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Sampling, storage and stability

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Introduction

Appropriate selection, sampling and proper storage of biological evidence are important, yet sometimes overlooked, steps in forensic toxicology. These factors, in combination with drug stability, can profoundly impact the interpretation of results and the outcome of forensic casework. Criteria surrounding each of these are presented and discussed in the material that follows. Further reference to tissue sampling will also be found in other chapters in this book and will be cross-referenced within the text where appropriate.

Specimen selection considerations

Selection of the appropriate specimen is a critical component of any toxicological investigation. Circumstances surrounding the case, the availability of specimens, the nature of the investigation and even legal or statutory issues may dictate which specimens are selected, and for what purpose. The role of the toxicologist is to recommend the most appropriate samples to be collected and tested for specific investigations with various guidelines available on how to collect different sample types. The Royal College of Pathologists has published guidelines for handling medicolegal specimens and preserving the chain of evidence, the Faculty of Forensic and Legal Medicine has published recommendations for the collection of forensic specimens from complainants and suspects, and the European Workplace Drug Testing Society has published guidelines on the collection of urine, oral fluid and hair.

Timing is an important factor in specimen collection, particularly in antemortem cases where some drugs have short detection times and therefore limited detection windows; examples include detection of an elevated concentration of Δ^9-tetrahydrocannabinol (THC) in blood from an impaired driver, or of gamma-hydroxybutyric acid (GHB) following an alleged drug-facilitated sexual assault.

Postmortem specimens pose additional challenges owing to autolytic and putrefactive changes. Timing is also important in death investigations because it becomes increasingly difficult to obtain good-quality specimens as the time between death and sampling (postmortem interval) increases. Factors such as embalming of the body, decomposition or burial can further complicate interpretation if tissues have been preserved, if specimens are putrefied, or if exhumation is necessary. In order to be able to

Table 13.1 Specimen selection considerations

• Ease of use
• Ease of specimen collection
• Presence of interferences
• Matrix effects
• Parent drug and/or metabolites
• Detection time
• Stability of the drug(s) in the specimen
• Putrefaction
• Potential for automated analysis
• Sample volume
• Indication of short-term or long-term drug use
• Reference data
• Interpretive value

select the appropriate specimen(s) the toxicologist should have access to the case history, autopsy records/pathologist's report and all other relevant documents.

Some of the important specimen selection considerations are listed in Table 13.1.

Specimen containers

It is important that the specimen container is appropriate for the intended use and does not compromise the analytical findings. Container size should be appropriate for the volume or weight of the specimen so that headspace is minimised. Typical specimen collection quantities are given in Table 13.2. Excessive headspace in the container can increase the chance of oxidative loss, volatilisation of analyte (e.g. ethanol and other low-boiling-point compounds) or salting out, which may occur if preservatives are present. Some analytes have a tendency to adhere to plastic or glass surfaces depending on their physicochemical properties. Silanisation of glassware can reduce adsorptive losses for drugs that are present at trace levels (10 µg/L or less). Although glass containers are preferred by many, disposable plastic containers are used routinely for a wide variety of postmortem tissues and antemortem samples, particularly urine.

If glass containers are used, it is important to make use of appropriate racks for storage and transportation. One of the major disadvantages of glass is the possibility of breakage, particularly during storage at low temperatures. In order to minimise sample loss, glass containers are preferred if volatile analytes such as solvents or anaesthetic gases are suspected. Plastic containers are more susceptible to interferences by plasticisers such as phthalates that might interfere with the analysis. The use of an inert plastic such as Nalgene decreases the likelihood of chemical interference, but it is good laboratory practice to evaluate all new specimen containers prior to routine use in the laboratory. If plastic containers are chosen, their integrity at low temperatures should be evaluated. Polystyrene is more susceptible to cracking at frozen temperatures than polypropylene vessels. Rubber septa or liners in screw-cap containers should be avoided and replaced with inert liners (e.g. polytetrafluoroethylene (PTFE), or Teflon) to reduce leakage and minimise drug adsorption.

Antemortem blood samples are generally collected into evacuated glass tubes such as Vacutainer or Venoject for forensic toxicology purposes. Collection of blood into similar glass collection vessels is also good practice in postmortem blood sampling. These tubes allow the sample to be collected into a vessel that already contains necessary additives to stabilise and preserve the matrix. Proper mixing is necessary when sodium fluoride or other additives are used to ensure that dissolution is complete.

Blood

Blood is one of the most important specimens of toxicological interest as it provides unique advantages over other matrices in terms of the wide variety of analytical methodologies available, the vast amount of published reference data for both antemortem and postmortem drug concentrations, and the interpretive value of the matrix from a pharmacological standpoint. However, antemortem and postmortem blood samples are notably different, and the site of the postmortem blood draw (central or peripheral) can be

Table 13.2 Typical specimen collection quantities

Postmortem		Antemortem	
Specimen	**Quantity**	**Specimen**	**Quantity**
Blood, heart	25 mL	Blood	10–20 mL
Blood, peripheral	10–20 mL	Urine	25–100 mL
Urine	All	Amniotic fluid	5–30 mL
Bile	All	Breast milk	10–20 mL
Vitreous humour	All	Meconium	All
Cerebrospinal fluid	All	Hair	Pen-size lock
Gastric contents	All	Saliva	1–5 mL
Liver	50 g	Sweat	Microlitres (insensible sweat); 1–5 mL (sensible sweat)
Kidney	50 g		
Spleen	50 g		
Brain	50 g		
Lung	50 g		
Hair	50 g Pen-size lock (150–200 hairs or 50 mg)		

Sources: Dinis-Oliveira *et al.* (2010); Hepler and Isenschmid (2007); Kerrigan (2002); Kidwell *et al.* (1998); Skopp (2004); SOFT/AAFS (2006).

of critical importance. Determination of parent drug and metabolite concentrations (and their ratios) may also yield useful information pertaining to acute or chronic use. A summary of the common advantages and disadvantages of various specimens is given in Table 13.3.

Antemortem blood is collected by venepuncture, typically from the antecubital region of the arm, using a syringe or evacuated container (e.g. Vacutainer, Venoject). Prior to collection, an antiseptic wipe is often used to clean the collection site. Non-alcohol-containing antiseptic wipes such as Betadine (povidone–iodine) are preferred to avoid any contamination that could interfere with alcohol analysis. Although evacuated blood tubes are typically glass, plastic tubes have also been evaluated (Karinen *et al.* 2010).

Postmortem blood

Postmortem blood collected at autopsy is quite different from antemortem blood collected by venepuncture from both qualitative and quantitative standpoints. Postmortem blood may be more viscous, may contain numerous small clots or sedimented cells, has a lower pH (as low as 5.5 owing to protein degradation), may contain 60–90% water, and is subject to varying degrees of haemolysis. The site of blood collection should be clearly identified on postmortem specimens and blood from different sources should never be combined.

Table 13.3 Advantages and disadvantages of antemortem (AM) and postmortem (PM) biological specimens

Specimen	Advantages	Disadvantages
Amniotic fluid	• Determination of prenatal drug exposure • Not readily adulterated • Minimal sample preparation • Relatively few interferences	• Invasive collection • Risk of complications • Limited reference data • Collection by medical personnel
Bile	• Ease of detection of certain drugs (accumulation) • Particularly useful for conjugated drugs	• Complex matrix • Interferences due to bile salts and fats • Requires sample preparation/pretreatment • Limited reference data
Blood (AM)	• Widely accepted matrix • Determines recent drug use (hours–days) • Related to pharmacological effect • Not readily adulterated • Extensive reference data	• Invasive collection • Collection by medical personnel • Shorter detection time
Blood (PM)	• See above (AM) • Reference data widely available • Central/peripheral blood drug ratios known for some drugs • Cardiac blood typically in plentiful supply but requires caution with interpretation	• Susceptible to postmortem redistribution (central) • Susceptible to postmortem artefacts and interferences • Susceptible to contamination (e.g. trauma) • Quality of specimen highly dependent on collection protocol • Limited volume of peripheral blood
Brain	• Particularly useful for lipophilic drugs, volatiles and centrally acting drugs	• Non-homogeneous matrix • Drug concentrations vary by region • Complex matrix • Requires sample preparation/pretreatment • Limited reference data
Breast milk	• Determination of neonatal drug exposure • Not readily adulterated • Many drugs present	• Privacy, invasive collection • Limited reference data • Interferences due to high lipid content • Drug content varies with milk composition • Variable matrix
Cerebrospinal fluid	• Determines recent drug use (hours–days) • Minimal sample preparation • Relatively few interferences	• Invasive collection • Limited reference data
Gastric contents	• Identification of acute ingestion/delayed absorption • Identification of pill fragments possible • Particularly useful for orally administered drugs/poisons	• Non-homogeneous matrix • Complex matrix • Requires sample preparation/pretreatment • Requires total specimen collection for interpretation
Hair	• History of drug use (months) • Readily available, easy collection • Low potential for donor manipulation • Useful for drug and non-drug analytes, e.g. metals	• New technology • Recent drug use not detected • Environmental contamination • Potential for ethnic bias • Limited reference data
Kidney	• Particularly useful for non-drug analytes, e.g. metals	• Complex matrix • Requires sample preparation/pretreatment

(continued)

Table 13.3 (*continued*)

Specimen	Advantages	Disadvantages
Liver	• Ease of detection of certain drugs (accumulation) • Interpretive value for some drugs • Reference data available	• Complex matrix • Requires sample preparation/pretreatment
Lung	• Particularly important for volatile analyses	• Complex matrix • Requires sample preparation/pretreatment
Meconium	• Long-term window of drug exposure • Non-invasive sample collection	• Non-homogeneous matrix • Complex matrix (waxy) • Interferences • Requires sample preparation/pretreatment • Limited reference data
Nails	• Easy collection • History of drug use (months) • Particularly useful for metals	• Limited data • New technology • Not yet widely accepted • Recent drug use not detected • Environmental contamination
Saliva (oral fluid)	• Readily available, easy collection • Parent drug present • Related to free drug concentration in plasma • Minimal sample preparation • Many drugs determined • Indicates recent drug use	• New technology • Short drug detection time • Small sample volume (1–5 mL) • Potential for oral contamination • Collection method influences specimen pH and drug content
Spleen	• Particularly useful for certain analytes if no blood is available	• Complex matrix • Requires sample preparation/pretreatment • Limited data for most analytes
Sweat	• History of drug use (weeks) • Cumulative measure of drug use • Parent drug present • Non-invasive collection • Less frequent drug testing required • Not readily adulterated	• Newer technology • Potential for environmental contamination • High inter-subject variability • Requires special collection device • Skin irritation and discomfort • Small sample volume • No pharmacological interpretation possible • Non-homogeneous matrix (sweat/sebum)
Urine	• Widely accepted matrix • Easy collection • Plentiful supply • Amenable to automated analysis • Longer detection window than blood (days–weeks)	• Potential for donor manipulation • Minimal parent drug • Not useful for quantitative analysis • Not related to impairment or pharmacological effect
Vitreous humour	• Determines recent drug use (hours–days) • Related to pharmacological effect • Resistant to putrefaction • Interpretive value for ethanol-related investigations • Minimal sample preparation • Relatively few interferences • Useful for postmortem chemistry	• Limited data compared with blood • Small sample volume

Central blood

Cardiac blood samples are ideally collected following opening of the pericardial sac, removal of the pericardium, and removal of the blood from the left or right chamber after the heart has been dried. Collection of central blood by insertion of a needle through the chest wall ('blind stick') is practised but is discouraged. Although central blood collected in this manner may be identified to the laboratory as 'heart blood', it may be contaminated with pericardial fluid, fluid from the pleural cavity, or blood that has drained from the pulmonary vein or artery or the inferior vena cava (Jones 2007). Blood collected in this manner is considered non-homogeneous. Central blood may contain elevated drug concentrations as a result of postmortem redistribution or contamination (diffusion) from other body compartments (Prouty and Anderson 1990; Yarema and Becker 2005), especially following blunt force trauma. Passive drug release from reservoirs such as the gastrointestinal tract, liver, lungs and myocardium may occur immediately after death; later, cell autolysis and the putrefactive process participate in redistribution (Pelissier-Alicot *et al.* 2003). Drug properties such as volume of distribution, lipophilicity, protein binding and pK_a play a role in the site- and time-dependent mechanisms responsible for postmortem redistribution. Drugs with high volumes of distribution and basic character appear particularly susceptible to postmortem redistribution and their cardiac blood concentrations should be interpreted accordingly. Postmortem redistribution can account for central/peripheral blood drug concentrations that differ by 10-fold or more. Redistribution is time and concentration dependent and is very difficult to predict. Cardiac blood is typically more plentiful than peripheral blood. Although cardiac blood can be a very useful specimen for screening purposes, the relationship between cardiac blood drug concentrations and antemortem blood drug concentrations is complex. Many toxicologists therefore advise against the use of cardiac blood for quantitative and interpretative work.

Peripheral blood

Femoral blood is the best specimen for use in postmortem testing (Chapter 7) and should be sampled wherever possible. Blood collection from a ligated vein that has been 'tied off' is least likely to be contaminated by other sources of blood or a result of release of drug from tissues and organs. Typically, however, a 'femoral stick' involves the collection of femoral blood from an unligated femoral vein in the groin area. Only a small volume of blood should be collected to avoid 'milking' the vein and drawing blood from other sources. Typically 10–20 mL of femoral blood can be collected. Over-sampling of blood from the femoral vein will draw blood from the inferior vena cava, and hence the liver, and from the larger iliac vein. Although sampling from a ligated vein is generally preferred, a comparison of drug concentrations in clamped and unclamped femoral vessels showed good correlation for eight drugs including selective serotonin reuptake inhibitors, benzodiazepines, antihistamines and one opioid (Hargrove and McCutcheon 2008). If femoral blood is not available, subclavian or iliac blood may be an alternative.

Blood clots

Following a fall or blunt trauma to the head, a victim may survive with circulation intact for several hours. Owing to the decreased circulation in the damaged region of the brain, drug or alcohol concentrations in blood clots (e.g. subdural, subarachnoid and/or epidural) may be influenced by incomplete metabolism. It has been suggested that intracranial blood clots may serve as 'time capsules' prior to death, because they may reflect drug concentrations several hours prior to death, when an injury may have taken place.

Vitreous humour

Direct aspiration of vitreous humour using a hypodermic syringe may yield 2–3 mL of fluid per eye. The needle should be placed in the central globe and aspirated with gentle suction. Preservation with sodium fluoride is generally recommended. The eye is located within the protective environment of the orbit and, being essentially outside the body, is remote from other tissues. Vitreous fluid is therefore a particularly useful specimen owing to its anatomical isolation, affording it notable resistance in terms of microbial invasion and degradation, as well as being remote from the central organs and subsequently less

susceptible to postmortem redistribution phenomena. Vitreous humour is particularly useful for cases involving digoxin or hydrophilic analytes including paracetamol (acetaminophen) and salicylates. The equilibrium that exists between blood and vitreous fluid is slower than with other extracellular fluids, which can result in a slight delay in uptake. Furthermore, only free drugs are able to leave the blood and enter the vitreous humour. Since eye fluid is sterile and less susceptible to microbial contamination and hence postmortem alcohol production, it is routinely used for ethanol determination owing to its interpretive value from the standpoint of postmortem alcohol production and the determination of the pre- or post-absorptive phase of ethanol use (Honey *et al*. 2005). Vitreous humour is particularly useful for postmortem analysis of glucose, urea nitrogen, uric acid, creatinine, sodium and chloride. These are important analytes for the evaluation of diabetes, degree of hydration, electrolyte imbalance, postmortem interval and the state of renal function prior to death (Coe 1977, 1993). Sodium, calcium and chloride concentrations in vitreous humour during the early postmortem interval can be used to estimate antemortem serum concentrations. It is therefore important that sodium fluoride is not added to specimens requiring vitreous chemistries. For that reason, vitreous humour is frequently collected into two separate containers: one preserved (for drug and alcohol testing) and one unpreserved (for clinical purposes).

Cerebrospinal fluid

Cerebrospinal fluid (CSF) can be collected either by lumbar puncture at the base of the spine using a hypodermic syringe or by withdrawal of cisternal fluid by puncturing the base of the neck. Although there are limited published reference data for quantitative drug concentrations in CSF, this clear fluid comprising mostly water is amenable to most routine methods of toxicological analysis. CSF may be of particular importance in alcohol-related cases where no vitreous humour is available, particularly if postmortem alcohol production is suspected. Like vitreous humour, CSF is anatomically isolated and less prone to contamination and bacterial invasion. Although it is more plentiful than vitreous humour, the lack of plentiful reference data limits its usefulness. However, CSF may be particularly useful in surgical death investigations.

Bile

Bile is generally aspirated from the gallbladder using a hypodermic syringe. It may be necessary to tie off the gallbladder prior to collection if contamination appears to be an issue. Bile should be collected prior to the liver specimen to avoid contamination. Many drugs of forensic interest accumulate in the bile, particularly those that are heavily conjugated, such as opiates, benzodiazepines and cannabinoids. Bile may also be used in cases where chronic heavy-metal poisoning is implicated. However, owing to the presence of bile salts and fats, drug extraction from this matrix can be complicated and extensive extraction and clean-up procedures are often required.

Gastric contents

Gastric content is a potentially valuable specimen for analysis in postmortem and clinical cases. Unabsorbed drug or tablet fragments in the gastric contents may provide valuable information concerning ingested compounds and provide an excellent material for preliminary screening (Chapter 7) owing to the potentially large amounts of drug that may be present. The absence of a drug in the gastric contents does not necessarily preclude oral administration. Odours emanating from the gastric content can provide valuable clues about what may have been consumed, e.g. pesticides and cyanides. The entire contents of the stomach should be collected and weighed. Gastric contents are non-homogeneous and should be homogenised prior to sampling. Quantitative drug determinations should be interpreted within the context of the entire contents (total quantity, rather than concentration) and it is important to take into consideration the differing absorption rates of drugs based on their physicochemical properties as well as their formulations and coatings.

The presence of a drug in gastric contents, particularly at low concentration, does not necessarily indicate oral administration. Drugs may be absorbed

into the stomach via gastric juices that are in equilibrium with blood or as a result of intranasal drug use. Basic drugs are more susceptible to this because they have a tendency to become trapped in the gastric compartment owing to the low pH. If heavy metals are suspected, gastric contents should be collected, together with intestinal contents. In cases of suspected poisoning where the patient may have survived for a few days in hospital prior to death and where drugs may have been metabolised and eliminated from the body prior to death, any stomach content collected and retained by medical staff may provide valuable information concerning drugs or poisons consumed.

Urine

In antemortem settings, a mid-stream urine sample is usually collected into a plastic container containing sodium fluoride as preservative. In some settings it may be necessary to take precautions against specimen adulteration. In postmortem settings, urine is collected by insertion using a hypodermic syringe directly into the bladder under visualisation. Puncture of the abdominal wall should be avoided to reduce the possibility of contamination. Urine is a valuable specimen for both antemortem and postmortem drug testing because it is a relatively uncomplicated matrix. However, the multiplicity of factors influencing urine drug concentrations (e.g. urine volume, clearance, metabolism, pH and time of last void) generally means that, in isolation, these results have limited quantitative value. Exceptions to this rule may include ethanol determination in a second void. Care must be exercised when considering the interpretation of urine GHB concentrations as GHB is present as an endogenous compound formed as a by-product of metabolism and may also be produced as a postmortem artefact as a consequence of the breakdown of succinic acid semialdehyde.

Tissues

When tissues are sampled they should be collected quickly and placed immediately into airtight containers. This is particularly important if volatiles or inhalants are suspected. Liver, kidney, brain, lung and spleen are the most frequently collected postmortem tissues.

Liver

Liver is a particularly important organ because of the very large number of drugs that undergo hepatic metabolism and the fairly extensive published reference data that exist. To reduce the possibility of drug diffusion from the small bowel, tissue from deep within the right lobe is preferred (Drummer 2004). The concentrations of drugs and metabolites in liver are often elevated; hence this specimen has limited interpretive value. However, liver is particularly useful for highly protein-bound drugs and the comparison of liver/blood drug ratios may allow the differentiation of acute overdose from chronic drug use for some drugs.

Kidney

Most drugs pass through the kidney as a result of urinary elimination. Kidney is an important specimen in cases of suspected heavy-metal poisoning owing to accumulation in this tissue. The presence of heavy metals or ethylene glycol during toxicological tests may be accompanied by structural changes to the kidney that can be documented using histological tests.

Spleen

Spleen is an important specimen for cyanide or carbon monoxide analyses, particularly in fire-related deaths where blood may be compromised or unavailable.

Lung and brain are valuable specimens in cases involving volatiles or inhalants.

Brain

Brain tissue is lipid rich and has a tendency to concentrate some drugs, particularly lipophilic analytes, narcotics and halogenated hydrocarbons (Skopp 2004). If quantitative drug brain concentrations are used, it is important to know the location of the specimen because the brain is a non-homogeneous matrix. Drug concentrations within the brain may vary several-fold from one region to another owing to its complex structure and differing composition. Brain is not widely used in routine toxicological analysis.

Muscle

Muscle is not routinely encountered, despite the fact that it frequently contains relatively high drug concentrations, particularly for substances with high volumes

of distribution. Perfusion rates between sites and drug concentrations are not consistent, and drug concentrations must be interpreted accordingly. Muscle is encountered more frequently for ethanol determination in the absence of blood, or during the investigation of a suspected injection site.

Hair

Hair has been used in a variety of antemortem toxicology settings to provide a history of drug exposure and has therefore found applications in workplace drug testing, in monitoring of persons on probation or on parole for drug use, in insurance testing to verify the truthfulness of statements made by applicants relating to whether they use drugs or are smokers, in child endangerment, in re-granting of driving licences following suspension for drug misuse, in drug-facilitated sexual assault and in other types of criminal casework (Nakahara 1999; Kintz *et al*. 2006; Curtis and Greenberg 2008). Further discussion relating to the scenarios in which hair testing is employed is provided in Chapters 6 and 7. One of the major advantages is the long drug detection window compared with many other specimens. Hair may allow drug exposure over several weeks or months to be determined, depending on the length of the hair. Segmenting the hair by length may allow an approximate timeline for exposure to be determined based on head hair growth rates of approximately 1 cm per month (Clauwaert *et al*. 2000). Hair should be cut as close as possible to the scalp from the posterior vertex region of the head, since this region shows least variation in growth rate. Typically a lock of hair equivalent to the thickness of a pen or pencil is collected. The colour, length, sampling site and any obvious cosmetic treatment of the hair should be recorded. The root (proximal) and tip (distal) sections of the hair should be clearly identified. Although head hair is the preferred specimen, hair from other sites (e.g. pubis, axillae) may be used, but interpretation of analytical findings may be more complex. The lock of hair is typically tied, wrapped in aluminium foil and stored under dry conditions in the dark at room temperature.

Hair is also a useful specimen in postmortem investigations where arsenic or heavy metals are suspected. Although postmortem hair analysis is not yet widespread, there is growing interest because it may provide valuable interpretive information pertaining to the chronological sequence of toxin exposure (Cirimele *et al*. 2002). Hair has also proved to be useful in cases where exhumation is necessary (Tsatsakis *et al*. 2001). If hair is collected post mortem, it should be sampled at the very beginning of the examination to reduce the risk of contamination.

Hair can provide complementary toxicological information. Issues with drug testing in hair include external contamination, ethnicity and pigmentation, chemical treatment and the use of appropriate cut-off concentrations. Contamination of the hair with drugs from other sources (external deposition, environmental contamination, sweat or sebum) is generally minimised by pretreatment of the sample using a variety of aqueous and organic rinses or wash steps prior to analysis.

Other keratinised specimens such as nails can also be used to determine long-term exposure to drugs or poisons, in particular heavy metals such as thallium, arsenic or lead. However, drugs are deposited into nails at a much slower rate. External decontamination procedures should be performed prior to analysis (see Chapters 6 and 7).

Injection sites

Excision of skin and tissue (muscle) may be necessary in postmortem investigation of a suspected injection site. Typically a cube of muscle and skin is removed for this purpose. However, it is important to compare the drug concentrations in the suspected injection site with those in a control specimen from the same individual where there is no evidence of injection. Injection sites are not always reliable indicators of drug administration since the presence or absence of drugs in injection site tissue is dependent on the type and depth of the injection. If the injection is made directly into a blood vessel, little drug is likely to remain in the surrounding tissue.

Entomological specimens

The potential use of insects for detecting drugs and other toxins in decomposing tissues has been demonstrated and reviewed (Introna *et al*. 2001). If insects or larvae are collected from human remains they

should be frozen as soon as possible. Larvae rapidly eliminate drugs when removed from the food source. Drugs, metals and pesticides have been identified in entomological specimens including larvae and pupae. Following wash steps to remove external contamination, entomological specimens are homogenised and analysed in a manner similar to that for tissues.

Saliva

Saliva or oral fluid can be collected non-invasively by expectoration, by aspiration, by vacuum or by saturation of an absorbent swab (Kidwell *et al*. 1998). Detection times are comparable to those in blood. As much as 1.5 L of saliva per day is produced by the submandibular, parotid and sublingual glands inside the mouth. Secretions from a specific gland may be collected using a special device or by cannulation, but this is uncommon. Although specific gland secretions are advantageous from a standpoint of saliva:plasma ratio and reduced oral contamination, mixed saliva is typically collected for routine drug-testing purposes. Oral fluid can be collected non-invasively, conveniently and without invasion of privacy and is the most commonly collected fluid from the oral cavity for the determination of drugs. Chewing an inert substance, such as Teflon tape or a rubber band, may increase salivation for the purpose of specimen collection. It should be verified that no adsorption takes place between the drug and the chewed substance. Acidic sweets or citric acid have also been used to stimulate glandular secretions. Care must be taken that residual food, drink or interfering substances inside the mouth do not interfere with the analysis. This is particularly important for drugs that are ingested orally or smoked. Following eating or drinking, a 10-minute wait period is recommended before sample collection.

Owing to the ease and non-invasive nature of specimen collection, oral fluid is of particular interest in workplace drug testing, for insurance testing and, more recently, for roadside impairment testing. The placement of an absorbent pad into the oral cavity will collect oral fluid, a mixture of predominantly saliva and small amounts of gingival crevicular fluid, cellular debris and blood. Saliva contains serous fluid derived from plasma. This ultrafiltrate of interstitial fluid contains the unbound fraction of drug at concentrations that are typically proportional to those measured in plasma. However, the predictable relationship that theoretically exists between saliva and plasma drug concentrations is influenced by many factors such as saliva flow rate, which can complicate pharmacological interpretation (Crouch 2005). A more detailed discussion on saliva and oral fluid testing is provided in Chapter 6.

Sweat

Moisture loss via the skin and elimination of insensible (non-visible) sweat take place during normal breathing at a rate of 0.3–0.7 L/day. Sensible sweat refers to perspiration that is actively excreted during stress, exercise or extreme temperature, at rates of 2–4 L/h. About half the total volume of sweat is eliminated from the trunk of the body. The remaining fluid is lost from the legs or upper extremities and head in approximately equal amounts (Kidwell *et al*. 1998).

Sweat is usually collected using an adhesive absorbent patch that is placed on the surface of clean skin or by wiping the skin with a swab or gauze. Careful preparation of the skin is necessary prior to placement of a sweat patch to minimise external drug contamination or bacterial degradation of the drug once it has been retained. Use of a semipermeable membrane to cover the absorbent pad prevents non-volatile components in the environment from penetrating the pad externally, but allows oxygen, water and carbon dioxide to diffuse through. Salts, solids and drugs that pass through the skin are trapped in the absorbent pad, where they are temporarily stored *in situ*, until the patch is removed.

Owing to the relatively small volume (μL) of insensible sweat secreted from a small absorbent area (typically 3 × 5 cm), patches are typically worn for several days on the outer portion of the upper arm or back. In practice most skin wipes or sweat patches contain a mixture of sweat and sebum, the oily secretion from the sebaceous glands. As with saliva, increased flow rates can influence the quantity of drug eliminated into sweat. This specimen is particularly useful for compliance testing or monitoring long-term exposure (weeks), which might be desirable in probation or parole settings.

Amniotic fluid

Amniotic fluid has been used to investigate prenatal drug exposure. Its collection (amniocentesis) typically takes place between weeks 16 and 20 of pregnancy. A needle is inserted through the abdomen into the uterus where there is the least chance of touching the placenta or the fetus. The collection of amniotic fluid is typically performed in conjunction with ultrasound visualisation in order to reduce the risk of damaging the developing fetus. Although complications are rare, miscarriage occurs in a very small percentage of women. Typically 5–30 mL of amniotic fluid is removed during the procedure.

Breast milk

During pregnancy, oestrogen and progesterone, secreted in the ovary and placenta, cause milk-producing glands in the fatty tissue of the breasts to develop and become active. The pituitary hormone prolactin stimulates the production of fluid (600–1000 mL/day) by the milk-secreting cells. Contraction of the myoepithelial cells surrounding the alveoli allows the milk to be expressed. For specimen collection purposes, a breast pump can be used. The matrix is somewhat non-homogeneous. Colostrum, a creamy white to yellow pre-milk fluid, may be expressed from the nipples during the last trimester of pregnancy and shortly after delivery. Many drugs are excreted into breast milk and the scientific and medical literature contains numerous citations of the presence of drugs in this matrix. Drugs that are extensively protein bound may not readily pass into the milk, but emulsified fats contained in the milk may concentrate highly lipid-soluble drugs. The high lipid content and natural emulsifying agents present in breast milk mean that some sample pretreatment is often required.

Meconium

Meconium formation begins between weeks 12 and 16 of gestation. As the first faecal matter passed by the neonate, it is typically collected within 1–5 days of birth. Analysis of drugs in meconium may provide a relatively long-term history of drug exposure during pregnancy, in particular the last 20 weeks of gestation. It provides more complete and long-term information on drug exposure than neonatal urine or cord blood. The specimen is complex and non-homogeneous. All available samples should be collected and homogenised prior to analysis. Meconium and other important matrices involved in maternal–fetal medicine have been reviewed (Gray and Huestis 2007; Lozano *et al*. 2007).

Sample handling

Sample handling is an important consideration during the pre-analytical phase. Unlike a clinical setting, where the time between sample collection and testing is often very short, significant delays are common in a forensic setting. The pre-analytical phase may be considerable, spanning the time of death and/or discovery of a victim, autopsy and collection of specimens, sample storage, transport to the laboratory and subsequent storage prior to analytical testing. In antemortem toxicology settings, the time delay between an alleged offence and specimen collection may be short (e.g. minutes to hours in the case of most impaired driving cases) or long (e.g. hours to days in the case of some sexual assault cases). Following collection, antemortem specimens may be subject to similar delays due to shipping or transport of specimens, requests for testing made by the submitting agency and storage of samples prior to actual testing. Although the toxicologist must consider the time delay between the event (i.e. death, or committing or being the victim of an offence) and collection of a specimen for interpretation purposes, these delays are beyond the control of the laboratory. Measures can be taken, however, to preserve and maintain the integrity of specimens after collection. Sample quality plays an important role in the validity or usefulness of subsequent analytical determinations. Inappropriate sample preservation or storage may have a deleterious effect on qualitative and quantitative determinations.

Preservation and storage

Specimens should be stored at appropriate temperatures, with adequate preservative and in an

environment accessible only to authorised personnel to ensure security and integrity. Short-term storage at refrigerated temperature (4°C) is recommended for most samples, or frozen (−20°C or lower) during long-term storage (more than 2 weeks). Exceptions to this include hair, nails or dried blood swatches on filter paper, which can be stored at ambient temperatures.

Whereas clinical specimens are typically unpreserved, the use of a chemical preservative is often warranted in forensic specimens. Preservation of blood samples with sodium fluoride (2% w/v) is routine in most laboratories. Commercial evacuated blood collection tubes (e.g. grey-top tubes) contain sodium fluoride as the preservative and potassium oxalate as the anticoagulant. These are the preferred evacuated blood tubes for antemortem forensic toxicology casework. Inhibition of microorganisms and enzymes with sodium fluoride is important for commonly encountered analytes such as ethanol, cocaine and others. Fluoride acts as an enzyme inhibitor and helps prevent glycolysis.

Commercial blood tubes may contain a wide variety of additives (citrate, heparin, EDTA, thrombin, acid citrate dextrose mixtures, clot activator, etc.). Although these tubes are designed for a variety of clinical uses, they are not the preferred specimen containers for drug-testing purposes. Laboratories frequently encounter these blood tubes when they are submitted from a hospital setting and special care must be taken when interpreting their results (LeBeau *et al.* 2000; Toennes and Kauert 2001). If an anticoagulant is to be used, potassium oxalate is preferred rather than alternatives such as EDTA, heparin or citrate. Antioxidants such as ascorbic acid (0.25% w/v) or sodium metabisulfite (1% w/v) are sometimes used to prevent oxidative losses, but these agents have the potential to act as reducing agents towards some drugs, in particular *N*-oxide metabolites, which may be transformed into the parent drug. In a similar fashion, adjustment of specimen pH is not generally favoured routinely, because, just as some drugs are alkali labile (e.g. cocaine, 6-acetylmorphine), others are acid labile. Sodium azide (0.1% w/v) is sometimes used as a preservative and antimicrobial agent in urine samples. Sodium azide should not be used if samples are to be analysed by enzyme-linked immunosorbent assay because it can interfere with horseradish peroxidase-mediated colorimetric detection.

Although the addition of preservative should be routine for most antemortem and postmortem blood samples, an aliquot of unpreserved postmortem blood is sometimes collected. For example, fluoride preservatives should not be used if organophosphorus chemicals are suspected since this accelerates chemical degradation (Skopp and Potsch 2004). Some drugs are known to be photolabile (e.g. ergot alkaloids such as lysergic acid diethylamide and the phenothiazines). Specimens known to contain photolabile drugs should be stored in amber vials or foil-covered containers, or otherwise protected from direct sources of light. Storage of tightly sealed appropriate containers at low temperature further inhibits sample loss. Short-term storage at refrigerated (4°C) and frozen (−20°C) temperatures is commonplace in most laboratories and repeated freeze–thaw cycles should be avoided.

Labelling and specimen transfer

All samples should be properly marked for identification with the case number, donor name, date and time of collection, signature or initials of the collector and specimen description. Tamper-proof containers and/or tape bearing the collector's initials and date of collection should be used. Specimens should be forwarded to the laboratory in appropriate leak-proof and tamper-proof packaging/shipping materials with all appropriate documentation (chain-of-custody forms, requisitions for testing, special requests, case information, medications list, police report, donor information/identifier such as date of birth or social security number, agency case number, pathologist/police officer name and contact information). Improperly packaged or identified materials should be returned to the submitting agency. Documentation accompanying the specimen(s) should list all of the specimens that were collected or available for testing. Once received by the laboratory, the specimens should be inspected and appropriately documented in terms of condition and quantity during the accessioning process.

Contamination

There are a variety of contamination sources for both antemortem and postmortem specimens. In addition to the potential contamination issues that may result from the use of containers and external factors,

a number of important exogenous and endogenous sources of contamination should be considered.

Exogenous contaminants

Specimens collected into plastic containers are sometimes susceptible to phthalate interferences. Numerous plasticiser interferences such as dibutylphthalate may co-extract and interfere with analytical detection by gas-chromatographic or mass-spectrometric techniques, yielding characteristic phthalate ions. All plastic containers should be evaluated prior to widespread implementation. It should be noted that contamination from phthalates may occur during the analytical process through use of disposable pipette tips, solvent containers, solid-phase extraction cartridges, tubing and numerous other sources. However, environmental exposure to these substances from household items, food, beverages and other sources can produce detectable quantities of phthalate esters or their metabolites in biological specimens including blood, serum, urine and breast milk (Silva *et al*. 2005; Högberg *et al*. 2008).

Embalming fluids, which typically contain a variety of alcohols and aldehydes, are a potential source of contamination in postmortem casework. These fluids not only dilute any remaining fluid in the body, but also alter drug distribution in remaining tissues. Another potential source of contamination comes from reusable syringes and containers for postmortem specimen collection. Some cleaning fluids that are used for syringes may contain alcohols that can compromise the analysis of volatiles. This highlights the importance of analysing specimens from multiple sites and using disposable syringes wherever possible.

The principal concern with antemortem contamination arises from the intentional manipulation of the sample to mask the presence of drugs. This typically involves the substitution, dilution or adulteration of the biological specimen with a foreign substance. Donor manipulation occurs most frequently with urine samples in workplace drug-testing situations (see Chapter 5). As a result, specimen validity testing is required in some drug-testing programmes such as for federal employees under US Department of Health and Human Services (DHHS) guidelines (or EWDTS or UKWDT guidelines). Initially, adulteration of urine for drug-testing purposes involved the use of crude household items such as soap, bleach, vinegar, ammonia or cleaning fluids. Although these substances met with some success, a wide variety of commercial adulteration reagents and kits is now widely available (Dasgupta 2007). A summary of *in vitro* adulteration agents is provided in Table 13.4. Some of the most popular commercial products

Table 13.4 ***In vitro*** **adulteration agents**

• Ascorbic acid
• Alcohols
• Amber-13 (hydrochloric acid)
• Ammonia
• Bleach
• Clear Choice (glutaraldehyde)
• Detergent or soap (surfactant)
• Drano
• Ethylene glycol
• Gasoline
• Glutaraldehyde
• Hydrogen peroxide
• Klear (potassium nitrite)
• Lemon juice
• Liquid soap
• Lime-A-Way
• Mary Jane Super Clean 13 (surfactant)
• Salt
• Stealth (peroxide/peroxidase)
• THC-Free (hydrochloric acid)
• UrinAid (glutaraldehyde)
• Urine Luck (chromium VI, oxidant)
• Vanish
• Vinegar
• Visine
• Water
• Whizzies (sodium nitrite)

Source: Kerrigan and Goldberger (2005).

contain glutaraldehyde (fixative), pyridinium chlorochromate (PCC) or chromium(VI)-containing species (oxidant), nitrite (oxidant) or peroxide/peroxidase. In general, *in vitro* adulteration can interfere with presumptive immunoassay tests, with the intention of producing false-negative results. However, some agents have the potential to interfere with confirmatory tests such as gas chromatography–mass spectrometry (GC-MS) as well. Although this is less likely, studies have shown that some reagents may produce lower than expected or negative results for some analytes. Adulteration-detection products are available commercially. On-site or dipstick tests are available for nitrite, glutaraldehyde, pH, specific gravity, creatinine, bleach, PCC and oxidants.

Specimen dilution or *in vivo* adulteration by ingestion of a substance to mask the presence of drugs is also encountered. This is commonly achieved by the ingestion of large quantities of fluid prior to the test or by administration of a diuretic. Examples of *in vivo* adulteration agents are given in Table 13.5. Urine specimen substitution or dilution can be detected if specimen validity tests are performed. A specimen may be considered invalid if the pH is between 3 and 4.5 or between 9 and 11. It may be adulterated if the pH is less than 3 or greater than 11. The normal temperature range is 32–38°C. A specimen is considered dilute if the creatinine concentration is less than 200 mg/L and the specific gravity is less than 1.003. (Numbers differ slightly under EWDTS or UKWDT guidelines.)

Other sources of contaminants or unexpected analytes include pyrolytic breakdown products due to thermal degradation of drugs. These may be present due to pyrolysis during administration of the drug (e.g. anhydroecgonine methyl ester following crack cocaine use) or occasionally *in situ* during analysis if conditions are not properly controlled or evaluated. Other sources of contamination may arise from pharmaceutical impurities or adulterants and cutting agents that are incorporated into illicit drugs prior to sale.

Table 13.5 ***In vivo*** **adulteration agents**

Diuretics
Prescription
• Thiazides **and** thiazide-like drugs (e.g. hydrochlorothiazide, metolazone)
• Carbonic anhydrase inhibitors (e.g. acetazolamide)
• Loop diuretics (e.g. bumetanide, furosemide, torsemide)
• Osmotic diuretics (e.g. mannitol)
Over the counter (OTC)
• Aqua-Ban
• Diurex
• Fem-1
• Midol
• Pamprin
• Premsyn PMS
Other
• Alcoholic beverages
• Xanthines (e.g. caffeine, theophylline, 8-bromotheophylline)
• Herbals and aquaretics (e.g. golden seal root, juniper)

Source: Kerrigan and Goldberger (2005).

Medical artefacts

Clinical therapy can sometimes produce medical artefacts that complicate toxicological findings. Medical artefacts are most common in postmortem cases where infusion pumps may continue to run after death, introducing high concentrations of drug in local body compartments. Access to hospital records and case information, and collection of peripheral blood, vitreous fluid and liver are particularly important in these types of cases. Other sources of medical artefacts may include organ harvesting drugs such as the calcium-channel blocker verapamil, or papaverine, which is used to inhibit vasoconstriction during transplantation surgery.

If living patients are administered fluids (e.g. saline) during clinical care, blood is only contaminated (diluted) with the infusion solution if it is collected downstream from the intravenous line. Blood circulation and equilibrium with tissues

is rapid, so the administration of fluids does not usually influence drug or alcohol concentrations in blood if normal precautions are taken. If downstream collection is suspected, careful review of the medical records and/or measurement of the haematocrit to determine specimen dilution may be necessary.

Endogenous contaminants, artefacts and interferences

By their very nature, all biological specimens are subject to endogenous interferences, regardless of whether or not they are derived from living or deceased persons. More complex biological specimens such as blood, tissue or meconium will require more extensive sample preparation to remove these interferences than less complex matrices such as vitreous humour, cerebrospinal or oral fluid. In general, however, antemortem specimens are somewhat less susceptible to endogenous artefacts or contaminants. Ethanol, GHB, carbon monoxide, cyanide and other short-chain alcohols can be metabolically produced *post mortem* (Skopp 2004). The formation of toxicologically significant concentrations of cyanide in postmortem tissue (Lokan *et al*. 1987) has been attributed to the conversion of thiocyanate to cyanide and the breakdown of protein (Curry *et al*. 1967). Although in some circumstances ethanol can be produced *in situ* in unpreserved antemortem fluids, the same is true to a far greater extent in postmortem specimens, particularly blood. Likewise, GHB is present in antemortem fluids at very low concentrations in the absence of a serious genetic disorder such as GHB-uria (Knerr *et al*. 2007). Differentiation of exogenous and endogenous GHB is complicated by specimen type, storage conditions, preservative and other factors. Many laboratories use a cut-off concentration to help differentiate the two, for example 10 mg/L in urine (Kerrigan 2002; LeBeau *et al*. 2007). Concentrations of GHB may increase in urine during storage, upon collection and storage of unpreserved blood, or in citrate-buffered antemortem blood (LeBeau *et al*. 2000). Although preserved antemortem blood GHB concentrations are typically lower than those in urine, numerous studies have shown forensically significant concentrations of GHB in postmortem blood. Postmortem urine and vitreous fluid appear to be less susceptible to this increase.

Major changes that occur after death produce autolytic changes and putrefaction by microorganisms. Invasion of microorganisms, particularly from the gastrointestinal tract into tissues and body fluids, occurs within hours at ambient temperature. Lipids, carbohydrates and proteins are hydrolysed by microbial enzymes, the pH of blood steadily increases, and the putrefactive amines, tyramine, tryptamines, phenethylamines and other endogenous substances are liberated.

Trauma is a non-preventable source of contamination in postmortem forensic toxicology. Rupture of organs or compartments within the body can compromise quantitative drug analyses owing to the mixing of fluids (e.g. of gastric contents with blood) or from the microbial action that occurs as a result. Postmortem alcohol production can also result in detectable quantities of ethanol as an artefact. Glycolysis and the presence of yeasts and microorganisms can convert a variety of postmortem substrates to ethanol. Although concentrations are typically low (<0.7 g/L), concentrations of 2 g/L and higher have been reported (Zumwalt *et al*. 1982; O'Neal and Poklis 1996). Postmortem alcohol production is influenced by many factors, including the time between death and sampling, environmental conditions (temperature, humidity, location), external factors (traumatic injury, incineration), the availability of an ethanol substrate and the extent to which microorganisms are available. Vitreous humour and urine provide complementary information that may assist with the interpretation of results. This highlights the importance of collecting a variety of specimens *post mortem*. Other short-chain alcohols can be produced by microorganisms *in situ*. Isopropanol has been documented as a postmortem artefact, particularly in drowning victims. Putrefaction can also complicate carbon monoxide determination in postmortem blood. Increases in the apparent concentration of carboxyhaemoglobin have been documented owing to the formation of methaemoglobin, a decomposition product that can interfere with spectrophotometric determination. Preservation of postmortem blood (and storage in the dark at 4°C or lower) has been recommended (Skopp 2004).

Stability

Drug stability can be influenced by many factors including the physicochemical properties of the drug, characteristics of the specimen or matrix, tendency to conjugate/deconjugate, specimen collection procedure (e.g. contamination with microorganisms), container selection (e.g. oxidation, adsorption), and the use of preservatives or other additives.

The majority of published drug stability studies focus on antemortem or non-biological matrices. Scientific findings for biological specimens are complex because drug instability is often matrix dependent and influenced by factors such as specimen pH and the presence of other substances in addition to external factors. In general, drug instability in any toxicological specimen is due to metabolic degradation, chemical transformation, or a combination of both (e.g. cocaine).

Drug stability in postmortem matrices poses an added level of complexity because conditions are less controlled and degradation of analytes may be accelerated owing to putrefactive decomposition, microbial invasion and the increased presence of bacteria, for example, the bacterial enzymatic conversion of morphine glucuronides to free morphine in blood (Carroll *et al*. 2000), and the bioconversion of nitrobenzenes by enteric bacteria (Robertson and Drummer 1998).

After a specimen has been collected, enzymes may remain active and continue to degrade or transform the drug *in vitro*. This process may take place *post mortem* inside the body, or after postmortem or antemortem specimens have been collected, during transportation to the laboratory and during storage. This is particularly important with esterases, which may further hydrolyse drugs post-collection unless they are inhibited by a preservative.

In general, drug instability arises as a result of moieties or functional groups that are susceptible to transformation, such as esters (e.g. 6-acetylmorphine, cocaine, acetylsalicylic acid), sulfur-containing drugs, photolabile drugs (e.g. phenothiazines, midazolam, lysergic acid diethylamide) or those with functional groups that are readily oxidized or reduced. Although instability typically leads to decreases in drug concentration, this is not always the case. Conjugated drugs, such as the glucuronides may deconjugate under some conditions, increasing the concentration of free drug. Depending on the collection, storage conditions, use of preservative, container type, matrix and other factors, the concentration of a drug at the time of assay may not be identical to the concentration at the time of collection. All toxicological results should be interpreted within this context.

Some drugs also exhibit a degree of thermal instability. This is a consideration for drugs that are subjected to elevated temperatures during administration (e.g. by smoking) and during analysis (e.g. by GC-MS). Pyrolysis products may be indicative of smoking if it can be shown that they are not produced during analysis, for example: anhydroecgonine methyl ester (AEME) following the use of crack cocaine; 1-phenyl-cyclohexene following phencyclidine (PCP) use; and transphenyl propene following methamfetamine use.

Ideally, drug stability should be evaluated in a number of ways: long-term stability in the specimen or matrix of interest; the effect of freeze–thaw cycles; short-term stability (typically refrigerated); and bench-top (room temperature) stability. The kinetic variables governing instability are often matrix and temperature dependent and an understanding of any one of these (e.g. long-term stability of a particular drug in blood) does not necessarily imply predictable results under different conditions (i.e. short-term storage of the drug in urine).

It should be noted that, although there are many published studies and reviews of drug stability, these tend to focus on long- or short-term storage in common matrices such as blood, urine, serum or plasma. As the number of drugs of interest continues to grow and the variety of specimens becomes more diverse, the scientific literature becomes somewhat limited in terms of drug stability. The stability of drugs in non-traditional and non-biological matrices is beyond the scope of this discussion, but investigations in this area are ongoing. These include studies of the stability drugs in dry stains of biological origin (DuBey and Caplan 1996), in formaldehyde solutions following embalming (Tracy *et al*. 2001), and in hair fibres after exposure to cosmetic treatment such as bleaching, perming or straightening (Pötsch and Skopp 1996). Drug stability data are often confounded by the fact that a considerable number of studies report instability as an incidental or anecdotal finding, rather than as part of a formalised and well-designed investigation

of stability. Guidelines for conducting stability experiments and the statistical evaluation of the results have been reviewed (Peters 2007).

Of all of the most commonly encountered drugs, cocaine is certainly the most notorious in terms of instability. Nevertheless, the vast majority of drugs are relatively stable or moderately so to the extent that one can make reasonable assumptions when interpreting the results. Stability for some of the most frequently encountered drugs is summarised below.

Amfetamines

Methamfetamine and amfetamine in urine samples preserved with sodium fluoride (1% w/v) were stable during long-term storage at −20°C for at least a year (Moody *et al*. 1999). A long-term study of whole blood stored over 5 years at room temperature showed erratic but significant decreases in methamfetamine between 3 months and 5 years of storage, ranging from 9% to 38% (Giorgi and Meeker 1995). Results suggested that amfetamine was perhaps less stable than methamfetamine. Samples were collected into 10-mL grey-top Vacutainer tubes with sodium fluoride (100 mg) and potassium oxalate (20 mg). Although both drugs are considered to be moderately stable, storage of blood samples at room temperature is not advised owing to the production of interfering substances.

The overall stability of the amfetamine class extends to many of the designer amfetamines, for example, 3,4-methylenedioxymethamfetamine (MDMA), 3,4-methylenedioxyamfetamine (MDA) and 3,4-methylenedioxyethylamfetamine (MDEA). Stability of these drugs was investigated in urine, blood and water for 21 weeks at −20°C, 4°C and 20°C (Clauwaert *et al*. 2001). Although all drugs were stable at −20°C, results were compromised in blood samples at 5 and 13 weeks when stored at 20°C and 4°C, respectively, owing to matrix degradation and interfering substances.

Cannabinoids

Decreases in cannabinoid concentrations in refrigerated and frozen samples are largely attributed to oxidative losses, temperature effects or lipophilic binding to containers. THC may decompose when exposed to air, heat or light. It can undergo hydrolysis to cannabidiol, or be oxidised to cannabinol as a result of exposure to air or acidic conditions. THC has been reported to be stable in refrigerated blood for 6 months and at room temperature for 2 months (Johnson *et al*. 1984). Binding of THC to hydrophilic surfaces, such as storage containers or rubber stoppers should be considered. For example, THC stored in blood collected in unsilanised glass tubes was stable for 4 days at room temperature and 4 weeks at −20°C. By comparison, similar samples stored in polystyrene tubes showed 60–100% decreases in concentration (Christophersen 1986). The principal metabolite shows greater stability than the parent drug. In one study, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) was stable in frozen urine preserved with sodium fluoride (1% w/v) for a year (Moody *et al*. 1999). However, this study used silanised glassware for urine sample storage, which may not be typical of storage containers for actual casework. Loss of THCA in urine is largely attributed to adsorption, or from foaming of the sample, which can account for losses as high as 89% (Dextraze *et al*. 1989). The use of a de-foaming agent (e.g. 2-octanol) can reverse these losses, but such use is not routine. It has been suggested that, although the adsorptive losses of THCA from urine stored in plastic containers take place quickly (within an hour of collection), they are not significant enough to compromise the analysis (Stout *et al*. 2000). Furthermore, adsorptive losses of THCA in urine may be pH dependent, with greater losses occurring at acidic pH (Jamerson *et al*. 2005).

Deconjugation of cannabinoids, such as THCA-glucuronide, to THCA should also be considered (Skopp and Potsch 2004). Hydrolysis of the acyl-glucuronide results in increased concentration of the unconjugated metabolite. THCA-glucuronide was unstable in urine at 4°C and above, and at increasing urinary pH (Skopp 2004). Studies suggest that THCA-glucuronide is less stable in plasma than in urine (Skopp and Potsch 2002). Although no significant losses in THCA-glucuronide were seen in plasma or urine stored at −20°C, instability was documented following storage of plasma and urine at refrigerated and room temperature, in some cases within 2 days (Skopp and Potsch 2002).

Opioids

6-Monoacetylmorphine (6-MAM) is a labile metabolite of diamorphine due to hydrolysis of the ester bonds. It may undergo deacetylation to morphine during storage. Buprenorphine, codeine, fentanyl, hydromorphone, methadone, morphine, oxycodone, oxymorphone and tramadol were all stable in frozen plasma stored for almost 3 months and subjected to two freeze–thaw cycles (Musshoff *et al*. 2006). Free concentrations of morphine, codeine and methadone were moderately stable in frozen urine preserved with sodium fluoride over a year (Moody *et al*. 1999). However, total morphine concentrations under similar conditions may be less stable (Moriya and Hashimoto 1997). Long-term storage in preserved whole blood stored at room temperature showed significant increases and decreases (Giorgi and Meeker 1995) over 1 to 5 years. These results suggest important differences in stability between free and conjugated species.

The stability of glucuronidated morphine is of importance because ratios of free and total morphine are sometimes used for interpretive purposes. Marked differences in glucuronide stability exist between antemortem and postmortem blood. Morphine-3-glucuronide was stable in refrigerated antemortem blood preserved with sodium fluoride, but unstable in postmortem blood under the same conditions (Carroll *et al*. 2000). Hydrolysis of the glucuronidated species to free morphine increases with temperature, storage time and degree of putrefaction. Other studies have confirmed the stability of morphine-3-glucuronide in refrigerated antemortem blood and plasma for up to 6 months (Skopp *et al*. 2001). Storage of postmortem specimens at −20°C prevented *in vitro* hydrolysis of the glucuronide.

Phencyclidine

Studies suggest that PCP is a relatively stable drug, even when stored in blood at room temperature for up to 18 months (Levine *et al*. 1983). However, significant decreases in concentration were measured in preserved blood at room temperature over 5 years (Giorgi and Meeker 1995).

Cocaine

Of all of the common drugs of abuse, cocaine is certainly notorious in terms of stability. Both chemical and enzymatic transformations occur to produce hydrolytic products. Spontaneous conversion of cocaine to benzoylecgonine (BE) via the ester linkage occurs at physiological and alkaline pH. At pH 5, there were no measurable decreases in cocaine concentration at 40°C after 21 days, compared with a decrease of 40 to 70% in urine at pH 8 (Baselt 1983). Ester linkages have a tendency to be alkali labile, and as a result the chemical transformation of cocaine to BE is increasingly favourable as the pH of the matrix increases. Although the addition of preservative does not prevent chemical hydrolysis, the kinetics can be inhibited by storage of samples at low temperature, or by pH adjustment. The latter is not favoured in routine casework because of the possibility that acid-labile drugs might also be present. Liver methylesterases are largely responsible for the enzymatic transformation of cocaine to BE and plasma pseudocholinesterase for the conversion of cocaine to ecgonine methyl ester (EME). Addition of a cholinesterase inhibitor such as sodium fluoride and reduced temperature are important precautions. Both BE and EME undergo further transformation to ecgonine, a polar metabolite. In unpreserved blood, hydrolysis of the phenyl ester predominates, yielding EME. Addition of sodium fluoride inhibits the production of EME but does not prevent chemical hydrolysis of cocaine to BE. Cocaine in blood stored at 4°C was undetectable within 3 days in the absence of preservative, and only 40 to 60% of the cocaine was detected in preserved blood and plasma after 21 days (Baselt 1983). There have been numerous studies on the stability of cocaine and its metabolites in various media, and comprehensive stability studies are available (Isenschmid *et al*. 1989). In unpreserved blood, cocaine is hydrolysed to EME, whereas transformation to BE predominates in preserved blood. Although BE exhibits greater stability than cocaine, decreases in concentration are largely due to further hydrolysis to ecgonine. BE was shown to be stable in preserved urine for a period of at least 1 year when stored at −20°C (Moody *et al*. 1999).

Chemical hydrolysis of cocaine during analysis should also be considered. Liquid–liquid and solid-phase extractions of cocaine and metabolites from biological specimens routinely employ alkaline conditions during extraction or elution steps. These conditions may result in chemical hydrolysis of cocaine to BE as an artefact of analysis. Minimising the duration of exposure and appropriate use of deuterated internal standards is recommended.

Antidepressants

Studies in serum have shown the tricyclic antidepressants to be moderately stable. Amitriptyline, imipramine, clomipramine and doxepin are relatively stable in serum samples stored at −25°C for 3 to 6 months or at 4°C for 7 days (Rao *et al*. 1994). Newer antidepressants, including reboxetine, sertraline and venlafaxine, were also stable in frozen plasma. Significant decreases in concentration were seen for sertraline, desmethylsertraline and reboxetine when stored at room temperature for more than 7 days (Heller *et al*. 2004). Atomoxetine, citalopram, fluoxetine, mirtazepine and paroxetine have also shown moderate long-term stability when frozen (Peters 2007).

Neuroleptics

Among the newer atypical neuroleptics, quetiapine and olanzapine have been shown to exhibit significant instability at room temperature (Heller *et al*. 2004). Although both were stable for a few days, quetiapine concentrations decreased by as much as 50% after 14 days and olanzapine was undetected in some samples. However, the majority of neuroleptic drugs, including olanzapine and quetiapine, were stable in frozen plasma at −20°C for 1 month after three freeze–thaw cycles (Kratzsch *et al*. 2003). Phenothiazines exhibit a pH-dependent photosensitivity. Furthermore, increases in chlorpromazine and thioridazine concentrations in patient samples have been documented and may be attributed to a conversion of the metabolite back to the parent drug during storage (Davis *et al*. 1977; Holmgren *et al*. 2004).

Benzodiazepines

Benzodiazepines including alprazolam, chlordiazepoxide, clonazepam, diazepam, flunitrazepam, flurazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, temazepam and triazolam were stable in plasma for 1 month at −20°C (Kratzsch *et al*. 2004). Diazepam was found to be stable in blood stored at room temperature or refrigerated over a period of 5 months (Levine *et al*. 1983). In contrast, diazepam and temazepam were unstable in postmortem blood under putrefying conditions. In general, benzodiazepines with a nitro group (e.g. clonazepam, nitrazepam, flunitrazepam) are among the most unstable owing to reduction of the nitro group. Additives that inhibit reduction (e.g. 2% w/v sodium metabisulfite) slow the degradation. Postmortem conversion of nitrobenzodiazepines to their respective 7-amino breakdown products may also occur as a result of anaerobic bacterial action (Robertson and Drummer 1995, 1998). Chlordiazepoxide, which contains an *N*-oxide functionality, is also unstable in whole blood. At room temperature, chlordiazepoxide rapidly decreased and was undetectable by day 8 (Levine *et al*. 1983). Sodium fluoride inhibits the degradation of chlordiazepoxide to nordiazepam and demoxepam, but does not completely prevent it. Storage at low temperatures, preferably frozen, is recommended (Drummer and Gerostamoulos 2002; Peters 2007).

Lysergide

Lysergide (LSD) is photolabile, and specimens suspected of containing LSD should be protected from the light. Decreases in drug concentration have been documented in blood, serum and urine, with and without sodium fluoride. In one study, however, LSD concentrations in urine were stable for 4 weeks at room and refrigerated temperatures (Francom *et al*. 1988).

Ethanol

During storage, ethanol concentrations may increase or decrease. Ethanol losses are largely attributed to evaporation, chemical oxidation and microbial

consumption, whereas increases are largely due to microbial conversion of substrates to ethanol. Although measured increases in blood ethanol concentrations have been documented under some conditions, this is inhibited by the addition of sodium fluoride as preservative and storage at refrigerated temperatures. Ethanol was stable in fluoridated blood for 2 months at room temperature (Glendening and Waugh 1965). Even after storage for 1 to 3 years at room temperature, average decreases at room temperature were 0.4 g/L. Average losses following storage of blood at room temperature for 3 and 6.75 years were 0.19 and 0.33 g/L, respectively (Chang *et al*. 1984). In one study, loss of ethanol was evident in blood contaminated by *Pseudomonas*. Although this was not prevented by 1% sodium fluoride, increasing the quantity of preservative to 2% did prevent ethanol loss (Dick and Stone 1987).

Urine is less susceptible than blood to *in vitro* ethanol production except in rare instances. Urine samples treated with microorganisms known to produce ethanol did not produce increases in ethanol concentration greater than 0.2 g/L, even following incubation at 37°C (Blackmore 1968). The use of preservative and refrigeration of urine samples is effective in terms of maintaining ethanol stability. Exceptions have been noted, but are rare. For example, a dramatic increase in ethanol concentration was documented in the urine from a diabetic patient found to contain *Candida albicans*. The increase in ethanol concentration was also accompanied by a significant decrease in glucose concentration (Ball and Lichtenwalner 1979).

Gamma-Hydroxybutyric acid

In addition to GHB being present in a variety of biological specimens as an endogenous substance, *in situ* production of GHB during storage has been documented and widely studied. In general, increases in GHB concentration are more pronounced in postmortem specimens. The concentration of GHB in an unpreserved postmortem blood sample stored under refrigerated conditions for 4 months approached 100 mg/L. GHB increases in postmortem urine are less pronounced and typically an order of magnitude lower, even in the absence of preservative (Berankova *et al*. 2006). Antemortem samples are much less susceptible to *in situ* production over time (Kerrigan 2002; LeBeau *et al*. 2007). Nevertheless, storage at refrigerated temperature, use of sodium fluoride as preservative and analysis at the earliest possible interval are recommended wherever possible.

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