

La Lettre

LABORATORY DETECTION OF ADDICTIVE BEHAVIOR



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In the same way that a slave was ad dictus, from the Latin ad-dicere, i.e. 'spoken to' his master, in the sense of attributed or given to, we may consider that an alcoholic is given to alcohol, a smoker to tobacco or a drug addict to a drug. Addictive behavior is not always associated with a xenobiotic substance but also describes certain behavioral patterns such as disorders of dietary behavior, a passion for gambling (and particularly for games of pure chance) and, why not, an excessive commitment to sexuality or work. An addiction is thus defined both with respect to the nature of the object coveted and by the intensity of the bond between it and the subject: a simple weakness or attachment, an ingrained habit, or a compulsive and irresistible craving.

In medical terms, pharmacological dependence (psychological and sometimes physical) is most frequently accompanied by acquired tolerance.

ADDICTIVE BEHAVIOR

The medical laboratory is currently, more frequently than in the past, requested to screen for licit or illicit substances under various circumstances. Screening consists in testing for the incriminated product or its metabolites in a biological medium or investigating for a biological indicator reflecting abuse (chronic alcoholism).

Screening for chronic alcoholism and monitoring withdrawal are prescribed under various circumstances involving vehicle driving (road accidents, driving license withdrawal, monitoring compliance with withdrawal treatment, etc.).

Investigating for smoking as a major cardiovascular risk factor has become fundamental for the medical commissions of banks and insurance companies. Testing for passive smoking may sometimes be indicated in asthmatic children.

Screening for narcotics in the context of occupational health may be prescribed to detect

addictive behavior in subjects with safety and/or security responsibilities. Screening is conducted on hiring, after having informed the subject of the type of test to be conducted, and also, in certain circumstances, without prior warning. Testing is obviously prescribed by methadone centers to check compliance with the replacement treatment and the discontinuation of opioid use. It also measures the impact of the residual multiple-drug addiction. Lastly, screening has become increasingly frequent in minors at the request of parents who are worried by the increase in the prevalence of drug abuse in recent years (strong media impact of newspapers, television, etc.).

At epidemiological level, some recent figures collected by the French Drug and Drug Addiction Monitoring Organization (OFDT) show that, in France, alcohol is consumed by 8 million people, 12 million people smoke, psychotropic drugs are taken by 2 million people and cannabis by 350,000 people daily and 3 million occasionally.

Cocaine and ecstasy are consumed by 150,000 people occasionally and opioids by 150,000 to 200,000. Opioid-dependence treatments have been prescribed for 75,000 patients (buprenorphine) and 15,000 patients (methadone).

The gradual change in the status of drugs from socially unacceptable to increasingly socially acceptable (increased consumption by adolescents, legalization of cannabis in certain countries), is tending to make drug screening a common occurrence. Today, it may be said, almost without risk, that nothing distinguishes the patient attending the laboratory for medical tests from the patient referred to the laboratory for illicit drug screening.

In the relationship which unites the subject, his/her environment and the substance consumed there is still the risk that, depending on the circumstances, the switch from recreational use to abuse, then harmful abuse (dependence, acquired tolerance, dose escalation) may result in social exclusion and a marginal lifestyle.

Dependence is a compulsive behavior pattern consisting in searching for and consuming the drug. Dependence may be psychological (habituation) and/or physical but, in reality, that distinction is little justified since the state of craving is not fatal. Acquired tolerance gives rise to the need to escalate doses to procure the same effects. The tolerance breaks down during drug withdrawal, sometimes giving rise to overdoses if consumption is resumed at the dosage used before withdrawal.

The type of product used largely depends on the circumstances of use: ecstasy and crack use is more to be found in party settings (raves, night clubs) where the drug is used to resist tiredness and sleep. Opioid use characterizes an individual search for isolation.

In contrast, cannabis seems to be used under many circumstances.

WHICH SPECIMEN?

Urine is the most appropriate biological matrix for evidencing regular or occasional use of a narcotic or psychotropic agent. Urine has several advantages as a screening medium: the specimen is obtained non-invasively and the window of detection is larger than that for blood. For that reason, there is a greater variety of commercially available reagents for that matrix. Reagents are available for solid media (enabling immunochromatographic screening for 1 to 10 different substances on a given medium) and for automated immunoassays in liquid media. However, a few disadvantages inherent to the medium and immunological methods necessitate identifying the drug or confirming positive results using a 'reference' physicochemical method.

Since metabolism or uptake rapidly clears substances from the blood (or serum), the latter is only of value in correlating a clinical state or behavior that is addictive at the time of sampling or in the few hours immediately preceding sampling.

Screening tests using alternative biological matrices to increase the window of detection (hair) or facilitate sampling, sample shipment or testing by non-medical personnel (sweat, saliva) are now also available.

Hair takes up xenobiotic substances and, in particular, narcotics. Testing on hair increases the window of detection (growth of about 1 cm/month). It is, in particular, possible to determine a consumption profile (compliance with withdrawal), and distinguish between a single dose and regular use.

SPECIAL FEATURES OF URINE COLLECTION

In order to avoid disputes with patients who wish to conceal a positive result, a certain number of precautions may be taken with the aim of avoiding cheating.

Urination is to take place in the laboratory in a toilet with no water source. The water in the toilet itself is dyed with an additive.

The temperature of urine immediately after emission is greater than 30°C.

Creatinine determination may be of value since, if the result is < 0.3 g/L, the urine is excessively dilute (excessive intake of beverages in the hour preceding urination). Reservations are then to be formulated with respect to drug screening results.

Reagent sticks are also available to determine density, pH and creatinine, and to detect the presence of exogenous adulterants such as nitrites, aldehydes and oxidation products.

If the urine density determination yields a result < 1.005, the specimen may have been diluted with a liquid after urination. Similarly, a pH result outside of the range 6.5 to 7.5 may result from addition of an exogenous substance post-urination with a view to falsifying and/or disturbing an illicit drug screen.

AUTOMATED ASSAY METHODS

The automated immunoassays in liquid media used in laboratories are frequently dedicated to a specific equipment:

The reagents available only enable screening by drug type, i.e. molecular series. Reagents for



Suppliers	Method	Equipment
Abbott Diagnostics	FPIA = Fluorescence Polarization immunoassay	AxSYM, TDx, FLx
Beckman Coulter France	EMIT-DAT = homogeneous phase immunoenzymatic method	Synchron CX and LX20
Dade Behring SA	EMIT = enzyme-multiplied immuno technique	EMIT II reagents may be used on various autoanalyzers
Microgenics	CEDIA = cloned-enzyme donor immunoassay	Hitachi and rela- ted analyzers
Roche Diagnostics	KIMS = kinetic interaction of microparticles in solution	Integra range autoanalyzers

* * from: "Dépistage des stupéfiants par immunoanalyse en milieu liquide". - Spectra Biologie 2004 ; No. 140:49-56

amphetamines and amphetamine derivatives, opioids, cannabinoids, cocaine, methadone, phencyclidine, LSD, buprenorphine and propoxyphene are the most widely used. The reference molecules for those reagents (used for calibration and determination of the positive cutoff) are parent molecules such as delta-amphetamine, morphine, methadone or metabolites such as benzoylecgonine (major metabolite of cocaine) or d-9-THC-COOH (major metabolite of tetrahydrocannabinol).

Opioid screening reagents enable detection of natural opium alkaloids (morphine, codeine) and semi-synthetic opioids (heroin and its metabolite monoacetylmorphine, pholcodine, codethyline). They do not enable detection of the synthetic opioids used in analgesic therapy or the replacement products used to treat opioid dependence such as bupre-

norphine and methadone. A positive screening result must necessarily be followed by identification of the substance or substances present in order to relate the presence to medical treatment (pholcodine, codethyline, codeine) or illicit drug use (heroin).

The **cocaine** screening reagents enable detection of benzoylecgonine, the most abundant urinary metabolite. The reagents used to screen for **cannabis** also detect the presence of the major metabolite, THC-COOH. A positive result gives no information on how the drug is consumed (cannabis smoked or swallowed and cocaine snorted or smoked).

The amphetamine screening reagents, in most cases, enable detection of **amphetamine**, **methamphetamine** and **ecstasy** (MDMA). It is indispensable to confirm a positive result using a

physicochemical method, due to the numerous cross-reactions that have been demonstrated or that are potential. The cross-reactions differ depending on the reagents used.

The detection cutoffs recommended by the suppliers are a compromise between a minimum number of false-negative and false-positive results. The lower the cutoff, the lower the risk of not detecting addictive behavior but the higher the number of false-positives requiring confirmation.

Opioids	300 ng/mL
Amphetamines	500 - 1000 ng/mL
Cocaine	300 ng/mL
Cannabinoids	50 - 100 ng/mL
Methadone	250 - 300 ng/mL
LSD	0.5 ng/mL
Phencyclidine	25 ng/mL

The confirmation of a positive result by a physicochemical method (gas or liquid chromatography, usually with mass spectrometry) is always to be recommended since screening only consists in use of an antibody. For positive results with opioids, the reference method consists in an identification rather than a confirmation, since result interpretation is modulated by the type of drug detected.

CHROMATOGRAPHIC METHODS FOR CONFIRMATION AND IDENTIFICATION

The physicochemical methods employ, in succession, chromatographic separation and detection of the substances using UV spec-



*Gas chromatography - mass spectrometry (GC-MS) system
Photograph taken at Laboratoire Pasteur Cerba*

trophotometry or mass spectrometry. These methods thus include high performance liquid chromatography with diode-array UV detection (HPLC-DAD), gas chromatography with mass spectrometry (GC-MS), the most widespread, and an increasing number of applications in which liquid chromatography is used with tandem mass spectrometry (LC-MSMS). These methods (except LC-MSMS) require pre-analytical stages of various lengths (hydrolysis of the urine, liquid/liquid or liquid/solid extraction, derivatization, etc.), a higher sample volume than that used in immunoassay and a complex and expensive system. These methods are therefore only envisaged in the second line to confirm a positive screening result obtained using qualitative immunoassay.

Using GC-MS, it is possible to identify and quantify the following

opioids:

- morphine
- codeine
- codethyline
- pholcodine
- 6-monoacetylmorphine (6-MAM)

Only the presence of 6-MAM, the metabolite of heroin, enables

confirmation of heroin (diacetylmorphine) use. Morphine detected alone may derive from therapeutic morphine, opium use or codeine (or even pholcodine) metabolism. If codeine is identified, its presence does not supply information on the quantities used or on the frequency of use (as an antitussive or as a heroin replace-

ment). Codeine is a licit substance. Pholcodine detected alone or in the presence of a small quantity of morphine confirms therapeutic use. GC-MS, following a positive urinary screen for **benzoylecgonine**, enables identification and quantitation of benzoylecgonine as the main metabolite, ecgonine methyl ester, as another metabolite, and, sometimes, cocaethylene when cocaine and alcohol were used concomitantly. In contrast, while cocaine use in the form of crack induces formation of anhydroecgonine, the product is very rapidly hydrolyzed in vitro and can no longer be detected.

Quantitation of **THC-COOH** in a urine sample screening positively for cannabis confirms active consumption of a cannabinoid product. A certain number of subjects screening positively argue that they have been contaminated by passive inhalation in the presence of people smoking cannabis. Studies conducted by American scientists have shown that a level greater than 10 ng/mL does not occur in the context of passive smoking.



*Liquid chromatography - tandem mass spectrometry system (LC-MSMS).
Photograph taken at Laboratoire Pasteur Cerba.*

The identification of **amphetamines** using GC-MS on a positively screened sample enables identification of the currently most widely used derivatives: amphetamine, methamphetamine, ecstasy (MDMA) and its metabolite MDA, MDEA, MBDB, etc. Customs and police seizures in recent years have shown the development of a large number of synthetic 'designer drugs' in the amphetamine series for which laboratories do not necessarily have a reference product enabling identification and quantitation.

CANNABIS

Cannabis is the most widely used illicit drug. Surveys of middle-school and high-school students (source: OFDT) show that at age 18, 2 boys out of 3 and half of the girls have already experimented (at least once) and that 1 boy out of 5 and 1 girl out of 15 regularly uses cannabis (at least 10 times per month). Among the numerous derivatives extracted from cannabis resin (some 60 cannabinoids), only d-9-tetrahydrocannabinol (THC) is recognized as the main psychoactive agent. THC is classified as a psychodysleptic substance (i.e. which disturbs the central nervous system) as are hallucinogens, volatile solvents and ethanol.

THC content varies depending of the source of the plant and the type of preparation:

- the powdered and dried leaves, stems and seeds constitute 'grass' (or 'marijuana', 'weed', 'pot') and contain less than 5% THC for the indica variety and less than 0.5% for hemp;

- threshing the dry flower heads and leaves yields a greenish brown or black powder that is pressed into slabs or rods and constitutes 'hashish' (or 'hash', 'shit') containing 10 to 20% THC;

- cannabis oil obtained from the resin by extraction in a solvent may contain up to 60% of THC.

Cannabis is generally smoked alone, e.g. marijuana in bongs, or mixed with tobacco ('grass' or 'hash') and smoked in cigarettes ('joint', 'reefer'). Oral cannabis intake following incorporation of the drug in food (baked goods, 'space cakes') is frequent in North Africa.

Following inhalation, pulmonary absorption is fast and the peak plasma concentration occurs in 8 to 10 minutes. THC is very rapidly distributed to many tissues, but, above all, to those with a high lipid content (in particular the brain) where it accumulates. The THC accumulated in fat is gradually released and metabolized by the liver. Thus, as a function of the THC concentration of the product used, frequency of use and quantity of adipose tissue, THC release and ongoing elimination of metabolites in the urine may occur over periods from one to several weeks.

The liver rapidly converts THC, mainly to 11-OH-d-9-THC, which is then oxidized to 11-nor-d-9-THC-carboxylic acid (THC-COOH). Approximately half of the dose taken is metabolized by that route. THC-COOH rapidly becomes present in the urine and constitutes the main urinary metabolite. The metabolite is also found in sweat and breast milk.

The psychotropic effects vary between subjects but also in the same subject depending on the psychological state at the time of use and the quantity used:

- at low doses, cannabis has euphoric properties: inebriation, good feeling, enhanced sensory perceptions, distortion of time, etc. This effect rapidly disappears but alertness may be compromised for 24 hours;

- at high doses, cannabis has hallucinogenic properties and may induce depersonalization and panic attacks.

Repeated use of cannabis induces persistent behavioral changes with loss of intellectual and memory faculties, emergence of an amotivational syndrome and impaired psychomotor performance. In certain users, this psychotoxic effect of cannabis may induce a clinical picture of gradual onset similar to a negative-symptom schizophrenic process. Adolescents rendered fragile by a disturbed familial or social environment are the most sensitive. Cannabis use by drivers increases the risk of accidents. Cannabis only induces weak physical dependence.

Screening for cannabis use is currently based on evidencing the metabolite, THC-COOH, using a urine sample. The most frequently proposed positive cutoff (50 ng/mL) rules out passive cannabis smoking, which is frequently cited in attempts to dissimulate active consumption. In the event of a dispute, confirmation of the presence of THC-COOH by a chromatographic method removes any doubt since studies have shown that, in the event of passive smo-

king, urinary concentrations do not exceed 10 ng/mL determined by GC-MS.

OPIOIDS

The term 'opioids' covers a series of products of natural or semi-synthetic origin (opiates) or synthetic origin (opioids) used therapeutically as analgesics and antitussives. Only heroin (diacetylmorphine) is not included in the pharmacopoeia and is an illicit drug. Opioids are psycholeptics or central nervous system depressants.

The main product is morphine. Morphine and other alkaloids such as codeine, thebaine and papaverine are contained in the latex obtained by incising the green capsule of the Indian poppy (*Papaver somniferum*). The latex, when dried in air, constitutes opium.

Codeine (extracted from opium) together with codethyline and pholcodine (semi-synthetic derivatives of morphine base) are used as antitussives in numerous proprietary medicinal products (syrups and tablets) available over the counter and are not subject to prescription.

In contrast, morphine, opium-derived preparations and agonist synthetic derivatives such as pentazocine, pethidine, oxycodone, fentanyl and buprenorphine are used for their analgesic properties in the treatment of pain. They are included in the narcotics list or in list I.

Heroin is clandestinely produced from morphine base. Outside of India, the only country allowed to grow poppies for production of medicinal morphine, poppies are grown in the Golden Triangle (Thailand, Laos, Burma), the Golden

Crescent (Afghanistan, Pakistan), in the Near-East and in Colombia. Illicit poppy growing supplies the starting material for conversion of morphine to heroin that may subsequently be shipped to North America and Europe.

Illicit opioid use may present in several manners: heroin or opium use has fallen markedly in the last decade while fraudulent use of replacement products (buprenorphine), major opioid analgesics and OTC opiates (codeine) has increased.

Heroin is essentially injected by the parenteral route. Only exceptionally is it smoked or swallowed. In contrast, opium is smoked or swallowed, but its use in those traditional ways mainly occurs in certain communities originating from the Far East.

Following IV injection, heroin is very rapidly cleared from the blood, in a few minutes. Heroin is metabolized to 6-monoacetylmorphine (whose plasma half-life is some 20 minutes), then to morphine. Morphine undergoes marked hepatic glucuroconjugation (about 70% morphine-3-glucuronide) or N-demethylation (about 20% normorphine). Codeine is also formed during metabolism.

In consequence, only the urine of the first few hours following heroin use contains traces of the specific metabolite, 6-monoacetylmorphine. Morphine and its metabolites continue to be eliminated in the urine over 3 to 4 days.

Codeine is metabolized in the same way, undergoing conjugation to codeine-6-glucuronide and norcodeine. Codeine is also metabolized by O-demethylation to morphine. The metabolic pathway (morphine

to codeine and codeine to morphine), together with the rapid clearance of 6-monoacetylmorphine in the event of heroin use, very frequently renders interpretation of the molecules identified in the urine problematic (isolated therapeutic intake or therapeutic intake in order to mask addictive behavior). The semi-synthetic opiates such codethyline (ethylmorphine) and pholcodine have morphine as a minor metabolite.

Opioids bind to membrane receptors present in numerous tissues (opiate receptors). The receptors have natural endogenous ligands (endorphins). IV injection of heroin induces a phase of euphoria followed by a state of great wellbeing ('rush'). This phase is followed by a stage of drowsiness and apathy that may last for several hours. Tolerance phenomena develop very rapidly giving rise to a tendency to marked dose escalation to prevent withdrawal phenomena and maintain the feeling of wellbeing.

Opioids and heroin are endowed with numerous pharmacological properties: they induce respiratory depression and even respiratory arrest in the event of overdose; they have an analgesic action (by raising the pain perception threshold inducing indifference and sedation) and an antitussive action; they induce hypotension and syncope; they reduce secretion and induce constipation.

Opioids induce marked physical and psychological dependence inducing. On discontinuation of the drug, the withdrawal syndrome emerges. The symptoms vary (mydriasis, tachycardia, perspiration, diarrhea and vomiting, agitation, insomnia, instability and anxiety).

The use of methadone (synthetic opioid) in the treatment of opioid dependence enables, at a suitable oral dose, prevention of the withdrawal symptoms while not procuring the 'rush' phase induced by IV heroin injection. The withdrawal syndrome that follows discontinuation of methadone is reported to be mild. However, buprenorphine is currently the main product for the replacement treatment of opioid dependence. Opioid-replacement treatment aims to stabilize the dependence so as to gradually wean the patient from the drug, eradicate the craving and socially reintegrate the patient by withdrawing him/her from illegal supply circuits.

Natural and semi-synthetic opiates (morphine, heroin, codeine, codeine, codeine and pholcodine) and their metabolites may be detected by urinary screening over the 3 to 4 days following therapeutic intake or illicit use. The positive cutoff for screening is 300 ng/mL. A positive screening result must obligatorily be followed by identification by a chromatographic method so as to identify the drugs involved (therapeutic or illicit opioids and their metabolites). Opioid screening methods do not enable detection of synthetic derivatives (dextromethorphan, dextropropoxyphene, pethidine, fentanyl, nalbuphine, pentazocine) or opioid-replacement treatments (methadone and buprenorphine).

COCAÏNE

Cocaine (methylbenzoylecgonine hydrochloride) is an alkaloid extracted from the leaves of the coca plant: *Erythroxylon coca*. The plant is a shrub grown in South America on the slopes of the Andes in nume-

rous countries (Colombia, Bolivia, Peru, Ecuador, North-West Brazil). Locally, coca leaves have been chewed by the inhabitants for many centuries. The leaves are mixed with chalk or ash.

The various stages of alkaloid extraction from the freshly harvested leaves yield cocaine base and its sulfates. Cocaine hydrochloride obtained from cocaine base consists in fine white crystals (ice, snow) and is the most widely consumed form. The drug is inhaled ('snorted'). The drug may also be mixed with heroin and injected. Cocaine base or 'crack' is obtained by precipitation of the hydrochloride. The drug is in the form of 'rocks' and is mixed with tobacco and smoked in waterpipes or cigarettes. This new form of cocaine has extended the previously-elite market toward a large number of cannabis smokers.

Following intranasal administration, the peak plasma concentration occurs in 30 to 40 minutes. Following inhalation, the peak plasma concentration occurs in 10 to 20 minutes. Cocaine is rapidly distributed to numerous storage tissues (half-life of 30 to 90 minutes). However, its metabolism and elimination are relatively slow. Cocaine is mainly converted to benzoylecgonine and ecgonine methyl ester, devoid of psychotropic activity, which are eliminated in the urine.

Metabolism takes place in the liver and in the blood mediated by circulating esterases (cholinesterase). Traces of benzoylecgonine may be detected in the urine 2 to 4 days after cocaine use. Small quantities of ecgonine and norcocaine resulting from demethylation may also be eli-

minated in the urine. Concomitant intake of ethanol results in the formation of cocaethylene by trans-esterification of cocaine at hepatic level. Metabolism of cocaethylene yields ecgonine ethyl ester, benzoylecgonine and norcocaine. Crack smoking, a particular form of consumption, induces formation of anhydroecgonine methyl ester via pyrolysis of cocaine base. Anhydroecgonine methyl ester, due to its cholinergic properties, strongly contributes to the particular toxicity of crack.

Cocaine stimulates alertness and is classified as a psychoanaleptic substance together with amphetamines and their derivatives, caffeine and nicotine. The stimulation is a result of release of neuromediators, dopamine and serotonin. Similarly, cocaine induces release of norepinephrine, which is responsible for the sympathicomimetic effects. Cocaine procures a brief state of artificial wellbeing with intellectual stimulation and intense exaltation accompanied by hallucinations.

Psychological dependence is strong and of rapid onset. The anxiety and apathy which follow the euphoric phase may give rise to psychiatric complications. There is no physical dependence. The placental barrier crossing and excretion of cocaine in breast milk have harmful effects on the unborn fetus and neonate: mental retardation and difficulties at school related, overall, to hypoxia. Synthetic local anesthetics long ago superseded cocaine for medical use.

Testing for addictive behavior in the form of cocaine consumption is conducted using immunochemical reagents that enable screening

on urine samples. The antibodies are generally against benzoylecgonine.

The usual positive cutoff is 300 ng/mL. A method of identification and confirmation using gas chromatography with mass spectrometry may be necessary in the event of a positive screening result.

In whole blood samples, testing for cocaine and its main metabolites (benzoylecgonine, ecgonine methyl ester, ecgonine, cocaethylene) may be conducted for subjects suspected of cocaine use and presenting with signs of paranoid psychosis.

AMPHETAMINE AND DERIVATIVES



Amphetamine and amphetamine derivatives are a series of synthetic central nervous system stimulants now considered to have addictive potential. The main amphetamine derivatives (and the most widely used) are:

- D-amphetamine (or speed)
- methamphetamine (or ice, crystal, meth)
- methylenedioxyamphetamine (MDA) (or love drug)
- methylenedioxymethamphetamine (MDMA) (or ecstasy, 'E', Adam)
- methylenedioxyethamphetamine (MDEA) (or Eve)
- methylbenzodioxylbutanamine (MBDB) (or Eden).

By extension, cathinone, the alkaloid extracted from khat (an African shrub) and its syn-

thetic homologue, methcathinone (or Jeff, cat), also belong in this series due to their chemical structures and psychotropic properties. Similarly, ephedrine and pseudoephedrine, alkaloids derived from Ephedra, and used as nasal decongestants, may be clandestinely converted to methcathinone.

Amphetamines are subject to very many chemical transformations and the 'designer drugs' thus obtained have similar or different pharmacological properties including that of feeling attuned to one's inner self (entactogenic drugs such as MDMA, MDEA, MDA, MBDB).

The amphetamines, ephedrine, phenylpropanolamine, phenylephrine and pseudoephedrine are sympathomimetic amines due to their structural and functional similarities to the catecholaminergic transmitters (epinephrine, norepinephrine, dopamine). However, the psychostimulant and anorexigenic properties of amphetamines dominate the alpha-constrictor (decongestant effect) and bronchodilatory properties.

Therapeutic use of amphetamines is exceptional and restricted to the treatment of infantile hyperkinesia and narcolepsy.

Until the middle of the 1970s, amphetamines were widely used as stimulants by students, athletes, military personnel and businessmen, or as appetite suppressants in weight-loss diets (slimming pills). Inclusion of those drugs on the narcotics list put an end to those uses and the drugs are now considered to have addictive potential. Similarly, a number of proprietary medicinal products containing structural analogs of ampheta-

mines and proposed as anorexigenic agents (amfepramone, fenfluramine, clobenzorex, etc.) were withdrawn from the market in 1999 in France.

Amphetamine and amphetamine derivatives may be administered orally, intravenously or, sometimes, by inhalation. IV administration induces fast-onset and more intense effects than the oral route: a phase of excitation and exaltation ('rush') follows injection. The subject has the impression of being able to think more clearly and remember things more easily. He has a reassuring feeling of enhanced potency, no longer suffers from tiredness and experiences euphoria and a deep decrease in the desire to sleep and eat. This psychological status is accompanied by hypertension, tachycardia, bronchodilatation and mydriasis. After the excitation phase, there is a very unpleasant depressive state ('the down') which encourages the subject to take more of the drug. The subject becomes anxious and irritable. He experiences great psychological and physical weariness. Dependence is above all psychological and emerges rapidly irrespective of the method of administration. Physical dependence is weak. Overdoses induce intense agitation, hallucinations, hyperthermia and hypertension with seizures and loss of consciousness, sometimes complicated by cardiac disorders. In the initial stage of poisoning, the intense thirst experienced (particularly with ecstasy) may induce water poisoning with irreversible cerebral edema and hyponatremia.

In HIV-positive patients, the combination of ritonavir and ecstasy is dangerous. The action mechanism of the two

drugs in combination is due to release of the neurotransmitters, dopamine, norepinephrine and serotonin.

Since amphetamine derivatives are relatively stable molecules, a fraction of the drug ingested can be detected in the urine in unmetabolized form. The percentage unchanged drug in 24-h urine is higher, the lower the urinary pH. Thus, the level may increase from 2% at alkaline pH to 75% at acid pH.

Part of the dose is degraded in the liver to yield various compounds, depending on the metabolic pathway:

- oxidative deamination yields benzoic acid, then hippuric acid after glucuroconjugation;
- hydroxylation yields hydroxynorephedrine.

At normal urinary pH, about 30% of the dose is excreted in the 24-h urine in the form of unchanged amphetamine and 25% in the form of benzoic and hippuric acids. The same applies to methamphetamine: at normal urinary pH, about 40% is excreted as unchanged methamphetamine and 4 to 7% as amphetamine derived from demethylation of the drug.

Testing for amphetamines and amphetamine derivatives in the urine may be conducted by an immunological method. Depending of the commercially available reagents and the specificity of the antibodies used, drugs related to D-amphetamine and methamphetamine are recognized to a variable extent. Thus, MDMA or 'ecstasy' is detected by most reagents. However, the anorexigenic analogs are not detected, nor is cathinone or methcathinone.

A gas chromatography - mass spectrometry (GC-MS) method

of identification is indispensable following positive screening in order to identify the main amphetamines. This method is also of value in testing for amphetamines and amphetamine derivatives in the blood, the only appropriate biological medium for detection of recent intake and enabling, through quantitative analysis, determination of the concentration in order to assess the impairment of alertness.

Amphetamine and its derivatives can be detected in the urine over approximately 2 to 4 days post-intake. However, the detection window may vary as a function of individual metabolism, urine output, dosing frequency and the doses used or the type of drug taken (MBDB can only be detected in the urine of the first 24 hours).

LSD

Lysergic acid diethylamine (LSD) is the most potent known hallucinogen. It is classified as a narcotic but does not induce dependence or tolerance phenomena. Obtained by synthesis from lysergic acid, which is produced with other alkaloids by rye ergot, a parasitic fungus.

LSD is generally sold in the form of small squares of blotting paper or stamps on which it has been applied and rendered insoluble. LSD is taken by the oral route and induces variable effects depending on the

subject and quantity taken: euphoria or anxiety, hallucinations, consciousness disturbances which may include loss of inhibition resulting in transient attacks of madness.

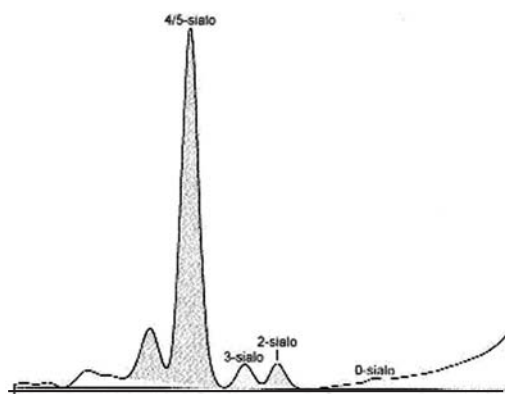
LSD is rapidly metabolized (blood half-life of 2 to 5 hours) and eliminated in the urine over 24 hours. The urinary screening methods have a positive cutoff of 0.5 ng/mL.

ALCOHOL

Chronic alcoholism consists in regular intake of a large quantity of alcohol, rarely sufficient to induce drunkenness but whose somatic and metabolic consequences are excessively serious after a number of years: pancreatitis, alcoholic hepatitis, cirrhosis, cancer of the gastrointestinal tract, psychological disorders with anxiety, insomnia, depression, memory disorders, mental disorders, cardiovascular diseases, etc.

Electrophoresis of transferrin isoforms

(method conducted using SEBIA CAPILLARYS capillary electrophoresis)



Isoform	%
4/5-sialo	92,0
3-sialo	3,7
CDT	4,3 >
2-sialo	3,9
0-sialo	0,4

Electrophoretogram of transferrin isoforms

Among the markers of continuous heavy drinking, several have been suggested for the detection of alcohol abuse: γ -GT, ALT, AST, MCV, HAA (hemoglobin-associated acetaldehyde), EDAC (early detection of alcohol consumption or score calculated on 36 routine laboratory parameters) and CDT (carbohydrate-deficient transferrin).

The latter parameter currently appears the most pertinent in terms of specificity.

Transferrin is a glycoprotein synthesized by the liver. The polypeptide chain normally bears 2 polysaccharide chains terminating in 2 or 3 negatively charged sialic acid residues. The predominant form in healthy subjects is tetrasialotransferrin. The glycosylated fraction of the protein confers its electric charge (isoelectric point: pHi of 5.2 to 5.7).

Prolonged daily consumption of 50 to 80 g of pure alcohol (a liter of 12% wine contains 96 g of pure alcohol) modifies the distribution of the molecular forms of transferrin. Ethanol intoxication induces inhibition of the N-glycosylation mechanisms with, as a consequence, an increase in the non-sialo, monosialo and disialo forms in heavy drinkers. The forms are collectively known as carbohydrate-deficient transferrin (CDT).

CDT concentration is positively correlated with alcohol consumption. Daily drinking of 50 to 80 g of pure alcohol over 1 to 2 weeks induces an elevation in CDT levels. The diagnostic efficacy of CDT as a laboratory marker of alcohol consumption is probably not markedly greater than the other biological markers (sensitivity: 0.6 to 0.8). In contrast, its specificity of

greater than 90% makes the marker, when elevated, an indispensable control for satisfactory compliance with alcohol withdrawal treatment since CDT falls over withdrawal in all patients with an initially high CDT. The time to return to baseline is about 2 to 4 weeks.

The baseline, which is individual to the patient, also enables detection of relapses even though the levels remain within the usual range of the method. CDT is increased in non-alcohol drinking patients presenting with non-alcohol-related liver disease: primary biliary cirrhosis, chronic hepatitis and hepatocellular carcinoma.

The assay is only to be conducted on serum since both EDTA and heparin act on the pHi of the proteins and interfere with the separation phase of the assay. Several methods may be used: isoelectric focusing, immunoturbidimetry, HPLC and capillary electrophoresis. Capillary electrophoresis has the advantage over the immunoturbidimetric methods of showing the presence of carboxy-deficient isoforms and the presence of genetic variants (B, C and D, each with several subtypes) on the electrophoretogram.

TOBACCO AND SMOKING

Cigarette smoke contains numerous substances of which nicotine, carbon monoxide, thiocyanates, cadmium, irritants (phenols, acrolein, aldehydes) and carcinogenic tars (polycyclic aromatic hydrocarbons, nitrosamines). It is now firmly established that smoking gives rise to serious diseases: cancer, coronary artery

disease, stroke, peripheral vascular diseases, chronic respiratory diseases. Smoking kills 66,000 people each year in France. The impact of cigarette smoking during pregnancy on the fetus has also been demonstrated (increased prevalence of intrauterine growth retardation) and on the neonate and infant (increased risk of cot death and increased frequency and duration of obstructive sleep apnea).

Nicotine is responsible for the pharmacological dependence but is not a psychoactive substance: it stimulates dopamine release in the cortex but not or very little in the nucleus accumbens, the site of the reward system. Smokers suffer from psychological dependence related to social habits that have given rise to veritable conditioned reflexes (the desire for intellectual stimulation, alleviation of tension or anxiety, etc.) and physical dependence on nicotine. Withdrawal gives rise to irritability, a feeling of malaise, reduced concentration, drowsiness, and episodes of bulimia, etc.

A cigarette contains 1 to 3 mg of nicotine, of which almost 100% is absorbed and reaches the nicotinic receptors located in the adrenal glands and CNS within a few seconds. Only nicotine and, a fortiori, its metabolites are specific markers of smoking. The half-life is very short (30 to 120 minutes). Nicotine is rapidly metabolized by the liver, kidneys and lungs to cotinine (20% of the metabolites), the only parameter of real interest with regard to assay, due to its elimination half-life (16 to 22 hours).

Urinary cotinine assay is conducted by immunoassay or by chromatographic methods.

These methods are generally more sensitive (with a limit of detection of 10 µg/L) and enable demonstration of passive smoking due to a smoky atmosphere (level between 10 and 50 µg/L). Nicotine, following inhalation or oral intake (gums and chewing-gums) or transdermal delivery (nicotine patch), is abundantly present in the urine in the form of cotinine. Concentrations between 800 and 3000 µg/L are usually observed in heavy smokers.

Nicotine is excreted in breast milk and may thus be found in the body of breast-fed infants.

Urinary cotinine assay is rarely used to demonstrate passive smoking except for neonates and infants. Concentrations of up to 50 µg/L have been detected in infants suffering from respiratory diseases (asthma) and living with smokers. Monitoring nicotine replacement treatment using a patch is not conducted in practice. Assay may be used to check that withdrawal is effective but is particularly conducted in the context of screening for risk behavior. Demand for the latter has markedly increased in recent years under the pressure from insurance companies. The 'tobacco test' or urinary cotinine assay is conducted on applicants for substantial loans to document cardiovascular status. Smoking is considered a cardiovascular risk factor. Given the elimination half-life of cotinine, it is necessary to refrain from smoking for several days to 'clear' urinary cotinine.

OTHER PLANTS

In the last few years, consumption of other substances of plant origin (toxic

and/or medicinal plants, hallucinogenic plants and fungi) for recreational purposes in order to procure sensations (mainly visual) has increased. Such substances induce hallucinations at non-toxic doses but generally do not induce habituation. The context of their use is more experimental (in small groups of initiated people) rather than due to an imperious need to consume them.

Apart from LSD, a semi-synthetic derivative, many of the substances have been known since Antiquity and are sometimes still used in ancestral cultural and religious contexts (shamanism). However, the migration of people (particularly to large European cities), the difficulty of controlling the increasing quantities of products imported, and e-business have facilitated the arrival of such products in our cities.

The vast majority of clinicians (emergency care physicians) and laboratory scientists are not equipped to test for those substances. Testing is more indicated in the context of a clinical emergency (overdose) than in the context of addiction. The products encountered may be common in our own countries or on other continents: mescaline, atropine, scopolamine, psilocybin, harmaline, harmine, dimethyltryptamine, kavaine, etc. No commercial immunoassay is currently available and only a limited number of laboratories are able to test for those substances using chromatographic methods.

VOLATILE SUBSTANCES

The regular inhalation of volatile organic compounds by 'sniffers' induces dependence

on the product and, particularly, a risk of severe acute and chronic poisoning which may be fatal. The products are used directly from their container (gas cylinders, tubes of glue, solvent bottles, etc.) or after having been placed in a bag (moistened cloth or spraying) or used directly on a moistened cloth placed over the nose.

In the majority of cases, the products have been diverted from their domestic, industrial or medical use (theft of nitrous oxide (N₂O) cylinders from hospitals). The collective behavior pattern is known as volatile substance abuse (VSA). The products are not illicit substances. The products include anesthetic gases or solutions (nitrous oxide, ether, chloroform), quick-drying adhesives (due to the solvents contained in them such as petroleum hydrocarbons, ketones, alcohols, acetates, chlorinated aliphatic hydrocarbons, etc.), antifreezes (ethylene glycol), alkane-based stain removers, correction fluids (Tipex®), fuels (gasoline), nail varnish removers, paint diluents, cleaning products, etc.

The products are used for their short-term effects: euphoria, drunkenness, colored visual sensations and frequently as a means of fleeing difficult social conditions (children in Brazil).

The methods of investigation and assessment of the risks run may be based on the usual methods used in monitoring laboratory markers and markers of occupational exposure to solvents.

	CANNABIS	COCAINE	OPINOIDS	AMPHETAMINE AND DERIVATIVES	LSD
Most widespread methods of use	Mainly smoked by mixing the hashish form (resin) with tobacco in a cigarette (joint)	Nasal route (snorting) in the form of the hydrochloride or smoked after mixing with tobacco in the form of cocaine base (crack)	Heroin injected by the IV route Morphine taken by the oral or IV route after dissolution Codeine by the oral route	Tablets taken by the oral route	Sublingual administration
Desired effects	Inebriated feelings with euphoria, alleviation, pleasure and relaxation. But it induces a decrease in alertness, sedation and hallucinations (at high doses)	Stimulation of the reward circuit (fundamental emotions and behavior) Feeling of intellectual and physical potency Indifference to pain and tiredness	Effect desired with IV injection (orgasmic rush with appeasement, euphoria, feeling of ecstasy) is fleeting (a few tens of seconds) and followed by a phase of sedation, drowsiness and sleep	Stimulation by: - increase in alertness, performance, concentration and self-confidence - decrease in the feeling of tiredness Change in social relationships through the entactogenic and hallucinogenic effect	Hallucinations , feeling of well-being, uncontrollable laughter
Dependence	Psychological (±) depending on the individual	Psychological (very intense when smoked)	Physical (+++) and psychological	Psychological (±) depending on the drug and individual	No dependence
Duration of urinary detection	2 to 3 days in occasional users 2 to 3 weeks in regular users	2 to 3 days for benzoylecgonine	2 to 3 days	2 to 3 days	24 hours (under protection from light)
Duration of blood detection	2 to 3 hours for THC 12 to 24 hours for the metabolite, THC-COOH	12 hours	Heroin: a few minutes 6-MAM: 1 to 2 hours Morphine and codeine: 12 to 15 hours	24 hours	< 6 hours
Risk of fatal overdose	Not if used alone	Particularly if injected or smoked	Especially with heroin	With ecstasy particularly and even at low doses	
Neuronal toxicity	No	Yes	No	Yes	No
Risk of psychiatric disorders	Yes	Yes (neurological manifestations of the paranoiac psychosis type)	No	Yes	Yes
Systemic toxicity	In the long term, emergence of an amotivational syndrome and decreased intellectual, motor and cognitive performance	Cardiac arrhythmia	Respiratory insufficiency with loss of consciousness and death	Paradoxical toxicity. The increased self-confidence induces irrational behavior and risk taking	Risk related to impaired consciousness