix-based calibrators, and the analysis of unknown samples, ensuring the traceability, accuracy, and reproducibility of results. Application of the operating conditions described is essential to ensure the reliability of the calibration curve, the correctness of quantification, and the overall validity of the analytical method. In detail:

- Prepare working solutions, matrix-based calibrators, and quality controls (high, medium, and low concentration) from stock solutions.
- Store working solutions at -20 °C until use.
- Prepare a blank sample (biological sample free of the analyte(s) of interest and of the internal standard), a negative sample (biological sample free of the analyte(s) of interest but containing the internal standard), calibrators, and quality controls in the biological matrix according to a procedure that, where possible, mirrors that of the unknown sample.
- Add the internal standard to the blank sample, the negative sample, the calibrators, the quality controls, and the unknown samples in the same amount.
- Perform extraction of the blank sample, the negative sample, the calibrators, the quality controls, and the unknown samples according to a validated procedure.
- Analyse the samples in a single sequence comprising: instrumental blank, matrix blank, negative sample, calibrators in ascending order, quality controls, and, after an instrumental wash to avoid carryover, the unknown samples.
- Record and archive the analytical data in compliance with data integrity requirements.

Determination of Psychoactive Substances in Biological Matrices

Below are recommended SOPs for the determination of psychoactive substances in different biological matrices, developed on the basis of scientific evidence and good practice in toxicology.

The flow charts attached to Appendix A summarize, in an operational format, an example of the main stages of the analytical procedures used for the determination of psychoactive substances in the various biological matrices. Each flow chart schematically represents the essential steps of the analytical workflow, including sample pretreatment, extraction, any derivatization, resuspension, and final instrumental analysis. The aim is to provide operators with an immediate and easy-to-consult support tool, useful for quickly identifying the sequence of operations and the critical points of the process, in order to guarantee procedural uniformity, reproducibility of results, and traceability of the entire analytical pathway.

Emphasis is placed on NPS, an extremely heterogeneous group comprising numerous chemical classes. The rapid evolution of this phenomenon, the ease of structural modification, and the continuous introduction of new molecules onto the market make NPS particularly difficult to detect using conventional analytical methods. This context requires the adoption of highly sensitive and selective protocols, the constant updating of spectral libraries, and the use of advanced chromatographic and spectrometric techniques, often indispensable for the identification of substances not yet fully characterized from a chemical and toxicological standpoint.

Finally, periodic consultation of the national and international scientific literature is recommended in order to ensure continuous updating of knowledge on the chemical characteristics, analytical challenges, and most appropriate methodologies for the determination of drugs of abuse and NPS (1-27).

Quantitative Data Analysis

Quantitative data analysis makes it possible to process the instrumental signals obtained during analysis in order to accurately determine the concentration of analytes in the samples examined. This process is based on the construction and validation of the

calibration curve, the application of appropriate regression models, and verification of specific acceptability criteria, which are essential to guarantee the reliability and robustness of analytical results.

a) Construction of the calibration curve:
   - Plot a graph with the calculated concentrations of the calibrators on the X-axis and the ratio between the analytical signal of each analyte and that of the corresponding internal standard on the Y-axis.
   - Construct a separate calibration curve for each analyte under study.
   - Assess the most appropriate regression model in order to obtain a linear relationship between concentration and instrumental response.
   - Determine the equation of the calibration curve for each analyte.

b) Quantification of analytes using the calibration curve:
   - Determine the concentration of the analyte(s) of interest in unknown samples by applying the equation of the corresponding calibration curve.

c) Verification and validation of analytical results. For results to be considered valid, it is necessary to ascertain:
   - The presence of the internal standard in all samples analysed.
   - The absence of the analytes in the negative sample(s).
   - The presence and correct concentration of all analytes in the calibrators and quality control samples.

d) Sample can be considered positive for a specific analyte only if the following criteria are met:
   - Presence of a chromatographic peak for each of the selected ions at the retention time corresponding to that observed in the calibrators, with a tolerance of ± 0.5%.
   - Analytical signal above the LOD for qualitative analysis or the LOQ for quantitative analysis of the method.
   - Correspondence of the ratio between the analytical signals of the selected ions for each analyte with those obtained in the calibrators or quality control samples, within a tolerance of ± 20%.

For further details, reference should be made to COMMISSION DECISION 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (21).

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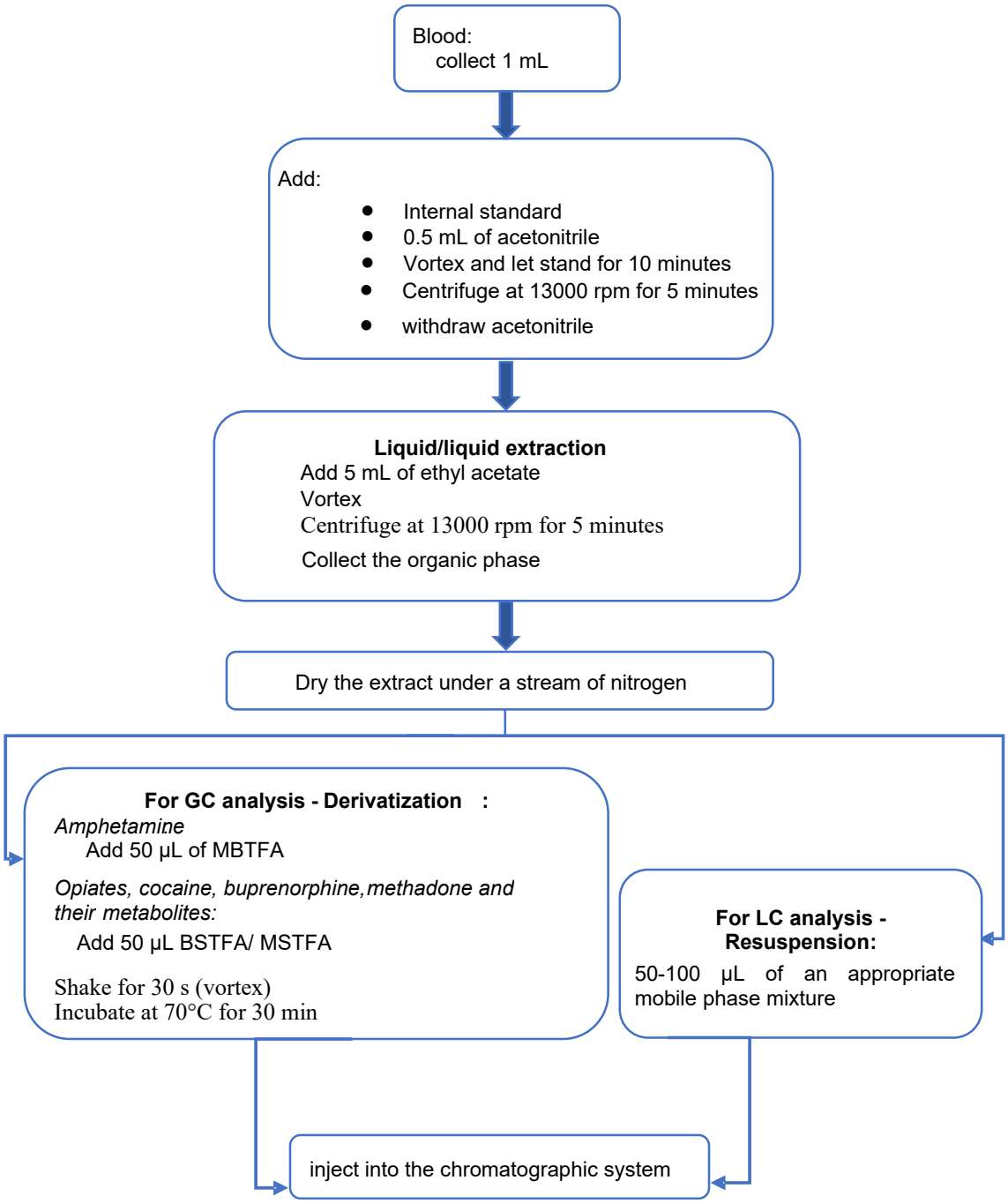
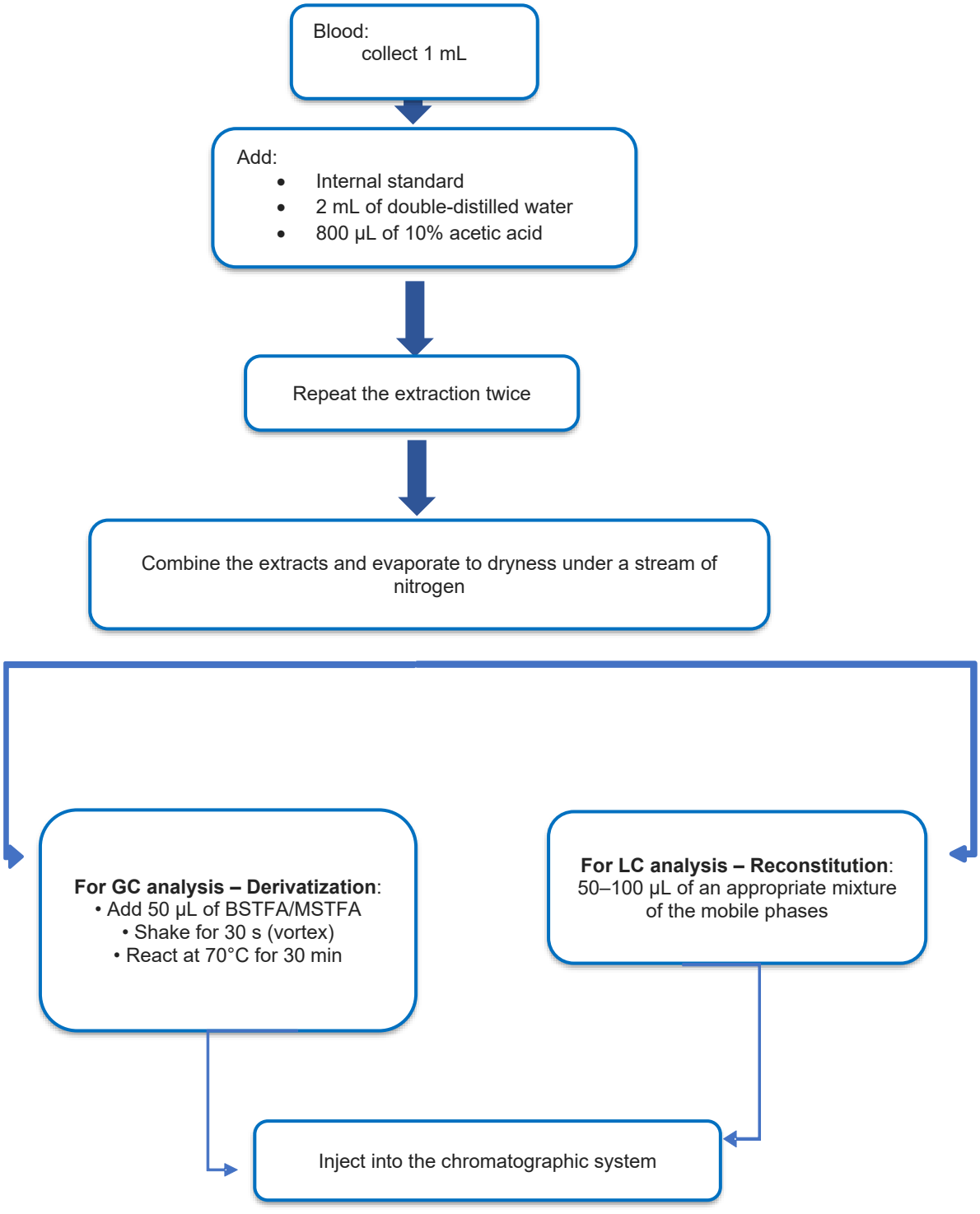
Annex A1

Example of the determination of opiates, cocaine, buprenorphine, methadone, amphetamines and their metabolites in blood



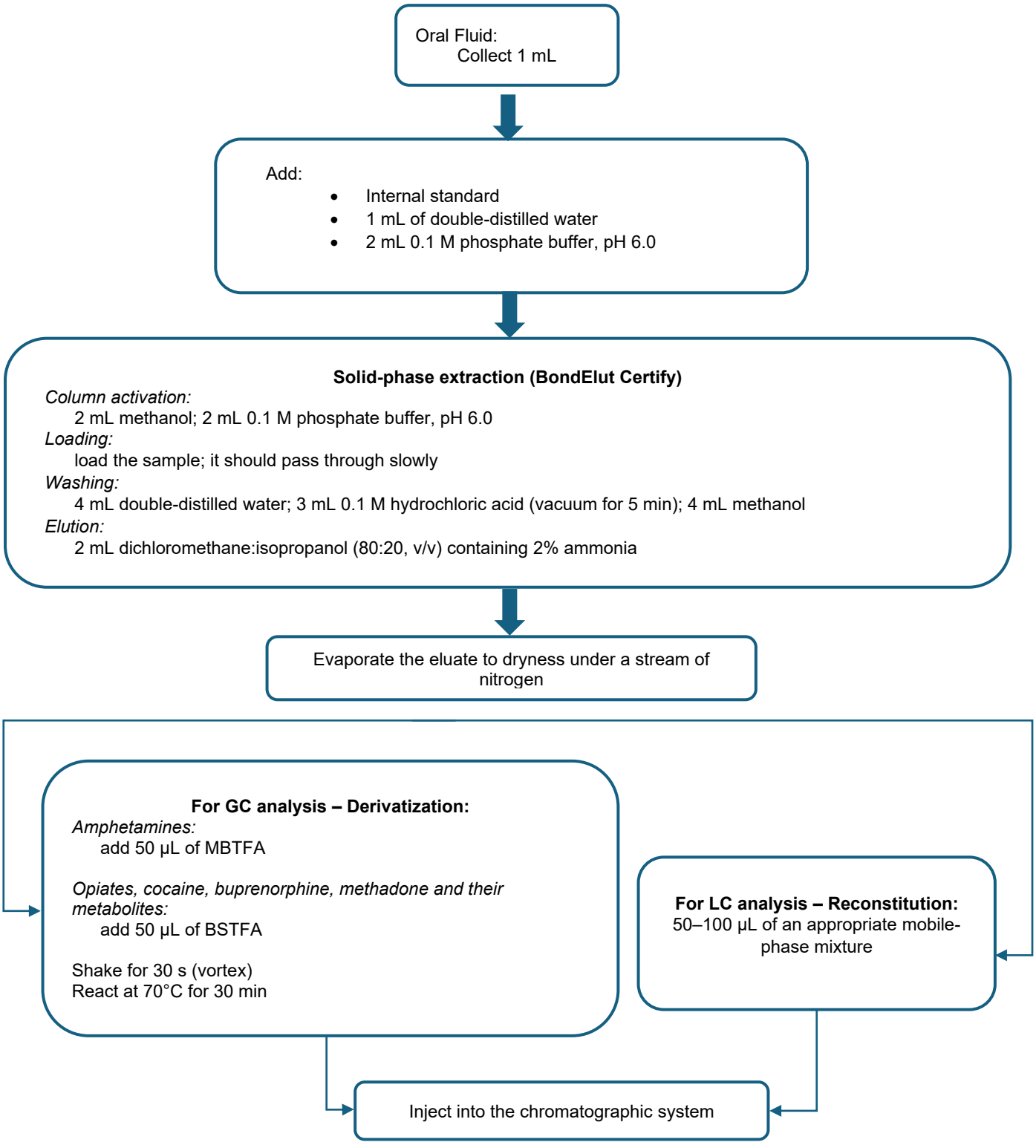
Annex A2

Example of the determination of delta-9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and 10-hydroxy-delta-9-tetrahydrocannabinol (10-OH THC) in blood



Annex A3

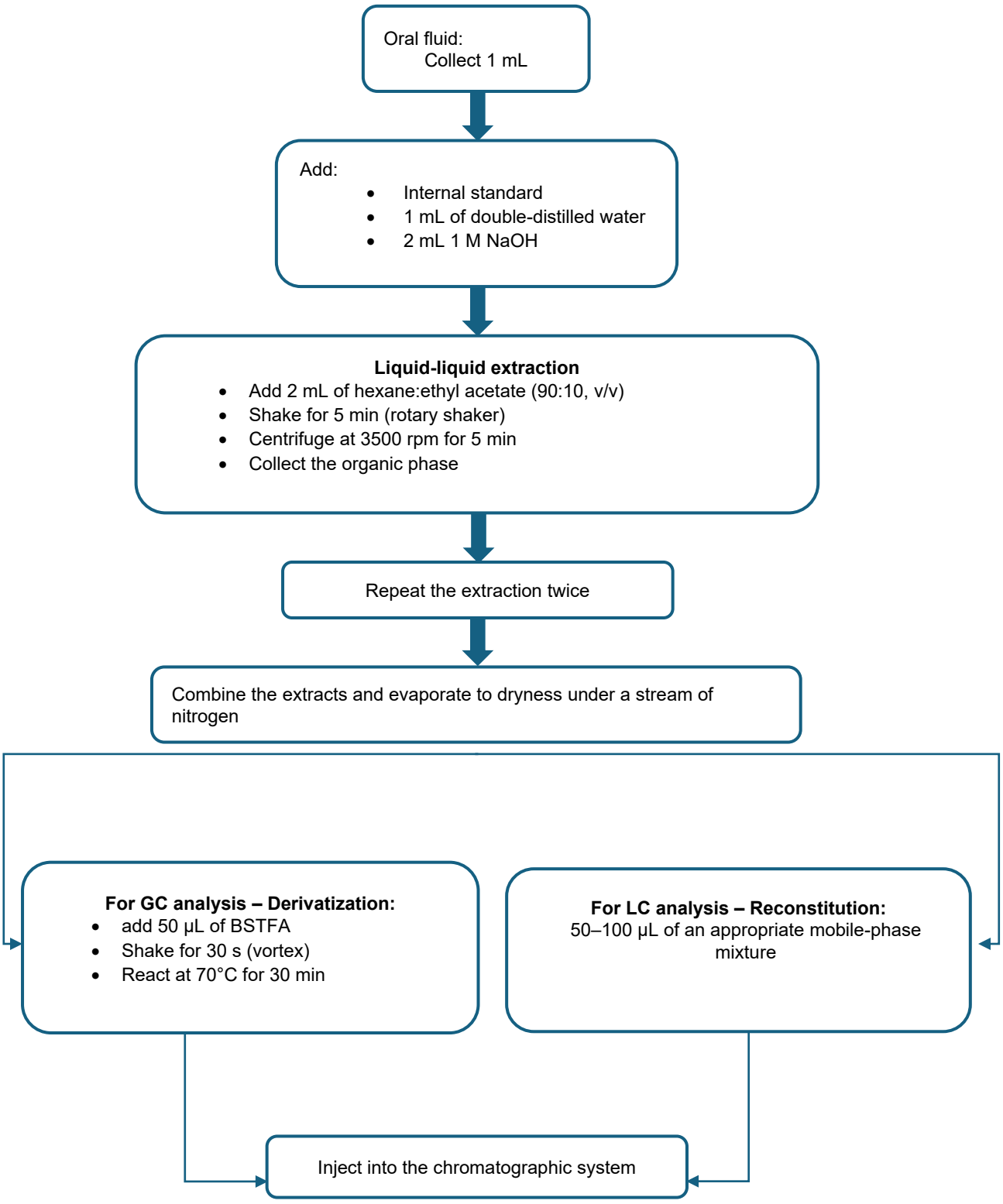
Example of the determination of amphetamines, opiates, cocaine, buprenorphine, methadone and their metabolites in oral fluid*



* LC-based analytical procedures currently use the "dilute and shoot" approach, which involves simple dilution of the sample followed by direct injection into the instrumental system.

Annex A4

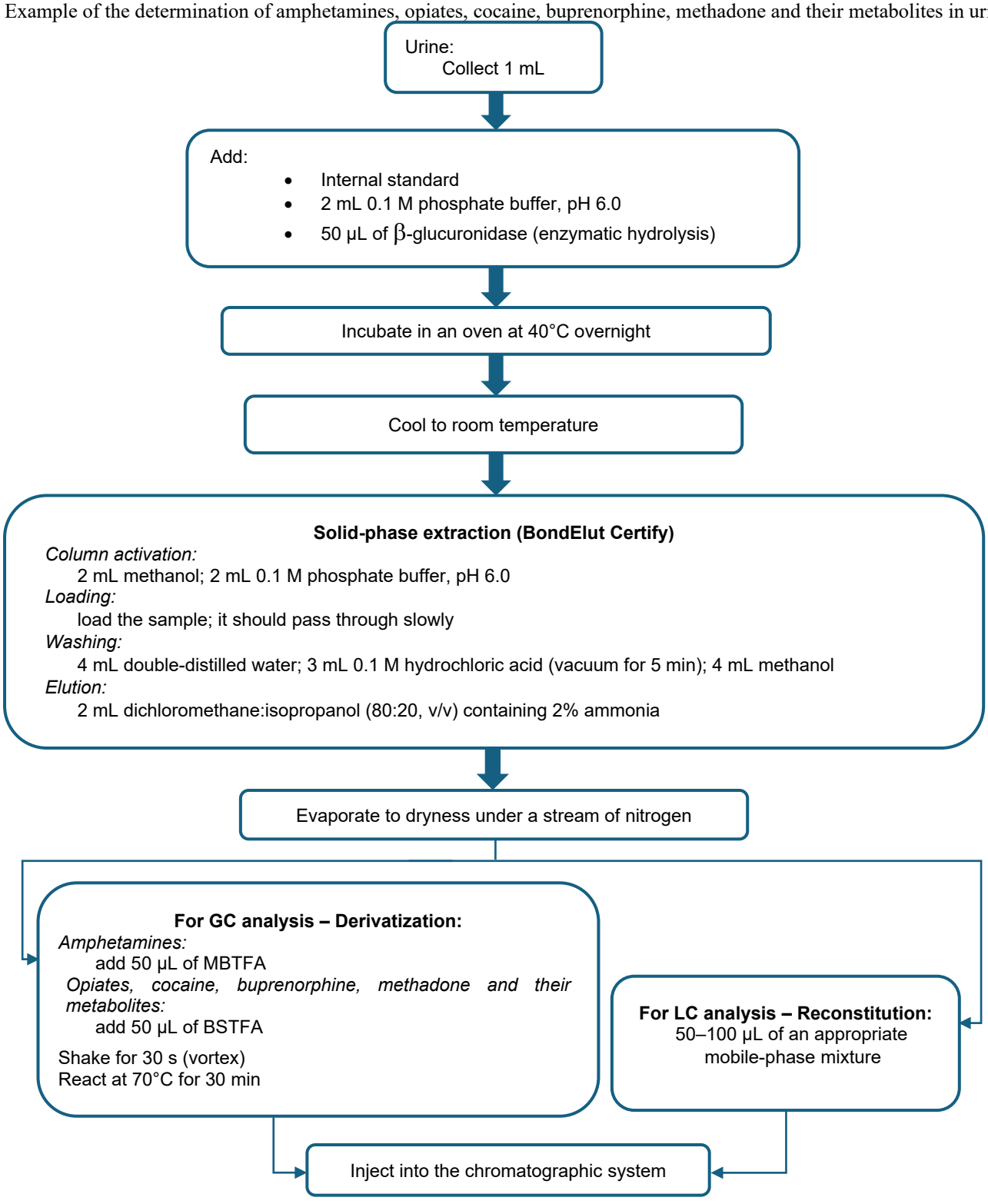
Example of the determination of THC in oral fluid*



* LC-based analytical procedures currently use the "dilute and shoot" approach, which involves simple dilution of the sample followed by direct injection into the instrumental system.

Annex A5

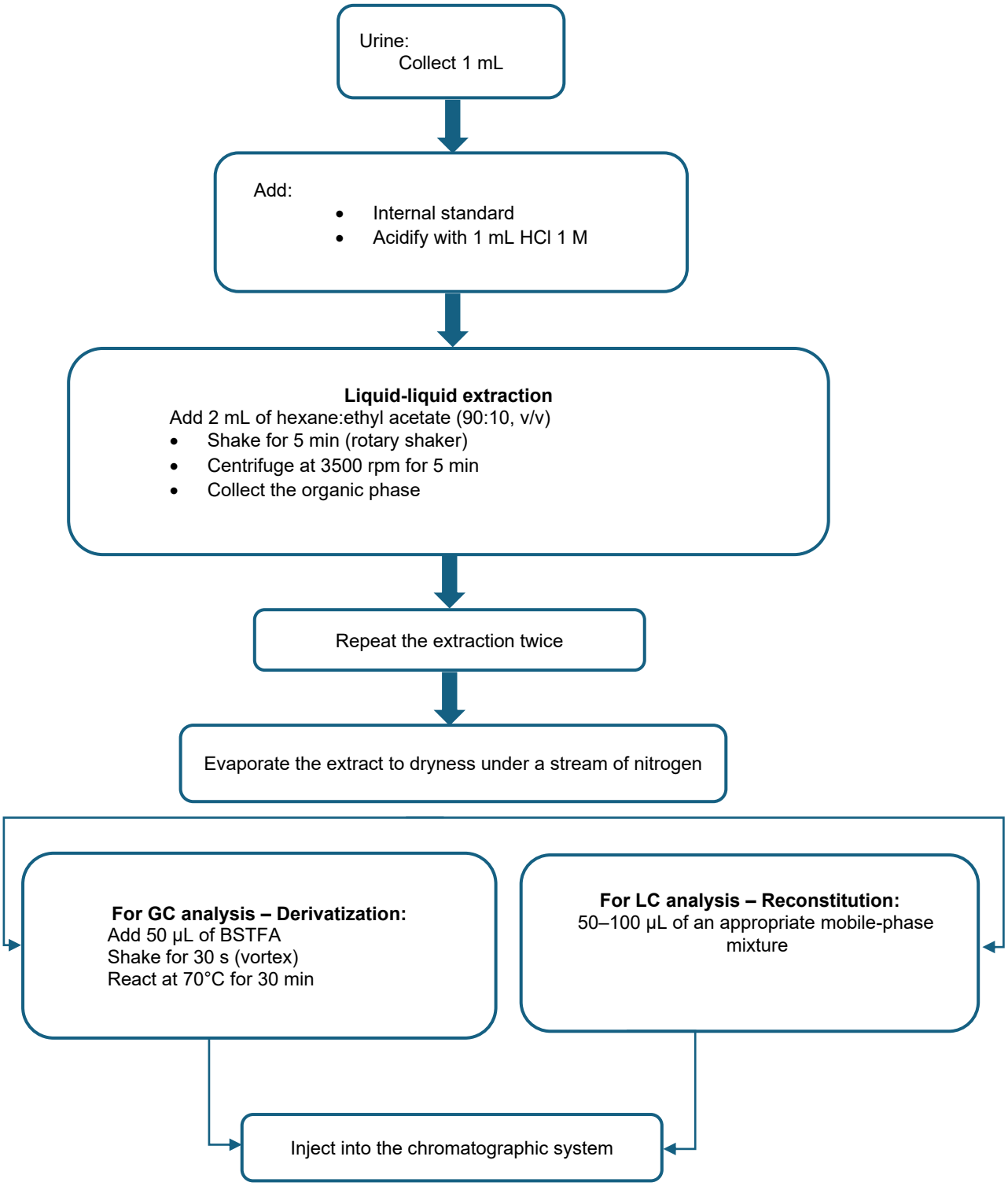
Example of the determination of amphetamines, opiates, cocaine, buprenorphine, methadone and their metabolites in urine *



* LC-based analytical procedures currently use the "dilute and shoot" approach, which involves simple dilution of the sample followed by direct injection into the instrumental system.

Annex A6

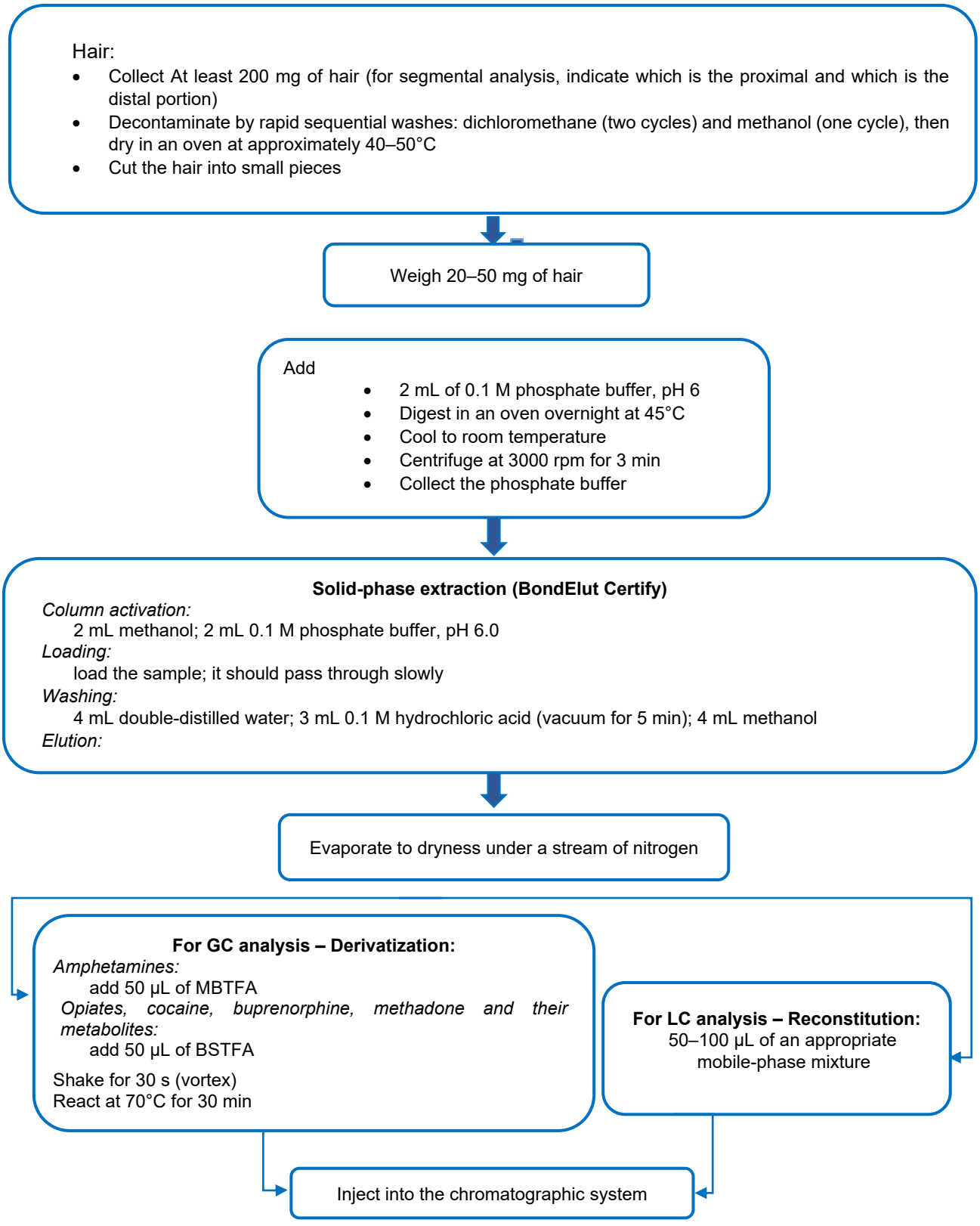
Example of the determination of THC-COOH in urine*



* LC-based analytical procedures currently use the "dilute and shoot" approach, which involves simple dilution of the sample followed by direct injection into the instrumental system.

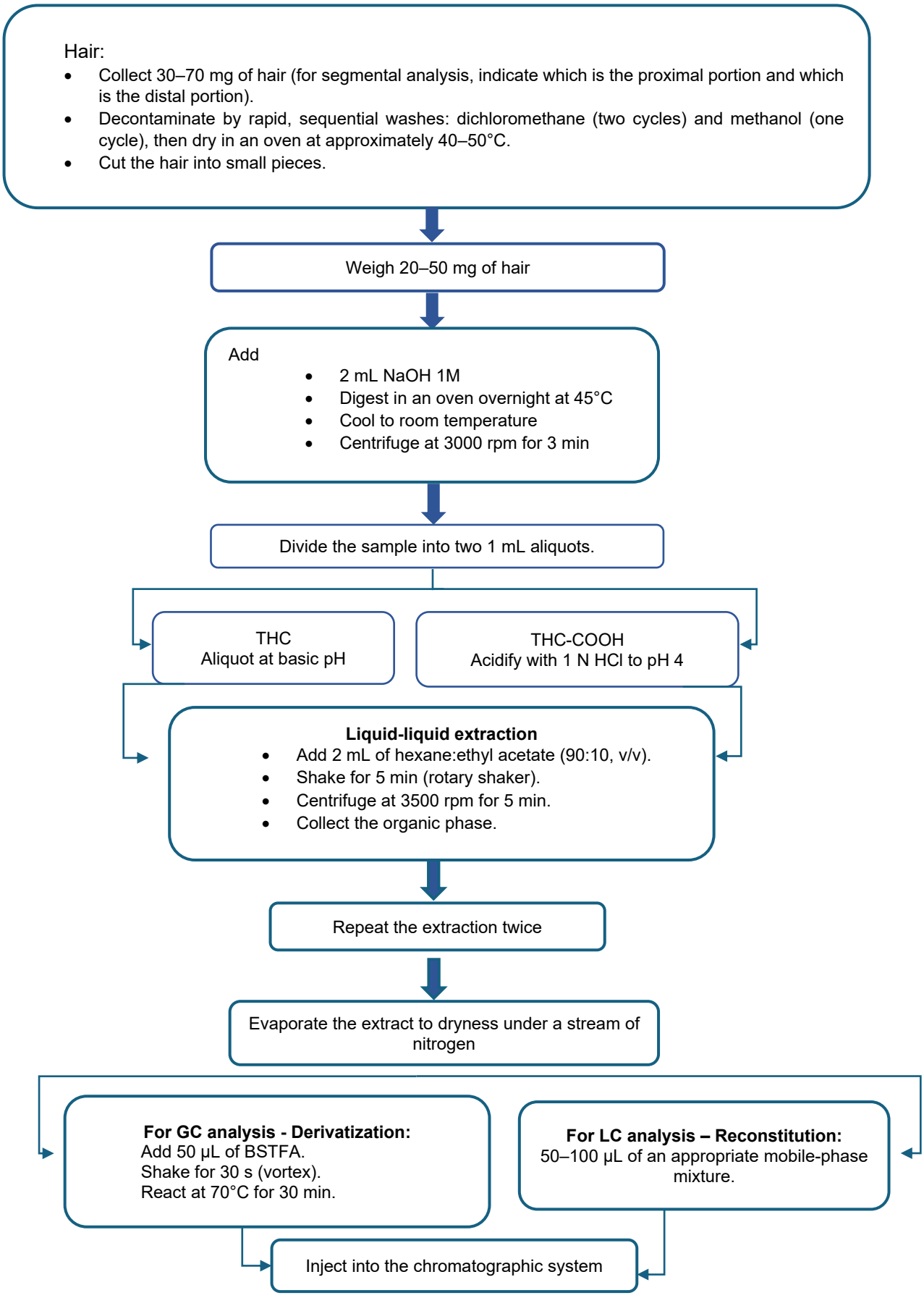
Annex A7

Example of the determination of amphetamines, opiates, cocaine, buprenorphine, methadone and their metabolites in hair



Annex A8

Example of the determination of THC, THC-COOH and 11-OH THC in hair



APPENDIX B

Examples of forms

B1. Example of informed consent form

INFORMED CONSENT FORM
Determination of Drugs of Abuse and New Psychoactive Substances (NPS) in Biological Matrices

Facility/Laboratory Information:
Nome: _____
Indirizzo: _____
Telefono/E-mail: _____

Data Subject Information
Full Name: _____
Date of Birth: _____
Identity Document (type and number): _____
Address: _____
E-mail: _____

Purpose of the Examination
The requested examination involves the analysis of one or more biological matrices (hair, urine, blood, saliva, others) in order to identify the possible presence of drugs of abuse and/or new psychoactive substances (NPS).
This examination carried out for the following purposes:

- □ Clinical
- □ Diagnostic
- □ Forensic / Medico-legal
- □ Administrative / Employment-related
- □ Other (please specify): _____

Procedure Description
Sample collection will be performed by qualified healthcare personnel in compliance with good clinical practice standards and health and safety regulations. The amount of sample collected will be limited to the minimum necessary to perform the analyses.
All samples will be labeled, registered, and stored according to procedures designed to ensure their integrity, traceability, and security.

Possible Risks and Discomforts
The risks associated with the procedure are minimal:

- In the case of blood collection: mild pain, redness, or a small hematoma at the puncture site;
- In the case of hair collection: no significant risk;
- In the case of urine or saliva collection: no risk.

Confidentiality and Data Processing
Personal data and analytical results will be processed in compliance with the applicable legislation governing personal data protection (European Regulation EU 2016/679 - GDPR) and will be used exclusively for the purposes indicated above. They may also be disclosed to competent authorities where required by law.

Consent

- The undersigned declares:
- to have read the privacy notice regarding the processing of personal data (pursuant to Article 13 of Regulation (EU) 2016/679 of the European Parliament and of the Council), to have understood its content, and to have been given adequate time for consideration before expressing this declaration;
- to have been informed in a clear and comprehensible manner about the purposes, procedures, and risks associated with the examination;
- to understand that refusal to provide the sample may result in legal or administrative consequences in cases provided for by applicable legislation.

and voluntarily consents to:
□ the collection and analysis of biological samples;
□ the storage of the sample for the period necessary to complete analytical verification procedures;
□ the communication of results to the authorized entities/personnel indicated.

Signature of the Subject
I declare that I have carefully read this form, received satisfactory explanations, and fully understand its contents.
Signature: _____
Date: ___ / ___ / __

In the Case of a Minor or Legally Disabled Person
I_____, acting as (parent/legal guardian/support administrator), hereby consent to the collection of a biological sample from the minor/disable person _____ for the purposes described above.
Signature: _____
Date: ___ / ___

Signature of the Authorized Operator
Name and Surname:
_____
_____
Signature:
_____
_____ Date: ___ / ___ / __

B2. Example of a privacy information and consent form

PRIVACY NOTICE AND INFORMED CONSENT FORM
Detection of Drugs of Abuse and Novel Psychoactive Substances (NPS) in Biological Matrices

Data subject information:
Full name: _____
Address: _____
Telephone/E-mail: _____

Purpose of examination:
□ Clinical and diagnostic testing
□ Medico-legal investigations
□ Authorized scientific research
□ Health monitoring

Personal Data:
Data subject information

- Full name: _____

Biological Specimens Collected:

- □ Blood
- □ Oral Fluid
- □ Urine
- □ Keratinized matrices (hair and/or nails)
- □ Other (please specify):

Data Processing:
Personal data and biological sample data will be processed in either paper-based or electronic format by authorized personnel, ensuring confidentiality, integrity, and security.

Disclosure of Data:
Data may be disclosed solely to authorized healthcare professionals, accredited laboratories, or competent health authorities, where required by applicable law.

Data Retention:
Personal data and related analytical results will be retained in accordance with current legal and regulatory requirements and only for the period necessary to fulfill the purposes for which they were collected.

Rights of the Data Subject:
The subject has the right to access, rectify, erase, restrict, or object to the processing of personal data, as well as the right to data portability and to withdraw consent at any time, without affecting the lawfulness of processing carried out prior to withdrawal.

Consent:
I hereby declare that I have received, read, and understood the Privacy Notice. I voluntarily consent to the collection and analysis of my biological specimens and to the processing of my personal data for the purposes specified above.

☐ Yes, I consent ☐ No, I do not consent

Place and Date: _____
Subject's Signature: _____
Signature of Responsible Operator: _____

B3. Example of biological sample collection report

SAMPLE COLLECTION FORM
Determination of Drugs of Abuse and New Psychoactive Substances (NPS) in Biological Matrices

Facility/Laboratory Information:
Name: _____
Address: _____
Telephone/E-mail: _____

Data Subject Information
Full Name: _____
Date of Birth: _____
Identity Document (type and number): _____
Address: _____
E-mail: _____

Type of Matrix Collected

□ Blo
Tube type: □ EDTA □ Heparin □ Fluoride/oxalate □ Other
Collected volume: _____ mL
Time: _____
Date: _____

□ Urine
Collected volume: _____ mL
Temperature upon delivery: _____ °C
Time:
Date:

□ Saliva
Device used: _____
Time: _____
Date: _____

□ Hair
Collection site: _____
Sample length: _____ cm
Approximate quantity (weight/locks): _____
Time: _____
Date: _____

Any anomalies observed: _____

Storage and Transport

Sample / Label Number:
_____

Destination laboratory:
_____
_____

Storage method:
□ Room temperature
□ Refrigerated (4 °C)
□ Other:
_____
_____

Additional notes:
_____
_____

Signature of the Subject

I declare that the sample collection was carried out in my presence and that the information provided is true and correct.
Signature of the Subject: _____
Date: ___ / ___ / __

In the Case of a Minor or Legally Disabled Person
I, _____, acting as (parent/legal guardian/support administrator), declare that the sample collection from the minor/legally disabled person was carried out in my presence and that the information provided is true and correct.
Signature of the Legal Representative:
_____
Date: ___ / ___ / __

Signature of the Authorized Operator

I, the operator authorized to collect the sample, declare that the sample was collected according to standardized procedures, ensuring traceability, integrity, and chain of custody.
Name and Surname: _____
Operator's Signature: _____ Date: ___ / ___ / ___

B4. Example of Chain of Custody Form

CHAIN OF CUSTODY FORM
Determination of Drugs of Abuse and New Psychoactive Substances (NPS) in Biological Matrices

Type of Examination:
_____

Type of Matrix:
□ Blood □ Urine □ Saliva □ Hair □ Other: _____
Unique Sample Code:
_____

Date and Time of Collection: ___ / ___ / _____ _____ :
Condition of the Sample at Collection:
□ Intact □ Not intact (specify): _____

Packaging:
□ Sterile container □ Paper bag □ Aluminum foil □ Other: _____

Activity	Date and Time	Delivery (name, signature)	Received (name, signature)	Sample Condition	Notes
Collection → Delivery 1					
Delivery 1 → Delivery 2					
Delivery 2 → Laboratory					
Internal Analysis					
Storage/Archiving					
Disposal (if applicable)					
Transport					

Transport method:

□ Manual □ Courier □ Cold chain □ Other: _____

Transport temperature (if applicable): _____ °C

Any anomalies: _____
Additional notes:
_____
____

Signature of Final Responsible Operator

I declare that all operations relating to the sample were carried out in compliance with the procedures and that traceability was ensured at every stage.

Name and Surname: _____

Operator's Signature: _____ Date: ___ / ___ / ___

B5. Example of a reporting form

ANALYTICAL REPORT FORM
Determination of Drugs of Abuse and New Psychoactive Substances (NPS) in Biological Matrices

Facility/Laboratory Information:
Name: _____
Address: _____
Laboratory Manager: _____
Telephone/E-mail: _____

Data Subject Information
Full Name: _____
Date of Birth: _____
Identity Document (type and number): _____
Address: _____
E-mail: _____

Type of Sample Analyzed
□ Urine □ Blood □ Saliva □ Hair □ Sweat □ Other: _____

Sample Code: _____

Date and Time Received at Laboratory: ___ / ___ / _____ _____ : _____

Date and Time of Collection: ___ / ___ / _____ _____ : ____

Analytical Methodology
Technique Used:
□ LC–MS/MS □ GC–MS □ GC–FID □ Immunochemistry □ Other: _____
Validated Method No.: _____

Limit of Detection (LOD): _____
Limit of Quantification (LOQ): _____

Analytes Tested
(Complete list or select categories)
□ Opioids
□ Cocaine and metabolites
□ Amphetamines / Methamphetamines
□ Cannabis (THC, THCA, metabolites)
□ Benzodiazepines
□ Alcohol / EtG / EtS
□ New Psychoactive Substances (NPS): _____
□ Other: _____

Analysis Results
(Indicate concentrations, units of measurement, or qualitative result) Preliminary interpretation:

Analyte	Result	Reference Value / Cut-off	Unit of Measurement	Measurement Uncertainty

Interpretation and Conclusions
Clearly indicate the outcome of the analysis.
□ Negative result: no substance detected above the cut-off.
□ Positive result: presence of _____
• Detected levels consistent with _____
□ Result cannot be interpreted due to:
□ Insufficient sample
□ Sample degradation
□ Analytical interferences
□ Other: _____

Signature of the Authorized Operator
I, the operator authorized to collect the sample, declare that the sample was collected according to standardized procedures, ensuring traceability, integrity, and chain of custody.
Name and Surname: _____
Operator's Signature: _____ Date: ___ / ___ / ___