Anal Bioanal Chem (2007) 388:1495–1503
DOI 10.1007/s00216-007-1238-7

REVIEW

Requirements for bioanalytical procedures in postmortem toxicology

Olaf H. Drummer

Received: 18 January 2007 / Revised: 25 February 2007 / Accepted: 1 March 2007 / Published online: 23 March 2007

Abstract The application of analytical techniques in postmortem toxicology is often more difficult than in other forms of forensic toxicology owing to the variable and often degraded nature of the specimens and the diverse range of specimens available for analysis. Consequently, analysts must ensure that all methods are fully validated for the particular postmortem specimen(s) used. Collection of specimens must be standardized to minimize site-to-site variability and should if available include a peripheral blood sample and at least one other specimen. Urine and vitreous humor are good specimens to complement blood. In some circumstances solid tissues such as liver are recommended as well as gastric contents. Substance-screening techniques are the most important element since they will determine the range of substances that were targeted in the investigation and provide initial indication of the possible role of substances in the death. While immunoassay techniques are still commonly used for the most common drugs-of-abuse, chromatographic screening methods are required for general unknown testing. These are still predominately gas chromatography (GC) based using nitrogen/phosphorous detection and/or mass spectrometry (MS) detection, although some laboratories are now using time-of-flight MS or liquid chromatography (LC)–MS(MS) to cover a sometimes more limited range of substances. It is recommended that laboratories include a second chromatographic method to provide coverage of acidic and other substances not readily covered by a GC-based screen when extracts do not include all physiochemical types. This may include a gradient high-performance liquid chromatography (HPLC) photodiode array method, or better LC-MS(MS). Substance-specific techniques (e.g., benzodiazepines, opiates) providing a second form of identification (confirmation) are now divided between GC-MS(MS) and LC-MS(MS) procedures. LC-MS(MS) has taken over from many methods for the more polar compounds previously used in HPLC or in GC methods requiring derivatization. Analysts using LC-MS will need to obtain clean extracts to avoid poor and variable sensitivity caused by background suppression of the signal. Isolation techniques in postmortem toxicology tend to favor liquid extraction; however solid-phase extraction and solid-phase microextraction methods are available for many analytes.

Keywords Postmortem toxicology · Bioanalytical methods · Minimum standards · Artifacts · Systematic toxicological analysis · Initial testing

O. H. Drummer (✉)
Victorian Institute of Forensic Medicine,
Department of Forensic Medicine, Monash University,
57–83 Kavanagh Street,
Southbank, VIC 3006, Australia
e-mail: olaf@vifm.org

Introduction

The postmortem environment produces a number of additional challenges to the forensic analyst. The specimens are often less than ideal and may even be substantially degraded or nonexistent. Moreover, it is expected that analysts produce results that can be of use in the case investigation since many of these cases are of forensic interest and may involve criminal prosecutions. Subsequently, it is expected that any bioanalytical procedures and results that stem from these investigations can meet the rigor of court cross-examination and medicolegal scrutiny.

The application of robust and properly validated analytical methods is just as important in postmortem toxicology

as it is in other branches of the science. The application of the most appropriate procedures will provide the basis for the most correct interpretation of toxicology findings.

The term "bioanalytical procedures" covers both the appropriate selection of the sample and the application of appropriate analytical methods to achieve the intended purpose of the investigation.

In this paper, important considerations in the selection of the sample or specimen and in the selection and application of appropriate analytical methods will be reviewed. The focus will be on methods published in the last 10 years. This information will hopefully assist the forensic toxicologist in providing the most reliable and useful information to their clients.

Purpose of conducting postmortem toxicological analysis

The purpose of conducting toxicological analysis in decedents will of course vary from case to case and will depend on the policy of the jurisdiction. In some case types it may only be appropriate to test for a specific drug. For example, in the death of an elderly person in a nursing home on digoxin when the cause of death is likely to be associated with the consequences of heart failure it may only be necessary to check the digoxin concentration to ensure no component of the death was due to the treatment. However, in another context the death of an elderly person where there is no medical details of treatment or the person's health in the days to weeks leading up to death it will be appropriate to perform (amongst other things, e.g., an autopsy) a comprehensive toxicological evaluation to assess the role if any of drugs or poisons.

A psychoactive drug screen would include ethanol (alcohol), common drugs-of-abuse (e.g., amphetamines, benzodiazepines, cocaine, cannabis, and opiates), and a range of other substances, including anticonvulsants, antidepressants, antipsychotics (neuroleptics), sedating antihistamines, and a number of other opioid drugs, such as methadone and meperidine (pethidine). In some jurisdictions variations of these drugs-of-abuse classes might occur owing to local usage, e.g., γ-hydroxybutyrate (GHB), phencyclidine, fentanyl, and muscle relaxants (e.g., carisoprodol).

A comprehensive drug screen should include the psychoactive drugs of interest to the jurisdiction but should also include a range of less common psychoactive drugs and other substances likely to exhibit behavioral changes in a person, or even be toxic and therefore be potentially fatal. These substances might include GHB (and its precursors), acetaminophen (paracetamol), diuretics, cardiovascular drugs (at least some), barbiturates, and antidiabetic drugs. Testing might include other therapeutic substances, volatile substances and some heavy metals, depending on the type of case and possible risk. In some circles this is called systematic toxicological analysis (STA) or general unknown screening (GUS) and ensures as far as it is possible that a series of tests have been conducted to cover a large range of possible substances.

Whatever policy is applied in a particular jurisdiction it should consider that whenever possible a reasonably comprehensive toxicological analysis should be conducted on most case types since this provides a greater assurance that no relevant substance has been missed. The desire to conduct no toxicological analysis or very limited toxicological analysis based on what substances the person was known to consume will very frequently miss relevant substances. This can lead to misinterpretations and even miscarriages of justice.

Selection of sample

In postmortem toxicology the selection of the most appropriate specimens is far more important than in other branches of forensic toxicology [1].

Wherever possible blood is the preferred specimen and allows concentrations to be compared with clinical and pharmacokinetic data. Importantly, blood does need to be taken from a peripheral site to avoid excessive postmortem changes due to redistribution. Reviews illustrating the extent of changes for drugs can be found in [1–4]. It should be recognized that some changes in drug concentration will occur for all drugs even when blood is taken from a peripheral site, particularly for lipid-soluble drugs. Moreover, in cases of putrefaction further changes are likely owing to changes in the composition of the blood, instability of the substance, and diffusion of other fluids from neighboring sites [5].

For these reasons it is never necessary to quote drug concentrations to any degree of accuracy, e.g., three significant figures; one or two significant figures is sufficient. However, in order to properly interpret blood concentrations in a postmortem specimen it is preferable to quantify the substance in another specimen. The specimen will depend on the availability of the specimen and the substances present in the case [6].

In the case of ethanol, vitreous humor is the preferred second specimen. Quantitative analysis of ethanol in blood and vitreous humor enables (in most cases) a proper interpretation of the presence of alcohol in the decedent [7]. Vitreous humor has not been used routinely for analysis of other drugs owing to the more complex distribution kinetics although all substances present in blood are also present in vitreous humor [6, 8].

Table 1 Selected bioanalytical screening procedures

Specimen	Drug/drug classes	Details of isolation and separation method	Details of detection method and comments	Reference
Blood	Acidic/neutral drug screen	Single step extraction, dual-column (NB-54, NB-1701) GC	Drugs detected by NPD; identified by their cubic spline retention indices using multidetector retention index standards and Micman software	[30]
Blood	All drug types (n=225)[a]	Ethyl acetate extraction of blood at pH 4.6. HPLC gradient using Superspher 10 RP-18 column and acetonitrile/triethyl ammonium phosphate buffer, pH 3.0	Retention indices measured using 1-nitroalkane scale	[47]
Blood	Acidic/neutral drug screen	HPLC gradient; acetonitrile precipitation. C18 column with acetonitrile/phosphate gradient	Detection by DAD; for barbiturates, anticonvulsants, diuretics, nonsteroidal anti-inflammatory drugs, sulfonylurea antidiabetic drugs, theophylline, and analgesic drugs	[86]
Blood	Basic drugs	Dual-column GC (HP-5 and DB-17 columns); extraction with butyl acetate	NPD detection. Absolute retention time, the relative retention time related to dibenzepin, and the internal retention index based on the alkylfluoroaniline series with retention time locking collectively showed CV<0.1%	[87]
Blood	All drug types[a]	Acetone precipitation. Gradient HPLC with ammonium formate/formic acid in methanol	Use of Q-trap and MRM to identify drug. Compared results with those from GC-MS and found 100% concordance	[12]
Blood	Basic drugs	Extraction with diethyl ether followed by back-extraction into 0.1 M HCl	Saturn GC-MS ion trap in full scan mode, DB-5 capillary column	[88]
Serum	All drug types[a]	SPE extraction using Extrelut NT1 columns—2 extracts for acidic and neutral drugs were extracted with DCM and combined. HPLC on Xterra MS C18 column using a linear gradient of acetonitrile/formate pH3.0	API 100 single quadrupole LC-MS with ESI, results compared to LLE and HPLC-DAD.	[10]
Plasma	All drug types[a] (n=301)	LLE (basic/neutral and acidic extracts) [89] or SPE extraction [90, 91]. Synergi Polar RP and gradient with formate/formic acid and acetonitrile	Use of Qtrap with MRM and EPI and library matching	[39]
Blood (and liver)	Basic/neutral drug screen	GC method with BP-5 column. Butyl chloride extraction from pH 9	NPD detector; can be used with split for dual NPD/MS detection of one injection	[29]
Blood and plasma	Basic drug screen	SPE Bond Elut-Certify extraction using GC	NPD detection. Urine can also be used with a 20% acetonitrile cleanup step	[92]

Table 1 (continued)

Specimen	Drug/drug classes	Details of isolation and separation method	Details of detection method and comments	Reference
Blood and plasma	Basic/neutral drug screen (n=311)	HPLC using pH 9.5 using chloroform/2-isopropyl alcohol/heptane (60:14:26, v/v/v), on a NovaPak C18 column (methanol/THF/pH 2.6 phosphate buffer (65:5:30, v/v/v)	Detection by DAD, retention times and automatic UV spectra matching.	[49]
Blood and urine	All drug types[a]	SPE extraction on Oasis HLB cartridge; analysis with hybrid triple–quadrupole linear ion-trap LC-MS(MS) (Qtrap). HPLC using Xterra MS C18 column and linear gradient using pH 3.0 formic acid/formate and acetonitrile	Enhanced MS mode with dynamic subtraction of background noise and a dependent scan obtained in the enhanced product ion scan mode	[44]
Urine (2 mL)	Basic drugs	Mixed-mode SPE extraction, dual-plate over OPLC & UV spectra	Good for polar compounds, compared with GC-MS screening	[40]
Blood, plasma and urine	All drug types[a]	SPE differential extraction based on the method in Chen et al. [90] and derivatized with MSTFA/TMCS	GC-MS on Ultra HP-5 capillary column, semiautomated processing of data	[41]
Urine	Basic drugs	In-line extraction of urine with StrataX-CW and separation on a ternary system with 2 analytical columns (LunaSCX 150 mm×4.6-mm inner diameter, 5 μm) with DAD detection	Validation data provided for codeine, EDDP, MDA, morphine and scopolamine	[42]
Liver	All drug types	Homogenized liver with subtilisin digestion is subject to SPE (Bond-Elut Certify) and acid/base fractions differentially eluted. GC on an HP-1 column	Detection by NPD and FID	[93]
Liver	Basic drugs	LLE with butyl acetate after drying and filtration step with separation on a deactivated SE-52 capillary column	Detection by NPD	[94]
Gastric contents	Drugs with UV or fluorescence detectability (n=340)	HPLC gradient analysis, following dilution with 0.01 M HCl	DAD and florescence detection. Used in conjunction with GC-MS	[95]

Bioanalytical screening procedures primarily for postmortem specimens but also include some other selected methods

DAD photodiode array detector, *DCM* dichloromethane, *EDDP* 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene (methadone metabolite), *EPI* enhanced product ion, *ESI* electrospray ionization, *FID* flame ionization detection, *GC* gas chromatography, *HPLC* high-performance liquid chromatography *LLE* liquid–liquid extraction, *MDA* methylenedioxyamphetamine, *MRM* multiple reaction monitoring, *MS* mass spectrometry, MSTFA *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, *NPD* nitrogen/phosphorous detection, *OPLC* overpressured (thin) layer chromatography, *SPE* solid-phase extraction, *THF* tetrahydrofuran, *TMCS* trimethylchlorosilane

[a] Acidic, neutral, and basic drugs

Table 2 Essential elements of validated methods used in postmortem toxicology

Validation requirement	Anticipated weakness
Lower limit of detection	False identifications or missed analytes
Limit of quantitation	Poor accuracy at low concentrations
Upper limit of quantitation	Poor accuracy at high concentrations
Calibration and linearity	Poor accuracy generally
Recovery	Poor accuracy or undetected analytes
Selectivity	False identifications or missed analytes
Precision	Imprecision of results
Accuracy or bias	Poor accuracy
Stability of analytes	Poor accuracy or missed analytes

Liver has been one of the most common second specimens. Most case reports illustrating toxicology findings will include concentrations of the substance(s) in liver. This solid specimen is the major metabolic organ and is reasonably homogeneous and importantly can be readily homogenized to provide a useful medium for extraction techniques. A variety of homogenization techniques are available, but most simply a section of tissue is diced and homogenized with a mechanical device with 1–5 vol of water or dilute buffer. The use of a proteolytic enzyme (e.g., subtilisin) to remove fibrous tissue can improve homogeneity of the final fluid and increase recovery of drugs [9].

Urine is widely used in clinical toxicology for drug screening and indeed is also used in postmortem toxicology. When it is available in cases it can be quite useful to supplement blood-based screening procedures. Indeed many laboratories conduct drugs-of-abuse screening using immunological methods on this specimen in addition to blood-based or liver-based drug screening.

On occasions analysis of gastric contents (and other intestinal tissues) can be useful to determine if oral ingestion occurred within hours of a death, and if so to determine the remaining drug contents. While the results are not always conclusive, the presence of a large amount of a chemical substance in the gastric contents is pivotal in understanding the significance of a blood concentration.

Since resampling is rarely possible it is recommended to collect as a minimum set of specimens for postmortem toxicology two blood samples (at least one that is peripheral), vitreous humor, urine, section of liver, hair, and gastric contents. In some cases not all of these specimens are available owing to the state of the body and in some situations other specimens may be needed; hence, it is recommended that medical examiners/pathologists, and if necessary the police investigators or judicial authority, discuss the case with toxicologists in advance of collection to ensure the most appropriate specimens are collected.

Isolation techniques

The isolation of substances from postmortem matrices is in general more difficult than that from clinical specimens primarily owing to the range of specimens encountered and the inferior quality of many specimens received in the laboratory resulting from putrefaction or trauma of the body during the death process, or both. For example, it is almost impossible to obtain serum or plasma because of postmortem hemolysis; hence, whole blood is the most common specimen. Since blood is more viscous than serum/plasma, solid-phase extraction (SPE) techniques are often too difficult unless they are used for some forms of drug analyses in urine or vitreous humor.

Some published procedures adopt extraction methods that enable acidic, neutral, and basic drugs to be present in one chromatograph. This can occur most economically by combining two SPE-based eluates [10] or using polar solvents such as ethyl acetate at pH 4.6 [11] or acetone precipitation [12]. Clearly chromatography of separate extracts can also be performed, but this lengthens the analysis time for the case (Table 1).

Solid-phase microextraction has been used for the analysis of a number of specific substances; however, its application for GUS is limited since the absorption of each substance onto fibers needs to be optimized [13, 14]. Developments such as in-tube extraction or single-drop microextraction have allowed some exciting applications to be developed for specific substances [13, 15].

Solvent-extraction techniques for blood and urine vary substantially between publications. In essence the choice of solvent is determined by its selectivity or ability to have reasonable solubility for target drugs but not to be too polar and also allow extraneous or endogenous material to be also extracted. Safety and environmental concerns preclude use of diethyl ether, chloroform, and toluene. Useful solvents include 1-chlorobutane, hexane/isoamyl alcohol (98:2), butyl acetate, and dichloromethane/isopropyl alcohol/ethyl acetate (1:1:3) [16].

Extractive methylation has been successfully used for acidic compounds such as diuretics and uricosurics, stimulant laxatives and/or their metabolites, and nonsteroidal anti-inflammatory drugs [17–19], as well as for buprenorphine and metabolites [20].

Selection of analytical methods

The modern analyst has a wide array of techniques and procedures available to conduct toxicological investigations. Notwithstanding the issue of specimen type and quality discussed earlier, the analyst will first of all determine what form of testing is required. Is a compre-

hensive screen required or can some more limited form of toxicological analysis be conducted? As discussed earlier, most often the answer to this question should be to conduct a comprehensive toxicological investigation. This needs to cover as many substances as reasonably possible while still taking into consideration the circumstances of the case, including any substances thought to have been used by the decedent.

Analytical methods can be broadly classified into three types of procedures: (1) screening procedures, (2) confirmation procedures, and (3) specific methods for an analyte or for a narrow range of analytes.

Screening procedures

This is the most important part of the toxicological analysis since what is done defines the extent to which analytes are sought and detected. Procedures that are deficient owing to insufficient sensitivity or coverage of substances will devalue the toxicological investigations. While there is no one ideal method it is essential that more than one method is conducted to allow a sufficient coverage of substances.

Often a first step is a test for the presence of ethanol, ideally in blood. It is recommended that a gas chromatography (GC) method is adopted since it allows concomitant detection of other simple volatiles, including acetone and methanol [7]. Acetone is a product of metabolic disease, including diabetic ketoacidosis. Other volatile substances can be included if deemed necessary using similar GC methods [21].

Immunoassay screens for common drugs-of-abuse should be included on either blood or urine. Blood-based tests involving ELISA technology are preferred for blood analyses over test kits designed for urine analysis since this combination gives higher sensitivity and is less prone to interference, but nevertheless has proven successful [22–25]. Naturally, urine kits can be used for urine screening; however, urine is not always available in postmortem cases.

Additionally a comprehensive chromatographic screen(s) for basic or basic/neutral substances is required. In postmortem cases peripheral blood is the preferred specimen for GUS since it provides the best direct evidence of substances having pharmacological actions compared with urine. Various methods have been published for blood screening using GC–nitrogen/phosphorous detection(NPD), some of which are listed in Table 1. Naturally, GC–mass spectrometry (MS) is widely used [26–28]. The use of GC-NPD in combination with MS is a powerful technique and offers the advantage of nitrogen-selective detection on a capillary column with MS detection [29]. The use of automated procedures using retention indices reduces the time spent on postchromatographic analysis and improves turnaround times [30]. Furthermore, the use of two columns of different polarity from one extract improves the identification power if MS is not available [30, 31]. Reviews on the various merits of chromatographic methods are also available [16, 32–36].

Urine is used by some groups, and like clinical toxicology applications, can provide a reasonable specimen to determine prior exposure to foreign substances [37–42]. However, it is not recommended to use urine alone, but rather blood or liver plus urine if it is to be included in the analyses conducted. The use of GC-MS screening in blood combined with fully automated high-performance liquid chromatography (HPLC) methods such as Remedi™ on urine has also been shown to be quite effective in screening for a large number of possible analytes [43].

While it is more difficult to conduct GUS with liquid chromatography (LC)–MS than with GC-MS it has been successfully applied to a large range of compounds of diverse structure [10, 37, 44]. This method is further enhanced using sophisticated library searching algorithms or macros based on MS/MS data and has been shown to detect and identify large numbers of drugs in one single LC-MS/MS run [37, 39, 45]. The application of MS/MS techniques using GC or HPLC, or use of negative ion chemical ionization, affords greater sensitivity (and selectivity) but may limit the range of substances detected if these methods are used alone [46]. Nevertheless it does afford better detection of polar compounds and better analysis of macromolecules than GC-MS or other more traditional techniques (Table 1).

Photodiode or multiwavelength UV detection (DAD) coupled to HPLC has been widely used to detect acidic and neutral compounds, and even basic compounds, and is a useful adjunct to a GC-MS-based screening method (Table 1) [42, 47–53]. These acidic compounds include many diuretics, nonsteroidal anti-inflammatory drugs, antidiabetic drugs, barbiturates, and the benzodiazepines, as well as a number of pesticides and herbicides. Additionally, nonnarcotic analgesics such as acetaminophen and salicylate are easily detected using these HPLC techniques. Basic compounds can also be detected using HPLC-DAD and traditional extraction from basic solution as well as using in-line extraction techniques [42].

The use of fast gradient elution HPLC with DAD has been used to achieve analysis times of less than 3 min, including column reequilibration between analyses. A corrected retention index to account for day-to-day and column-to-column variations in retention time has been shown to produce a discriminating power and mean list length of 0.95 and 3.26 for a set of 47 target compounds [52, 53].

More recently, time-of-flight MS has been used successfully as a screening tool to identify drugs and drug metabolites in urine using their accurate molecular masses [38, 54].

In common practice is the use of databases to assist with the identification of drugs. A number of databases and algorithms are available for HPLC-DAD [42, 47–51, 53]. Similarly, GC-MS databases have been used successfully for many years and have formed an important basis of the identification of drugs and poisons in biological matrices (see the reviews in [16, 26–28] for details). Increasingly LC-MS databases are being used despite the greater difficulties in obtaining standard mass spectral patterns using this technique [10, 37].

Confirmation procedures

In many situations screening methods will provide confirmation of a suspected analyte, based on MS data. It is still recommended that another analysis be performed to confirm the assignment. This should use another chromatographic method to avoid repeating an earlier misidentification and ensures that sample contamination or injection port cross-contamination has not occurred. This second method may use selected ion monitoring with a deuterated internal standard if using GC-MS technology for a new extract, or it may use an entirely different procedure, e.g., LC-MS if GC-MS was previously used.

Additionally, it is advised that if results are likely to be important in the case and affect the outcome of an investigation or criminal trial, then the substance should also be detected in another specimen. This reduces issues associated with contamination of the specimen or extract of the sample and may improve the ability to interpret the concentrations [1, 4–6]. This may also include measurement of morphine conjugates with morphine (total morphine) in blood and 6-acetylmorphine in urine to ascertain the source and relevance of a morphine concentration.

Drug-specific procedures

Numerous procedures exist in the literature that detect and quantify specific drugs or classes of like drugs. It would be too difficult to review all of them here except to outline some GC-MS or LC-MS procedures for some of the more important drug classes. Recent reviews have been published [34, 35].

Blood-based methods for drug classes of most interest to forensic toxicologists engaged in death investigation include amphetamines [55, 56], antidiabetic drugs [57–59], benzodiazepines, including the non-benzodiazepine hypnotics zolpidem and zopiclone [60–62], and the neuroleptics, including antidepressants [63–67]. Procedures for anticonvulsants and a variety of narcotic analgesics also exist but tend to be more diverse owing to widely differing chemical structures of these classes [68, 69].

Increasingly chromatographic methods allowing simultaneous detection and quantitation of drugs-of-abuse are being published, mainly using LC-MS [68, 70]. This is in part driven by small sample volumes in oral fluid analysis [71, 72]. However, it is likely that this will extend to postmortem specimens when limited screens for psychoactive drugs are desired, e.g., persons involved in misadventures such as drownings and motor vehicle crashes.

Hair has also been increasingly used in postmortem analyses to assist in determining if the presence of a drug was related to longer-term exposure or if doses changed substantially in the months prior to death. Recent reviews on this topic are available [73, 74].

Limitations of analytical methods

One of the biggest and most common limitations of analytical methods used in postmortem toxicology is their lack of suitability for the complex matrix. This was discussed earlier. Simply adopting an isolation method for one specimen with another can lead to problems, such as poor recovery or interference from coextractants [75, 76].

With the advent of LC-MS as a routine analytical tool for drug analyses in biological specimens the usual criteria for identification based on GC-MS principles have changed. Typically, spectra seen with electron ionization (EI) do not occur with the various forms of LC-MS; hence, the spectral information is much more limited than with conventional EI GC-MS. While some spectral information can be obtained at high fragmentor voltages, the patterns are not consistent and vary between instruments; hence, the use of large-scale mass spectrum libraries is quite limited. This means that LC-MS does not easily lend itself to GUS or STA [77–80].

Additionally, the presence of coextractants near the substance of interest can affect formation of ionized droplets in the ionization stage, leading to a phenomenon known as ion suppression. This may reduce or even eliminate the signal due to the substance of interest. Electrospray ionization is most susceptible to this phenomenon [81, 82]. This can be reduced or even eliminated by proper cleanup procedures.

Validation of analytical methods

Validation requirements for analytical methods have been published before, with the more relevant examples for forensic toxicology listed in [83–85]. In essence, the international community through various organizations, international journals, and professional societies has reasonable concordant views that for any method to be adopted and applied in case work it must be properly

validated and shown to be an appropriate method for the intended application. Indeed laboratories seeking accreditation under the International Organization for Standardization standard 17025 or 15189 require laboratories to demonstrate such validation prior to its application in real cases.

This review will not detail the requirements; this can be found in a recent paper [83]. However, a number of features that play a dominant role in forensic toxicology are often overlooked or underplayed in the analysis of postmortem specimens. Table 2 lists the essential elements of a validated method and the expected outcome if a particular validation requirement is not complete.

The two major outcome of incomplete validation are poor quality of the quantitative result and the possibility that an analyte (e.g., drug or poison) has been misidentified or more likely even missed altogether.

Poor quantitative results may or may not affect the interpretation. This will of course depend on the result and the circumstances. An example might be an overestimation of a result for a drug with a narrow therapeutic index. What was therapeutic might now be toxic, or conversely what was toxic could now be regarded as nontoxic. A result in substantial error can be potentially misleading and lead to a misinterpretation of the results. This is clearly not desired.

Misidentifications can be corrected when the laboratory engages in a confirmation procedure subsequent to the initial test. A requirement in forensic toxicology is to confirm all drug identifications. If the initial assignment is not confirmed it has the net result of wasting valuable laboratory time, but also raises the issue of what this substance might have been.

Arguably of more importance is the possibility that a substance has been missed since its recovery or detectability in a particular specimen is so poor it will not be detected even when it is present in concentrations associated with toxicity. This will arise because the validation process has not considered the recovery of the analyte(s) when the matrix is different and/or the stability of the analyte in the matrix or drug-isolation procedure.

For example, validation was performed on blood for a range of analytes but did not consider what happens to drug recovery and stability when the blood is partially putrefied. Alternatively, validation was performed on blood as a specimen and it was assumed that the same extraction procedure will work for another specimen type. Validation will need to be conducted on all specimen types used in case work to ensure that the laboratory fully understands the limitations of the method.

It is just as important to employ properly qualified analysts who have the appropriate experience and who have been shown to be technically competent for the analytical aspects of their work. Competency is also required for the interpretation of any analytical result. Failure to use competent personnel can be lead to poor or even incorrect results. This has the potential to cause erroneous diagnoses and even miscarriages of justice.

Conclusions

The adoption of a comprehensive drug-screening approach in postmortem toxicology is preferred for many case types and should involve a combination of bioanalytical procedures to cover the widest range of substances possible. This should include a chromatographic screen for basic or basic and neutral substances together with a series of other tests for alcohol, common drugs-of-abuse, and other substances indicated in the circumstances but not covered in the screening procedures. Other chromatographic screening methods for acidic compounds are recommended and may include HPLC-DAD. LC-MS(MS) provides a very useful complementary technique to those listed earlier for targeted compounds, for confirmation, and for the analysis of acidic or polar compounds. Peripheral blood is the preferred specimen and can be used in combination with other specimens such as vitreous humor, urine, liver, and gastric contents.

References

1. Skopp G (2004) Forensic Sci Int 142:75–100
2. Barnhart FE, Bonnell HJ, Rossum KM (2001) Forensic Sci Rev 13:101–128
3. Drummer O, Forrest AR, Goldberger B, Karch SB (2004) BMJ 329:636–637
4. Drummer OH, Gerostamoulos J (2002) Ther Drug Monit 24:199–209
5. Drummer OH (2007) In: Rutty G (ed) Essentials of autopsy practice, vol 3. Springer, London
6. Flanagan RJ, Connally G (2005) Toxicol Rev 24:51–62
7. Kugelberg FC, Jones AW (2007) Forensic Sci Int 165:10–29
8. Mackey-Bojack S, Kloss J, Apple F (2000) J Anal Toxicol 24:59–65
9. The Pharmaceutical Press (2004) Clarke's isolation and identification of drugs, 3rd edn. The Pharmaceutical Press, London
10. Saint-Marcoux F, Lachatre G, Marquet P (2003) J Am Soc Mass Spectrom 14:14–22
11. Bogusz MJ (1999) J Chromatogr B 733:65–91
12. Herrin GL, McCurdy HH, Wall WH (2005) J Anal Toxicol 29:599–606
13. Dietz C, Sanz J, Camara C (2006) J Chromatogr A 1103:183–192
14. Mosaddegh MH, Richardson T, Stoddart RW, McClure J (2001) Ann Clin Biochem 38:541–547
15. Junting L, Peng C, Suzuki O (1998) Forensic Sci Int 97:93–100
16. Drummer OH (1999) J Chromatogr B 733:27–45
17. Beyer J, Peters FT, Maurer HH (2005) Ther Drug Monit 27:151–157
18. Beyer J, Bierl A, Peters FT, Maurer HH (2005) Ther Drug Monit 27:509–520
19. Maurer HH, Tauvel FX, Kraemer T (2001) J Anal Toxicol 25:237–244

20. Lisi AM, Kazlauskas R, Trout GJ (1997) J Chromatogr B 692:67–77
21. Streete PJ, Ruprah M, Ramsey JD, Flanagan RJ (1992) Analyst 117:1111–1127
22. Hino Y, Ojanpera I, Rasanen I, Vuori E (2003) Forensic Sci Int 131:148–155
23. Kroener L, Musshoff F, Madea B (2003) J Anal Toxicol 27:205–212
24. Gjerde H, Christophersen AS, Skuterud B, Klemetsen K, Morland J (1990) Forensic Sci Int 44:179–185
25. Bogusz M, Aderjan R, Schmitt G, Nadler E, Neureither B (1990) Forensic Sci Int 48:27–37
26. Maurer HH (1992) J Chromatogr 580:3–41
27. Neill GP, Davies NW, McLean S (1991) J Chromatogr 565:207–224
28. Weinmann W, Svoboda M (1998) J Anal Toxicol 22:319–328
29. Drummer OH, Horomidis S, Kourtis S, Syrjanen ML, Tippett P (1994) J Anal Toxicol 18:134–138
30. Ojanpera I, Rasanen I, Vuori E (1991) J Anal Toxicol 15:204–208
31. Fretthold D, Jones P, Sebrosky G, Sunshine I (1986) J Anal Toxicol 10:10–14
32. Maurer HH (2004) Clin Chem Lab Med 42:1310–1324
33. Maurer HH, Peters FT (2005) Ther Drug Monit 27:686–688
34. Maurer HH (1999) J Chromatogr B 733:3–25
35. Maurer HH (2005) Anal Bioanal Chem 381:110–118
36. Polettini A (1999) J Chromatogr B 733:47
37. Cheng WC, Yau TS, Wong MK, Chan LP, Mok VK (2006) Forensic Sci Int 162:95–107
38. Ojanpera S, Pelander A, Pelzing M, Krebs I, Vuori E, Ojanpera I (2006) Rapid Commun Mass Spectrom 20:1161–1167
39. Mueller CA, Weinmann W, Dresen S, Schreiber A, Gergov M (2005) Rapid Commun Mass Spectrom 19:1332–1338
40. Pelander A, Ojanpera I, Sistonen J, Rasanen I, Vuori E (2003) J Anal Toxicol 27:226–232
41. Polettini A, Groppi A, Vignali C, Montagna M (1998) J Chromatogr B 713:265–279
42. Schonberg L, Grobosch T, Lampe D, Kloft C (2006) J Chromatogr A 1134:177–185
43. Valli A, Polettini A, Papa P, Montagna M (2001) Ther Drug Monit 23:287–294
44. Sauvage FL, Saint-Marcoux F, Duretz B, Deporte D, Lachatre G, Marquet P (2006) Clin Chem 52:1735–1742
45. Dresen S, Kempf J, Weinmann W (2006) Forensic Sci Int 161:86–91
46. Maurer HH, Kraemer T, Kratzsch C, Peters FT, Weber AA (2002) Ther Drug Monit 24:117–124
47. Bogusz M, Wu M (1991) J Anal Toxicol 15:188–197
48. Maier RD, Bogusz M (1995) J Anal Toxicol 19:79–83
49. Tracqui A, Kintz P, Mangin P (1995) J Forensic Sci 40:254–262
50. Elliott SP, Hale KA (1998) J Anal Toxicol 22:279–289
51. Pragst F, Herzler M, Erxleben BT (2004) Clin Chem Lab Med 42:1325–1340
52. Stoll DR, Paek C, Carr PW (2006) J Chromatogr A 1137:153–162
53. Porter SE, Stoll DR, Paek C, Rutan SC, Carr PW (2006) J Chromatogr A 1137:163–172
54. Gergov M, Boucher B, Ojanpera I, Vuori E (2001) Rapid Commun Mass Spectrom 15:521–526
55. Peters FT, Schaefer S, Staack RF, Kraemer T, Maurer HH (2003) J Mass Spectrom 38:659–676
56. Frison G, Tedeschi L, Favretto D, Reheman A, Ferrara SD (2005) Rapid Commun Mass Spectrom 19:919–927
57. Maurer HH, Kratzsch C, Kraemer T, Peters FT, Weber AA (2002) J Chromatogr B 773:63–73
58. Hoizey G, Lamiable D, Trenque T, Robinet A, Binet L, Kaltenbach ML, Havet S, Millart H (2005) Clin Chem 51:1666–1672
59. Magni F, Marazzini L, Pereira S, Monti L, Galli Kienle M (2000) Anal Biochem 282:136–141
60. Kratzsch C, Tenberken O, Peters FT, Weber AA, Kraemer T, Maurer HH (2004) J Mass Spectrom 39:856–872
61. Laloup M, Ramirez Fernandez Mdel M, De Boeck G, Wood M, Maes V, Samyn N (2005) J Anal Toxicol 29:616–626
62. Quintela O, Cruz A, Castro A, Concheiro M, Lopez-Rivadulla M (2005) J Chromatogr B 825:63–71
63. Kratzsch C, Peters FT, Kraemer T, Weber AA, Maurer HH (2003) J Mass Spectrom 38:283–295
64. Kirchherr H, Kuhn-Velten WN (2006) J Chromatogr B 843:100–113
65. Gutteck U, Rentsch KM (2003) Clin Chem Lab Med 41:1571–1579
66. Goeringer KE, McIntyre M, Drummer OH (2003) J Anal Toxicol 27:30–35
67. Josefsson M, Kronstrand R, Andersson J, Roman M (2003) J Chromatogr B 789:151
68. Rook EJ, Hillebrand MJX, Rosing H, van Ree JM, Beijnen JH (2005) J Chromatogr B 824:213–221
69. Dams R, Benijts T, Lambert WE, De Leenheer AP (2002) J Chromatogr B 773:53
70. Dams R, Murphy CM, Choo RE, Lambert WE, De Leenheer AP, Huestis MA (2003) Anal Chem 75:798–804
71. Wood M, Laloup M, Fernandez M, Jenkins KM, Young MS, Ramaekers JG, Boeck GD, Samyn N (2005) Forensic Sci Int 150:227
72. Wylie FM, Torrance H, Anderson RA, Oliver JS (2005) Forensic Sci Int 150:191
73. Pragst F, Balikova MA (2006) Clin Chim Acta 370:17–49
74. Kintz P, Villain M, Cirimele V (2006) Ther Drug Monit 28:442–446
75. Bogusz M, Erkens M (1995) J Anal Toxicol 19:49–55
76. Bogusz M, Wijsbeek J, Franke JP, de Zeeuw RA, Gierz J (1985) J Anal Toxicol 9:49–54
77. Rivier L (2003) Anal Chim Acta 492:69–82
78. Weinmann W, Stoertzel M, Vogt S, Wendt J (2001) J Chromatogr A 926:199–209
79. Lips AGAM, Lameijer W, Fokkens RH, Nibbering NM (2001) J Chromatogr B 759:191–207
80. Hough JM, Haney CA, Voyksner RD, Bereman RD (2001) Anal Chem 72:2265–2270
81. Dams R, Huestis MA, Lambert WE, Murphy CM (2003) J Am Soc Mass Spectrom 14:1290–1294
82. Mallet CR, Lu Z, Mazzeo JR (2004) Rapid Commun Mass Spectrom R18:49–58
83. Peters FT, Drummer OH, Musshoff F (2007) Forensic Sci Int 165:216–224
84. Peters FT, Maurer HH (2002) Accred Qual Assur 7:441–449
85. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT, Yacobi A (2000) Pharm Res 17:1551–1557
86. Drummer OH, Kotsos A, McIntyre IM (1993) J Anal Toxicol 17:225–229
87. Rasanen I, Kontinen I, Nokua J, Ojanpera I, Vuori E (2003) J Chromatogr B 788:243–250
88. Paterson S, Cordero R, Burlinson S (2004) J Chromatogr B 813:323–330
89. Gergov M, Robson JN, Ojanpera I, Heinonen OP, Vuori E (2001) Forensic Sci Int 121:108
90. Chen XH, Franke JP, Wijsbeek J, de Zeeuw RA (1992) J Anal Toxicol 16:351–355
91. Muller C, Schafer P, Stortzel M, Vogt S, Weinmann W (2002) J Chromatogr B 773:47
92. Chen XH, Franke JP, Wijsbeek J, de Zeeuw RA (1994) J Anal Toxicol 18:150–153
93. Huang ZP, Chen XH, Wijsbeek J, Franke JP, de Zeeuw RA (1996) J Anal Toxicol 20:248–254
94. Eklund A, Jonsson J, Schuberth J (1983) J Anal Toxicol 7:24–28
95. Politi L, Groppi A, Polettini A, Montagna M (2004) Forensic Sci Int 141:115