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A review on biological matrices and analytical methods used for determination of drug of abuse

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ABSTRACT

Drug addiction is a chronic disease with a potential for fatality if not treated. The drugs with potential for abuse are mostly psychoactive drugs. Serious widespread medical and health consequences associated with drug abuse involve neurotoxicity, cardiovascular complications, impairment of the immune system function, and many other physiological effects. Illicit drug use remains the second most common mode of HIV infection. Various analytical techniques and number of biological matrices has been used for the detection of drug of abuse in cases such as drug addiction, driving under influence of drugs, neonatal drug exposure in case of drug abuse by pregnant women etc. Urine and blood sample remain the most widely used conventional biosample for the detection of drug of abuse. Various other alternative biological matrices such as saliva, hair, nails, tears and meconium have also been used for the same purpose. Number of analytical techniques such as liquid chromatography with mass spectrometry (LC-MS) and LC with tandem MS (LC-MS^2), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), electrospray ionization Time-of-Flight mass spectrometry (ESI-TOF), combination of ultra-performance liquid chromatography (UPLC) and TOF, fluorescence polarization immunoassay (FPIA) and enzyme multiplied immunoassay technique (EMIT) have been used for the detection of drugs of abuse in above mentioned biosamples. This review summarizes the conventional as well as alternative biological matrices and various analytical techniques used for the determination of drugs of abuse.

Key words: MTCT, TRIAGE, HAIRVEQ, Solid phase extraction, liquid-liquid extraction, Fluorescence polarization immunoassay.

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INTRODUCTION

Drug abuse has been defined as a pattern of problem use that results in health consequences, social problems, or both. Drug addiction is a chronic disease of the brain that involves relapse, progressive development, and the potential for fatality if not treated. Addiction cannot be cured but can be brought into remission through a program of treatment, abstinence from all psychoactive substances, and supported recovery (Seymour and Smith, 2002). The drugs involved in abuse of drugs are comprised of the group called "psychoactive" drugs. Psychoactive drugs have their primary effect on the brain and central nervous system (CNS). This class of drugs includes opioids, sedative–hypnotics, stimulants, and hallucinogens and recent additions such as performance-enhancing drugs, for example steroids, and combinations producing the effects of several drug groups. Inaba and Cohen (2000) list six levels of drug use and abuse namely abstinence, experimentation, social/recreational, habituation, drug abuse, and addiction. These levels may not be progressive from one to the next, but will indicate in a progression context if the

individual is developing a drug problem. Psychoactive drugs are chemically similar to chemicals called neurotransmitters that occur naturally in the human brain. Because of the similarity, psychoactive drugs pass through the blood–brain barrier that exists to protect the brain from foreign materials and once in the brain, produce their effects by stimulating the release, inhibiting the release, blocking the reuptake, or imitating the brain's own neurotransmitters.

With a few notable exceptions, the bulk of psychoactive drugs that are abused by human beings fall into four general categories. These categories are: 1) opioid/analgesic drugs; 2) sedative–hypnotic drugs; 3) stimulant drugs; and 4) hallucinogenic drugs. There are drugs of abuse that either fall outside these basic categories, such as ether and other general anesthetics and steroids, or are considered to have attributes of more than one category, and these include the stimulant hallucinogens.

The use of illicit drugs is associated with serious widespread medical and health consequences. Among the potential consequences of illicit drug use are neurotoxicity from cocaine and methamphetamine, cardiovascular complications from cocaine, impairment of the immune system function, and many other physiological effects (Khalsa, 2004). Illicit drug use remains the second most common mode of HIV infection and drugs such as amphetamines, cocaine, marijuana, and opiates serve as cofactors for susceptibility to HIV infection, disease progression and also mother to child transmission of HIV.

Various analytical techniques and number of biological matrices has been used for the detection of drug of abuse. In the past few years many international forensic toxicology groups have evaluated several alternative biological matrices as diagnostic tools for drug-testing (Fucci et al., 2006). In recent decades, growing interest has been noted in determination of drugs in alternative biological materials, mainly promoted by intensive development of highly sensitive and selective techniques, especially liquid chromatography with mass spectrometry (LC-MS) and LC with tandem MS (LC-MS^2). The most important unconventional biosamples include hair, oral fluid, sweat, meconium, nails and tears (Madej, 2010).

Following is the review of conventional as well as alternative biosamples and analytical techniques that has been used for the determination and quantification of drug of abuse in human body.

DRUG ABUSE AND HIV

The gestational drug exposure is an issue of rising interest because of the negative effects of drugs on the foetus. Several risks of drugs abuse in pregnancy are reported in literature such as abruptio placentae, spontaneous abortion, haemodynamic changes, malformations, low birth weight, behavioral anomalies, encephalographic and electrocardiographic anomalies and AIDS (Forman et al., 1992). It has been well documented that HIV-infected women who use illicit drugs during pregnancy had a higher risk of transmitting HIV to their infants as drugs such as amphetamines, cocaine, marijuana, and opiates serve as cofactors

for susceptibility to HIV infection and disease progression (Ellis et al., 2003).HIV-1 infects peripheral blood mononuclear cells (PBMCs) such as macrophages and CD4+ T lymphocytes by binding to co-receptors such as CXCR4 and CCR5. It is known that drugs of abuse modulate expression of chemokines in CD4+ lymphocytes. Although clinical reports indicate association between HIV/AIDS and drug use, the molecular mechanism of infection susceptibility and disease progression remains unclear.

Pandhare (2011) hypothesized that drugs of abuse may regulate gene expression in CD4+ lymphocytes via epigenetic modifications. Based on this hypothesis, an effect of drug abuse-induced epigenetic changes can be envisioned at several steps of HIV life cycle. Although, the literature on drug abuse-associated epigenetic changes in CD4+ T cells is still in infancy, it is important to point out that CD4+ T lymphocytes undergo extensive changes in chromatin structure via epigenomic modifications during cytokine production. Therefore, efforts to understand drug abuse-associated epigenetic modifications and their implications in HIV-1 replication will bridge a major gap in drug abuse and HIV/AIDS field.

In the United States, approximately 5.1% pregnant women use illicit drugs and 16.4% use tobacco. Drugs of abuse used by pregnant mothers may increase mother-to-child transmission (MTCT) of HIV through several mechanisms. Indeed use of illicit drugs (heroin or other opiates, cocaine, methadone, or other injection drugs) has been linked to increased rate of MTCT of HIV in the pre-HAART era. Recently two large epidemiologic studies from Western Europe (France and UK/Ireland) point out that despite use of HAART, MTCT of HIV could be as high as 6–7% in some women (Warszawski et al., 2008).

Drugs of abuse have the potential to increase MTCT of HIV in the presence of HAART by inflicting injury to placenta, inducing preterm birth, and increasing maternal plasma viral load. Drugs of abuse may increase maternal viral load by: a) promoting HIV mutation through non-adherence to HAART; b) impairing the efficacy of HAART through drug–drug interaction; and c) promoting HIV replication in monocyte/macrophages. Drugs of abuse may promote HIV replication by 1) increasing the expression of CCR5 receptors; 2) decreasing the expression of CCR5 receptor ligands; 3) increasing the expression of CXCR4 receptors; 4) increasing the expression of DC-SIGN; and 5) possibly inducing epigenetic changes (Purohit et al., 2011).

Two methods are used to asses gestational drugs exposure, namely, self-report/interview methods and analytical methods. Analytical methods grouped under two classes, i.e. analysis of mother tissues/fluids and analysis of newborn tissues/fluids. In order to reduce the possibility of misinterpreting the phenomenon, most of the studies reported in the literature make use of two or more coupled methods. The most common samples used for maternal drug screening are urine and hair, although breast milk was used in one case. Meconium, hair and urine samples are used when screening is performed on the new-borns.

Amniotic fluid, umbilical cord blood, nails and gastric fluid are other samples which are rarely used. One study shows

cerebrospinal fluid to be useful in analyzing the concentration of monoamine precursors and metabolites following cocaine exposure in the new-born (Sabina, 1999).

BIOLOGICAL MATRICES AND ANALYTICAL METHODS

Urine and blood as matrix

Traditionally, urine was the sample of choice for the screening and identification of unknown drugs due to high concentration of drugs in urine. However, improvements in sample preparation, chromatography and in detector techniques have made blood accessible as a screening matrix. Both identification and quantification can be performed in one matrix. As physiological parameters vary within only narrow limits, blood as a matrix is relatively homogeneous. Another great advantage is that drugs can be detected just after intake prior to metabolism and/or filtration. The most relevant matrices to be analyzed are serum, plasma and whole blood. Difficulties arise when only aged or hemolyzed blood is available (Moeller et al., 1998).

In case of urine screening, immunoassays (IA) are used to differentiate between negative and presumably positive samples. Positive results must be confirmed by a second independent method that is at least as sensitive as the screening test and that provides the highest level of confidence in the result such as GC–MS as it provides high levels of specificity and sensitivity (Goldberger and Cone, 1994).

Making use of the IAs developed for urine samples, some authors established IA prescreening methods for blood samples. Lillsunde et al. (1996) used an immunological screening method after acetone precipitation of the plasma proteins. They found sufficient sensitivity for opiates, amphetamines and cocaine / cocaine metabolites. The drugs were quantitated after extraction and derivatization with heptafluorobutyric anhydride (HFBA) by GC–MS. Perrigo and Joynt (1995) tested the enzyme-linked immunosorbent assay (ELISA) technique on whole blood samples for COC and metabolites, cannabinoids, amphetamines and opiates. Moriya and Hashimoto (1996) reported a screening with TRIAGE (Merck, Germany; or Biosite Diagnostics, San Diego, CA, USA) after protein precipitating in whole blood with sulfosalicylic acid.

Being highly efficient, very less sample volume requirement and rapid analysis capillary electrophoresis (CE) has been proven to have great utility in the analysis and detection of drugs of abuse. But CE is not sensitive enough to be applied to trace analysis due to low injection volumes and limited detection path lengths. Sample preconcentration can provide an alternative approach for sensitivity enhancement. Techniques, such as solid-phase extraction (SPE), liquid–liquid extraction and solid phase microextraction can be used in combination with CE to improve sensitivity. These extraction procedures can be performed through the use of sequential injection (SI) techniques. SI, a second generation flow injection technique involves the reduction of sample and reagent volume from milliliters to microliters. In addition, the full automation of the technique enables the entire process to be rapid and precise. An issue in the analysis of drugs of abuse by CE is the need to modify electrolyte composition in order to separate a wide variety of acidic, basic and neutral compounds, which may be present in biological fluids. Electrolyte additives have been used to modify the mobility of analytes, altering the electro-osmotic flow (EOF) and improving solubility (Baryla and Lucy, 2001).

The work by Ahmed Alnajjar et al. (2007) describes the development of a CE method for the screening of human urine for 19 drugs of abuse. The proposed method involves a combination of β-CD and organic solvents to permit the development of highly selective separations. Sample treatment was performed using a SI-SPE manifold, in which matrix clean-up and analytes extraction and preconcentration were performed onto a C18 cartridge. The automation and miniaturization of SI-SPE permits a rapid, robust and cost-effective procedure. Our results show the SI-SPE preconcentration process combined with sample stacking provides a sensitive method with detection levels in the low ng ml^{-1} range.

The conventional sample pretreatment techniques for drugs of abuse analysis in urine samples are liquid–liquid extraction (LLE) and solid phase extraction (SPE). However, they are rather laborious, time consuming and using large amounts of toxic solvents. Therefore, solventless sample preparation techniques such as liquid-phase microextraction (LPME), supercritical fluid extraction (SFE) and solid phase microextraction had already been proposed for the analysis of drugs of abuse. Stir bar sorptive extraction (SBSE) which was developed from SPME is a kind of novel and solvent-free sample pretreatment technique with high concentration factors, good reproducibility and high sensitivity. Only poly (dimethysiloxane) (PDMS) coating is commercially available for SBSE now, and it has some inherent shortcomings such as low recovery for relative high polarity compounds and the limited tolerance of pH range, which have limited the application of SBSE technique to a certain extent, especially for the analysis of polar compounds and basic compounds. To overcome the above-mentioned limitation and to extend the application field of SBSE, Lidan Lan et al. (2010) prepared an organic–inorganic hybrid titania-hydroxy-terminated silicone oil (titania-OH-TSO) stir bar coating by sol–gel method. The experimental results revealed that the titania-OH-TSO coated stir bar exhibited highly pH-resistant ability, good preparation reproducibility, superior selectivity and high extraction efficiency for the target compounds. Based on this fact, a new method of titania-OH-TSO coated stir bar sorptive extraction (SBSE) combined with high performance liquid chromatography (HPLC)–ultraviolet visible (UV) detection was developed for the analysis of five drugs of abuse in urine samples.

Oral fluid sample

Oral fluid (OF) has been used in testing drugs of abuse due to many advantages over blood and urine. Collection of OF is easy and non-invasive and there is a lower risk of infection than in drawing blood. OF samples are more difficult to adulterate as these can be collected under supervision. Detection time of drugs in OF (5–48 h) is similar to that in blood (1–2 days) whereas the detection times in urine can be much longer. Due to short detection

times, OF is a feasible matrix for confirmation analysis of driving under the influence of drugs (DUID) cases, where indications of recent drug use is required. At the moment, OF is used by the police for on-site screening in a number of countries, but in the state of Victoria, Australia, OF samples have been used for confirmation analysis of amphetamines and cannabis since 2004, and in Belgium a law on random OF testing, which allows police to screen for drugs in OF and collect an OF sample for confirmation analysis, has been implemented in October 2010 (Blencowe et al. 2010)

Sample volumes of oral fluid are smaller compared to the conventional sample matrix blood, thus the concentrations of some drugs can be much lower. This imposes some restrictions on the analysis method, which has to be able to detect and quantify multiple analytes from a small sample volume at low concentrations. A sensitive multi-component method for quantitative determination of 50 drug compounds from oral fluid samples collected with the StatSure SalivaSampler™ device was developed by Langel et al. (2011). The compounds analyzed included cannabis, cocaine, amphetamines, opioids, benzodiazepines and other psychoactive medicines. Both liquid–liquid-extraction (LLE) and solid-phase-extraction (SPE) were employed in the sample pretreatment and the samples were analyzed using gas chromatography–mass spectrometry (GC–MS) with the mass selective detector (MSD) operating in either electron ionization (EI) or negative-ion chemical ionization (NICI) mode. The method was fully validated. Stability of the collected samples during storage at -18° C was also studied, and even after over a year's storage all analyte concentrations were more than 60% of the original concentrations. The described method is suitable for routine analysis of oral fluid samples and it has been applied to analysis of more than 4000 oral fluid samples collected anonymously from volunteer road users in Finland during 2007–2009 as a part of the EU project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines). At the moment the developed method is the most comprehensive validated analysis method for oral fluid samples.

Analysis of drugs of abuse in Hair

Blood and urine concentrations only reflect dosages of several hours and several days respectively thus hair analysis has become very important as it provides much information about consumption over a long period and can also provide evidence of the lack of use of drugs of abuse.

Drugs are fixed inside the hair matrix, therefore a digestion procedure is necessary before the extraction of drug from the matrix. As demand grew, an automatic solid-phase extraction method was developed by Girod and Staub (2000). The use of a robot ASPEC allowed to drop certain fastidious manipulations, and to treat a large number of samples at a time. The method is used along with analysis by gas-chromatography-mass spectrometry (GC/MS) in selected ion monitoring mode (SIM), for the following drugs: codeine, 6-monoacetylmorphine (6-MAM), morphine, cocaine, methadone, ecstasy (MDMA) and Eve (MDE). This requires prior derivatization with propionic anhydride. Analysis of some real cases is also performed by an ion trap GC/MS in chemical ionization mode (GC/IT/CI /MS) in order to demonstrate the usefulness of this technique as a complement to routine analysis. Analysis by GC/ IT/CI/MS indeed avoids the risk of false–positive results by the identification of metabolites.

The Istituto Superiore di Sanita` of Rome, Italy, in cooperation with Institut Municipal d'Investigacio` Me`dica of Barcelona, Spain, set up an external quality control program (HAIRVEQ) to evaluate reliability in hair testing for drug abuse by laboratories from the Italian National Health Service (Pichini S et al., 2004). Samples included in the program were real hair samples from drugs consumers. Prior to sending, hair samples were reduced to powdered form, mixed to ensure homogeneity and tested with GC/MS by four Reference Laboratories. Outcomes of the study suggested that guidelines should be provided by Italian authorities for method validation as well as set of recommended cut-off concentrations to orientate laboratories in their quality objectives when developing analytical methodologies as tools to improve reliability and consequently performance of hair analysis.

Francesca et al. (2009) developed and validated a liquid chromatography–electrospray ionization ion trap mass spectrometry method for the analysis of 16 drugs (cocaine and its metabolites, opiates and some stimulants) in human hair. In particular the advantages of ion trap use were greater sensitivity, good mass resolution and scan speed. The ion trap spectrometer afforded to work over the entire mass range in full scan mode, in MS/MS and MS^n mode. The qualitative and quantitative method proved suitable for routine use in the Antidoping Laboratory, resulting specific, accurate and precise across the calibration range. Recently, electrospray ionization Time-of-Flight mass spectrometry (ESI-TOF) has proven to be a powerful tool for multi-residue screening of pharmaceuticals and drugs of abuse in biological samples. Nielsen MKK et al. (2010) developed a simple, sensitive and reproducible UPLC–TOF-MS method using buffer extraction to determine 52 drugs in hair. The combination of ultra-performance liquid chromatography (UPLC) and TOF provides significant advantages concerning sensitivity, selectivity and speed. Even though, selectivity provided by full scan of accurate mass by the TOF instrument is less than the selectivity provided by monitoring MS/MS transitions, the UPLC generates narrow peaks, which reduces the likelihood of unwanted interferences. Hair analysis for drugs is, however, not a simple routine procedure and needs substantial guidelines throughout the testing process, i.e., from sample collection to results interpretation (Pragst, 2006).

Analysis of meconium, nails and tears

In comparison with routinely-used urine or blood samples, samples such as hair, nails and meconium are characterized by a larger detection window. This considerably increases their application range.

Meconium

Meconium is the first fecal matter passed by a neonate, and is commonly characterized by its dark-black color and lack of

the odor of regular feces. It is formed between the 12th and the 16th weeks of gestation, and then accumulated and confined in the fetal bowels until birth. Its analysis allows detection of drugs or other substances to which the fetus was exposed in uterus during about the last 20 weeks of gestation. Urine is the most widely tested biological fluid for the determination of drug exposure during pregnancy. However, it is a difficult sample to collect from newborns, and is only indicative of recent drug exposure (occurring within a few days of birth). Therefore the false negative rate is high when urine drug testing is used. Many authors have concluded that meconium is a superior sample to neonatal urine for the purposes of determining drug use in pregnancy (Moore et al., 1998)

Meconium sampling is easy and completely non-invasive. Drugs are stable in meconium for up to 2 weeks at room temperature and for at least a year when stored frozen. Sample is achieved by scraping the contents (minimum 0.5 g) of the soiled diaper into a special collection container. Meconium analysis generally requires a thorough, preliminary clean-up procedure, including solid-phase extraction (SPE) and sometimes liquid-liquid extraction (LLE), prior to any analytical assays. Weighed amount of meconium is homogenized in methanol, a mixture of methanol and acetonitrile or a suitable buffer, and centrifuged. The supernatant is then removed and evaporated, and the residue is reconstituted with a suitable buffer and then subjected to an appropriate extraction procedure. In some cases, alkaline or enzymatic hydrolysis is necessary to release target compounds from more complex organic combinations. When using gas chromatography (GC) as the determination method, a derivatization step may also be needed.

Radioimmunoassay (RIA), fluorescence polarization immunoassay (FPIA) and enzyme multiplied immunoassay technique (EMIT) and enzyme-linked immunosorbent assay (ELISA) has all been described as useful analysis methods for screening meconium specimens. Overall, FPIA and RIA have been shown to be more sensitive than EMIT for the detection of cocaine metabolite (benzoylecgonine) in spiked meconium samples. Other comparative research has shown that the CAC Cocaine RIA is the most sensitive assay for meconium screening. Presumably this is because there is significant cross reactivity with cocaine which is often present in meconium, compared to various other immunoassays which are specific for benzoylecgonine (Gareri et al., 2006)

In the 1980s, Ostrea became the first researcher to publish and patent procedures for the screening of drugs of abuse in meconium. In 1994 Lewis patented and published a new method, Fluorescence polarization immunoassay (FPIA), for screening meconium samples for cocaine, cannabinoids, amphetamines and opiates.

Meconium analysis is a very sensitive tool for assessing the risk of gestational exposure to drugs and other xenobiotic agents in newborn infants. One study has provided evidence of the exposure of the fetus in American women to a wide spectrum of illicit drugs and commonly prescribed medicines. This study covered 98 randomly selected infants and demonstrated that 82.7% infants tested positive for xenobiotics.

Meconium was also analyzed to assess fetus exposure to tobacco smoke (Madej KA, 2010). Cotinine, a metabolite of nicotine, was found in meconium obtained from newborns whose mothers were active or passive smokers, but no cotinine was detected in meconium obtained from infants whose mothers did not smoke.

Nails

The sampling procedure of fingernails and toenails is rather simple. Nail samples are usually obtained by cutting the excess overhang of the nail plate using cosmetic nail clippers. The samples of each person examined are pooled and stored (e.g., in sealed plastic bags) at room temperature with limited light exposure until required for analysis.

The four key steps in preparing nail samples are -
1) Decontamination;
2) Cutting into small segments;
3) Digestion/Hydrolysis (alkaline, acidic or methanolic) and,
4) Extraction (usually LLE).

In some cases, SPE (together with LLE) as well as derivatization (when GC is used) may be applied. For surface decontamination, nails are usually washed in an appropriate mixture of reagents (e.g., water, methanol, acetone or surfactant SDS) using an ultrasonic bath. A variety of licit drugs (b-blockers, sedatives, anticoagulant agents, antidepressants and antipsychotics) and illicit drugs (cocaine, cannabinoids, morphine and AM related compounds, including their metabolites) has been detected and determined in nails.

Irving et al. (2007) described a screening method for nine sedatives [zopiclone and eight benzodiazepines (alprazolam, clobazam, clonazepam, diazepam, midazolam, oxazepam, temazepam and triazolam)] and their selected metabolites in human nails and hair employing LC-MS[2]. The usefulness of nails as an alternative matrix to blood and urine for detecting illicit drugs exposure was also demonstrated in procedures for determinations of cocaine, cannabinoids, opiates and MA-related compounds, including their metabolites. Fingernail and toenail samples obtained from 18 suspected cocaine users were subjected to qualitative and quantitative analysis for nine cocaine analytes (anhydroecgonine methyl ester, benzoylecgonine, cocaine, cocaethylene, ecgonine ethyl ester, ecgonine methyl ester, m-hydroxybenzoylecgonine, norbenzoylecgonine and norcocaine) by GCMS. Cocaine analytes were present in 14 (82.3%) of subjects, while only 5 (27.7%) had been found positive in conventional post-mortem analysis.

Lemos et al. (2000) also evaluated the usefulness of fingernails as analytical specimens in identifying and quantifying morphine in heroin users. RIA method was used for screening and an HPLC method for confirming morphine. Positive RIA results were obtained with nails from 25 of the 26 heroin users with mean morphine concentration of 1.67 ng/mg. HPLC results were positive

for 22 of the 26 nail samples with mean morphine concentration of 2.11 ng/mg. Based on the results obtained, the authors concluded that nails could become a powerful alternative to hair for detecting past heroin use in forensic cases.

In a comparable study, 18 post-mortem toenails and hair samples obtained from drug abusers were analyzed for presence of opiates and cocaine (Cingolani M et al., 2004). The results revealed that both cocaine and morphine were more concentrated in toenails than in hair. Mean concentrations were 0.99 ng/mg (toenails) versus 0.48 ng/mg (hair) for cocaine and 1.27 ng/mg (toenails) versus 0.79 ng/mg (hair) for morphine.

Tears

Sampling tears is the chief problem to producing precise, reproducible analytical results. The two main procedures for collecting tears are:

1) direct sampling and
2) indirect sampling.

Direct sampling comprises collecting tears with capillary tubes and requires previous stimulation, which facilitates withdrawal of lachrymal secretions and may be conducted in three main ways namely chemical (e.g., fumes of liquid agents, such as ethanol, formalin or ammonia); physical stimuli (e.g., intensive light); or physiological stimuli (e.g., sneezing or yawning stimulation).

An alternative procedure involves instilling various amounts of liquid (e.g., 20–100 μl saline solution), but this technique is rather limited to qualitative examinations.

The main disadvantages of direct sampling are major dilution of tears induced by stimulation, lack of a standardized time required to collect a sufficient volume of tears and the difficulty of collecting samples from specific sites (e.g., under the eyelid).

Indirect sampling uses absorbing supports that are very similar to Schrimer strips (classically used to diagnose dry-eye syndrome). These strips are made of cellulose filter paper and possess precise characteristics to promote good tolerance. The different components from this strip are generally released out by impregnating with an appropriate solvent (e.g., mobile phase when LC is the analytical technique). Total removal of the compounds may be facilitated by agitating with ultrasound. The liquid receiver can be frozen before analytical measurement (Madej, 2010).

Preparation of tears for analysis is not complicated, and often only one step is required i.e. either dilution with an appropriate solvent; precipitation of proteins (e.g., with acetonitrile or perchloric acid) or LLE.

Fucci et al. (2006) used vitreous humor, another alternative biological fluid, as diagnostic tools for drug-testing. The vitreous humor sample is a low volume, low concentration (for exogenous substances) sample and it is therefore necessary to use a high sensitivity screening method which is also capable of using a very low volume of sample.

One hundred and forty-six vitreous humor samples were collected from autopsies authorized by the judge: 35% coming from road accidents, 10% from overdoses, and the remaining from other causes of death. The vitreous humor samples (average volume 2 ml) were immediately frozen without preservatives and kept at -20^{0}C until analysis. All samples were screened using the on-site Cozart1 RapiScan System without any pre-treatment, except a centrifugation step sufficient for direct analysis. The cartridge used, screened for the presence of amphetamines (Amp), methadone (Met), opiates (Opi), benzodiazepines (Benzo) and cocaine (Coc).

The Cozart1 RapiScan immunoassay cartridge is based upon a reaction between the drug present in the sample and the anti-drug antibody conjugated with gold. Within the cartridge there is a specific sequence of immobilized drugs. The drug present in the sample competes with the immobilized drug for binding antibody and inhibits the colorimetric reaction. Absence or reduction of the colour in the specific drug position indicates the presence of drug which is interpreted by the Cozart1 RapiScan reader. All samples positive to Met, Opi, Coc or Amp by the screening, were then confirmed by GC/MS. Also 20% of negative samples were confirmed negative with the same techniques.

The detection of drugs of abuse in fingerprints using Raman Spectroscopy

Edwards et al. (2004) published a paper which describes the application of Raman spectroscopy to the detection of exogenous substances in latent fingerprints. The scenario considered was that of an individual handling a substance and subsequently depositing a contaminated fingerprint. Five drugs of abuse (codeine phosphate, cocaine hydrochloride, amphetamine sulphate, barbital and nitrazepam) and five non-controlled substances of similar appearance, which may be used in the adulteration of drugs of abuse (caffeine, aspirin, paracetamol, starch and talc), were studied in both sweat-rich and sebum-rich latent fingerprints. The substances studied could be clearly distinguished using their Raman spectra and were all successfully detected in latent fingerprints. Photobleaching was done to reduce the fluorescence background in the spectra of some substances. Raman spectra obtained from the substances in sweat-rich latent fingerprints were of a similar quality to spectra that obtained from the substances under normal sampling conditions. The most difficult aspect of the detection of these substances in latent fingerprints was visually locating the substance in the fingerprint in order to obtain a Raman spectrum.

Edwards HGM et al. (2004) extended the research to include the Raman spectroscopic detection of the same exogenous substances in fingerprints which have been enhanced by cyanoacrylate fuming, a technique which is being adopted of the forensic crime scene examination of invisible fingerprints. Latent fingerprints are invisible and must be enhanced in some way before they can be visually detected. Cyanoacrylate fuming is a technique in which the latent fingerprint is exposed to vapours of cyanoacrylate monomer, a liquid adhesive sold commercially under numerous trade names, including Superglue®. The cyanoacrylate monomer vapour polymerises on the material

comprising the latent fingerprint to form a layer of white polymer, which increases the contrast between the fingerprint and the background, thus enhancing its visibility. The polymer layer also protects the fingerprint, preventing it from being smudged.

A study by Went and West (2009) involved the application of Raman spectroscopy for the analysis of drugs of abuse in latent fingerprints for fingerprints that had been treated with powders and also subsequently lifted with adhesive tapes. The application of powders to latent fingerprints is a simple, common and long established method for their development. Powders used for this purpose are aluminium milled flake, or various grades of graphite or magnetic powders which are based on metals or their oxides that are ferromagnetic. Application of magnetic powders is via a magnetic applicator. The advantage of such powders is that there is no contact between the applicator and the fingerprint thus reducing the chance of damage being sustained to the fingerprint detail.

Wide range of different types of tapes designed for lifting powdered fingerprints from a range of surfaces are available. A short length of tape is pressed down onto the fingerprint with care to ensure no air bubbles are present. The tape is then removed slowly and transferred to a backing sheet. The resulting fingerprints are then examined under the Raman microscope and spectra are obtained from small crystals of the substance observed within the deposited latent fingerprint.

CONCLUSION

Determination of drug of abuse in biological matrices plays very crucial role in cases such as road accidents due to driving under influence of drug, health problems, social problems, morbidity, injuries, unprotected sex, violence and deaths due to drug addiction and also mother to child transmission of HIV due to drug abuse. Type of biosample, its size, concentration of drug in biosample as well as sensitivity and accuracy of analytical method is also important and should be taken into account while choosing biosample and analytical method for deretmination of drug of abuse.

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